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Dynamics of Antibodies on Planar Model Membranes

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Introduction

Antibodies are proteins that are synthesized and secreted by immune cells (specifically, B cells) in response to the presence of foreign matter (antigens). All antibodies have a common three-dimensional structure, which consists of two Fab regions that bind to specific molecular sites on the antigenic material and an additional Fc region that binds to antibody receptors on various cell types (Figure 1). The binding of antibodies to cell membrane surfaces, through the Fab regions and/or the Fc region, plays a key role in a variety of immune processes, such as phagocytosis, antibody-dependent cell-mediated cytotoxicity, complement activation, B cell regulation, and cellular secretion of inflammatory reagents and allergic mediators. The association of antibodies with surfaces is also of importance in the design and function of biosensors and immunodiagnostic devices.

Several physical phenomena special to surface–solution interfaces may be important in immunological processes that require antibody–membrane interactions (Figure 2): (1) the equilibrium between antibodies in solution and antibodies bound to membranes may depend on the physical and biochemical properties of the membrane, on the antibody structure and flexibility, on the valency of antibody–membrane association, and on the degree of surface saturation; (2) the kinetics of antibody binding to membranes may be coupled to the rate of solution transport and/or the spatial geometry; (3) the translational diffusion, rotational diffusion, and segmental flexibility of membrane-bound antibodies may depend dramatically on local structural details. The coupling of reaction and transport during antibody–membrane binding and the role of antibody–membrane dynamics in various immune responses are not yet well understood.

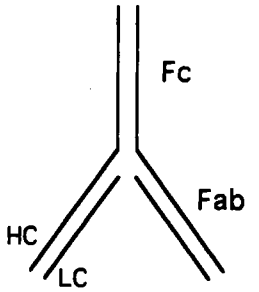

Figure 1. Antibody structure. Immunoglobulin molecules are formed from two identical heavy chains (HC) of MW ≈ 50K (depending on class) and two identical light chains (LC) of MW ≈ 25K. The intact molecules contain two antigen binding regions (Fab) and one Fc region. Different heavy chains result in antibodies of different "class" (i.e., IgG or IgE).

Elucidating the dynamics of antibody–membrane interactions is facilitated by the use of model phospholipid membranes deposited on transparent planar substrates and techniques in laser-based, time-resolved fluorescence microscopy.[1-4] Antibodies may bind either through their Fab regions to specific chemical determinants incorporated into planar model membranes (e.g., antigenic molecules) or through their Fc regions to membrane-embedded Fc receptors that have been

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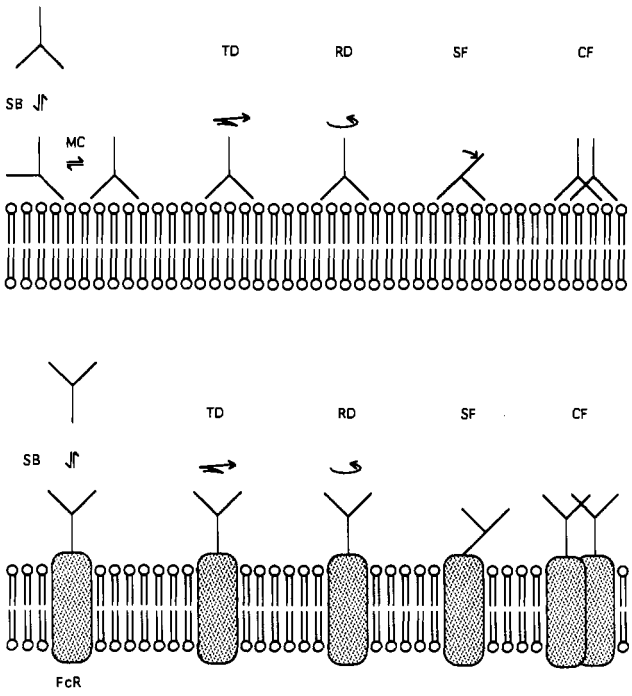

Figure 2. Reaction and transport modes for antibodies at membrane surfaces. (Top) Antibodies are bound to a membrane surface through their antigen binding regions. (Bottom) Antibodies are bound to a membrane surface through their Fc region. Abbreviations are as follows: SB, surface binding; MC, membrane conversion; TD, translational diffusion; RD, rotational diffusion; SF, segmental flexibility; CF, cluster formation.

purified from immunological cells and reconstituted into planar membranes. A wide variety of dynamic fluorescence microscopy techniques may be used to examine quantitatively the structure, organization, and dynamics of antibodies in, on, or near the planar membranes.

