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Research Article

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Neurological Dysfunction and Hyperactive Behavior Associated with Antiphospholipid Antibodies

A Mouse Model

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Abstract

Antiphospholipid antibodies (aPL) have been associated with various neurological manifestations, but the underlying mechanism has not been elucidated. We assessed mice with induced experimental antiphospholipid syndrome (APS) for neurological and behavioral changes. After immunization with monoclonal human anticardiolipin antibody (H-3), female BALB/c mice developed elevated levels of circulating anti–negatively charged phospholipids (aPL), anti–β_2-glycoprotein I (aβ_2GPI), and anti–endothelial cell antibodies (AECA), along with clinical manifestations of APS like thrombocytopenia and fetus resorption. APS mice were impaired neurologically and performed several reflexes less accurately compared to the controls, including placing reflex ($P < 0.05$), postural reflex ($P < 0.05$), and grip test ($P = 0.05$). The APS mice also exhibited hyperactive behavior in an open field, which tests spatial behavior ($P < 0.03$), and displayed impaired motor coordination on a rotating bar. aPL in combination with aβ_2GPI and AECA is probably involved in the neurological and behavioral defects shown in mice with experimental APS. (*J. Clin. Invest.* 1997. 100: 613–619.) Key words: antiphospholipid syndrome • anticardiolipin antibodies • autoimmunity • behavioral defects • autoimmune experimental model

Introduction

Antiphospholipid syndrome (APS)[1] is an autoimmune disorder defined in patients by the occurrence of characteristic clinical manifestations including recurrent thromboembolic events (venous or arterial), repeated spontaneous abortions, thrombocytopenia, and the presence of elevated titers of circulating antiphospholipid antibodies (aPL) or lupus anticoagulant (1–3). The syndrome can appear secondarily to another autoimmune disease (most commonly systemic lupus erythematosus [SLE]), to malignancy or infection, or as a drug-induced condition (2). In the absence of an underlying disease, APS is defined as a primary syndrome (2). A wide spectrum of other clinical manifestations has been reported recently in association with APS, including valvular heart disease (4), dermal complications (2), and diverse neurological disorders involving mainly the central nervous system (CNS) (2, 5–9). A significant association has been reported between aPL and recurrent ischemic events, strokes, or transient ischemic attacks (7–10). Likewise, several studies have reported the existence of a link between aPL and seizures in patients with SLE or in patients with the primary syndrome (11, 12). Ocular disturbances (amaurosis fugax [2, 13] and optic atrophy [2]), multiinfarct dementia, dementia of nonischemic origin (6, 14, 15), and chorea (16) have also been reported in patients with primary APS. Controversial reports exist regarding a similar association with nonspecific and migraine headaches (17, 18), transverse myelitis (3, 19), and Guillain-Barre syndrome (20).

Thromboembolism, due to the procoagulant state exerted by the aPL, is considered to be the main pathophysiologic process. In experimental studies, aPL have been shown to cause an enhanced thrombus formation in an in vivo thrombosis model (21), and to activate monocytes in vitro (22). However, thrombosis can explain only part of the neurological clinical manifestations, and a direct interaction between aPL and brain tissue phospholipids has also been proposed, since aPL interfere with astrocyte proliferation and functioning (23).

An experimental model for APS could be valuable in resolving some of the remaining problems regarding APS and could provide more direct evidence of a pathogenic role for aPL. MRL/lpr (24) and NZB × SxSB F1 mice (25) early in life develop spontaneous manifestations resembling APS secondary to SLE. In MRL/lpr mice one can find elevated aPL levels, thrombocytopenia, vascular thrombosis, and reduced litter size compared to their congenic strain MRL/++ (24). In addition, MRL/lpr have been found to be cognitively and neurologically impaired, but the correlation of these defects with anticardiolipin (aCL) levels has not been ascertained (26, 27). However, immunization of MRL/++ mice with β_2-glycoprotein I (β_2GPI, known to be an essential cofactor for aPL binding [28]) has yielded acceleration of neurological as well as renal damage (29).

