Binding of macrophages and phospholipid flip-flop in supported lipid bilayers

Mamoru Nakanishi*, Kazuko Matsumoto and Seizo Takahashi

*Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113 and Department of Chemistry, Japan Women's University, Mejirodai, Bunkyo-ku, Tokyo 112, Japan

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Subclass-specific antibody-dependent interactions (binding and triggering) between macrophages and supported lipid bilayers have been studied. Percentages of mouse macrophage binding (J774 cell line) to the lipid bilayers were dependent on mouse monoclonal IgG subclasses. The efficiencies were as follows: IgG1 = IgG2a > IgG2b > IgG3. Furthermore, macrophage triggering (spreading) was more efficient on IgG2a- or IgG1-coated lipid bilayers than on IgG2a, IgG3, or non-specific rabbit IgG. The present experiments show also that phospholipid molecules are able to flip-flop from one side of a supported planar bilayer membrane to the other with a half-life of 10 h–1 day at 25°C.

Supported bilayer *Planar membrane* *Flip-flop* *Antibody subclass* *Macrophage* *Fc receptor*

1. INTRODUCTION

There is much current interest in the development of suitable model membrane systems for immunological studies [1,2]. The substrate-supported monolayer system, which has been developed by McConnell and his colleagues, provides an ideal configuration for visualizing molecular and cellular events that take place at the interface between a cellular membrane and a reconstituted membrane [1,3–5]. One of the limitations of this system, however, is the difficulty of reconstituting transmembrane proteins into monolayers [5–7]. For this purpose, a substrate-supported phospholipid bilayer system has been recently developed and a physical property (lateral diffusion of phospholipids) characterized by a fluorescence photobleaching method [8].

Abbreviations: DMPC, dimyristoylphosphatidylcholine; PBS, phosphate-buffered saline; TNP-Cap-DPPE, trinitrophenylaminocaproylphosphatidylethanolamine; FCS, fetal calf serum; NBD-DPPE, *N*-(7-nitro-2,1,2-benzoxodiazol-4-yl)dipalmitoylphosphatidylethanolamine

The question has arisen here as to whether the biochemical properties (binding and triggering of cells) or other physical properties of a substrate-supported bilayer are similar as a phospholipid vesicle or a substrate-supported monolayer. For this reason we have been motivated to study subclass-specific antibody-dependent binding of macrophages to supported phospholipid bilayers and also the flip-flop rate of phospholipids in supported lipid bilayer membranes.

A second motivation for this work is to clarify subclass-specific antibody-dependent triggering (spreading) of macrophages on supported lipid bilayers. Although a number of workers have investigated at the subclass specificity of IgG molecules [9–12], subclass-specific antibody-dependent triggering of macrophages has not yet been well defined [13,14].

2. MATERIALS AND METHODS

Mouse monoclonal antibodies (IgG1, IgG2a, IgG2b, IgG3) for anti-trinitrophenyl residue (TNP) were provided by Dr M. Ueda (Kyoto University). The preparation procedures were

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described in [15]. The class and subclass of antibodies were checked by ELISA and the Ouchterlony methods. TNP-Cap-DPPE was provided by Dr T. Yasuda (University of Tokyo). DMPC and DPPC were purchased from Sigma (St. Louis, MO). NBD-DPPE was purchased from Avanti.

J774 cell line was grown in RPMI 1640 (Gibco, NY) that was supplemented with 10% (v/v) FCS (M.A. Bioproducts, MD) (heat-inactivated at 56°C for 30 min).