Substrate-Supported Planar Model Membranes

One method of constructing substrate-supported models of cell membranes is to use Langmuir–Blodgett film technology. Phosphatidylcholines, which are the most abundant lipid in many natural cell membranes, readily form monomolecular layers at air–water interfaces and are most commonly used to model cell membranes. Phosphatidylcholine monolayers may be transferred from an air–water interface onto transparent, hydrophobic solid supports (e.g., alkylated glass or quartz) so that the acyl chains face the substrate and the head groups face an adjacent, aqueous phase.[5] Substrate-supported phosphatidylcholine bilayers may also be formed by two sequential passes of a transparent hydrophilic substrate (e.g., unalkylated glass or quartz) through a phosphatidylcholine monolayer at an air–water interface. In supported bilayers, the phospholipid head groups face both the substrate and the adjacent water, with the acyl chains forming the membrane interior.[6]

Natural cell membranes contain not only phospholipids but also proteins. However, attempts to reconstitute purified transmembrane proteins into phospholipid Langmuir–Blodgett films have not been widely or successfully adopted, primarily because proteins can denature or oxidize at the air–water interface and because large quantities of protein are required. An alternative method for incorporating transmembrane proteins into supported planar membranes is to reconstitute the proteins into phospholipid vesicles in solution by conventional means; the vesicles may then be deposited on hydrophilic surfaces where they fuse to form a uniform coating of phospholipids and proteins.[7] This type of planar membrane may also be formed using membrane fragments that are derived from whole cells.[8]

Physical Properties of Supported Planar Membranes

A measure of the area that is removed from a phospholipid monolayer at an air–water interface during deposition onto a substrate by the Langmuir–Blodgett method gives an estimate of the amount of material transferred; usually, an area that is slightly greater than the area of the substrate is removed.[5,6,9,10] The result that this "transfer ratio" is approximately equal to 1 provides evidence that single monolayers are deposited during each pass of the substrate through the air–water interface. The number of phospholipid lamellae in supported planar membranes made by the vesicle-fusion technique has not been unequivocally established. However, for some chemical compositions, measurements with radioactive phospholipids as tracers have shown that the vesicle-fusion method results in the deposition of single phospholipid bilayers.[11]

Fluorescent lipids such as nitrobenzoxadiazole-conjugated phosphatidylethanolamine (NBD-PE), nitrobenzoxadiazole-conjugated phosphatidylcholine (NBD-PC), and dioctadecyltetramethylindocarbocyanine (diI) are readily incorporated as tracers in supported planar membranes. In fluid-like Langmuir–Blodgett films, fluorescent lipids are usually uniformly distributed within optical resolution.[4,5,10,12] In Langmuir–Blodgett films that are not fluid-like, fluorescent lipids often do not have uniform spatial distributions. In these samples, the spatial inhomogeneities are most likely a consequence of unequal probe partitioning between coexistent fluid-like and solid-like phospholipid phases.[13–16] In fluid-like planar membranes made by the

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vesicle-fusion method, fluorescent lipids are uniformly distributed except for sparse, bright features of size ≤1 μm^2; these nonuniformities may be due to vesicles that adsorb but do not fuse with the supported membrane.[17-19]

Further evidence for long-range structural integrity in supported planar membranes has been acquired by using polarized total internal reflection fluorescence microscopy (P-TIRFM).[20] In this technique, an evanescent field is created at the surface by a totally internally reflected laser beam. The fluorescence arising from lipid probes is measured as a function of the evanescent field polarization and provides information about the orientation distribution of the fluorophore absorption dipoles. These measurements have shown that both NBD-PE and diI are highly oriented in phospholipid Langmuir–Blodgett films.[20-22]

