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Oxidized low-density lipoprotein (Ox-LDL) but not LDL aggravates the manifestations of experimental antiphospholipid syndrome (APS)

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SUMMARY

Ox-LDL is thought to play a major role in atherogenesis. The mechanisms mediating the deleterious influences of Ox-LDL include foam cell formation and cell cytotoxicity. The production of anti-Ox-LDL antibodies results in the formation of immune complexes which are taken up at enhanced rate by macrophages, leading to foam cell formation. APS is characterized by repeated venous and arterial thromboembolic phenomena, recurrent fetal loss and thrombocytopenia, associated with the presence of antibodies to negatively charged phospholipids (aPL) (i.e. cardiolipin, phosphatidylserine). Phospholipids bear structural resemblance to LDL, and several studies have indeed proved that aPL display cross-reactivity with anti-Ox-LDL antibodies. In this study we assessed the capacity of oxidized and native forms of LDL to aggravate the clinical picture of experimentally induced APS in naive mice. Mice were actively immunized intradermally with anticardiolipin antibodies and developed a clinical picture resembling APS in humans. Subsequently, the mice were infused with either Ox-LDL, native LDL or PBS, and similar regimens were applied to controls. APS mice infused with Ox-LDL were found to exhibit a significantly more severe form of the disease in comparison with native LDL- and PBS-infused mice, expressed by lower platelet counts (261 000/mm^3, 535 000/mm^3 and 455 000/mm^3, respectively), longer activated partial thromboplastin time (aPTT) (99 $\pm$ 12 s, 63 $\pm$ 8 s and 74 $\pm$ 8 s, respectively) and higher fetal resorption rates (72·7%, 34·4% and 32·6%, respectively). The results of this study show that Ox-LDL, compared with native LDL, aggravates the clinical manifestations of experimental APS and suggest that cross-reactivity of Ox-LDL with phospholipids may provide a pathogenic explanation for this effect.

Keywords low-density lipoproteins antiphospholipid syndrome anticardiolipin antibodies autoimmunity autoantibodies

INTRODUCTION

APS is characterized by recurrent arterial or venous thromboses, fetal wastage and thrombocytopenia, accompanied by increased titres of antiphospholipid antibodies [1,2]. However, the syndrome constitutes a wider clinical spectrum, encompassing cardiac valve involvement [3], neurologic manifestations [4], renal impairment [5], and various cutaneous expressions [6]. APS may either occur as a distinct clinical entity (termed primary) or coexist with other disease states, examples of which include autoimmune disorders (i.e. systemic lupus erythematosus (SLE)) and malignancies, and thus be referred to as secondary [7].

The etiopathogenesis of the thromboembolic manifestations displayed in APS, although not yet fully understood, has been subjected to intensive research in recent years. It is gradually being realized that antiphospholipid antibodies (aPL) may precipitate thromboemboli following the formation of a complex interaction, not yet completely resolved, with phospholipids, in the presence of β_2-glycoprotein I (β_2-GPI), a phospholipid-binding plasma protein [8,9]. One of the most substantial proofs for the pathogenicity of aPL has been exemplified by models of experimentally induced APS in animals, by which a clinical picture resembling APS in humans was either accomplished following passive transfer of anticardiolipin antibodies (aCL) to naive mice [10] or by active immunization of mice with the antibodies [11].

The antigens bound by aPL are not confined to phospholipids, but also constitute other negatively charged substances, such as glycosaminoglycans, heparin [12], vascular heparan sulfate proteoglycan [13], placental anticoagulant protein I [14] and Ox-LDL [15]. LDL is composed of a surface structure containing apolipoprotein B-100 and

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a mixed phospholipids and cholesterol monolayer. Oxidative modification of LDL has been associated with various disease states, the hallmark of which is atherosclerosis [16–18]. Several mechanisms have been proposed to explain this effect, including enhanced uptake of Ox-LDL by macrophages, chemotactic potential for circulating monocytes, inhibition of the motility of tissue macrophages, and alteration of the coagulation pathways [16,19]. Ox-LDL has been shown to be cytotoxic to various cells such as vascular cells, monocytes and macrophages [20,21] by mechanisms not yet sufficiently characterized. It does seem conceivable, however, that Ox-LDL may exert some of its deleterious effects, owing to its immunogenicity, with the resultant formation anti-Ox-LDL antibodies and generation of immune complexes. Alternatively, it has been suggested that Ox-LDL may inactivate endothelial-derived relaxing factor (EDRF) (i.e. nitric oxide) following its release from endothelial cells, thus inhibiting EDRF-mediated vasodilatation [22].