To study the mechanism underlying the neurological changes associated with APS, we induced a primary experimental APS in naive BALB/c mice through idiotypic manipulation by immunization with a pathogenic monoclonal human aCL (30–32). In this study, the animals were assessed for their behavioral and neurological functions, and other clinical and

1. *Abbreviations used in this paper:* aβ_2GPI, anti-β_2GPI antibodies; aCL, anticardiolipin; AECA, anti–endothelial cell antibodies; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; β_2GPI, β_2-glycoprotein I; H-3, anticardiolipin antibody; H5V, transformed mouse endothelial cell line from hearts of C57BL/6 mice; HUVEC, human umbilical vein endothelial cells; pNPP, *p*-nitrophenylphosphate disodium.

serological manifestations. APS mice with elevated aPL levels, anti–endothelial cell antibodies (AECA) and anti-β₂GPI (aβ₂GPI) antibodies displayed diverse neurological and behavioral defects compared to the controls.

Methods

Animals

Female BALB/c mice (25–30 g), 8–10 wk old, were obtained from Tel-Aviv University Medical School. The mice were housed in groups of 10 per cage, and raised under standard conditions, $23 \pm 1°C$, 12:12 h light–dark cycle with ad libitum access to food and drink.

Antibodies, cell lines, and reagents

Monoclonal human anticardiolipin antibody (H-3). H-3 is a natural human monoclonal aCL antibody of the IgM isotype generated from a healthy subject and immunized with diphtheria and tetanus (33). It was found to carry an idiotype which is common in autoimmune diseases (33) and when injected into naive female BALB/c mice induced primary APS (31). It was purified from the supernatant of our hybridoma cell line, and donated by Dr. M. Sutjita (Flinders Medical Center, South Australia) (31, 33). Phospholipids, BSA, and other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Purified human β₂GPI was provided by Dr. Angela Tincani (Servizio di Immunologia Clinica, Spedali Civili, Brescia, Italy).

Endothelial cell cultures. Human umbilical vein endothelial cells (HUVEC) were isolated from human umbilical cord veins by collagenase perfusion and cultured under standard conditions (34). Cells were passaged on gelatin-coated culture dishes. Cells of multiple donors were pooled to eliminate the influence of blood group antigens (35). The second passage culture was used for cyto-ELISA to detect AECA in the mouse sera. H5V is a transformed mouse endothelial cell line from hearts of C57BL/6 mice (36), grown in our laboratory and used for cyto-ELISA.

Induction of experimental APS by active immunization with aCL (H-3)

Female BALB/c mice were immunized intradermally in the hind footpads with either 10 μg H-3 ($n = 25$) or normal human IgM ($n = 25$), emulsified in CFA (Difco Laboratories Inc., Detroit, MI). A boost injection with 10 μg H-3 or normal IgM in PBS was administered 3 wk later (31).

Serological and clinical evaluations of the mice

Randomly selected animals were bled from the retroorbital plexus in 1-mo intervals ($n = 5$). Sera were separated by centrifugation and stored at $-20°C$ until assessed. The sera were then tested by ELISA for the presence of different autoantibodies, as described previously (31, 32, 37). Sera samples were tested in 1:100–1:400 dilutions for aCL, antiphosphatidylserine (aPS), antiphosphatidylinositol (aPl), antiphosphatidylcholine (aPC), aβ₂GPI, anti-BSA (aBSA), anti-dsDNA, and AECA.

Antiphospholipid antibodies were measured as follows: 96-well polystyrene microtiter plates (Nunc Inc., Roskilde, Denmark) were coated with cardiolipin (CL), phosphatidylserine (PS), phosphatidylinositol (PI), or phosphatidylcholine (PC) at 50 μg/ml in ethanol, then left open at 4°C until evaporation, or were left empty. 5% bovine serum in Tris buffer saline (TBS) was used as blocking agent, and 2% bovine serum in TBS as sample diluent. Washings were performed with TBS. The antibody binding was quantitated using alkaline phosphatase–conjugated goat anti–mouse IgG or IgM (Jackson ImmunoResearch Labs., Inc., West Grove, PA), followed by *p*-nitrophenylphosphate disodium substrate (pNPP; Sigma Chemical Co., St. Louis, MO). OD was read at 405 nm.

