Subclass-specific antibody-dependent binding of macrophages to supported planar lipid monolayer membranes

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Subclass-specific antibody-dependent binding of macrophages to supported planar lipid monolayers has been studied. The binding of the P388D$_1$ macrophage-like cell line to planar DMPC or DPPC monolayers was dependent on IgC subclasses and hapten concentrations. The binding efficiencies were as follows on both 'solid' and 'fluid' lipid monolayer membranes; IgG1 = IgG2a > IgG2b > IgG3 = IgA. These results suggest that the present system is very useful for studying the mechanisms of the transmembrane signal triggered by Fc receptors of macrophages.

Lipid monolayer *Planar membrane* *Lipid hapten* *Macrophage* *IgG subclass* *Fc receptor*

1. INTRODUCTION

The process of recognition among cells of the immune system provides one of the most interesting molecular problems in membrane biology today [1,2]. To solve such problems, supported planar lipid monolayer membranes containing specific antigens or lipid hapten (haptenated phospholipid) have been introduced into this field [3–8]. These reconstituted membranes have several advantages compared with the reconstituted lipid bilayer membranes which are in the form of multilamellar liposomes or single-shell vesicles. That system provides an ideal configuration for visualizing molecular and cellular events that take place at the interface between a cellular membrane and a reconstituted membrane.

In the case of polyclonal IgG bound to lipid haptens in these phospholipid monolayers supported on alkylated glass coverslips, the specific adherence and subsequent triggering of macrophages have been observed [3,9]. These experiments naturally generated an interest in studying how the hapten-IgG subclass affects the interaction of macrophages with lipid monolayer membranes. Since the composition and physical properties of supported lipid monolayers can be precisely defined, they can be expected to give us new information on the physicochemical properties of Fc receptors bound to an antigen-antibody complex in the membranes.

2. MATERIALS AND METHODS

Monoclonal antibodies (IgG1, IgG2a, IgG2b, IgG3 and IgA) for anti-trinitrophenyl residue (TNP) were provided by Dr M. Ueda (Kyoto University) and prepared by the following procedures [10]. BALB/c mouse immunized by ovalbumin conjugated with trinitrobenzenesulfonate and Freund complete adjuvant: antigen was

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Abbreviations: DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; PBS, phosphate-buffered saline; TNP-Cap-DPPE, trinitrophenylaminocaproylphosphatidylethanolamine; FCS, fetal calf serum; ELISA, enzyme-linked immunosorbent assay

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boosted after 3 weeks, and spleen cells were fused with myeloma cells (NS-1) with polyethylene glycol 1500. Antibody-producing clones were selected by ELISA using trinitrophenylated bovine serum albumin and rabbit anti-mouse Ig antibody conjugated with horseradish peroxidase. The class and subclass of antibody were checked by ELISA and Ouchterlony methods. Antibody from the peritoneal cavity was purified by ammonium sulfate precipitation (20–50%). TNP-Cap-DPPE was provided by Dr T. Yasuda (University of Tokyo).

The $P388D_1$ cell line was obtained from Dr Y. Takeda (University of Tokyo) and grown in RPMI 1640 (Gibco, NY) that was supplemented with 10% (v/v) FCS (heat-inactivated at 56°C for 30 min) (M.A. Bioproducts, MD).

Trypsinization was carried out by incubating 10^7 cells/ml PBS with 0.1 mg/ml TPCK-trypsin (Sigma, MO) for 20 min at 25°C following the method of Lane and Cooper [11].

Alkylated glass coverslips (1.8 × 1.8 cm) were obtained as in [6,7]. Monolayers (DMPC or DPPC) were spread at an air-water interface by allowing lipid in hexane and ethanol (9:1), containing TNP-Cap-DPPE, to reach the surface of the water. The monolayers (single layer) formed at a pressure of 40 dyn/cm^2 were transferred to alkylated glass coverslips using the techniques in [3,5]. Each coverslip was attached to a glass slide. The coverslip and glass slide were separated by two narrow strips of double-stick tape, 250 μm thick.

For the binding experiments, a 50 μl solution of anti-TNP monoclonal antibody (1.5×10^{-6} M) was first added to the monolayers which were kept at 4°C for 30 min. After washing the unbound antibody with PBS (+ 1% FCS), macrophages ($P388D_1$ cell line) in PBS (+ 1% FCS) was introduced between the cover glass and slide, and allowed to settle on the lipid monolayers. 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 monolayers was calculated by photographing several fields on a slide at random and counting both cells in the focal plane of the monolayers and cells in the focal plane of a bare glass slide.

Fluorescence measurements were made on a Hitachi MPF-4 spectrophotometer with double monochromators on excitation and emission.