A recent attractive study [15] demonstrated cross-reactivity of aPL with anti-Ox-LDL antibodies, which have been presumed to account for the accelerated atherosclerosis observed in SLE patients, nearly half of which are aPL-positive.

Our study was based upon the above observations of cross-reactivity between aPL and anti-Ox-LDL [15,23], and on the knowledge that lipoproteins contain mixed amounts of phospholipids which may serve as targets for aCL binding.

We have been able to show that Ox-LDL, but not LDL, have an aggravating effect on the clinical findings in naive mice, induced to develop experimental APS. Additionally, it is proposed that cross-reactivity of Ox-LDL with aPL could be responsible for these observations.

MATERIALS AND METHODS

Mice

BALB/c mice (8–10 weeks old, 20 mice per group) were purchased from the animal house of the Sackler School of Medicine (Tel-Aviv University, Israel). Mice were fed maintenance pelleted diet and water *ad libitum*, and were kept in a room with a constant temperature and humidity.

Antibodies

H-3 is a human IgM anticardiolipin MoAb [24], generated following fusion of peripheral blood lymphocytes of a healthy subject immunized with diphtheria and tetanus with GM 4672 lymphoblastoid cell line. The H-3 has previously been used by us to induce successfully, by active immunization, a clinical picture resembling APS (manifested by elevated titres of mouse antiphospholipid antibodies, thrombocytopenia, prolonged activated partial thromboplastin time (aPTT) and increased resorption rate of embryos) in BALB/c mice [11].

Induction of experimental APS

The mice were immunized intradermally in the hind footpads with 10 μg H-3, MoAb or irrelevant IgM in Freund's complete adjuvant (FCA; Difco Labs, Inc., Detroit, MI), and 3 weeks later boost injections were administered with the same amount of antibodies in PBS in the hind footpads. It was shown, in this model of APS, that administration of aCL was followed by the production of mouse aCL, coinciding with the clinical picture of APS in humans [11].

LDL isolation, oxidation and characterization

Blood for lipoprotein isolation was collected in EDTA (1 mg/ml) after 12 h of fasting. LDL (density 1·019–1·063 g/l) was isolated from plasma as previously described (18], after density adjustment with Kbr^{-}, by preparative ultracentrifugation at 50 000 ***g*** for 22 h, using type 50 rotor. LDL preparations were washed by ultracentrifugation, dialysed against a pH 7·4, 0·15 mol/*l* EDTA, passed through Acrodisc filter (0·22 μm pore size) to remove aggregates, and stored under nitrogen in the dark.

LDL oxidation was performed by incubation of pre-dialysed LDL (1 mg of protein/ml in EDTA-free PBS) with copper sulfate (10 μM) for 24 h at 37°C. Lipoprotein oxidation was confirmed by analysis of thiobarbituric acid-reactive substances (TBARS) which measures malondialdehyde (MDA) equivalents by the lipid peroxidation test and also by analysis of the conjugated diene content of the lipoprotein.

Infusion of immunized mice with LDL/Ox-LDL

The mice were infused intravenously with either Ox-LDL, native LDL or PBS (200 μg/mouse every fifth day during 3 weeks from the day in which pregnancy was observed). This scheduled regimen was applied in order to evaluate the effect of Ox-LDL on the severity of clinical manifestations of the female mice, induced to develop experimental APS (consisting of fetal loss, prolonged aPTT and thrombocytopenia), spanning the date of delivery. The regimen was determined following a preliminary study (data not shown) in which various regimens of Ox-LDL and LDL were tested in mice with experimental APS, and the current dosage was found to have a non-toxic effect.

Control groups consisted either of mice immunized with irrelevant human IgM antibodies or of non-immunized mice. Both control groups were infused with either Ox-LDL, LDL or PBS, similar to the regimens administered to the mice immunized with anti-cardiolipin antibodies.