We described supported monolayer preparations in [12]. Supported lipid bilayers were prepared by similar procedures according to Tamm and McConnell [8]. The first DMPC monolayer (without TNP-Cap-DPPE) was spread at the air-water interface in a Langmuir trough. The lipid monolayer was then compressed to 40 dyn/cm^2. A clean hydrophilic piece of glass coverslip was then immersed vertically through the monolayer into the trough. No significant change in surface pressure was observed at this step. The glass coverslip was then pulled out at a speed just slow enough to permit water to drain from the surface [8]. A substantial drop in surface pressure was then observed. The loss of surface area was approximately equal to twice the area of the substrate. Then, the substrate was pushed through the second DMPC monolayer (with TNP-Cap-DPPE) at the air-water interface horizontally. The surface pressure decreased and recompression yielded an area decrease corresponding to roughly 1.5-times the area of substrate [8]. Each coverslip was attached to a glass slide under water. The coverslip and glass slide were separated by 2 narrow strips of double stick tapes, 250 μm thick. When 2 mol % NBD-PE was incorporated into a DMPC bilayer, the epifluorescence revealed a surface that was a uniformly fluorescent.

For binding experiments, a 50 μl solution of anti-TNP monoclonal antibody (1.5×10^{-6} M) was first added to the bilayers (or monolayers) which were kept at 4°C for 30 min. After washing the unbound antibody with PBS (+ 1% FCS), macrophages (J774 cell line) in PBS (+ 1% FCS) were introduced between the coverglass and glass slide, and allowed to settle on the lipid bilayers (or monlayers). Following incubation at 37°C for an appropriate time, the slide was inverted and examined using a Nikon VFD-R microscope with a camera. The percentage of macrophages bound to bilayers (or monolayers) was calculated by photographing several fields on a slide at random and counting both cells in the focal plane of the bilayers and cells in the focal plane of a bare glass slide. Spreading experiments were done by photographing spread cells on bilayers without inverting the slide after incubation.

For flip-flop experiments, supported lipid bilayers were prepared as follows: a DMPC monolayer (with TNP-Cap-DPPE) was first coated on a clean glass coverslip. Then, the first DMPC monolayer (with TNP-Cap-DPPE) was coated with the second DMPC monolayer (without TNP-Cap-DPPE).

3. RESULTS

3.1. *Macrophage bindings to supported lipid bilayers*

Mouse macrophages (J774 cell line) bound specifically to the lipid bilayers containing lipid

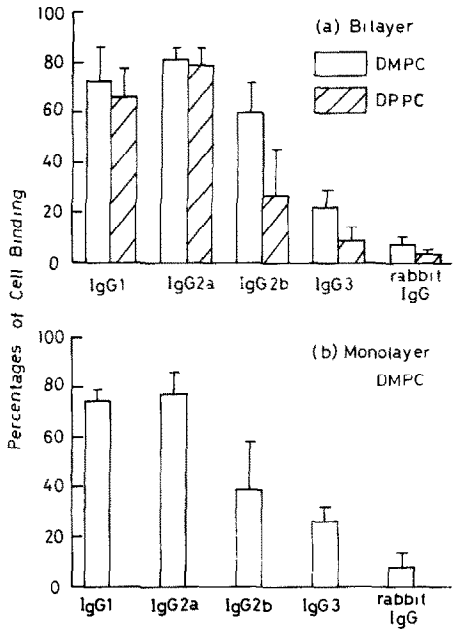

Fig.1. Percentages of macrophages (J774) bound to supported lipid bilayers or monolayers coated with subclass IgG. Bars are standard deviations. Measurements were taken after 15 min incubation at 37°C. (a) DMPC bilayers containing 1% TNP-Cap-DPPE. (b) DMPC monolayers containing 1% TNP-Cap-DPPE.

haptens (99% DMPC + 1% TNP-Cap-DPPE). The binding of J774 cells to supported lipid bilayers was dependent on antibody subclasses for anti-TNP residues as shown in fig.1a. The binding efficiencies are as follows: IgG1 = IgG2a > IgG2b > IgG3. For comparison we also measured the binding efficiencies of J774 cells to lipid 'monolayers'. The results for 'monolayers' were similar to those for 'bilayers' as shown in fig.1a and b. These results are consistent with our previous binding experiments for supported lipid monolayers using a different mouse macrophage cell line (P388D1) [12]. Since the binding equilibrium constants between lipid hapten (TNP-Cap-DPPE) and subclass IgG used here are almost the same [12], subclass-specific antibody-dependent binding of macrophages to the lipid bilayers is mostly due to the binding affinity differences between macrophage Fc receptors and subclass IgG molecules.