3. RESULTS

Macrophages ($P388D_1$ cell line) bound specifically to the lipid monolayers containing lipid haptens (TNP-Cap-DPPE). The binding of $P388D_1$ cells to planar lipid monolayers was dependent on IgG subclasses and hapten concentration as shown in fig.1. At 1% hapten, macrophages bound preferentially to 'fluid' DMPC lipid monolayers coated with IgG1 (94%) or IgG2a (96%) (fig.2). For convenience, we use the term 'fluid' and 'solid' lipid monolayer membranes throughout. By definition, a fluid membrane is one in which the lateral diffusion coefficients of fluorescent lipid probes are in the range 10^{-7}–10^{-8} cm^2/s. Solid membranes are those in which these lateral diffusion coefficients are of the order of 10^{-10} cm^2/s or less [3]. Macrophages also bound to the planar lipid monolayers coated with IgG2b, however, the percentage of cell binding (49%) was less than that of IgG1 or IgG2a. Then macrophages bound less efficiently to the lipid monolayers coated with IgG3 and IgA, the percentages of cell binding being 22 and 24%, respectively. Thus, the binding efficiencies are as follows: IgG1 = IgG2a > IgG2b > IgG3 = IgA. Substituting specific anti-TNP antibodies for nonspecific rabbit IgG, almost all cells became unbound from the monolayers (3%) as shown in figs 1 and 2. In addition, (a) without TNP-Cap-DPPE and IgG or (b) without IgG subclasses, macrophages did not bind to the monolayers (percentages of nonspecific cell binding 6 and 4%, respectively).

Such kinds of subclass specific binding were also found for solid DPPC monolayers. The percentages of cell binding were roughly equal for both fluid and solid monolayers as shown in fig.2 (15 min at 37°C). This means that under this condition enough hapten-IgG complexes already exist in the region of the monolayer-macrophage contact.

At low hapten concentration (0.1%), the percentages of cells bound for both fluid DMPC and solid DPPC monolayers were somewhat smaller than those at higher hapten concentration (1%). The percentages of cells bound to fluid DMPC monolayers were roughly equal to those of the solid DPPC monolayers (for both cases; 15 min at 37°C) (fig.3). At 0.1% hapten, macrophages

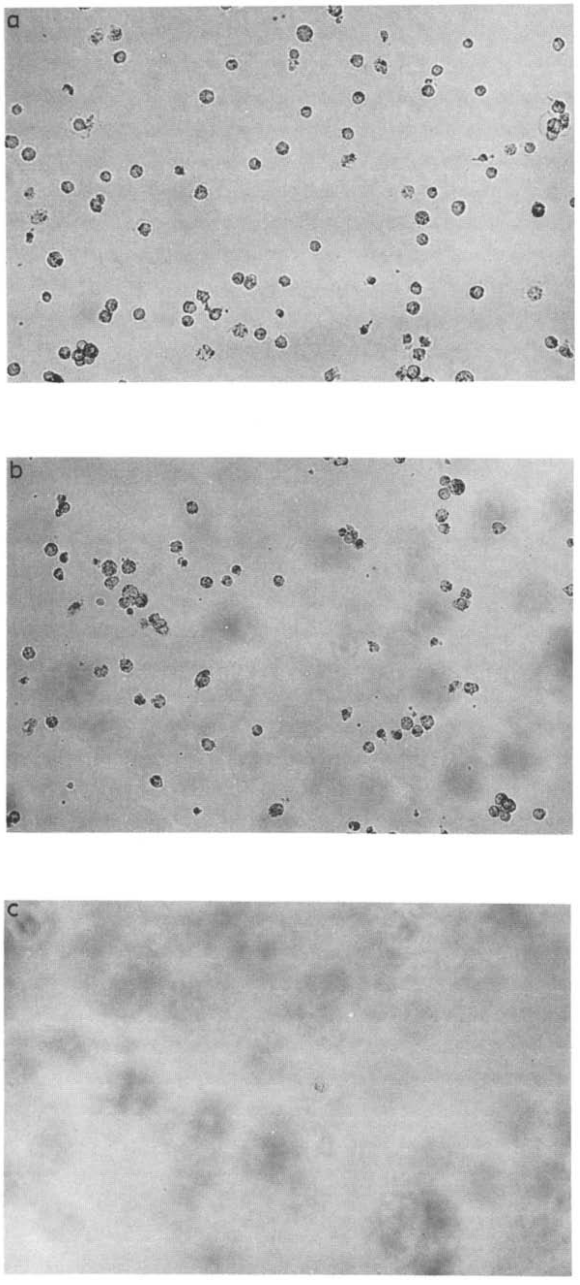

Fig.1. Phase contrast photomicrographs of macrophages ($P388D_1$) bound to planar lipid monolayers. (a) Bound macrophages on DMPC lipid monolayers coated with IgG2a. (b) Bound macrophages on DMPC lipid monolayers coated with IgG2b. (c) DMPC lipid monolayers treated with nonspecific rabbit IgG. Almost all cells became unbound from the monolayers. Measurements were taken after 15 min incubation at 37°C. Monolayers contained 1% TNP-Cap-DPPE.