Detection of antiphospholipid antibodies

Antiphospholipid binding of sera from the immunized mice was detected by ELISA [11]. Briefly, 96-well ELISA plates (Nunc, Roskilde, Denmark) were coated with phospholipid (cardiolipin, phosphatidylinositol, phosphatidylserine, phosphatidylcholine) (Sigma Chemical Co., St Louis, MO) at a concentration of 50 μg/ml in ethanol. Following evaporation the plates were blocked with 5% bovine serum (BS) in PBS. Serial dilutions (1 : 200, 1 : 3600) of mice sera in PBS + 2% BS were followed by incubation for 2 h. Bound antibodies were detected using 1 : 1000 dilution of goat anti-mouse IgG conjugated to alkaline phosphatase (Sigma) and the addition of its substrate *p*-nitrophenyl phosphate. Colour was read in a Titertek (S.L.T. Laboratory Instruments, Vienna, Austria) ELISA reader at an optical density (OD) 405 nm. Between each step extensive washings were performed.

Detection of anti-single-stranded DNA antibodies

Anti-single-stranded (ss) DNA antibodies were determined as described previously [11]. Briefly, polystyrene ELISA plates (Nunc) were coated sequentially with poly L-lysine (50 μg/ml in water), calf thymus ssDNA–2·5 μg/ml Tris base-buffered saline (TBS) and poly L-glutamate (50 μg/ml). Washing between steps was performed using TBS with 0·05 Tween-20 to minimize non-specific binding. Serial dilutions of the tested sera (1 : 200, 1 : 3600) of the mice sera were followed by incubation for 2 h. Detection of the antibodies in sera was done as described for anticardiolipin antibodies.

Inhibition studies of binding of aCL to Ox-LDL or LDL

In order to show cross-reactivity of aCL with Ox-LDL, inhibition

Table 1. Autoantibody titres in sera of experimental APS mice given ox-LDL or LDL

	Anticardiolipin			Human IgM			None		
Imunization treatment	PBS	OX-LDL	LDL	PBS	OX-LDL	LDL	PBS	OX-LDL	LDL
Antibody to:									
Cardiolipin	1436 ± 253	1387 ± 240	1299 ± 313	212 ± 67	198 ± 21	165 ± 72	176 ± 12	215 ± 58	147 ± 51
Phosphatidylserine	1389 ± 343	1402 ± 328	1365 ± 277	132 ± 45	151 ± 39	179 ± 63	184 ± 47	243 ± 48	162 ± 62
Phosphatidylcholine	341 ± 124	236 ± 77	313 ± 56	167 ± 45	138 ± 27	151 ± 24	178 ± 38	156 ± 33	191 ± 38
ssDNA	212 ± 45	289 ± 51	202 ± 39	232 ± 42	199 ± 25	167 ± 37	183 ± 47	174 ± 29	173 ± 50

The sera were tested at dilution of 1 : 200. The values are expressed as means ± s.d., 3 months after disease induction, 3 weeks after infusion, 20 mice in a group.

No differences were found by statistical analysis (ANOVA test) between the group of experimental APS infused with Ox-LDL and other groups of APS mice ($P > 0{\cdot}5$).

Differences were found to be statistically significant ($P < 0{\cdot}002$) between the group of experimental APS compared with other groups.

assays were performed to detect specific binding of the sera from the immunized mice before and after infusion of Ox-LDL or LDL. Sera, at a dilution that gave 50% of maximal binding to cardiolipin, were preincubated with different concentrations of Ox-LDL, LDL or cardiolipin. After overnight incubation of the sera with the different inhibitors at 4°C, remaining activity to cardiolipin was tested by ELISA, as detailed above.