Antibodies to proteins (β₂GPI, BSA) were detected by similar ELISA methods. Briefly, purified human β₂GPI (5 μg/ml) or BSA (2.5 μg/ml) in 0.05 M NaHCO₃, pH 9.5, were coated for 16–18 h at 4°C, followed by three washes with PBS/0.05% Tween 20, which was also used for all subsequent washings between steps. For blocking and sample dilution, 1% BSA/PBS/0.05% Tween 20 was used.

Anti–endothelial cell activity in the animal sera was measured in cyto-ELISA with unfixed HUVEC or H5V cells according to a previously described protocol (34, 35) in microtiter plates coated with confluent HUVEC or H5V endothelial cells. Briefly, HUVEC or H5V cells were seeded in gelatin-coated (for HUVEC, or empty for H5V cells) 96-well microtiter plates (Nunc, Inc.) at 2.5×10^4 cells/well, and allowed to grow to confluence for 1 or 2 d. Cells were washed with HBSS and incubated with a blocking buffer (HBSS/0.5% BSA) for 30 min at 37°C to prevent nonspecific binding. After an additional washing, cells were exposed to the tested sera or control antibody diluted in HBSS/10% FCS (dilution 1:25–1:200 of serum) for 60 min at room temperature. Cells were washed again and incubated with a second antibody, alkaline phosphatase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Labs.) followed by pNPP as a substrate. OD was read at 405 nm in an ELISA plate reader (model EAR 400 AT; SLT Labinstruments, Vienna, Austria).

Blood cell counts were determined using a single optical cytometer (HC Plus Cell Control; Coulter Corp., Hialeah, FL).

Neurological assessment

2 mo after boost injection and before mating, mice were evaluated neurologically by scoring their performance of a series of reflexes, according to an established protocol (38), as follows. 1. *General behavior.* The mouse is observed for awareness, feeding, curiosity, grooming, and spontaneous movements. 2. *Equilibrium tests.* The mouse is placed head down on a platform tilted at 30°, and climbing upwards is observed. 3. *Ear reflex.* A puff of air is blown through a rubber tube to the ear, and head shaking is observed. 4. *Front leg placing.* Holding the dorsum of the front paws close to a table edge causes placing of palms on the table. 5. *Hind leg placing.* Lowering one hind leg from the table (with the three other legs on the table) causes lifting of the leg to the table. 6. *Auditory startle.* When the mouse is quiet on a level surface, a loud hand clap causes it to flex its forelimbs, extend its hindlimbs, and arch its body. 7. *Righting reflex.* When the mouse is put on its back, it turns over immediately. 8. *Flexion reflex.* The mouse is picked up, and the toes are pinched with forceps, resulting in foot withdrawal. 9. *Corneal reflex.* Touching the cornea with a hair causes the mouse to close the eye. 10. *Placing reactions.* (*a*) The mouse is held by the tail over a table until its whiskers get near. The paws are then put on the table. (*b*) Same as *a*, but the whiskers do not touch the table: by sight the mouse tries to touch the table. 11. *Postural reflex.* Lifting the mouse by its tail causes hindlimb abduction, spreading of the toes, arching of the back, and elevation of the head. 12. *Grip test.* A neurologically normal mouse should be able to hang suspended on a stationary bar for at least 30 s. Animals were scored normal, abnormal, or severe abnormal. Statistical significance was assessed by Fisher's exact test.

Open field

Female BALB/c mice, 6–8 mo old, injected with H-3 ($n = 18$), or human normal IgM ($n = 9$), were tested for spatial behavior in a novel environment in an open field. The apparatus comprised of a black colored box ($60 \times 60 \times 20$ cm) placed in an air-conditioned (20°C) lighted room. Twenty-five equal squares (locales) were marked on the box floor, and were used for following the location of the mice during the experiment (see Fig. 2 *A*).

Procedure. The protocol was performed double-blind, according to Eilam et al. (39, 40). 1 wk before testing, the mice were handled daily. The tests were conducted during the light phase of the day–night cycle. 2 h before testing, the mice were brought to the experimental room. Each mouse was removed gently from its cage, placed individually at the center of the field, and videotaped with a camcorder (model CCD-V700; Sony Corp., Park Ridge, NJ) for 30 min. Video records included digital and binary time-code signals (Telecom Research, Burlington, Ontario, Canada) which enabled both the observer and a computer to identify each frame (25 frames/s) in the videotapes.

