Biochimica et Biophysica Acta, 735 (1983) 193–195
Elsevier

BBA Report

BBA 70126

MAGNETOLIPOSOMES: ANOTHER PRINCIPLE OF CELL SORTING

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(Received May 9th, 1983)
(Revised manuscript received August 1st, 1983)

Key words: Magnetoliposome; Cell sorting; Antibody; Liposome

Liposomes bearing anti-fibronectin antibodies and associated with ferromagnetic particles bound firmly to the surface of mouse embryo fibroblasts. Upon binding magnetoliposomes, the cells could be sorted in a magnetic field.

Cell sorting is a procedure widely used in modern laboratory practice to select cells with certain surface antigens. Cell sorting requires complex electronic instruments to deal with cells labelled with fluorescent antibodies. Here we suggest another, much easier way of cell sorting using immunomagnetoliposomes, i.e., antibody-bearing liposomes associated with ferromagnetic particles.

Liposome-cell interactions are studied in many laboratories in order to introduce exogenous molecules into the cytoplasm or plasma membranes or to simulate cell-cell interactions [1–3]. Cell-liposome binding can be made efficient and specific if immunoglobulins are inserted into the liposomal membrane [4,5]. We have used this technique to prepare magnetosensitive liposomes bearing specific anti-fibronectin immunoglobulins capable of binding to the cell surface.

Magnetoliposomes were prepared from distearoyl- or dipalmitoylphosphatidylcholine, as these 'solid' (at 37°C) vesicles firmly bind to the cell surface and can be removed by trypsin treatment [2,3,6]. 10 mg of dipalmitoyl- or distearoylphosphatidylcholine (Sigma) were dissolved in chloroform/methanol (2 : 1) in a round-bottomed flask. 1–2 mg of ferrite powder (the mean size of a particle is approx. 500 Å) was added to the same flask and the solvent was evaporated in a rotary evaporator, so that a thin lipid film with embedded ferrite particles was formed. 1 ml of Hanks' solution containing 0.5 mg of rabbit antibodies against human plasma fibronectin was added to the flask which was then vigorously shaken. The emulsion was sonicated in a UDZN-2 sonicator (30 s at maximal output, 50°C and 60°C for dipalmitoyl- and distearoylphosphatidylcholine, respectively). Liposomes were separated from free antibodies by centrifugation and resuspension in Hanks' solution (3×3 min $5000 \times g$, final lipid concentration 5 mg/ml). This procedure resulted in the formation of magnetosensitive liposomes, i.e., closed vesicles which can move along the gradient of magnetic field. The integrity of the vesicles was evidenced by their ability to entrap and retain sodium fluorescein. Then magnetoliposomes were formed in sodium fluorescein-containing solution and the unentrapped dye was removed by centrifugation, a substantial amount of dye was retained by magnetoliposomes and attracted by a magnet. No fluorescence was found in association with the magnet if the fluorescent dye

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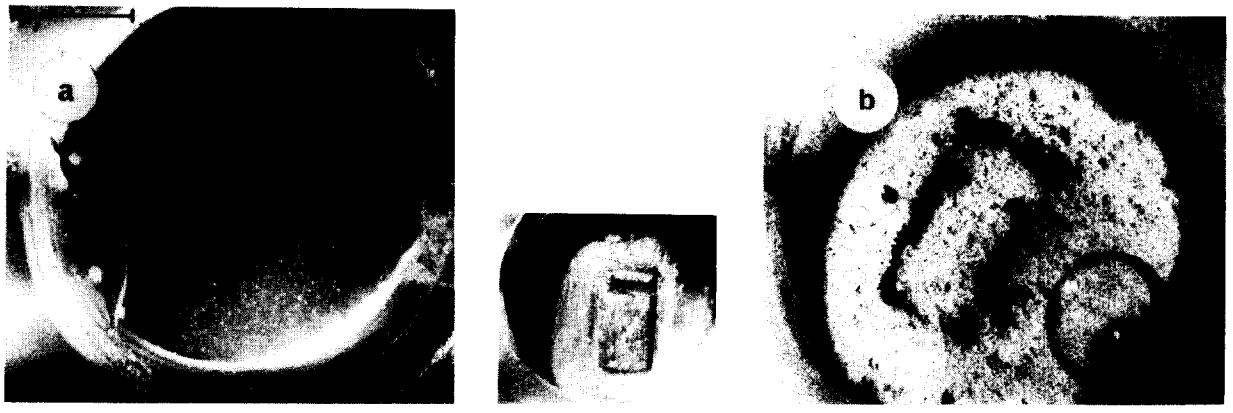

Fig. 1. Immunomagnetoliposome-treated cells concentrated by a magnetic field. (a) Inititally, the cells were randomly distributed in the chamber. The electromagnet, with a rectangular core (small photograph), is located under the chamber and is not visible behind a paper screen. Bar = 5 mm. (b) After the magnetic field is applied, the cells are concentrated along the magnetic core. To visualize the distribution of the cells at low magnification, they were labelled with carmin particles.

and ferromagnetic particles were mixed with 'empty liposomes'. The ultrastructure of magnetoliposomes and the mode of their association with ferrite particles were not studied.

