Chapter

Anti-Non-Bilayer Phospholipid Arrangement Antibodies Trigger an Autoimmune Disease Similar to Systemic Lupus Erythematosus in Mice

Albany Reséndiz-Mora, Alonso Tescucano, Giovanna Barrera-Aveleida, Anahi Sotelo-Rodríguez, Christian-Irene Nevárez-Lechuga, Iván Galarce-Sosa, Isabel Wong-Baeza, Isabel Baeza and Carlos Wong-Baeza

Abstract

Anti-lipid antibodies are present in some infectious and autoimmune diseases, such as Systemic Lupus Erythematosus (SLE). Particularly, anti-non-bilayer phospholipid arrangement (NPA) antibodies have been detected in patients with SLE, and these antibodies trigger a disease similar to human lupus in mice. NPA are lipid associations different from the lipid bilayer of cellular membranes and, since they are transient, they are not immunogenic. However, if NPA are stabilized by drugs, they induce an immune response with the production of anti-NPA antibodies, which bind to NPA on cell membranes and generate cell lysis. As a result, intracellular antigens are exposed and trigger an immune response that generates more auto-antibodies. In this chapter, we describe the formation and stabilization of NPA, the induction of B cell responses to generate anti-NPA antibodies, and the characteristics that the disease caused by these antibodies in mice shares with human lupus.

Keywords: non-bilayer phospholipid arrangements, anti-lipid antibodies, B cells responses, autoimmunity, mouse model of lupus

1. Introduction

1.1 Cell membranes

The cell membrane is the structure that gives cells their individuality by separating them from the extracellular medium and from other cells. It regulates the transport of ions, molecules and signals towards the interior and exterior of the cell. Membranes

confer the selective permeability that maintains the differences in composition between the cytoplasm and the extracellular medium, which in turn regulates the cellular volume and the cellular response to the different signals it receives or generates. In eukaryotes, membranes divide the cell interior into compartments, or organelles [1]. Membranes are made of proteins, lipids, and carbohydrates. Proteins and lipids represent almost its entire composition, while carbohydrates represent less than 10% [2]. In particular, lipids represent from 25 to 80% of the membrane weight, and they belong to three chemical groups: phospholipids, glycolipids and sterols. All membrane lipids are amphipathic molecules that have an hydrophilic region and an hydrophobic region. Phospholipids and glycolipids spontaneously assemble into closed bilayers in aqueous mediums and constitute the membrane matrix [1, 3].

1.2 Molecular shape of membrane lipids and their molecular associations

X-ray diffraction studies of membrane lipids have shown that the area of their polar regions (A_O) significantly varies in comparison with the cross-sectional area of their hydrocarbon chains (A_H). These studies have revealed three molecular shapes: cylindrical (**Figure 1A**), conical (**Figure 1B**) and inverted conical (**Figure 1C**) [4]. In cylindrical lipids, the area of the polar region is almost equal to the cross-sectional area of the non-polar region, so the A_H/A_O ratio is close to 1. In conical lipids, the area of the polar region is less than the cross-sectional area of the non-polar and A_H/A_O is >1; while in the inverted conical lipids this relationship is inverse and A_H/A_O is <1 [5]. Cylindrical lipids, such as phosphatidylcholine (**Figure 1D**), phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, and sphingomyelin, associated in aqueous medium to form closed bilayers or liposomes (**Figure 1E**), and represent 60 to 70% of membrane lipids. Conical lipids, such as phosphatidate (**Figure 1F**), phosphatidylethanolamine, diacylglycerol, and cardiolipin, assemble into an hexagonal phase II (**Figure 1G**), which consists of hexagonally packed cylinders, with the polar regions directed towards the interior of the cylinder where they form an aqueous pore of around 50 Å in diameter [5]. Inverted conical lipids, such as lysophospholipids and gangliosides (**Figure 1H**), assemble into a micellar phase (**Figure 1I**), with the polar regions towards the outside and the non-polar regions towards the inside. The membrane has lipids with the three molecular forms, and the higher proportion of cylindrical lipids compared to conical and inverted conical lipids allows the association of these three kinds of lipids into bilayers. However, membrane lipids can also have different molecular associations. ^{31}P NMR studies have indicated the presence of lipids associated in non-bilayer phospholipid arrangements (NPA) (**Figure 1J**) in membranes with high metabolic activity, such as in cancer cells and in rat liver microsomes [6].

1.3 Supramolecular organization of cell membranes

Singer and Nicolson proposed the fluid mosaic model for cell membranes in 1972. In this model, the integral proteins are inserted in the lipid bilayer, which constitutes the mosaic, and it is fluid because the interactions between lipids and between lipids and proteins are non-covalent, which allows these molecules to move laterally across the membrane.