Translational Mobility of Fluorescent Lipids in Supported Planar Membranes

An established method for characterizing supported planar membranes is to examine the long-range (>10 μm) translational diffusion of fluorescent lipids using fluorescence pattern photobleaching recovery (FPPR).[23] In this technique, an area of the membrane is illuminated with a spatially striped intensity. After irreversibly photobleaching a fraction of the illuminated fluorophores, subsequent fluorescence recovery is observed as unbleached molecules from adjacent nonilluminated stripes move into the illuminated stripes via translational mobility. The rate of fluorescence recovery is related to the stripe periodicity and the translational diffusion coefficient.

Extensive studies with FPPR have shown that phospholipid Langmuir–Blodgett films are often "fluid-like", where the translational diffusion coefficients of fluorescent lipids are $\approx 10^{-8}$ cm^2/s and the fractional mobilities are approximately equal to 1.[5,6,10,12] The films may also be "solid-like", where only a fraction of incorporated fluorescent lipids are translationally mobile and the diffusion coefficient is $\leq 10^{-10}$ cm^2/s.[5,6,21,24] The fluidity of phospholipid Langmuir–Blodgett films is determined by at least five factors, including the lipid composition, the lipid density, the temperature, the nature of the supporting substrate, and the presence of bound proteins.[5,6,25,26] The majority of work with supported planar membranes made by the vesicle-fusion method has been carried out with lipid vesicles that are above their phase transition temperature and result in fluid-like supported membranes.[17-19] Fluorescent lipid translational mobility in supported planar membranes made from isolated cell fragments is low, perhaps due to incomplete vesicle fusion at the substrate surfaces.[27]

Antibodies Tightly Bound to Hapten-Conjugated Langmuir–Blodgett Films

For structural or dynamic studies of antibodies bound to membranes through their antigen binding regions, supported planar membranes must present the specific chemical groups to which an antibody binds. The most common approach in the design of these "antigenic" planar membranes has been to use phosphatidylethanolamine which has been conjugated at its free amino group with a small organic molecule, or "hapten", for which the antibody is specific. A body of work has demonstrated that anti-hapten antibodies specifically bind to phosphatidylcholine Langmuir–Blodgett monolayers and bilayers that contain hapten-conjugated phosphatidylethanolamine.[9,10,12,21,24,25,28-41] This result has been demonstrated for a variety of chemical systems, including those in which the hapten was dinitrophenyl, trinitrophenyl, nitroxide, dinitrophenyl nitroxide, or fluorescein; in which the antibodies were either of the IgG or of the IgE class; and in which the antibodies were either polyclonal or monoclonal. Membrane binding is minimal for antibodies that are not immunologically specific for the surface-associated haptens, for membranes that do not contain hapten-conjugated phospholipids, and in the presence of saturating amounts of solution-phase hapten.

In some (nonequilibrium) studies, hapten-presenting Langmuir–Blodgett films have been treated with solutions containing fluorescently labeled antibodies for defined durations (e.g., 15 min) and then washed with buffered solutions, leaving only tightly bound antibodies. This approach has been useful for subsequent measurements of antibody translational and rotational mobility, antibody crystallization, and cell binding (see below). The two-dimensional density of bound antibodies can be reproducibly controlled by altering the antibody solution concentration, the molar fraction of hapten-conjugated phospholipids in the planar mem-

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branes, or the time for which the films are treated with antibodies. The sensitivity of fluorescence microscopy in detecting bound antibodies is limited primarily by the photochemical bleaching rates of fluorophores; typical working densities of antibodies have been in the range of 10^3 molecules/μm^2, but densities as low as 20–50 molecules/μm^2 have also been detected.[12,17,24,42]

Antibodies Loosely Bound to Hapten-Conjugated Langmuir–Blodgett Films

Because antibodies are bivalent with respect to the Fab regions, membrane-bound antibodies (in general) may be attached either monovalently or bivalently (Figure 2). An understanding of the dynamic conversion between the two bound states requires measurement of loosely bound antibodies, while they are in equilibrium with antibodies in solution.