3.2. *Phospholipid flip-flop in supported bilayers*

We prepared supported lipid bilayers which contained lipid haptens (1 or 0.1% TNP-Cap-DPPE in DMPC) in the inner monolayer but not in the outer monolayer. Keeping these supported bilayers at 25°C for an appropriate time, we measured the spontaneous rotation of lipid haptens from one side of a monolayer to the other. The transition of a lipid hapten from the inner to the outer monolayer was measured by experiments on macrophage binding to these lipid bilayers with anti-TNP IgG2a. As shown in fig.2 macrophages were able to bind to the supported lipid bilayers mentioned above. The percentages of macrophage binding were dependent on time when bilayers were kept at 25°C after preparation. This phenomenon shows that phospholipid molecules (TNP-Cap-DPPE) can flip-flop from the inner to the outer monolayer in supported lipid bilayers. When these supported monolayers were kept at 25°C for 2–4 h after bilayer preparation, sufficient macrophages were able to bind these bilayers.

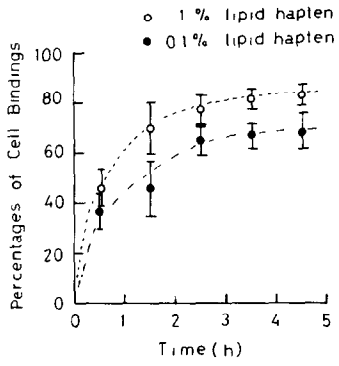

Fig.2. Percentages of macrophages (J774) bound to supported lipid bilayers coated with IgG2a. Here the supported lipid bilayers have lipid haptens (1 or 0.1% TNP-Cap-DPPE in DMPC) in the inner monolayers but not in the outer monolayers. The supported bilayers were kept at 25°C for the indicated time. Then the transition of a lipid hapten from the inner to the outer monolayer (flip-flop) was measured by macrophage binding experiments.

This does not mean, however, that the half-life of the flip-flop process in supported bilayers is 2–4 h, because macrophages can bind well to supported lipid bilayers even if TNP-Cap-DPPE in the outer surface of bilayers is 0.01%. Fig.3 shows the dependence of macrophage binding on the surface concentration of lipid hapten in supported bilayers. The binding efficiencies decreased abruptly, when lipid hapten concentration in the outer surface of bilayers (coated with IgG2a) was decreased from 0.01 to 0.001%. Thus we can conclude that the flip-flop rates of phospholipids are several times slower than the rates of appearance of lipid hapten in the external half of the bilayer as shown in fig.2. Therefore, phospholipid molecules are able to flip-flop from one side of a supported planar bilayer membrane to the other with a half-life of 10 h–1 day at 25°C. This is consistent with previous results for liposome vesicles [16,17].

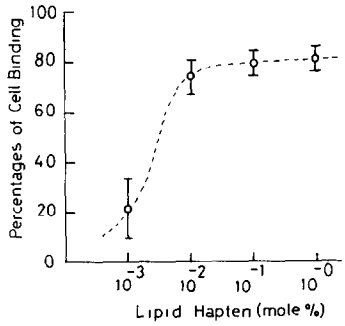

Fig.3. Percentages of the macrophage binding vs hapten concentrations in supported lipid monolayers. Bars are standard deviations. Measurements were taken after 15 min incubation at 37°C.