In cell membranes, the role of lipids is mainly structural, and the type of fatty acids they contain determines the fluidity of the membrane. The hydrophobic effect

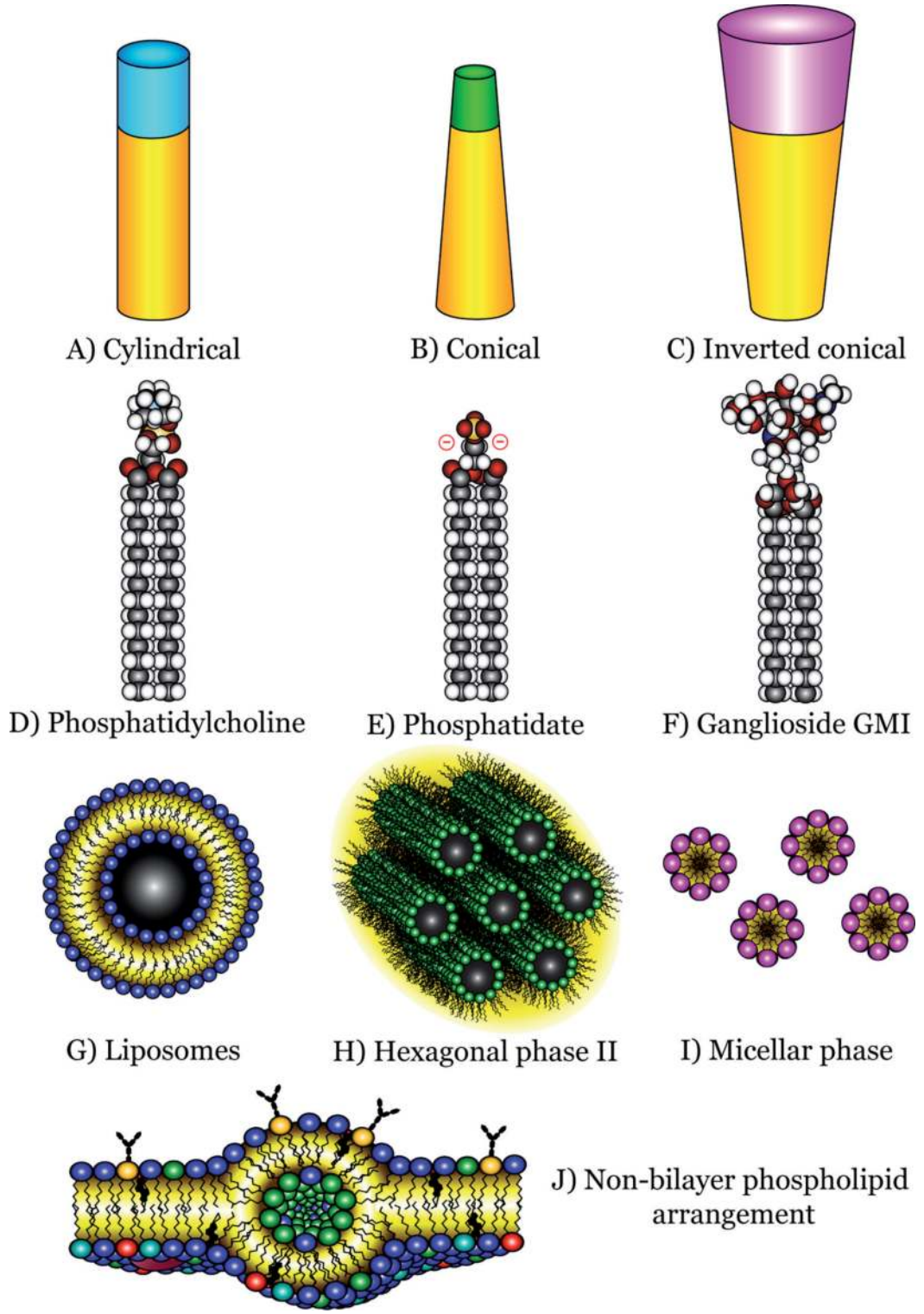

Figure 1.
Classification of membrane lipids based on their molecular shape. The lipid molecular shape (A–C) depends on its chemical structure (D–F). In addition, the three-dimensional association of lipids in an aqueous medium (G–I) depends on the lipid molecular shape. Diagram of lipids associated in a non-bilayer phospholipid arrangement (J).

is the main force that maintains the organization of proteins and lipids in this model. The thickness of cell membranes, with their peripheral and integral proteins, is around 100 Å [7, 8].

Although the fluid mosaic model explains multiple properties of cell membranes, it does not consider the cylindrical, conical and inverted conical molecular shapes of lipids, nor their functional role. Cullis and colleagues proposed the metamorphic mosaic model, where the bilayer is formed by lipids of the three molecular forms, and may temporarily have lipid associations different from the bilayer, such as NPA (**Figure 1J**). These structures, which form a microdomain, may participate in many cellular functions, including phagocytosis, membrane junctions, transport of ions and polar molecules, membrane fusion in exocytosis and endocytosis, protein insertion, and formation of polar pores and compartments [4]. The importance of this model lies in proposing lipid associations different than the lipid bilayer, like NPA, which participate dynamically in membrane functions. Therefore, they attribute a functional role to lipids, in addition to the structural role of Singer and Nicolson.