Behavioral analysis. The frequency and duration of progression or stopping in the open field was analyzed during playback of the video records by software custom-written by Dr. David Eilam (Life Sciences Department, Tel-Aviv University). The computer videocassette interface allowed assessment of frequency and duration of behavioral acts with a resolution of 1/25 s. The total distance traveled, locomotion time, speed of moving, spatial distribution of explorations and latency for establishing a home base (40), and duration of staying there were extracted. Differences between groups were analyzed by Mann-Whitney U and Wilcoxon rank sum W tests.

Rota-Rod treadmill

The degree of motor coordination of mice was determined using a standard Rota-Rod treadmill adjusted for mice (Ugo Basile, Varese, Italy), rotated at a speed of 20 rpm (41). Mice were acclimatized to the treadmill by placing them on it two to three times before the actual experiment. Treadmill scores were determined for APS mice ($n = 23$) and controls ($n = 19$) in the age of 10 mo, 7 mo after boost injection. Each mouse was placed on the rotating bar, and the duration it remained on it was measured, up to a maximum of 60 s. 13 sessions were conducted for each mouse at 5-min intervals.
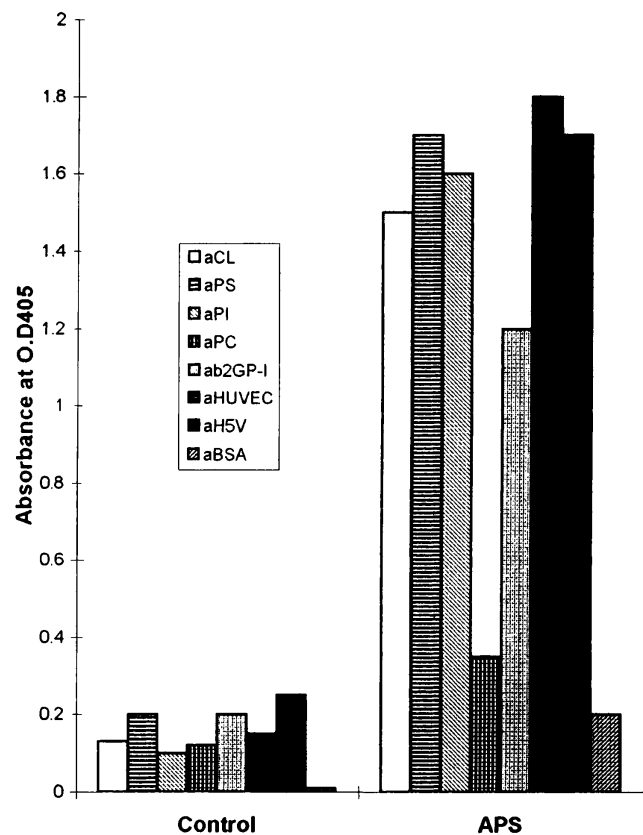
Results

Serological and clinical findings

Autoantibodies. 1 mo after the boost immunization with aCL H-3, mice developed elevated titers of autoantibodies detected in ELISA against CL, PS, PI, β_2GPI, HUVEC, and H5V endothelial cells. Results are depicted in Fig. 1. Mean levels of OD 405 units±2SD were 1.5, 1.7, 1.6 , 1.2, 1.8, and 1.7, respectively, significantly higher compared to controls: 0.13, 0.2, 0.1, 0.2, 0.15, and 0.25, respectively ($P < 0.05$ for all autoantibody specificities). No binding to PC or BSA was shown. Control mice elicited only anti–human IgM antibodies measured in ELISA. The levels of autoantibody activity remained elevated for 6–8 mo after immunization, as previously reported (36).

Cell counts. Blood samples taken from representative pregnant and nonpregnant mice revealed lower mean±2SD platelets in the H-3–injected mice, 653 ± 179 ($\times 10^3/\mu l$), compared to $1,076\pm217$ ($\times 10^3/\mu l$) in control mice showing thrombocytopenia ($P < 0.05$). Normal values of white blood cells ($10 \times 10^3/\mu l$) and red blood cells ($7 \times 10^6/\mu l$) were detected in APS and control mice.