The percentage of inhibition was calculated as follows:

$$\text{Percent inhibition} = \frac{\text{OD control} - \text{OD with inhibitor}}{\text{OD control}} \times 100$$

Purification of anticardiolipin which also binds to Ox-LDL

Mouse aCL were purified as follows. Pooled sera from 60 mice, immunized with aCL, were incubated overnight with silica beads coated with cardiolipin in the presence of 2% BS. The cardiolipin-coated beads were prepared by sonication of 10 mg silica beads (Sigma) in ethanol and then 500 μg/ml of cardiolipin were added and incubated overnight with shaking at 4°C. The beads, extensively washed with PBS (until the supernatant did not contain phospholipids by thin layer chromatography (TLC) running), were blocked with 5% BS and employed to purify aCL from the pooled mice sera. Elution of the aCL was performed with glycine HCl 0·2 M pH 2·5, followed by neutralization of the eluate with Tris pH 8 and extensive dialysis against PBS. Mice purified aCL were further studied for binding to Ox-LDL or LDL.

In order to deplete the mouse aPL from possible anti-Ox-LDL and anti-LDL activity, silica beads were coated with Ox-LDL or LDL as described for cardiolipin and blocked with β_2-GPI or 1% bovine serum albumin (BSA). Total mice aCL were incubated for 5 h with shaking with Ox-LDL- or LDL-coated beads, washed and eluted with glycine HCl 0·2 M pH 2·5, followed by neutralization of the eluate with Tris buffer and extensive dialysis against PBS.

Assessment of the severity of findings of experimental APS

Three parameters were used to estimate the severity of the experimental APS in all mice groups.

Blood cell counts. Platelet counts were quantified using a single optical cytometer (HC Plus Cell Control; Coulter Electronics Ltd, Luton, UK).

Detection of lupus anticoagulant. The presence of lupus anticoagulant was evaluated by prolongation of aPTT in a mixing test, adding one volume of plasma (whole blood mixed with Na-citrate 0·13 mol/*l*, in a 9 : 1 ratio) to one volume of actin (a rabbit brain thromboplastin; Sigma) and incubating for 2 min at 37°C. Another volume of 0·02 M $CaCl_2$ was added and the clotting time was recorded in seconds. The presence of the lupus anticoagulant was confirmed by the kaolin clotting time (KCT).

Evaluation of pregnancy outcome. The number of vaginal plugs (indicating mating), the number of pregnancies (indicating fecundity), and the number of live embryos per successful pregnancy were studied according to methods previously described [11].

Additionally, the number of resorbed embryos was recorded and the resorption index (%*R*) was calculated, as follows:

$$\%R = \frac{\text{no. of resorptions}}{\text{no. of resorptions} + \text{no. of live embryos}} \times 100$$

RESULTS

Characterization of the experimental APS

Following immunization with H-3 the mice developed elevated titres of mouse aPL (anticardiolipin, anti-phosphatidylserine and anti-phosphatidylcholine) compared with non-immunized mice or mice given irrelevant human IgM (Table 1). No significant differences were observed ($P < 0{\cdot}05$) between levels of aPL among APS mice given Ox-LDL compared with the ones infused with LDL or PBS. Similar results were obtained in control mice, namely lack of difference between levels of aPL between groups immunized with Ox-LDL, LDL and PBS.

Characterization of mice aCL

In order to study whether aCL recognize and bind Ox-LDL or LDL, we performed inhibition assays. Partial inhibition of binding of sera from immunized mice to cardiolipin (before infusion with Ox-LDL) was demonstrated, employing Ox-LDL as an inhibitor, in comparison with LDL or cardiolipin as controls (Fig. 1a).

No inhibitory effect of Ox-LDL (or LDL) was observed on binding of sera from mice with experimental APS infused with Ox-LDL to cardiolipin (Fig. 1b). Further characterization of mice aCL was accomplished by affinity purification of mouse aCL (by loading on cardiolipin-coated silica beads) from the experimental APS mice following Ox-LDL infusion. aCL from the APS mice infused with Ox-LDL and depleted of anti-Ox-LDL antibodies (following loading of mice aCL on Ox-LDL-coated silica beads)