Lipids are generally poorly immunogenic molecules [9]. From the two molecular associations that can occur in cell membranes, the lipid bilayer is considered to be the least immunogenic, because it constitutes the lipid matrix of all cell membranes. NPA are also poorly immunogenic, since they are transitory and therefore are not detected by the immune system; however, if they are stabilized by amphipathic molecules, an immune response is induced with the production of antibodies against these lipid structures [10]. Among the molecules that have been found to stabilize NPA are drugs that, as a side effect, induce a disease similar to Systemic Lupus Erythematosus (SLE) in humans. SLE is a chronic, multifactorial autoimmune disease with an unknown etiology. SLE patients present anti-nuclear, anti-histone, anti-cardiolipin and anti-DNA auto-antibodies that can form antigen-antibody complexes that damage multiple organs. This disease can affect the skin, joints, blood cells, kidneys and the nervous system [11]. The most common clinical manifestations are extreme tiredness, unexplained fever, skin rash, malar rash, and arthritis. Serious complications can also occur, such as lupus nephritis and autoimmune cytopenias [12]. SLE mainly affects women in a female to male ratio of 9:1 [13]. This disease can develop at any age; however, in most cases it occurs between the ages of 24 and 32 during the fertility peak, so female sex hormones are considered a key factor in the development of this disease [14]. According to the Lupus Foundation of America, at least 5 million people worldwide have lupus. There is a higher prevalence and incidence among the Hispanic, Asian, and African-American populations [15].

1.4 Drug-induced lupus in humans

Drug-induced lupus its generated by the chronic intake of certain drugs, which induce an immune response that triggers a disease that is very similar to, but less severe than SLE. There are about 38 drugs that cause drug-induced lupus, including hydralazine, procainamide and isoniazid, which are responsible for most of the cases [16]. The exact mechanism that leads to drug-induced lupus is not well understood; however, one factor that predisposes to its development is the rate at which drugs are metabolized, which is markedly decreased in patients with a genetic deficiency of N-acetyl transferase. These patients have a higher incidence of drug-induced lupus [17].

In addition, it has been reported that these lupus-inducing drugs can suppress central and peripheral tolerance, alter gene transcription in T and B cells, alter the balance and function of cytokines or their receptors, and modify the structure of

chromatin and self-antigens [17–19]. Another possible mechanism that would explain the involvement of these drugs in the development of lupus is the stabilization of NPA on cell membranes, which then become immunogenic. We have explored this mechanism in a mouse model of lupus induced by drug-stabilized NPA on liposomes [20].

1.5 Mouse models of lupus

Mouse models of lupus have been very important to understand the genetic, cellular and molecular mechanisms of this autoimmune disease [21]. B/W mice, MRL/*lpr* mice and BXSB mice have been the most frequently used mouse models of lupus. New Zealand Black (NZB) mice develop autoimmune hemolytic anemia in the early stages of their lives, with reticulocytosis, jaundice and splenomegaly. Anti-nuclear antibodies are found in these mice, although generally in low titers [21]. The cross between NZB mice and New Zealand White (NZW) mice produces B/W mice, which develop a more aggressive autoimmune disease than that of NZB mice; this disease has similar characteristics to human lupus. B/W mice have mutations in the major histocompatibility complex (MHC) genes, and present high titers of anti-nuclear and anti-DNA antibodies, and glomerulonephritis caused by immune complexes [22]. Females B/W mice are more severely affected than males [23].

Murphy-Roths large (MRL)/*lpr* mice have a mutation on the Fas gene, which leads to deficient B and T cell apoptosis. These mice present non-malignant lymphoid proliferation and manifestations of autoimmunity, including the production of anti-DNA and anti-ribonucleoprotein antibodies, glomerulonephritis, vasculitis, and arthritis [22]. BXSB/Yaa mice have a duplicated genome section that includes the toll-like receptor 7 (*Tlr7*) and the phosphoribosyl pyrophosphate synthetase 2 (*Prps2*) genes. These mice spontaneously produce anti-DNA antibodies and develop an immune complex-mediated glomerulonephritis that resembles the glomerulonephritis of SLE patients. In contrast with B/W mice and MRL/*lpr* mice, male BXSB/Yaa mice are more severely affected by the disease than females [24].

In B/W mice, MRL/*lpr* mice and BXSB mice, a genetic abnormality alters the regulatory mechanisms of the immune system and promotes the development of autoimmunity. In other mouse models, a lupus-like disease can be induced in mice that are not genetically susceptible to autoimmune disease. This category includes mice that develop lupus after receiving DNA/protein or RNA/protein complexes [25, 26], or after receiving pristane (2,6,10,14-tetramethylpentadecane) [27, 28], and it also includes the mouse model of lupus induced by drug-stabilized NPA on liposomes.

1.5.1 Mouse model of lupus induced by lipids associated in NPA

This mouse model of lupus can be developed by the administration of liposomes bearing drug-stabilized NPA, or by the administration of the NPA-stabilizing drugs alone; these drugs include chlorpromazine (anti-psychotic), hydralazine (anti-hypertensive - diuretic) and procainamide (anti-arrhythmic) [20]. Mice develop IgG anti-NPA antibodies, followed by anti-cardiolipin, anti-histone, anti-nuclear and anti-coagulant antibodies. They present moderate alopecia, symmetrical facial lesions similar to those observed in SLE patients, and immune complex deposits between the dermis and the epidermis, in the walls of the glomerular capillaries and in the glomerular mesangium, as occurs in SLE. The symmetrical facial lesions and the deposition of immune complexes between the dermis and the epidermis are unique features of this mouse model that have not been described in other mouse models of lupus.