Neurological findings

Generally, APS mice behaved differently than control mice, showing some hyperactivity and mild aggressiveness towards the experimenter. Postural responses, righting reflexes, and placing reflexes were significantly impaired in APS mice. The results are summarized in Table I.

Behavioral findings

Open field system. Mice with elevated aCL titers displayed hyperkinetic behavior with several characteristics: 4/10 APS mice, compared to 1/9 of controls, showed a tendency for paths stereotypy, a pattern of moving in one preferred route along the edges of the field rather than randomly (Fig. 2). APS mice displayed differently various locomotion parameters (Fig. 3),

Figure 1. Autoantibody levels in sera (diluted 1:200) of mice immunized with monoclonal aCL (H-3) and control mice immunized with normal human IgM. Results are expressed in OD_{405} units (mean±2SD) measured in ELISA assays. High levels of various anti–negatively charged PL antibodies, aβ_2GPI, and AECA (anti-HUVEC and anti-H5V) were elicited in APS mice 2 mo after boost injection. Low levels of anti-PC and anti-BSA were detected. *aPS*: antiphosphatidylserine; *aPI*: antiphosphatidylinositol; *aPC*: antiphosphatidylcholine; *aHUVEC*: anti-HUVEC; *aH5V*: anti–mouse endothelial cell line; *aBSA*: anti-BSA.

Table I. Neurological Examinations in BALB/C Mice Induced with APS Compared to Controls

Test	Primary site of CNS localization	APS mice
1. General behavior	Cerebral cortex	93% (NS)
2. Equilibrium	Cerebellum	87% (NS)
3. Ear reflex	Brain stem	100%
4. Front leg	Cerebral cortex	100%
5. Hind leg	Cerebral cortex	100%
6. Auditory startle	Brain stem	100%
7. Righting reflex	Brain stem	100%
8. Flexion reflex	Spinal cord	90% (NS)
9. Corneal reflex	Brain stem	100%
10. Placing reflex	Cerebral cortex	33% ($P < 0.001$)
11. Postural reflex	Brain stem	60% ($P < 0.05$)
12. Grip test	Brain stem	73% ($P = 0.05$)

Values are expressed as percentages correct on a subset of neurological examinations performed by APS mice at 5 mo when elevated levels of autoantibodies were detected, compared to the controls. Statistical significance was assessed by Fisher's exact test.
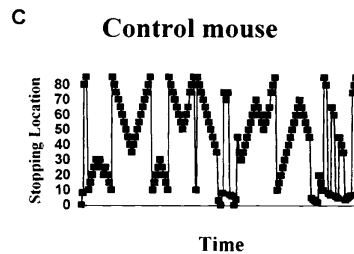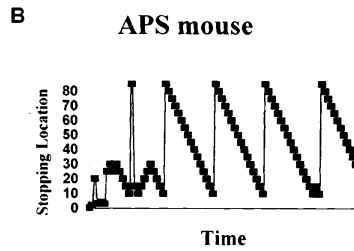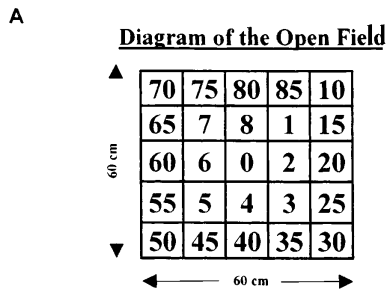
A
Diagram of the Open Field

70	75	80	85	10
65	7	8	1	15
60	6	0	2	20
55	5	4	3	25
50	45	40	35	30

60 cm ← → 60 cm

B
APS mouse

Time

C
Control mouse

Time

Figure 2. Spatial locomotion of APS and control mice in the open field is depicted as a sequence of locations during the exposure to the open field. (*A*) A diagram of the open field. 25 equal squares (locales) were marked on the field floor and used for following the location of the mice during the experiment. (*B*) A representative APS mouse immunized with aCL (H-3), with elevated levels of aCL displayed a regular stereotypic movement along edges of the field. (*C*) Control mouse immunized with normal human IgM moved randomly in the field.