One method for measuring the surface density of proteins weakly bound to surfaces is total internal reflection fluorescence microscopy (TIRFM).[43,44] In this technique, the thin (≈850 Å) evanescent field created by a totally internally reflected laser beam selectively excites membrane-bound fluorescent proteins. The evanescently excited fluorescence provides a measure of the surface density of bound proteins as a function of the protein solution concentration. The resultant equilibrium binding curve can then be used to obtain the apparent equilibrium association constant. The theoretically measurable range of association constants is lower-limited by the requirement that a significant fraction of the fluorescence arises from molecules bound to specific membrane sites rather than from nonspecifically bound molecules or from molecules in solution that are excited by the finite depth of the evanescent illumination.[3,18,19,27] Surface association constants as low as 10^5 M^{-1} have been measured by TIRFM with good accuracy and precision.[17,24,45]

TIRFM has been used to compare the apparent association constants of a fluorescently labeled anti-dinitrophenyl monoclonal antibody and its Fab fragment on solid-like Langmuir–Blodgett films composed of dipalmitoylphosphatidylcholine (75 mol %) and *N*-[[(dinitrophenyl)amino]caproyl]dipalmitoylphosphatidylethanolamine (25 mol %).[24] In this study, the apparent association constant of the intact antibodies was approximately 10-fold higher than the constant for the Fab fragments (Figure 3). Data analysis with simple theoretical models indicated that, at most surface densities of intact antibodies, 10–50% of the bound antibodies were attached to the membranes by only one antigen binding region. This surprising result conflicts with the notion that a primary biological function of antibody bivalency is to dramatically increase binding to antigenic membranes.

Binding Kinetics of Antibody Fabs on Hapten-Conjugated Langmuir–Blodgett Films

A better understanding of the mechanism and dynamics of antibody–membrane association requires information about binding kinetics. Surface binding kinetic rates (and surface diffusion coefficients) for proteins in equilibrium between solution and planar surfaces may be probed by using evanescent illumination with fluorescence photobleaching recovery (TIR-FPR).[46,47] In this technique, fluorescently labeled proteins are in equilibrium between solution and specific binding sites on the surface. Bound proteins are photobleached by a high-intensity pulse of an evanescent field, and subsequent fluorescence recovery is monitored as bleached molecules exchange with unbleached molecules from solution or from surrounding nonilluminated surface areas. The measured TIR-FPR recovery curves contain information about the intrinsic surface dissociation rates and about the surface and solution diffusion coefficients.

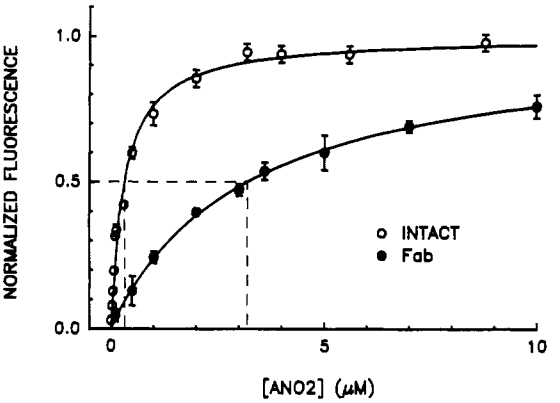

Figure 3. Equilibrium binding of antibodies and their Fabs to phospholipid Langmuir–Blodgett films. The surface-associated fluorescence of a labeled anti-dinitrophenyl IgG monoclonal antibody (ANO2) and its Fab on Langmuir–Blodgett films made from dipalmitoylphosphatidylcholine (75 mol %) and *N*-[[(dinitrophenyl)amino]caproyl]dipalmitoylphosphatidylethanolamine (25 mol %) was measured using TIRFM. The data were corrected for nonspecific binding and fit to the theoretical shape for a bimolecular reversible reaction between monovalent antibodies and monovalent surface sites. The best fits gave apparent equilibrium association constants equal to $\approx 3 \times 10^5$ M^{-1} for the Fabs and $\approx 3 \times 10^6$ M^{-1} for the intact antibodies. The fluorescence has been normalized so that the value at saturation is equal to 1. The data are from ref 24.