In this mouse model, drug-stabilized NPA become immunogenic and induce the production of anti-NPA antibodies, which are of the IgG class. Anti-lipid IgG antibodies have been detected in infectious diseases caused by mycobacteria [29] and in autoimmune diseases such as SLE [30]. For protein antigens, the development of IgG antibodies implies an isotype switch in germinal center reactions [31], but these events have practically not been studied *in vivo* for lipid antigens. Thus, this mouse model of lupus offers a unique opportunity to analyze the role of germinal centers, the extrafollicular reaction, and plasma cell development in response to lipid antigens.

2. Liposomes as model membranes to study the immunogenicity of lipids

Liposomes can be formed by the modified [10] reverse phase evaporation method [32], where the cylindrical lipid phosphatidylcholine and the conical lipid phosphatidate are used in a 2:1 molar ratio. The higher proportion of cylindrical lipids allows the liposome to form a lipid bilayer association (smooth liposome) (**Figure 2A**). When the NPA inducers chlorpromazine (**Figure 2B**), promazine, procainamide or hydralazine are added, they interact with the conical lipids and generate a lipid rearrangement that forms an inverted micelle that is the center of the NPA (**Figure 2C**); therefore, a liposome bearing NPA is formed (**Figure 2D**). These drugs are used to treat completely different disorders, but they all cause as a side effect a disease similar to SLE [17]. These drugs are amphipathic, so they have a high affinity for lipid bilayers. When the drug is inserted into the liposome bilayer, it diffuses freely until it interacts with a phosphatidate molecule. This interaction is facilitated because the drugs have a positive charge and a triangular shape, while the phosphatidate has a conical shape and two negative charges (**Figure 1B** and **E**).

2.1 Analysis of NPA formation on liposomes

The formation of NPA on liposomes can be detected by flow cytometry [33]. In this technique, the laser beam dispersion by a liposome gives information regarding the liposome size and membrane complexity. The reverse phase evaporation method described above was designed to obtain unilamellar liposomes. Therefore, the size of the smooth liposomes is very similar to the size of the liposomes bearing NPA. With the use of other techniques like the thin-film hydration method that produce multilamellar liposomes, liposomes with more heterogeneous sizes are obtained. The membranes of smooth liposomes have less complexity than the membranes of liposomes bearing NPA. This difference in membrane complexity is revealed because the laser beam dispersion is higher in liposomes bearing NPA than in smooth liposomes [20, 34].

2.2 Liposomes with drug-stabilized NPA induce a lupus-like disease in mice

The development of this mouse model of lupus is based on the generation of an immune response against NPA that leads to the formation of anti-NPA antibodies. These antibodies bind to the NPA that are naturally present in mouse cells and cause their lysis [10, 33]. This model of lupus can be induced in three ways [10, 20, 35]:

1. Direct administration of the drugs chlorpromazine, promazine, hydralazine or procainamide to mice. In this case, the drugs stabilize NPA on mouse cell membranes.