such as a longer total distance traveled in the open field during the experiment (Mann Whitney test, $P < 0.03$; Fig. 3 *A*), longer total moving time, and accelerated speed of locomotion (not shown). All the mice were bled at this point. Animals with aCL levels in the range of $OD_{405} < 0.8$ were significantly more affected in all locomotion parameters than those mice with higher aCL levels ($OD_{405} > 0.8$) ($P < 0.02$, Mann-Whitney test) (Fig. 3 *B*). In addition, APS mice showed more stopping events ($P < 0.03$, Fig. 3 *C*) lasting for shorter mean duration ($P < 0.02$, Fig. 3 *D*) at the home base location (40). 4/10 of APS mice, compared to none of controls, rested for a relatively extended period at the home base later than the controls (not shown). The correlation factor of hyperactivity and aCL in the range of moderate aCL levels was $r = 0.85$, but $r = 0.6$ in the range of high aCL levels.

Rota-Rod system. APS mice performed the Rota-Rod test poorly compared to controls, staying for a shorter mean duration on the rotating bar than control mice ($P = 0.09$, ANOVA with repeated measurements, group $\times$ session). A significant improvement over time was observed for both groups (ANOVA with repeated measurement, $P < 0.0001$). However, a slower rate of improvement was demonstrated by APS mice compared to control mice ($P = 0.07$, t test BETA for slope, not shown), suggesting motor incoordination and difficulties in learning.

The overlap of neurological and behavioral impairment manifested by individual mice in different tests is summarized in Table II.
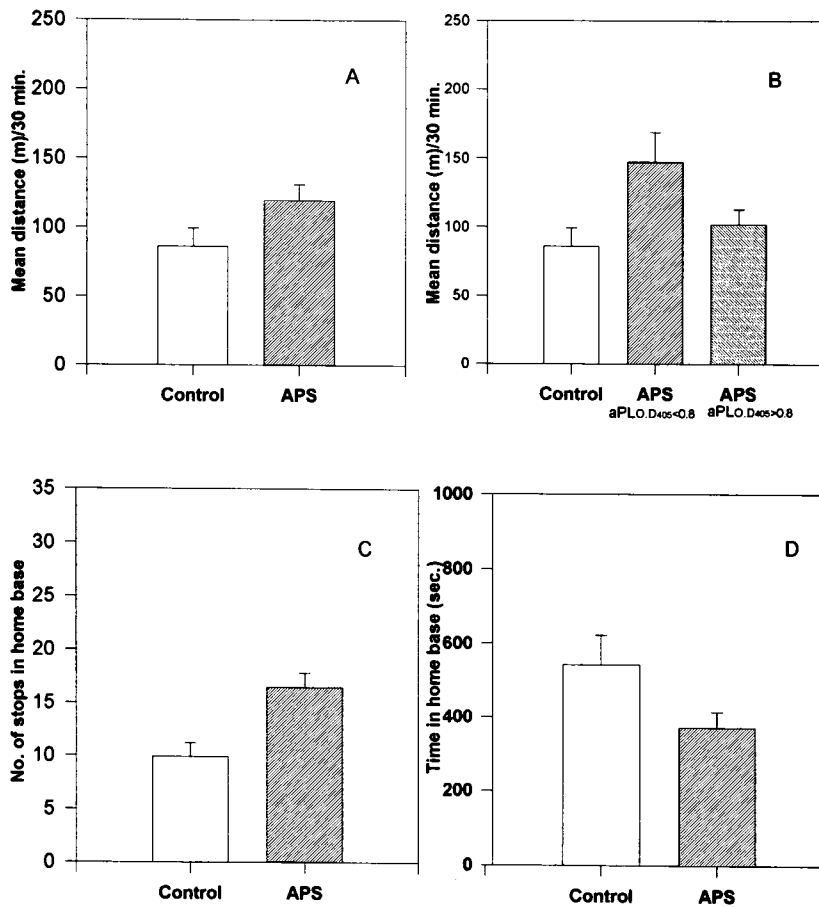

Figure 3. Different parameters of hyperkinetic locomotion in the open field were displayed by APS mice compared to controls, including (*A*) Longer total (mean±SD) traveled distance during the experiment ($P < 0.03$, Mann-Whitney U test). (*B*) Augmented hyperactive behavior demonstrated by APS mice having moderate levels of aPL ($OD_{405} < 0.8$) compared to mice with higher aCL levels measured in ELISA ($OD_{405} > 0.8$), or to controls ($P < 0.02$). (*C*) Higher (mean±SD) number of stopping events in the home base. (*D*) Shorter total duration in the home base ($P < 0.05$).