TIR-FPR has recently been used to examine the surface binding kinetics of fluorescently labeled anti-dinitrophenyl Fabs on solid-like Langmuir–Blodgett phospholipid monolayers (Figure 4).[35] The fluorescence recovery curves were triexponential, with two rates in the range of seconds and one much slower rate. The complex nature of the TIR-FPR data suggests that the mechanism of Fab–membrane association is less straightforward than a simple bimolecular reaction between monovalent Fabs and monovalent surface sites. Possible explanations for the complex TIR-FPR data include heterogeneous surface binding sites, heterogeneous Fab conformations in solution, orientation effects, and multistep Fab–hapten binding mechanisms.

Translational Mobility of Antibodies on Hapten-Conjugated Phospholipid Langmuir–Blodgett Films

Elucidating the factors that control the translational mobility of membrane-bound antibodies is required for

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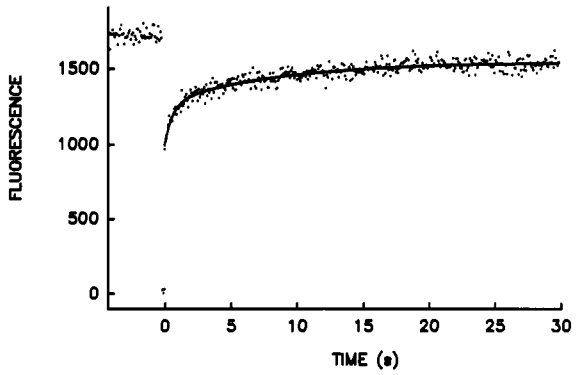

Figure 4. Surface binding kinetics of antibodies on phospholipid Langmuir–Blodgett films. The surface binding kinetics of labeled anti-dinitrophenyl IgG monoclonal Fabs (ANO2) on dipalmitoylphosphatidylcholine (75 mol %)/*N*-[[(dinitrophenyl)amino]-caproyl]dipalmitoylphosphatidylethanolamine (25 mol %) Langmuir–Blodgett films were examined using TIR-FPR. Nonlinear curve-fitting with multiexponential functions indicated that the recovery curves were best described as the sum of three exponentials. The best fit of the data to this functional form gave values for the three rates and fractional recoveries as $\approx 1\ s^{-1}$ (0.5), $\approx 0.1\ s^{-1}$ (0.3), and $\ll 0.1\ s^{-1}$ (0.2). The data are from ref 35.

understanding the physical nature of antibody-mediated membrane functions. Although a considerable body of theoretical work has addressed the translational diffusion of proteins in and on fluid-like phospholipid bilayer environments, very little data are available for testing these theories.[48] Thus, quantitative measurements of the translational diffusion coefficients of antibodies on supported planar membranes should not only assist in the understanding of membrane function in immunology but should also provide a paradigm for understanding other membrane-associated biological functions.

The translational diffusion of antibodies associated with hapten-conjugated phospholipid Langmuir–Blodgett films has been measured for several model membrane systems using FPPR.[10,12,21,24,25,30] These studies have demonstrated that the diffusion coefficients and the fraction of the bound antibodies that undergo diffusion can sharply depend both on the chemical composition of the membrane and on the antibody surface density. For solid-like Langmuir–Blodgett films, tightly bound antibodies are not appreciably mobile.[21,24] For fluid-like Langmuir–Blodgett films containing low densities of bound antibodies, the translational diffusion coefficient of the membrane-bound antibodies is comparable (within a factor of 2) to the measured diffusion coefficients of fluorescent lipids in the membranes, and a high fraction (≈ 1) of the bound antibodies undergo translational motion.[10,12,25,30] At intermediate antibody surface densities, both the antibody translational diffusion coefficient and the mobile fraction are decreased: in one study, multiple diffusion coefficients were observed, providing evidence for the formation of submicroscopic antibody or mixed antibody/lipid clusters;[12] in a different study, the reduction in translational mobility was correlated with the antibody surface density rather than the membrane composition.[10] When fluid-like Langmuir–Blodgett films are nearly saturated with bound antibodies, the antibody translational diffusion coefficient is dramatically reduced, sometimes to immeasurable levels ($\ll 10^{-12}\ cm^2/s$).[25,29,10] In some cases, this reduction in translational mobility has been accompanied by the appearance of nonuniform spatial distributions of antibodies.[30] The observation that the translational diffusion coefficient of bound antibodies is always less than or equal to the diffusion coefficient of fluorescent lipids in Langmuir–Blodgett films argues against the possibility that antibodies undergo translational motions independent of the motions of the lipids.