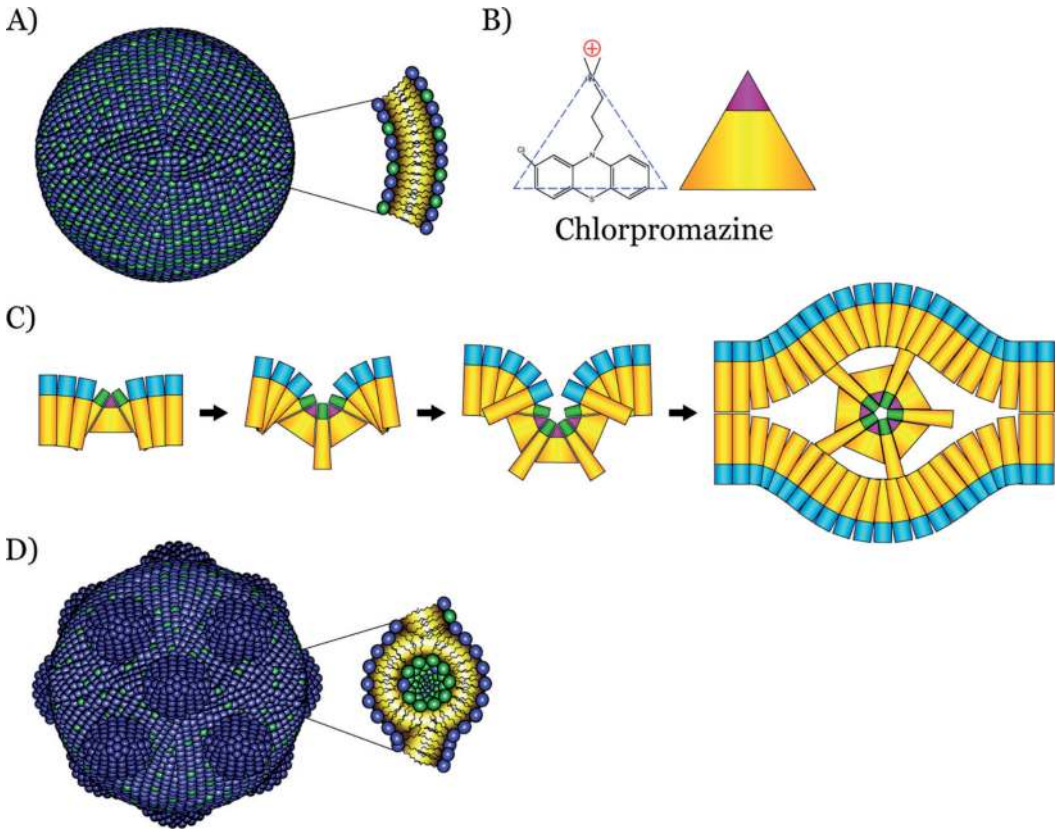

Figure 2.
Molecular structure of liposomes and NPA formation. Smooth liposomes made of phosphatidylcholine (blue polar head) and phosphatidate (green polar head) in a 2:1 molar ratio form a lipid bilayer (longitudinal view) (A). The drugs that induce NPA such as chlorpromazine, are amphipathic molecules with a triangular shape and a positive charge (B). When the NPA-inducer chlorpromazine (pink) is added to the smooth liposomes, it mainly interacts with phosphatidate, because this lipid has a conical shape with two negative charges. This interaction induces a lipid rearrangement that forms an inverted micelle that is the center of the NPA (C). In liposomes bearing NPA, most of the phosphatidate is forming inverted micelles inside NPA (longitudinal view) (D).

2. Administration of liposomes bearing drug-stabilized NPA to mice.

3. Administration of the H-308 monoclonal antibody to mice. This antibody is specific for NPA, and binds to the NPA on mouse cell membranes, causing their stabilization.

The administration of liposomes bearing drug-stabilized NPA is the method that generates the highest titers of anti-NPA antibodies, particularly when chlorpromazine is used as the NPA inducer, because these NPA are larger and their epitopes are more exposed [20, 35, 36].

During the formation of NPA, phosphatidate molecules are shifted (as a result of their interaction with an NPA-inducing drug) from a bilayer to an inverted micelle, which lodges between the two lipid monolayers and forms the center of an NPA [33, 37]. The insertion of the micelle spreads the phospholipids polar heads that surround it and exposes new epitopes to the immune system (**Figure 3A**). This open spatial arrangement of phospholipids may favor the activation of the adaptive immune system cells, thereby leading to the formation of antibodies against the phospholipids that form the lipid bulge, which is structurally different from the surface of a normal lipid bilayer, where

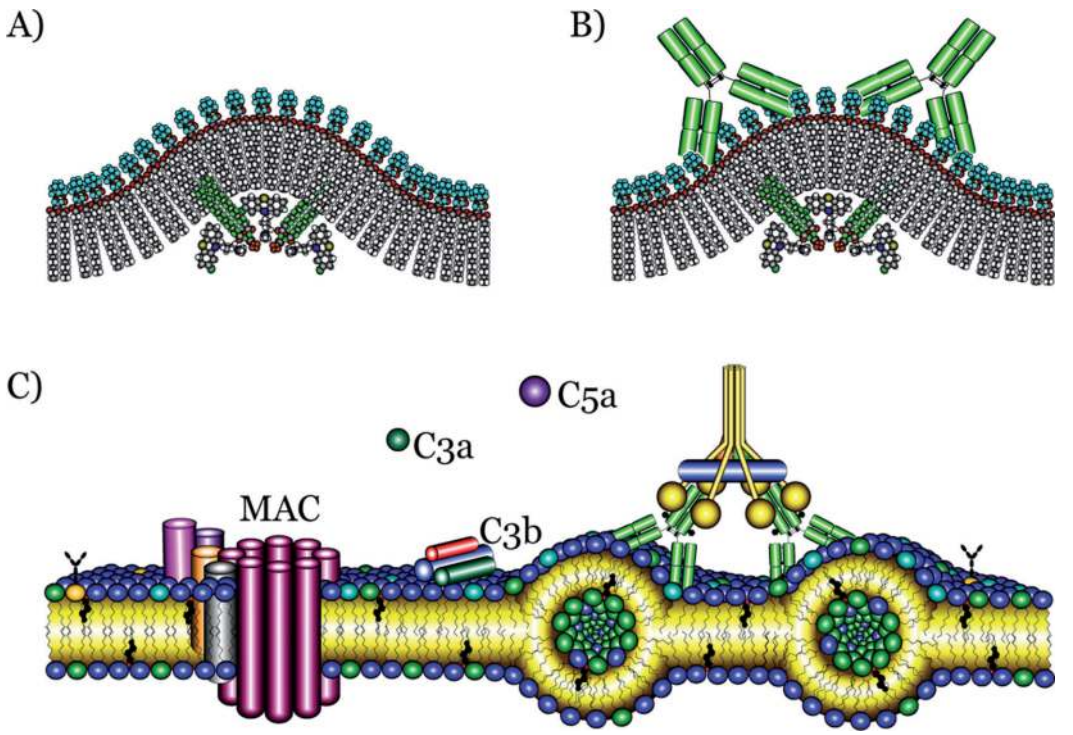

Figure 3.
Recognition of antigens by anti-NPA antibodies. Cross section of a liposome made of phosphatidylcholine (blue) and phosphatidate (green) bearing a chlorpromazine-induced NPA, which shows the spreading of the phosphatidylcholine molecules at the top of the NPA and the exposure of new antigens to the immune system (A). The immune system produces anti-NPA antibodies that recognize the polar heads of phosphatidylcholine (B). Anti-NPA antibodies bind to NPA on mouse cells and activate the complement cascade. Anaphylatoxins (C3a, C5a) are released to the medium, while opsonizing factors (C3b) bind to the cell; the membrane attack complex (MAC) is assembled and causes cell lysis.