APS mouse	Placing reflex	Grip test	Postural reflex	Open field stereotypy	Open field hyperactivity	Rota-Rod
1	+	−	+	+	+	ND
2	−	+	+	+	+	ND
3	+	−	−	+	+	ND
4	+	+	−	+	+	+
5	−	−	−	−	+	+
6	+	−	−	−	+	−
7	+	+	−	−	−	+
8	+	−	−	−	−	+
9	−	+	+	−	+	−
10	−	−	−	−	+	−

+, Impaired performance of the test. −, Correct performance of the test. *ND*, Not determined. The mouse was not assessed with this test.

Discussion

Our findings in the experimental model of APS support the notion that aPL are involved in the pathophysiology of neurological deficits associated with APS. There has been a controversy in previous reports on the pathogenic role of aPL in neurological deficits and the underlying mechanism (2, 9–20), specifically concerning whether aPL directly interact with structures in the brain, or indirectly through a thrombogenic effect, or both (21–23).

In this study, we induced experimental APS in naive female BALB/c mice through an idiotypic dysregulation (30–32) by active immunization with H-3, a monoclonal aCL carrying a pathogenic idiotype (33). As previously reported (30–32), upon stimulation with a pathogenic idiotype H-3 (Ab1), naive mice developed antiautoantibody (anti-Id = Ab2), and later, after 1–4 mo, generated an anti-antiautoantibody (anti-anti-Id = Ab3) which had similar binding characteristics to those of Ab1. In parallel, mice developed a respective overt autoimmune condition typically associated with the inducing autoantibody (Ab1). After immunization with H-3 (31), all the mice developed persistently elevated levels of antibodies against negatively charged phospholipids, and low levels of anti-PC and anti-dsDNA, along with mild thrombocytopenia, increased fetal resorptions and reduced fecundity rate. In this study, we found that additional binding specificities were present in those mice sera, including elevated levels of $a\beta_2GPI$ and AECA (Fig. 1).

Two approaches were taken to evaluate CNS involvement in our experimental model of APS. First, the neurological status of the animals was evaluated by a series of neurological tests (38). This examination is a gross technique for uncovering major neurological dysfunction, similar to a clinical neurological examination. The second approach was to assess behavioral ability by exposing animals to various behavioral tests, including the open field for measuring activity and spatial organization, and the Rota-Rod treadmill for evaluating motor coordination, along with assessment of other clinical and serological manifestations.

APS mice displayed impaired neurological ability shown in inaccurate performance of some neurological examinations, including placing reflex, which tests vision; grip test, which tests muscle strength; and defective performance of postural reflex (Table I), pointing to affected CNS function in the cortex level and brain stem. APS mice also showed a tendency for impaired general behavior and equilibrium.

To assess their behavior in a novel area, APS and control mice were examined in an open field (39, 40). When placed in an open field, a normal mouse alternates between locomotion and rest, with occasional stops or visits (40). In this situation, the APS mice exhibited hyperactivity, including limited routes of moving, i.e., a tendency to move along the edges of the field rather than in the center (Fig. 2); faster movement and longer duration of movement resulting in longer total distance traveled (Fig. 3 *A*); and abnormal spatial organization of behavior in regard to home base (Fig. 3, *C* and *D* and data not shown). The home base is the key location around which a normal animal organizes its behavior and in which it stops for the longest duration (35). The APS mice rested in their home base in a later phase of exposure to the new environment compared to controls (data not shown), and displayed more arrests in this location but with a shorter total duration, characteristic for hyperactive behavior (Fig. 3, *C* and *D*). The appearance of this altered spatial organization probably indicates emergence of altered cognitive processing, known to be one of the main cognitive defects found in patients with Alzheimer's disease.

