CELL HYBRIDIZATION AND CELL AGGLUTINATION

I. ENHANCEMENT OF CELL HYBRIDIZATION BY LECTINS

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SUMMARY

A great increase in hybridization frequency of cultured rodent cells was obtained when conventional cell fusion using 50% polyethylene glycol (PEG) was combined with a cell agglutination produced by plant lectins. The rate of appearance of hybrid colonies was found to be correlated with the extent of cell agglutination by lectin, as well as with cell fusion induced by subsequent PEG treatment. Phytohemagglutinin (PHA), wheat germ agglutinin, Wistaria floribunda agglutinin and concanavalin A were all active; the most effective was PHA. When parental cells in a monolayer were treated with PHA followed by PEG, the resulting hybridization frequency was very low because of markedly decreased viability, whereas the same cells in suspension yielded hybrid colonies at a higher rate.

These results suggest that the enhancement of hybridization by PHA/PEG treatment was brought about by the ability of lectin to agglutinate cells.

INTRODUCTION

Polyethylene glycol (PEG) has been used widely for fusion not only of plant protoplasts (Kao & Michayluk, 1974) but also of mammalian cells (Pontecorvo, 1975). However, PEG has a drawback in that it is cytotoxic (Norwood & Zeigler, 1982). PEG at 50% is most effective in yielding hybrid cells, though the treatment concomitantly results in extensive cell damage and loss of viability. Effective techniques have been developed for obtaining PEG-induced cell fusion with moderate toxicity (Gefter, Margulies & Scharff, 1977; Kennett, 1979). Mercer & Schlegel (1979) compensated for the decrease in fusion capacity by exposing cells to phytohemagglutinin (PHA) before exposure to 44% PEG, which resulted in a reduction of cytotoxicity. The combination of PHA and PEG has proved useful for the enhancement of fusion of liposomes (Wilson, Papahadjopoulos & Taber, 1977), plasma membrane vesicles (Poste & Nicolson, 1980), micro cells (McNeil & Brown, 1980) and cytoplasts (Mercer & Schlegal, 1980) with whole cells. Eglitis (1980) has reported that formation of tetraploid blastocytes from pairs of blastomeres can be accomplished by combined treatment with PHA and PEG.

This report shows that the treatment of cultured rodent cells with several lectins, including PHA, before cell fusion by 50% PEG is effective in forming hybrid cells, as well as inducing cell fusion. It is shown that this method is best used with cell suspensions.

Key words: cell hybridization, cell agglutination, lectin.
**MATERIALS AND METHODS**

**Cells and media**

The cell lines used in this experiment were as follows: FM3A-Br (FM3A) (Nakano, 1966) and Ehrlich-NH4 (Ehrlich) (Matsuya, Takahashi & Yamane, 1978), both of which are mouse mammary ascites tumour cells; LM (TK-') C1 D (C1 D) (Kit, Dubbs, Piekarski & Hsu, 1963) (mouse fibroblast) and V79 (Chu, Brimer, Jacobson & Merrian, 1969) (Chinese hamster fibroblast). FM3A and C1 D cells are resistant to 5-bromodeoxyuridine. Ehrlich and V79 cells are resistant to 8-azaguanine or 6-thioguanine. These cells were grown routinely in Dulbecco’s modified Eagle’s medium (E medium) supplemented with 7% calf serum (E-CS medium). Hybrid cells formed were selected in E-CS medium containing hypoxanthine, aminopterin and thymidine (E-HAT medium) (Littlefield, 1964).

**Materials**

All lectins were obtained from E. Y. Laboratories except phytohemagglutinin P, which was obtained from Difco Laboratories. Lectin solution was prepared with Ca2+ - and Mg2+-free phosphate-buffered saline. Sigma PEG 1000 (average M, 950–1050) purchased from Sigma Chemical Co. and Koch-Light PEG 1000 from Koch-Light Laboratories were used. PEG solution was prepared on a weight/weight basis by adding E medium to the autoclaved PEG.

**Lectin-induced agglutination**

FM3A (1 × 10⁵) and Ehrlich (1 × 10⁵) dispersed cells were mixed, centrifuged and suspended in 0.5 ml of E medium containing 3% calf serum and various concentrations (10 μg/ml or less) of lectins in a round-bottomed tube. Calf serum was added to prevent the attachment of cells to the glass surface. The tube was incubated for 20 min at 37 °C and centrifuged again. Agglutination was assessed by microscopic examination of cell aggregates formed. The percentage of cell agglutination was determined by counting the total number of cells and the number of cells in clumps of more than one cell.