the polar heads are not separated [1]. Anti-NPA antibodies bind to the phospholipids that are spread at the top of the NPA (**Figure 3B**), which in liposomes is the lipid phosphatidylcholine, and not to the conical phospholipids or the inducers that form the inverted micelle (this micelle is submerged between the two monolayers of phospholipids). The specificity of the anti-NPA antibodies has been demonstrated with the use of haptens that represent the polar region of phospholipids; these studies confirmed that anti-NPA antibodies recognize the polar region of phosphatidylcholine [37].

Anti-NPA antibodies are the first antibodies that can be detected by ELISA or by flow cytometry in the serum of mice that received liposomes bearing NPA [10, 20, 35]. Most of these antibodies are IgG, and their affinity increase over time [36]. Six weeks after anti-NPA antibodies are detected, anti-histone and anti-cardiolipin antibodies can be detected by ELISA in the serum of these mice [20, 36, 37]. The delayed appearance of auto-antibodies against intracellular antigens can be explained if anti-NPA antibodies cause the lysis of NPA-bearing cells (perhaps by activating the complement cascade) (**Figure 3C**) and lead to the exposure of intracellular antigens, which now become targets of the immune system.

Mice that received NPA-stabilizing drugs, liposomes bearing drug-stabilized NPA or the H-308 monoclonal antibody developed a lupus-like disease characterized by piloerection, anorexia, weight loss, moderate alopecia and symmetrical facial lesions resembling the rash described in human lupus (**Figure 4**). The alopecic skin shows atrophic epidermis, diffuse lymphocytic infiltrates, an accentuated decrease

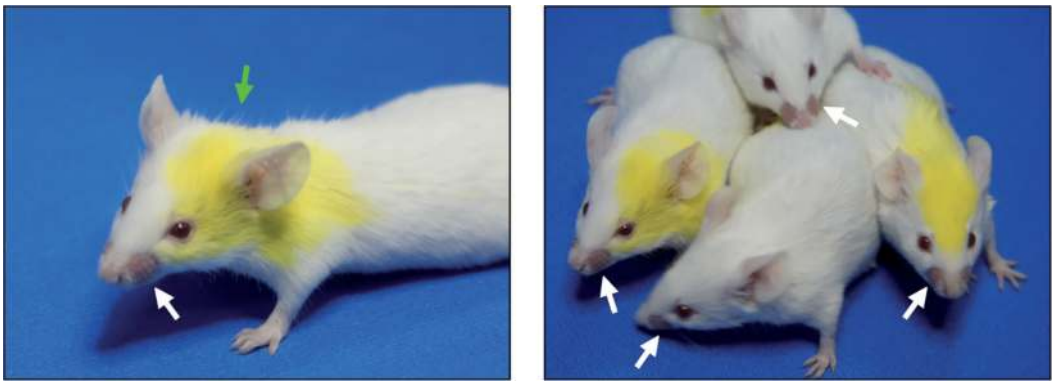

Figure 4.
Pictures of mice that develop a lupus-like disease after the administration of liposomes bearing NPA. Representative photographs of 6-month-old female BALB/c mice that received liposomes bearing chlorpromazine-stabilized NPA (mice are identified by marks made with a picric acid solution, which shows as yellow color). White arrows indicate the facial lesions. The green arrow indicates piloerection.

of terminal hair follicles, inflammation around the matrical cells of hair bulbs, and widening of the external fibrous sheath with massive disaggregation of matrical cells [10, 20]. The kidneys show a mild enlargement of the mesangial matrix with thickened capillary walls in the glomeruli [20, 37]. Immune complex deposits were also found between the dermis and the epidermis (similar to the lupus band described in SLE patients), as well as in the capillaries and the renal mesangium [10].

3. Elucidating the mechanisms that lead to the production of IgG anti-lipid antibodies

IgG anti-lipid antibodies have been detected in some infectious diseases, such as those caused by mycobacteria or in malaria, in individuals with autism-spectrum diseases and in individuals with autoimmune diseases, such as the anti-phospholipid syndrome and SLE [29, 30, 38, 39]. However, little is known about the mechanisms that lead to the production of these IgG antibodies. Understanding these mechanisms can help to understand the development of these diseases, and can lead to new therapeutic targets that improve the life quality of the patients. The mouse model of lupus induced by liposomes bearing NPA is particularly suitable to study the cellular and molecular mechanisms that lead to the production of IgG anti-lipid antibodies.

3.1 Identification of anti-NPA antibody-producing plasma cells

Plasma cells are the differentiation product of mature B cells [40]. Plasma cells can be short- or long-lived [41]. T-cell independent responses in the extrafollicular region of secondary lymphoid organs lead to the production of short-lived plasma cells, which in mice have a lifespan of less than a week. Long-lived plasma cells are generated in the germinal centers in T-cell dependent responses; after their generation, some of these cells migrate to the bone marrow and have a lifespan of months [42, 43].