To make the cells magnetosensitive, magnetoliposomes bearing anti-fibronectin antibodies were incubated for 15 min with confluent monolayers of mouse embryo fibroblasts (2 ml of liposome suspension per culture flask of base 3×5 cm). The nonbound liposomes were washed away with Hanks' solution. The liposome-treated cell monolayers turn grayish due to the binding of ferromagnetic particles entrapped in liposomes. The cells suspended by EDTA treatment were transferred to the chamber above the electric magnet (Fig. 1a). (For theoretical evaluation of magnetic fields necessary for cell sorting, see Ref. 7.) After the magnet was switched on, the cells assembled in 1–3 min along the perimeter of the magnetic core (Fig. 1b). It was necessary to agitate the chamber to facilitate cell movement along the field gradient. When non-immune magnetoliposomes were used, i.e., those without anti-fibronectin antibodies, only a small fraction of the cells was attracted by the magnet due to low liposome binding.

The cells assembled by means of the magnetic field could be collected from the chamber for further studies by a pipette. As tested by the Trypan blue exclusion test, the viability of the collected cells was 98.5%. These cells could be replated. For such experiments, the collected population was treated with trypsin (0.02%) to remove liposomes from the cell surface and to disaggregate the cells. After such treatment, the suspension consisted mainly of single cells, although a few large cellular aggregates still existed. No magnetic particles were observed in association with the cells after trypsin treatment. After replating, the cells attach to and spread on the substrate. At least 80% of the seeded cells remain spread on the surface of culture dishes, exhibiting normal morphology 6 h after plating.

To find out whether the described procedure could be used for cell sorting, we performed model experiments with mixed cultures. Mouse embryo fibroblasts labelled with carmin and treated with anti-fibronectin immunomagetoliposomes were mixed with a suspension of untreated cells. The mixed population was transferred to the chamber and the magnetic field was switched on. After the cells assembled in the bands along the magnetic core, the chamber was perfused with Hanks' solution. Magnetoinsensitive cells were washed away by the stream, while the cells forming the bands were collected and suspended. The number of labelled cells in the initial and collected population was evaluated. As shown in Table I, the collected population was approx. 5-times enriched with labelled cells. In some experiments, this value reached 15. The yield of the selected cells was high:

TABLE I

ENRICHMENT OF A MIXED CELLULAR POPULATION WITH MAGNETOSENSITIVE CELLS

The suspensions of immunomagnetoliposome-treated carmin-labelled cells were mixed with non-treated cells in different ratios. The mixture was transferred to the chamber. Cells assembled along the magnetic core were collected. The number of carmin-labelled and unlabelled cells was evaluated.

Expt. No.	Ratio of labelled to unlabelled cells	
	Before sorting	After sorting
1	0.8	3.6
2	0.7	4.0
3	0.9	5.1
4	0.3	1.4

70–90% of the initial population of labelled cells were collected from the chamber after cell sorting. In fact, both the enrichment and the yield of the sorted cells depend on how skillfully one manipulates the pipette when collecting the band formed by the cells.

The results do not depend on cell labelling. Similar enrichment was obtained when non-labelled cells were treated with immunomagneto-liposomes, while the nonmagnetic cells were pre-labelled with carmin particles. In this case, the final population was enriched with nonlabelled cells.

Thus, with the help of immunomagnetolipo-somes, the cells could in principle be sorted by a magnetic field according to their surface antigens. Such cell sorting does not require sophisticated and expensive instrumentation as does fluores-cence-activated cell sorting, and, if developed, could have in certain cases some advantages: for example for sorting cells in highly sterile conditions – in sealed flasks or in situ, e.g., in blood vessels.

References

1 Ryman, B.E. and Tyrrel, D.A. (1980) Essays in Biochemistry, pp. 49–98, Academic Press, New York

2 Pagano, R., Schroit, A. and Struck, D. (1981) in Liposomes: From Physical Structure to Therapeutic Application (Knight, C.G., ed.), pp. 332–348, Elsevier/North-Holland Biomedical Press, Amsterdam

3 Margolis, L.B., Neyfakh, A.A., Jr., Bergelson, L.D. and Vasiliev, J.M. (1980) Cell Biol. Int. Rep. 6, 131–136

4 Gregoriadis, G. and Neerunjun, E.D. (1975) Biochem. Biophys. Res. Commun. 65, 537–553

5 Margolis, L.B. and Dorfman, N.A. (1977) Bull. Exp. Biol. Med. 83, 53–57 (in Russian)

6 Pagano, R.E. and Takeichi, M. (1977) J. Cell Biol. 74, 531–546

7 Namiot, V.A. (1983) Biophisika 28, 152 (in Russian)

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