**Suspension of cells from monolayers**

Ehrlich, V79 and C1 D cells were grown in monolayers and then detached by dispersing agents in order to prepare cell suspensions. To minimize cell damage caused by the dispersing agents, cells in monolayers were first digested with 0.02% EDTA and 0.02% Pronase in phosphate-buffered saline for 20 s at room temperature, and the solution was immediately withdrawn. The monolayers were then incubated for 5 min on an ice-cold plate, followed by incubation for 3–5 min at room temperature. E medium was added to the dish and the loosened cells were suspended by pipetting gently. This procedure, based on the method of McKeehan (1977), is useful in preparing suspensions from monolayered cells with minimal cell damage.

**Fusion of cells in suspension**

In most experiments suspensions of 10⁵ FM3A and 1500 Ehrlich cells, and 10⁵ C1 D and 2 × 10⁴ V79 cells, were mixed together with lectins of varying concentrations, and the suspension was made up to 0.5 ml with E medium in a round-bottomed glass tube (12 × 100 mm). The tube was incubated at 37 °C in a 5% CO₂/air atmosphere for 10–90 min to allow cells to agglutinate: The tube was then centrifuged at 1200 rev./min for 5 min after an addition of 0.5 ml of E medium. The supernatant was removed carefully by aspiration, taking care not to disturb the cell pellet. An 0.1 ml sample of 50% PEG solution was added to the cell pellet and the tube was shaken gently for 1 min. A 1 ml sample of E medium was added gently and mixed with dilute PEG, and then a further 3 ml of E medium was added. The suspension was centrifuged and the pellet resuspended in 0.5 ml of E-HAT medium. The fused cells were then incubated for 20–24 h at 37 °C and harvested by trypsinization. The cells were plated in Petri dishes containing E-HAT medium. The dishes were fixed and stained with Giemsa after incubation for 7 days. Colonies that developed in the selective medium were confirmed to be hybrid cells by chromosome analysis.
Enhanced cell fusion by lectins

Fusion of cells in monolayer

Dispersed Cl1D (1 × 10^5) and V79 (2 × 10^4) cells were plated in a 24-well multidish (Nunc). Approximately 24 h after plating, the E-CS medium was removed and 0.5 ml of E medium containing 40 μg of PHA was added to each well. The plate was incubated for 90 min at 37°C. The medium was thoroughly pipetted off and 0.3 ml of 50% PEG added. After 1 min the PEG was aspirated, and the well was washed quickly three times with 1 ml of E medium. Then, 1 ml of E-HAT medium was added to each well and the dish was incubated for 20–24 h at 37°C. The cells were collected by trypsinization and plated in Petri dishes containing E-HAT medium. The subsequent procedure was the same as that for the fusion of cells in suspension.

Cytological procedure

To determine the effect of PHA treatment on the rate of formation of fused cells, the cells were plated onto coverslips and incubated for 16 h at 37°C in E-CS medium. The coverslips were then fixed and stained with Giemsa. The extent of cell fusion, expressed as the percentage of polykaryosis, was determined by counting the number of nuclei present in multinucleated cells. The term 'polykaryon' is used here to define any cell with more than two nuclei. The frequency of multinucleated cells was determined by counting 10^5 cells.

RESULTS

Correlation between cell agglutination and cell hybridization

In an FM3A × Ehrlich cross, PEG-induced fusion was performed after the cells had been brought into suspension by treatment with dispersing agents. Before this, agglutination by lectins was carried out, and the effect of this on hybrid formation was studied. As for plant lectins, 13 lectins including PHA were investigated to elucidate the relationship between the ability to produce cell agglutination and the ability to promote cell hybridization (Table 1). The maximum concentration of lectin was usually limited to 10 μg ml⁻¹, at which level accurate counts of the number of cells in aggregates could be obtained.

Table 1. Effect of lectins on agglutinability and hybridization of FM3A and Ehrlich cells

<table>
<thead>
<tr>
<th>Lectins</th>
<th>Cell agglutination (%)</th>
<th>Conc of lectins (μg ml⁻¹)</th>
<th>No. of hybrid colonies*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA</td>
<td>9.5</td>
<td>80</td>
<td>137</td>
</tr>
<tr>
<td>WFA</td>
<td>73.3</td>
<td>80</td>
<td>91</td>
</tr>
<tr>
<td>WGA</td>
<td>62.8</td>
<td>10</td>
<td>91</td>
</tr>
<tr>
<td>Suc-WGA</td>
<td>43.7</td>
<td>80</td>
<td>11</td>
</tr>
<tr>
<td>ConA</td>
<td>29.2</td>
<td>80</td>
<td>47</td>
</tr>
<tr>
<td>Suc-ConA</td>
<td>59.2</td>
<td>80</td>
<td>68</td>
</tr>
<tr>
<td>PWM</td>
<td>24.8</td>
<td>10</td>
<td>6.5</td>
</tr>
</tbody>
</table>