Anti-NPA antibody-producing plasma cells were identified by flow cytometry as cells that contained intracellular NPA-bearing liposomes (stained with a lypophylic dye) and intracellular IgG antibodies [36]. These specific plasma cells were found in the spleen, the inguinal lymph nodes and the bone marrow of mice that produce

anti-NPA antibodies. A higher number of NPA-specific plasma cells were found in the spleen than in the lymph nodes [36], perhaps because the spleen contains additional B cell subsets (B1 and marginal zone B cells) that can also generate plasma cells [40, 44]. Interestingly, NPA-specific plasma cells were also found in the bone marrow [36]. Bone marrow plasma cells are generally considered to be long-lived, because they receive BAFF (B-cell activating factor) family cytokines, which promote their long-term survival. These cells can secrete low levels of antibodies for months or even years after the antigen is no longer present, to provide immediate protection if there is a subsequent encounter with the same antigen [42, 43].

3.2 Determination of the NPA-specific B cell reaction pathway

B cells respond *in vivo* via the germinal center pathway or via the extrafollicular reaction pathway [45, 46]. In germinal centers, isotype switching, affinity maturation and memory generation occur. These T cell-dependent processes can increase the affinity and the specificity of the antibodies, and B cells with high-affinity antibodies differentiate into plasma cells or memory B cells [31, 45]. On the other hand, the extrafollicular reaction leads to rapid production of low-affinity antibodies, which is sometimes associated with antibody class change [46, 47].

NPA-specific B cells were identified by flow cytometry as cells that bind to the NPA-bearing liposomes with their extracellular antibodies. Mice that received liposomes bearing NPA had abundant NPA-specific germinal center B cells, which increased over time, in their spleens and draining lymph nodes. In contrast, low numbers of NPA-specific extrafollicular B cells were found in the lymphoid organs of these mice. The affinity of the IgG anti-NPA antibodies produced by these mice increased over time, which further suggests that their B cells responded to NPA mainly through the germinal center pathway [36]. In order to access the germinal center pathway, B cells require T cell cooperation. However, conventional helper T cells are believed to respond only to protein antigens, so other T cell subsets may provide cooperation to the NPA-specific B cells found in this mouse model.

3.3 Analysis of the cells that provide cooperation to the NPA-specific B cells

NKT cells are a group of thymus-dependent T cells characterized by the expression of αβ TCR and several NK cell markers (NK 1.1, NKPR1 or CD161). Their development and functions are different from those of conventional CD4 and CD8 T cells. NKT cells cooperate with B cells that react with lipid antigens, and this cooperation leads to B cell activation, proliferation and differentiation into plasma cells [48, 49]. A subset of NKT cells, known as invariant NKT follicular helper ($iNKT_{FH}$) cells, could provide cooperation to B cells, since their phenotype is similar to that of helper T cells [49]. These cells express CXCR5, Bcl-6 and CD40L, secrete cytokines and chemokines (IL-4, IFN-γ, IL-21 and BAFF) and are localized in germinal centers in response to the glycolipid α-galactosylceramide (α-GalCer) [50, 51] $iNKT_{FH}$ cells that are induced in response to other lipid antigens express CD40L, as occurs with follicular helper T cells (T_{FH}), which are crucial for the development of germinal centers [52]. This evidence suggests that $iNKT_{FH}$ cells could provide cooperation to NPA-specific B cells, leading to their proliferation, affinity maturation and class-switching to IgG in a germinal center reaction. It remains to be determined if $iNKT_{FH}$ cells are indeed the cooperating T cell in this mouse model. Our proposed model for the generation of high-affinity IgG anti-NPA antibodies is shown in **Figure 5**.