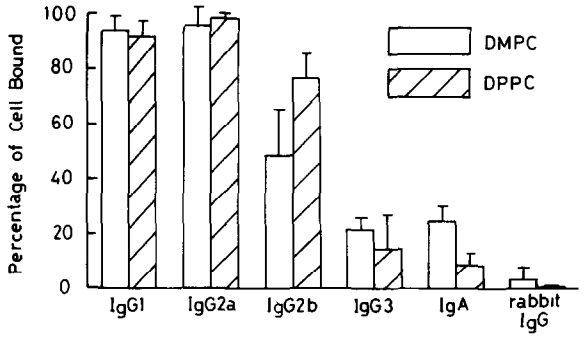

Fig.2. Percentages of macrophages bound to lipid monolayers coated with subclass IgG and IgA. Monolayers contained 1% TNP-Cap-DPPE. Bars are standard deviations. Measurements were taken after 15 min incubation at 37°C.

bound IgG-coated monolayers as follows: IgG1 = IgG2a > IgG2b = IgG3 = IgA.

Current evidence indicates that macrophages and macrophage-like cell lines have at least two distinct Fc receptors [12–18]. One of the receptors binds IgG2a with the highest affinity and is sensitive to degradation by trypsin. The second receptor binds IgG1 and IgG2b preferentially and is resistant to trypsin. We then treated $P388D_1$ cells with trypsin and subsequently tested their capacity to bind lipid monolayers. The binding percentage was slightly less efficient for trypsin-treated cells, however, binding of cells to the DMPC monolayers coated with IgG2a was not markedly inhibited under the conditions used [11]. The bind-

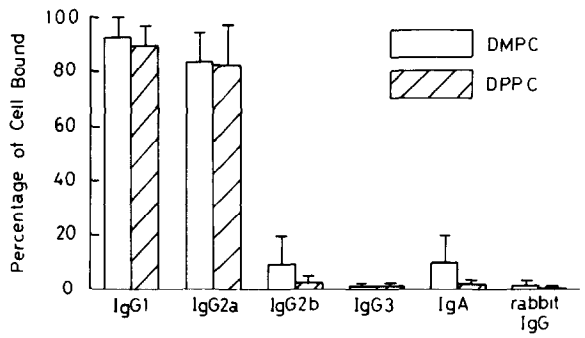

Fig.3. Percentages of macrophages bound to the lipid monolayers coated with subclass IgG and IgA. Monolayers contained 0.1% TNP-Cap-DPPE. Bars are standard deviations. Measurements were taken after 15 min incubation at 37°C.

ing percentages of trypsin-treated cells were as follows: 86% for IgG1, 85% for IgG2a and 47% for IgG2b (on the DMPC monolayers containing 1% TNP-Cap-DPPE).

Finally, we measured the binding equilibrium between TNP hapten and subclass IgG in solution. A solution of each subclass IgG was titrated with TNP-Cap-DPPE in PBS solution. Quenching of tryptophan fluorescence of IgG was measured after the binding of TNP-Cap-DPPE to IgG. The equilibrium constants for the hapten binding were determined using a Wang and Edelman plot [19]. The affinity constants for all the IgG used here are almost the same: $3.9 \times 10^6\ M^{-1}$ for IgG1, $5.2 \times 10^6\ M^{-1}$ for IgG2a, $1.2 \times 10^6\ M^{-1}$ for IgG2b and $7.8 \times 10^6\ M^{-1}$ for IgG3. These results support the view that subclass specific antibody-dependent binding of macrophages to lipid monolayers described here is not due to the different capacity of the affinity between lipid hapten and IgG, but due to the binding affinity between the Fc receptor of macrophage and the Fc domain of IgG.

4. DISCUSSION

Murine macrophages and macrophage-like cell lines such as $P388D_1$ have been shown to have at least two distinct Fc receptors, one specific for IgG2a and another for IgG2b (IgG1) [11–18]. Based on binding data, it has been suggested that IgG2b binds to a trypsin-resistant site, whereas IgG2a binding is sensitive to trypsinization.

The present results show that the macrophage-like cell line ($P388D_1$) binds IgG-coated lipid monolayers as follows: IgG1 = IgG2a > IgG2b > IgG3 = IgA. It is also shown that the $P388D_1$ cell line may bind mainly using one kind of Fc receptor (IgG2b receptor) to the lipid monolayer membranes.

Recently, it has been demonstrated that IgG2b receptor possesses phospholipase A activity whereas the IgG2a receptor does not [18]. As the supported planar lipid monolayers are ideal for biophysical and biochemical studies of 'cell-cell recognition' at the level of membrane-membrane binding and cell triggering, the present system will be very useful for studying the mechanism of biochemical signals transmitted by the distinct Fc receptors of macrophages.

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