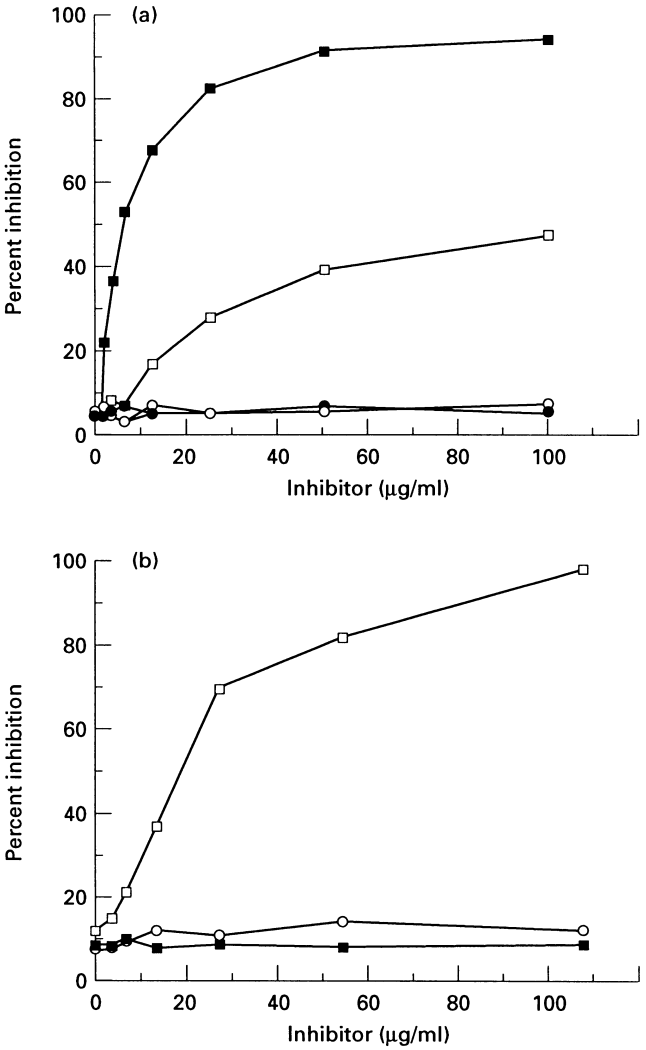

Fig. 1. (a) Inhibition of binding of sera of mice with experimental APS to cardiolipin with LDL (○), Ox-LDL (□) and cardiolipin (■). Inhibition test performed on sera of mice with experimental APS before infusion of Ox-LDL. Inhibition of the binding of mouse sera aCL to cardiolipin was challenged with Ox-LDL, LDL and cardiolipin. The Ox-LDL displayed partial inhibitory effect on binding, whereas native LDL did not have any effect. As expected, cardiolipin exhibited a dose-dependent inhibitory effect. ●, Control. (b) Inhibition of binding of sera with experimental APS infused with Ox-LDL to cardiolipin by LDL (■), Ox-LDL (○) and cardiolipin (□). Inhibition of binding to cardiolipin of mice sera which were infused with Ox-LDL disclosed lack of effect of either Ox-LDL or native LDL (transfused). The mouse anticardiolipin antibodies (aCL) (which were previously shown to bind Ox-LDL) were saturated with Ox-LDL, administered *in vivo*, and thus their binding to cardiolipin *in vitro* was not altered by its added concentrations.

exhibited significantly diminished cardiolipin binding on a solid-phase assay compared with binding before depletion (OD at 405 nm of 0·5 *versus* 1·4) (Fig. 2). The eluate obtained following loading mouse aCL on Ox-LDL silica beads was also shown to bind cardiolipin (OD at 405 nm of 0·6). Addition of β_2-GPI to the Ox-LDL-bound silica beads did not result in a considerably altered cardiolipin binding by the aCL (OD of 0·6). Furthermore, absorption of aCL from APS mice infused with Ox-LDL, through LDL-bound silica beads, did not change significantly their binding to cardiolipin (Fig. 2).