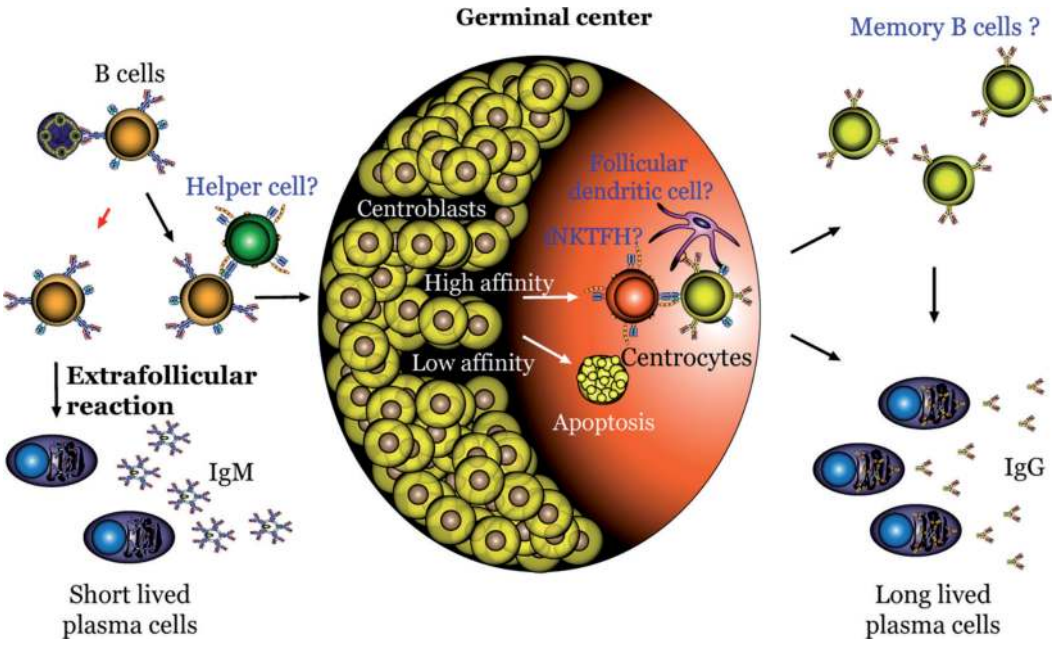

Figure 5.
Proposed mechanism for the production of anti-NPA antibodies. A naïve B cell recognizes the NPA antigen and responds. The B cell interacts with a helper T cell (possibly an iNKT cell) that induces its migration to a secondary lymphoid organ, in order to form a germinal center. In the germinal center, the B cell differentiates into a centroblast that proliferates and undergoes a series of mutations that generate low or high affinity antibodies. The centroblast then differentiates into a centrocyte, which no longer proliferates but undergoes antibody class-switching from IgM to IgG. B cells with high affinity antibodies are positively selected, while B cells with low affinity antibodies are negatively selected and die by apoptosis. When the B cell exits the germinal center, it differentiates into a memory B cell or a long-lived plasma cell that produces high affinity IgG anti-NPA antibodies. The B cells that do not receive T cell cooperation settle outside the follicles and differentiate into short-lived plasma cells that produce low-affinity IgM anti-NPA antibodies. Cell names in blue with a question mark indicate cells that have not yet been experimentally proven to participate in a germinal center specific to NPA.

4. Clinical implications of anti-NPA antibodies and future research directions

IgG anti-NPA antibodies were detected in the sera from patients that were positive for IgM or IgG anti-cardiolipin antibodies [53]. Some of these patients met four or more of the SLE criteria established by the American Rheumatism Association [54], others met the criteria for primary antiphospholipid syndrome [39], and others met the criteria for secondary antiphospholipid syndrome associated to SLE [39]. The presence of anti-NPA antibodies suggests that the NPA on the cell membranes of these patients had been stabilized; however, what led to this stabilization has not yet been identified. For example, in leprosy patients where anti-NPA antibodies have been detected, one factor that could induce the formation and stabilization of the NPA is the mycolic acid from the mycobacteria [55]. Since anti-NPA antibodies trigger a lupus-like disease in mice, it would be important to measure these antibodies in a larger number of SLE patients and in patients with other types of lupus, in order to identify if there is a relationship between the levels of these antibodies and the severity of the disease. If the anti-NPA antibodies are detected earlier than other autoantibodies (anti-cardiolipin, anti-histones, anti-coagulant) in patients with SLE, as it occurs in the mouse model, they could be used for the early detection of the disease, so that the patients could receive the appropriate treatment to prevent disease

complications [53]. It also remains to be determined if the levels of IgG anti-NPA antibodies are increased in patients with drug-induced lupus, compared to patients with non-drug related SLE, if high levels of anti-NPA antibodies correlate with a specific clinical phenotype of lupus, and if the levels of these antibodies could be used as biomarkers to monitor treatment response in these patients.

5. Conclusion

Non-bilayer phospholipid arrangements (NPA) are transient lipid associations different from the lipid bilayer. When they are stabilized by drugs, they induce the production, via germinal centers, of IgG anti-NPA antibodies; these antibodies lead to the development of a disease resembling human lupus in mice. This mouse model of lupus is suitable for the study of the molecular and cellular mechanisms that lead to the production of anti-lipid antibodies, which are currently poorly understood. In addition, this model allows us to propose that NPA and anti-NPA antibodies may contribute to the development of lupus in humans.

Acknowledgements

This study was supported by Instituto Politécnico Nacional, Mexico (grants SIP20210418 to IB, SIP20210382 to CWB and SIP2021 to ARM). Authors are fellows of Sistema Nacional de Investivadores, Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico.

Conflict of interest

The authors declare no conflict of interest.

DOI: http://dx.doi.org/10.5772/intechopen.106373

Author details

Albany Reséndiz-Mora[1], Alonso Tescucano[2], Giovanna Barrera-Aveleida[1], Anahi Sotelo-Rodríguez[1], Christian-Irene Nevárez-Lechuga[1], Iván Galarce-Sosa[1], Isabel Wong-Baeza[3], Isabel Baeza[1] and Carlos Wong-Baeza[1*]

1 Departamento de Bioquímica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional (Biochemistry Department, National School of Biological Sciences, National Polytechnic Institute), Mexico City, Mexico

2 Research Department of the Biological Pharmaceutical Chemistry Bachelor, University of Ixtlahuaca, Ixtlahuaca, Mexico

3 Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional (Immunology Department, National School of Biological Sciences, National Polytechnic Institute), Mexico City, Mexico

*Address all correspondence to: charlywong@icloud.com

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