It is notable that mice having moderate levels of aPL tested in ELISA ($OD_{405} < 0.8$) showed hyperactive behavior compared to those with even higher levels of aPL (Fig. 3 *B*). Decreasing aCL levels was reported also in SLE patients after thromboocclusive episodes (42). Based on our preliminary findings in histological evaluation of the APS mouse brain, thrombosis probably underlies some of the neurological defects exhibited by the APS mice. Capillaries occluded by microthrombi were evident in the cerebral cortex of those mice (our unpublished data).

The hyperkinetic movements observed in APS mice are reminiscent of chorea reported in association with the presence of elevated aPL in patients (16, 43), and thus may point to a defect in the functioning of the basal ganglia in APS mice.

Studies of the mechanisms for behavioral stereotypy and sensitization (44, 45) showed enhanced response and behavioral augmentation upon repeated exposure to dopaminergic stimulants such as amphetamine, resulting in hyperactivity, shortening of motor sequences, and decreased flexibility in motor performance. The mechanism by which autoantibodies, specifically aPL antibodies, may affect mice behavior is not clear. Hyperactivity observed in APS mice probably results from the combination of diverse autoimmune pathways in which aPL, AECA, or $a\beta_2GPI$ may be involved, affecting the dopaminergic system either by interfering in hemostasis leading to the occlusion of microvessels in the basal ganglia, or by direct binding of autoantibodies to the neurons.

Another neurological test to which the mice were exposed is the Rota-Rod, in which APS mice performed worse than the controls, reflecting motor incoordination (data not shown). In addition, cognitive deficit was also suggested by the tendency of APS mice to learn the task more slowly than the controls (data not shown). The underlying mechanism for motor inco-

ordination in the experimental model is not clear. A study made on the NZB/BINJ mice, genetically prone to develop autoimmune disease, reported accelerated impaired age-dependent learning and memory ability in combination with ataxia on a rotating bar, along with elevated levels of neuronal reactive antibodies (46). Since learning and memory abilities are mediated by cholinergic innervation, autoantibodies and probably aPL in our APS animals might also interfere with the cholinergic, as well as the dopaminergic processes. However, a detailed anatomical–histological evaluation is necessary to unravel the underlying processes leading to the various neurological manifestations in the experimental APS.

We suggest that an autoimmune-mediated mechanism underlies the neurological dysfunction observed in mice with experimental APS. The coexistence of aCL, $a\beta_2$GPI, and AECA activities in our mouse APS model (Fig. 1) may have pathological significance, as is the case in patients with APS where aPL, AECA, and $a\beta_2$GPI commonly coexist (47). The damage to the brain tissue of APS mice is probably due to interaction of the autoantibodies with endothelial cells (40) via binding to complexes of phospholipids with β_2GPI or other plasma proteins attached to the endothelial cells (48) or to activated platelets. Increased immunoglobulin binding to cerebral endothelium in patients with APS has been demonstrated also in in vitro studies reported by Hess et al. (49). aPL have been shown to activate endothelial cells in vitro (50) and to enhance platelet adherence to endothelial cells (51). Evidence for activation of endothelial cells in patients with APS has been reported recently by Brey et al. (52), who show that elevated aCL IgG and serum levels of various soluble adhesion molecules (ICAM-1, VCAM-1) correlated strongly with one another, and were temporarily related to clinical manifestations. The significance of $a\beta_2$GPI antibodies in the sera of SLE patients as a risk factor for thrombosis has been reported recently by Cabiedes et al. (53). They have shown that the clinical manifestations of APS associate more strongly with $a\beta_2$GPI antibodies than with aPL. High levels of $a\beta_2$GPI in our mouse APS model probably have a similar clinical significance.

In this study, we have shown that mice with experimental APS exhibit behavioral and neurological deficits along with elevated levels of aPL, AECA, and $a\beta_2$GPI, and other clinical manifestations of APS. These autoantibodies might have a crucial pathogenic role in the emergence of diverse neurological and behavioral deficits in experimental APS mice, presumably through diverse autoimmune-mediated processes leading to damage of brain tissue.

Acknowledgments

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