Rotational Mobility of Antibodies on Hapten-Conjugated Langmuir–Blodgett Films

The degree of rotational mobility and/or segmental flexibility that must occur for active sites on membrane-bound antibodies to become properly positioned for interaction with other molecules is of potential physiological importance. An experimental method for probing the rotational motions of antibodies bound to supported planar membranes is polarized fluorescence photobleaching recovery (PFPR).[49,50] This method is analogous to FPPR except that the excitation source is linearly polarized, and the illuminated area is large and spatially homogeneous to minimize contributions to the fluorescence recovery that might arise from translational motions. Fluorophores having absorption dipoles aligned parallel to the bleaching beam polarization are preferentially photobleached, creating an anisotropic, postbleach fluorophore distribution. If the rate of translational diffusion through the illuminated area is slow, subsequent changes in the postbleach fluorescence anisotropy reflect the rate of fluorophore rotational redistribution.

The rotational motions of anti-dinitrophenyl antibodies tightly bound to solid-like Langmuir–Blodgett monolayers composed of distearoylphosphatidylcholine (70 mol %) and dinitrophenyldioleoylphosphatidylethanolamine (30 mol %) have been examined with PFPR (Figure 5).[21] These measurements showed that the membrane-bound antibodies had characteristic rotational times that were extremely long (>seconds). Comparably slow rotational correlation times were also measured for the fluorescent lipid dil. This result is analogous to the observation that the translational diffusion coefficients of fluorescently labeled antibodies on planar membranes, at low antibody surface densities, are approximately equivalent to the translational diffusion coefficient of fluorescent lipids (see above).

The PFPR data also indicated that a significant fraction of both fluorescently labeled antibodies and fluorescent lipids were rotationally restricted. One possible explanation for the observed restriction is that both antigen binding regions are attached to the membrane over the time course of fluorescence recovery, so that the measured rotational motions reflect restricted segmental flexibility of the bound antibodies. However, because segmental fluctuations are expected to be rapid, a more plausible explanation may be that the antibodies report restrictions on lipid rotational mobility. Lipids in solid-like membranes could be rotationally restricted due to direct interactions with other lipids, interactions with the substrates, or the

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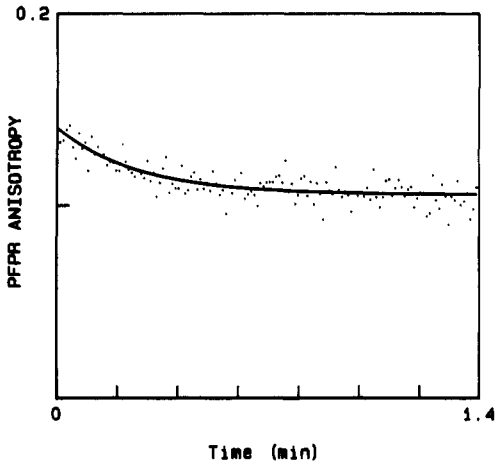

Figure 5. Rotational mobility of antibodies on Langmuir–Blodgett films. The rotational mobility of an anti-dinitrophenyl IgG monoclonal antibody (ANO2) on distearoylphosphatidylcholine (70 mol %)/dinitrophenyldioleoylphosphatidylethanolamine (30 mol %) Langmuir–Blodgett films was examined using PFPR. The best fit of the anisotropy function to an appropriate theoretical form gave a rotational correlation time of $\approx(100\ s)^{-1}$. The data are from ref 21.