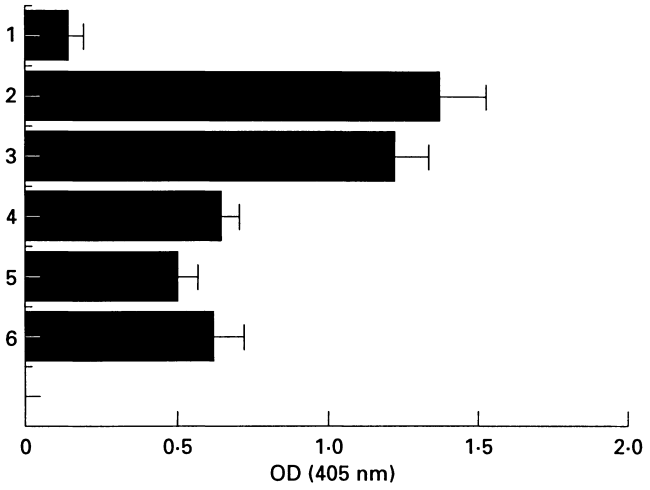

Fig. 2. Comparative binding of anticardiolipin antibodies (aCL) from mice with experimental APS to cardiolipin before and after Ox-LDL transfusion. The mouse aCL depleted of anti-Ox-LDL activity displayed a significantly diminished binding to cardiolipin compared with binding of aCL before *in vivo* infusion of Ox-LDL. This decreased binding to cardiolipin is due to cross-reactive population of antibodies binding to cardiolipin and Ox-LDL. Addition of β_2-glycoprotein I (β_2-GPI) to the Ox-LDL-bound silica beads did not alter the binding properties of mice aCL which was depleted of anti-Ox-LDL activity—namely, β_2-GPI did not have a significant effect on mice aCL binding to cardiolipin. 1. aPL from mice immunized with an irrelevant human IgM. 2. aPL derived from mice with experimental APS, before infusion of Ox-LDL. 3. aPL derived from mice with experimental APS, before infusion of Ox-LDL following anti-LDL depletion. 4. aPL derived from mice with experimental APS, before infusion of Ox-LDL, following depletion of anti-Ox-LDL and anti-β_2-GPI activity. 5. aPL from mice with experimental APS, before infusion of Ox-LDL, following depletion of anti-Ox-LDL activity. 6. aPL from mice with experimental APS following infusion of Ox-LDL.

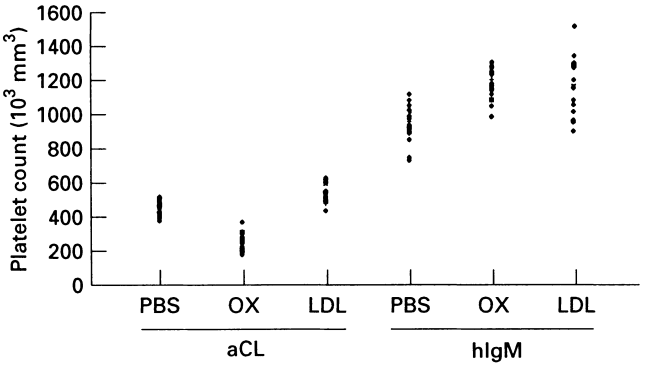

Fig. 3. Platelet count in mice with experimental APS, exposed to ox-LDL, LDL and PBS. Statistical analysis was made using the ANOVA test. Significantly decreased platelet counts were obtained in the Ox-LDL-transfused APS mice compared with ones given LDL ($P < 0{\cdot}04$) or PBS ($P < 0{\cdot}01$). No statistically significant differences were obtained when the group with experimental APS infused with LDL was compared with APS mice infused with PBS. $P < 0{\cdot}001$ was found in Ox-LDL-infused mice with experimental APS, compared with their littermates, which were immunized with human IgM (hIgM; Ox-LDL-infused). Each group represents mean ± s.d. of 16–20 mice.

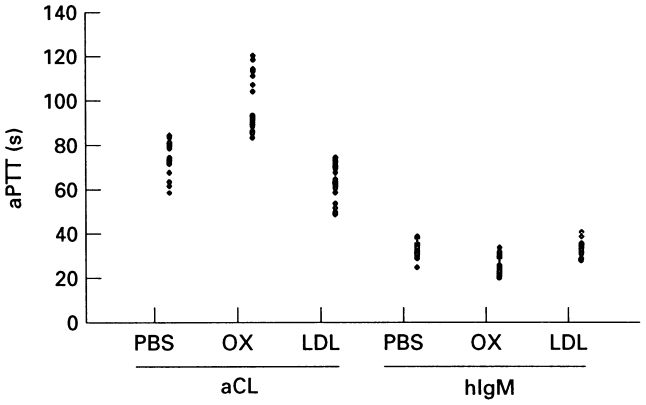

Fig. 4. Activated partial thromboplastin time **(**aPTT) in mice with experimental APS, exposed to ox-LDL, LDL and PBS. Each group represents mean ± s.d. of 16–20 mice. Statistical analysis was made using the ANOVA test. Significantly prolonged aPTT was found in the ox-LDL APS mice compared with the LDL APS mice ($P < 0{\cdot}05$) or the PBS-infused APS mice ($P < 0{\cdot}05$). No significant difference in aPTT was found between the LDL- and PBS-transfused APS mice.