* FM3A (1 × 10^5) × Ehrlich (1500) cells were incubated for 60 min in E medium containing each lectin before PEG-induced fusion; means for five experiments.
Among the 13 lectins, PHA, *Wisteria floribunda* (WFA), wheat germ agglutinin (WGA), concanavalin A (Con A), succinyl (Suc)-Con A and PWM (from *Phytolacca americana*) were observed to induce cell agglutination. WGA and PWM decreased cell viability. Apart from WGA and PWM, lectins having high agglutinating ability at 80 μg ml\(^{-1}\) enhanced hybridization; PHA especially had a pronounced effect. The lectins from *Pisum sativum*, *Glycine max*, *Ulex europaeus*, *Dolichos biflorus*, *Lens culinaris* and *Arachis hypogaea* did not induce cell agglutination and had no enhancing effect on hybridization. To examine the correlation between agglutination and hybridization in detail, further experiments with PHA and WGA were carried out. PHA is known to be a mixture of five isolectins containing erythroagglutinin (PHA-E) and leucoagglutinin (PHA-L), and mitogenic activities (Miller et al. 1973). The correlation between agglutination and hybridization was studied with particular reference to the effects of PHA-E and PHA-L.

As Fig. 1 shows, PHA was able to agglutinate Ehrlich cells even at a concentration of 1 μg ml\(^{-1}\), but did not induce the agglutination of FM3A cells at the same concentration. Accordingly, enhancement of the rate of formation of hybrid cells was not observed at 1 μg ml\(^{-1}\) of PHA. Even at 0.5 μg ml\(^{-1}\), PHA-L, obtained as a sub-fraction of PHA, increased both agglutination and hybridization. The effect of PHA-E on agglutination and hybridization was a little weaker than that of PHA. While WGA showed a marked drop in the agglutinability at less than 1 μg ml\(^{-1}\), it maintained high hybridization frequency even at 0.5 μg ml\(^{-1}\). When succinyl WGA (Suc-WGA) was used instead of WGA, the extent of agglutination was low, especially with Ehrlich cells. Consequently hybridization was enhanced only at 80 μg ml\(^{-1}\) or more (data not shown). Fig. 2 shows the correlation between the concentrations of lectins capable of

![Fig. 1. Effect of PHA, PHA-L and PHA-E on agglutination and hybridization of FM3A and Ehrlich cells. FM3A (1 × 10^6) and Ehrlich (1500) cells were incubated for 60 min in E medium containing various concentrations of PHA, PHA-L and PHA-E before PEG-induced fusion. (▲) Agglutination of FM3A; (△) Ehrlich; (■) hybrid colonies.](image_url)
Enhanced cell fusion by lectins

Fig. 2. Effect of various concentrations of lectins on hybridization of FM3A and Ehrlich cells. FM3A (1 × 10^5) and Ehrlich (1500) cells were incubated for 60 min in E medium containing various concentrations of lectins before PEG-induced fusion. (••) Hybrid colonies with PHA; (■■) with WGA; (□□) with WFA; (▲▲) with ConA.

agglutinating cells and hybridization frequencies. As mentioned before, WGA was found to be effective in inducing hybridization only at low concentration (1–5 μg), because it led to decreased viability at higher concentrations. The other three lectins showed that concentrations of 80 μg ml⁻¹ were effective and that, as the concentration of lectin decreased the enhancement of hybridization also decreased. Thus, it was evident that cell agglutination by the lectins is responsible for the enhancement of hybridization.

Effect of cell agglutination on cell fusion

The effect of agglutination on the process of cell hybridization was examined. Polykaryocytosis was used as a marker showing the rate of cell fusion, and the correlation between polykaryocytosis and hybridization frequency was determined. As Fig. 3 shows, it was found that in concentrations of PHA higher than 10 μg ml⁻¹ polykaryocytosis was parallel to the increase of hybridization frequency, though the increase of dikaryocytosis at more than 10 μg ml⁻¹ of PHA was not as great.

Comparison between suspension fusion and monolayer fusion

Hitherto, suspended cells have been used in all the experiments to determine the correlation between cell agglutination and hybridization. Parental cells (Cl1D and V79) grown in monolayers for 24 h were treated with PHA for 90 min, followed by
Fig. 3. Effect of concentrations of PHA on fusion and hybridization of FM3A and Ehrlich cells. FM3A ($1 \times 10^5$) and Ehrlich (1500) cells were incubated for 60 min in E medium containing various concentrations of PHA before PEG-induced fusion. (□—□) Polykaryon formation; (■—■) dikaryon formation; (○—○) hybrid colonies.