existence of small gel-phase domains which undergo joint rotational motion. A theory for interpreting PFPR data in systems where rotational mobility is restricted has recently been developed.[51]

Two-Dimensional Crystals of Antibodies on Planar Membranes

Under some conditions, antibodies on phospholipid Langmuir–Blodgett films spontaneously arrange into two-dimensional ordered arrays which have been called "two-dimensional crystals".[36–41] The formation of these ordered arrays depends on the chemical and physical structures of the films, the conditions under which the films are treated with antibody solutions, and the antibody structure. With the use of electron microscopy and optical diffraction techniques, at least three distinct types of patterned antibody arrays have been resolved for IgG and IgE antibodies on Langmuir–Blodgett films. These new crystallization and analysis methods are of considerable interest because they may prove to be broadly applicable for obtaining structural information about membrane-associated proteins.[52] Whether the tendency for antibodies to form long-range ordered arrays on phospholipid Langmuir–Blodgett films is related to the putative formation of antibody clusters on single-cell membranes or in the region of contact between two adjacent cell membranes is not yet clear.

Antibodies on Planar Membranes Containing Fc Receptors

Effector mechanisms of the immune system, which serve to destroy the pathogenic microorganisms recognized by the Fab regions of antibodies, are mediated by the Fc regions of antibodies. Antibody Fc regions bind to membranes via a family of cell surface Fc receptors on different cell types (e.g., macrophages, neutrophils, and B cells). A large body of recent work has provided information about the structure, expression, distribution, and function of different Fc receptor genes and their products.[53–55] For IgG, the most abundant antibody in blood, three structurally distinct forms of Fc receptors have been identified in mice and humans. Physical characterization of the interactions between IgG and these receptors has been limited, primarily because multiple receptor forms are present on different cell types and because the association of IgG with the receptors is often too weak to be directly measured with conventional methods.

One of the IgG Fc receptors, FcγRII,[56] is a glycosylated, single transmembrane-spanning, 50-kDa polypeptide. This receptor has been reconstituted into supported planar membranes by allowing detergent-dialyzed vesicles, derived from membrane fragments isolated from macrophage-related cells, to fuse onto quartz substrates.[27] TIRFM measurements showed that Fabs from an anti-FcγRII monoclonal antibody bound specifically to these planar membranes, indicating that the planar membranes contained the receptor. Using an indirect TIRFM assay in which the binding of fluorescently labeled anti-FcγRII Fabs was blocked by unlabeled IgG, the Fc-binding activity of the reconstituted FcγRII was demonstrated. However, the density of functionally active FcγRII in the planar membranes ($\approx$50 molecules/μm^2) was approximately 25-fold lower than the natural cell surface density and was too low for TIRFM to directly detect bound, fluorescently labeled IgG.

To increase the receptor density, supported planar membranes were formed with the vesicle-fusion technique, using phosphatidylcholine/cholesterol vesicles that contained purified FcγRII.[17] The density of FcγRII in these membranes was much higher (500–1500 molecules/μm^2), allowing bound, fluorescently labeled IgG to be directly detected with TIRFM (Figure 6). The fluorescence was significantly reduced for planar membranes that did not contain FcγRII, for antibodies from which the Fc region had been enzymatically cleaved, and in the presence of excess amounts of anti-FcγRII Fab. These results indicated that the IgG binding was biologically specific. The estimated equilibrium association constant for IgG and FcγRII ($2 \times 10^5\ M^{-1}$) agreed well with previous values obtained from indirect radioimmunoassays on cell surfaces.