3.3. *Macrophage triggering on supported lipid bilayers*

Finally, we studied subclass-specific antibody-dependent spreading of macrophages on the supported lipid bilayers. We prepared lipid bilayers containing 1% TNP-Cap-DPPE in the outer surface of bilayer membranes. Then anti-TNP monoclonal antibodies (IgG1, IgG2a, IgG2b or IgG3) or non-specific rabbit antibodies were added to the bilayers which were kept at 4°C for 30 min. After washing, macrophages (J774) were added to the bilayers which were kept at 37°C for 0.5–2.5 h. After incubation, macrophage triggering (spreading) was examined by a microscope with a camera. Macrophages spread uniformly in all directions on the bilayers and became large and thin as shown in fig.4. It appeared that macrophages spread more efficiently on IgG2a- or IgG1-coated lipid bilayers than on IgG2b, IgG3, or non-specific rabbit IgG.

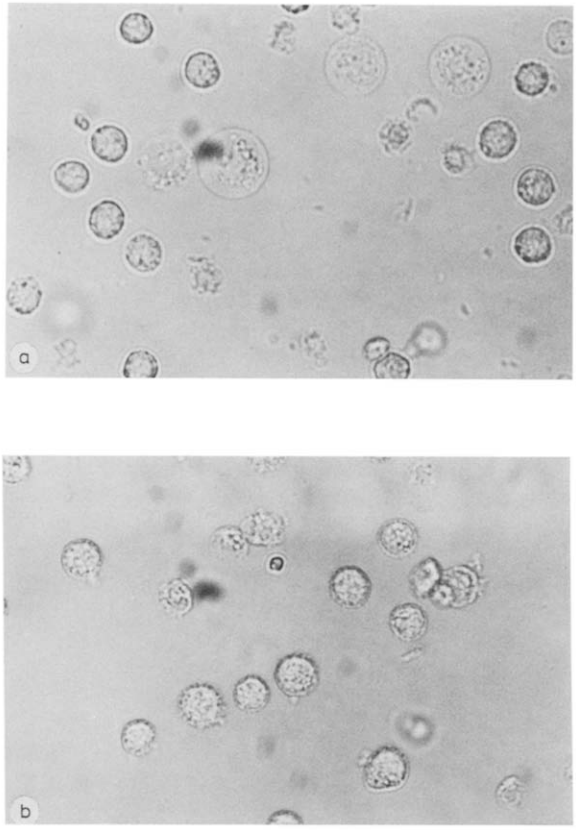

Fig.4. Phase contrast photomicrographs of macrophage (J774) spreading on the supported lipid bilayers. Measurements were taken after 50 min incubation at 37°C. Bilayers contained 1% TNP-Cap-DPPE + 99% DMPC. (a) Bilayers coated with IgG2a. Some macrophages spread. Macrophages also spread on IgG1-coated lipid bilayers as in this photograph. (b) Bilayers coated with IgG3. Most macrophages did not spread. Results were the same for IgG2b or non-specific rabbit IgG-coated bilayers.

4. DISCUSSION

The spontaneous rotation of lipids from one side of a supported planar bilayer membrane to the other was a very slow process. The half-life of phospholipid flip-flop was 10 h–1 h day at 25°C. This result was consistent with previous ones for unilamellar liposome vesicles [16,17]. This must be an indication that the physical properties of substrate supported lipid bilayers is very close to those of multilamellar liposomes or unilamellar vesicles.

Furthermore, the present results show that murine macrophage-like cell line (J774) was able to bind IgG-coated lipid bilayers as well as monolayers. The percentages of cell binding were as follows; IgG1 = IgG2a > IgG2b > IgG3. They also show that macrophages appear to spread more efficiently on IgG2a- or IgG1-coated lipid bilayers than on IgG2b, IgG3, or non-specific rabbit IgG.

Mouse macrophages have at least 3 different Fc receptors. One binds both IgG1 and IgG2, another is specific for IgG2a, and a third binds IgG3 [9–12]. It has been demonstrated that IgG2b receptor possesses phospholipase A activity, whereas IgG2a receptor does not [13]. Under the present experimental conditions, however, macrophages spread more efficiently on IgG1- or IgG2a-coated lipid bilayers. Therefore, the efficiencies of macrophage triggering (spreading) seem to be related to the affinity differences between macrophage Fc receptors and subclass IgG molecules.

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