PEG. During incubation at 37 °C extensive cell fusion occurred, which resulted in the formation of many multinucleated cell layers. Such treatment induced a remarkable decrease in the viability of fused cells. The viability of monolayered cells incubated for 24 h decreased to 12–14% when they were treated with PEG alone, and to 3–4% when they were pretreated with PHA (Table 2). Nevertheless, it was evident that the few surviving cells showed enhanced hybridization resulting from pretreatment with PHA before PEG-induced fusion and the hybridization frequencies are expressed as the number of hybrid colonies per $10^4$ V79 cells (means from five experiments) or per viable $10^4$ V79 cells.

**Table 2. Effect of PHA pretreatment on hybridization of Cl 1D and V79 cells in suspension or in monolayer**

<table>
<thead>
<tr>
<th>Fusion methods</th>
<th>Incubation time (h)</th>
<th>Pretreatment PHA</th>
<th>Time (min)</th>
<th>Viability (%)*</th>
<th>Hybridization frequency†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cl 1D</td>
<td>V79</td>
</tr>
<tr>
<td>Suspension</td>
<td></td>
<td>-</td>
<td></td>
<td>36</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 10</td>
<td></td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 90</td>
<td></td>
<td>33</td>
<td>21</td>
</tr>
<tr>
<td>Monolayer</td>
<td>24</td>
<td>-</td>
<td></td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>+ 90</td>
<td></td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

*Viability was defined as the plating efficiency of cells treated with PHA followed by PEG, relative to that of control cells not treated with both.
†Cl 1D($1 \times 10^5$) and V79($2 \times 10^4$) cells were incubated in E medium containing 80 µg ml$^{-1}$ of PHA before PEG-induced fusion and the hybridization frequencies are expressed as the number of hybrid colonies per $10^4$ V79 cells (means from five experiments) or per viable $10^4$ V79 cells.
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PHA. Table 2 also shows that the viability of parental cells treated in suspension was much higher than that of cells treated in monolayers, showing the marked effectiveness of PHA treatment in promoting hybridization. In view of the great amount of cytotoxicity produced by the combined use of PHA and PEG, the number of hybrid colonies was calculated after correcting for the relative plating efficiency.

The results showed that cells grown in monolayers and in suspension gave, respectively, 65 and 95 hybrid colonies per 10^4 viable V79 cells under optimum conditions. Therefore, the difference in the number of hybrid colonies derived from fusion between these cells is assumed to be due to the cytotoxicity resulting from the combined use of PHA and PEG.

DISCUSSION

Plant lectins bind to specific sugar receptors on the cell surface, thereby inducing cell agglutination. It is of interest to know which classes of sugar receptors are involved in promoting cell fusion. The physiological role of cell-surface glycoproteins and glycolipids in the process of cell fusion may be much more complex than in cell agglutination.

Mercer & Schlegel (1979) have developed an efficient cell-fusion method for reducing the toxicity of PEG, keeping a high fusion index by PHA treatment. Their technique (Mercer & Schlegel, 1979) is useful when cells are fused in suspension. We have also reported briefly that lectin treatment before cell fusion by PEG enhances the yield of hybrid cells (Matsuya, Takahashi & Yamane, 1979). In the hybridization experiments presented here, C11D and V79 cells in suspension gave a much higher viability and frequency of hybridization than the same cells in monolayers when treated with PHA/PEG. Taking the reduced viability into account, the difference between fusion in suspension and fusion in monolayers was found to be small.

The mechanism of the action of lectins on PEG-mediated cell fusion is not known. A simple and attractive hypothesis (Poste & Pasternak, 1978; Mercer & Baserga, 1982) is that cell agglutination by lectins creates conditions of close cell-to-cell contact that facilitate subsequent fusion. However, the possibility that lectins induce change in membrane structure cannot be completely ruled out.

Cell fusion by chemicals may at first occur in regions where the fluidity of the lipid bilayer is increased (Ahkong, Fisher, Tampion & Lucy, 1975). Mercer & Schlegel (1979) have suggested that PHA might act by producing a membrane perturbation that would render the cell more susceptible to fusion by PEG. The treatment of human lymphocytes with mitogens such as PHA and ConA produces a fluidization of membrane lipid, while non-mitogenic lectins are without effect on membrane fluidity (Barnett, Scott, Furcht & Kersey, 1974). Maximal fluidization of lymphocyte membranes by active lectins occurs between 15 and 30 min. Measurements of the microviscosity of the membrane lipid layer show that the membrane fluidity of murine T cells is increased remarkably by treatment with ConA, PHA and WFA (Toyoshima & Osawa, 1975; Yokoyama, Yano, Terao & Osawa, 1976), all of which were effective in inducing hybridization in our experiments.
Since the combined use of PHA and PEG increases fusion in suspended cells and the yield of hybrid cells, it is to be expected that it would be widely applicable as a method for the hybridization of many types of cells.

REFERENCES


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