Two questions of biological significance are whether occupation of IgG antigen binding regions can change the physical parameters of IgG–FcγRII interactions, and whether all monoclonal antibodies of a given IgG subclass bind with equal affinity to FcγRII. These questions have been addressed with supported planar membranes and TIRFM using several different anti-dinitrophenyl IgG antibodies.[45] The measured association constants obtained with TIRFM for the different IgG antibodies with FcγRII were approximately equivalent and did not differ in the absence or presence of saturating amounts of dinitrophenylglycine in solution. Antigenic ligands such as peptides or proteins have an antibody–ligand contact area that is larger and different

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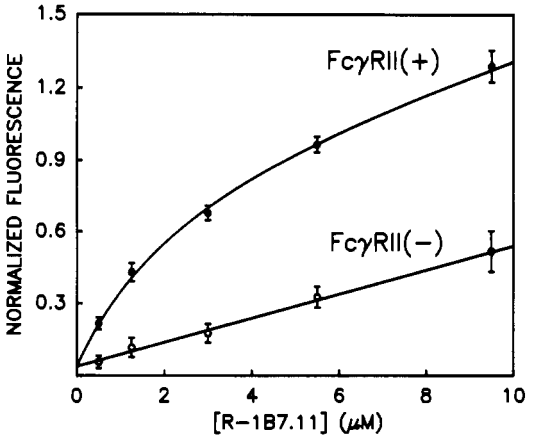

Figure 6. Equilibrium binding of antibodies to supported planar membranes containing FcγRII. The evanescently excited fluorescence of a monoclonal IgG antibody (1B7.11) on planar membranes containing FcγRII was higher than the fluorescence on membranes without FcγRII. The best fit of the difference between the two measured binding curves to the form for a simple reversible bimolecular reaction gave an association constant of $\approx 2 \times 10^5$ M^{-1}. The data are from ref 45.

in character than that of small, organic haptens and may have a larger effect on IgG–FcγRII interactions.

Immunological Cells on Antibody-Coated Planar Membranes

A potentially interesting application of planar model membranes is to use them to quantitatively define the molecular requirements for and effects of target recognition by immunological cells possessing the intrinsic ability to destroy antibody-coated matter. Several previous studies have shown that macrophages and macrophage-related cells specifically recognize phospholipid Langmuir–Blodgett monolayers or bilayers containing hapten-conjugated phospholipids and specifically bound IgG.[28-32] The bound cells respond to the IgG-coated planar membranes as though they were cellular targets, as evidenced by glucose oxidation, release of superoxide and lysosomal enzymes, and cellular spreading on the membrane. Immunological cells containing surface receptors for IgE antibodies may also specifically bind to antibody-coated supported planar membranes.[9]

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Summary and Future Directions

The use of model phospholipid membranes deposited on transparent substrates and techniques in laser-based, time-resolved fluorescence microscopy holds considerable promise for delineating the distributive and dynamic properties of membrane-associated antibodies. These measurements should facilitate a deeper understanding of the physical chemistry that underlies membrane function during antibody-mediated immunity. The results should also serve as a paradigm for other cellular processes involving the association of soluble proteins with membrane surfaces.

One current limitation of the described experimental approach is that reconstituted transmembrane proteins like FcγRII do not undergo significant (i.e., physiologically relevant) translational mobility.[17] Although the reasons for the restricted translational mobility are not well understood, it is plausible that direct interactions between the cytoplasmic regions of the receptors and the substrate significantly retard lateral motions. The future development of reconstitution methods that yield proteins with physiologically relevant degrees of translational mobility should expand the range of applicability of supported planar membranes as models of natural cell membranes.

Several other fluorescence microscopy techniques that can be used to probe macromolecular dynamics may also be applicable to supported planar membranes. Fluorescence correlation spectroscopy (FCS), which measures fluorescence fluctuations occurring within a small illuminated volume, gives information about the source of the fluorescence fluctuations (e.g., translational diffusion, rotational diffusion, flow, or chemical reaction between states with different fluorescence yields) and also contains unique information about the state of oligomerization of the fluorescent species under investigation.[57,58] The use of evanescent interference patterns with fluorescence photobleaching recovery (TIR-FPPR) is a recently developed method that may yield information about the translational diffusion coefficients of loosely bound proteins.[9,59-62] Instrumentation has been developed for characterizing energy transfer between donor and acceptor chromophores using fluorescence microscopy; this experimental method should prove useful for measuring structural parameters that describe the spatial organization of molecular components in or near supported planar membranes.[63] Finally, fast (psec-nsec), time-resolved fluorescence microscopy may yield new information on the rapid rotational and segmental motions of membrane-associated lipids and proteins.[64]

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