Clinical manifestations of APS

Statistically significant differences were detected in the platelet counts between APS mice infused with Ox-LDL, compared with the ones given LDL or PBS (261 000/mm^3, 535 000/mm^3 and 455 000/mm^3, respectively) (Fig. 3). No differences were noted in platelet counts between non-immunized mice and mice immunized with irrelevant IgM infused with either Ox-LDL, LDL or PBS (1089 000 mm^3, 995 000 mm^3, 1187 000 mm^3 in non-immunized mice and 1278 000 mm^3, 1184 000 mm^3, 979 000 mm^3 in the IgM group, respectively). APS mice infused with Ox-LDL were also found to have a more prolonged aPTT (Fig. 4) and higher fetal resorption rate (Table 2) (99 ± 12 s and 72·7% resorption rate, respectively), in comparison with mice given LDL or PBS (63 ± 8 s, 34·4% resorption rate and 74 ± 8 s, 32·6% resorption rate, respectively), all the above marking a more severe form of APS in Ox-LDL-administered mice.

DISCUSSION

The complex mechanisms leading to thromboembolic phenomena in APS are far from being completely resolved. It was formerly believed that interaction of aPL with phospholipids may by itself be the triggering event, an observation which was later challenged by authors demonstrating the requirement of yet an additional factor—β_2-GPI, for the binding to cardiolipin [8,25–27]. However, it is becoming gradually apparent that the interaction of aPL with phospholipids in the presence of β_2-GPI is not solely responsible for the thrombotic events in APS. As such, several other glycoproteins have been suggested as targets of aPL binding [12–15]. This study presents another candidate factor in this apparently 'multilateral' binding—Ox-LDL.

LDL in its modified (oxidized) form constitutes a key factor in the accelerated progression of atherosclerosis [16–18]. This effect is probably facilitated by its enhanced uptake by macrophages, possibly in the form of immune complexes with anti-Ox-LDL antibodies, resulting in an increased formation of foam cells [28,29]. Indeed, immune complexes consisting of Ox-LDL and its corresponding antibodies have been demonstrated within atherosclerotic plaques [30]. The presence of high titres of Ox-LDL antibodies has been documented in various disease states [31–34], yet the true pathogenic significance of this finding is not clear.

Recently, two studies presented compelling evidence of cross-reactivity between aPL and anti-Ox-LDL antibodies both in humans [15] and in rabbits [23]. In the former study [15], it was suggested that SLE patients, half of which were aPL-positive, are prone to develop accelerated atherosclerosis, presumably owing to the cross-reactivity of aPL with the Ox-LDL. This interaction may result in enhanced uptake of Ox-LDL by macrophages (through the scavenger receptor) as much as the complex Ox-LDL–anti-Ox-LDL does so [28,29]. These observations prompted us to explore the other aspect of this apparent cross-reactivity, i.e. the possible influence of Ox-LDL on the clinical manifestation of APS.

Very recently it was proposed that the procoagulant state in APS is generated following the exposure of a cryptic epitope within the plasma cofactor β_2-GPI [25,35], leading to its binding by aPL, with the subsequent clinical consequences. The exposure of this cryptic epitope was attributed to the binding of β_2-GPI by negatively charged substances or surfaces [25], and various phospholipids have been shown to be the principal binders. Since circulating lipoproteins are known to contain various amounts of phospholipids, and additionally, to bind β_2-GPI [36], we speculated that they might thus act to promote the binding of aPL, and result in exacerbation of the clinical manifestations of experimental APS.

We have chosen to evaluate the impact of Ox-LDL on a murine model of experimentally induced APS. This model provides a measurable means of estimating the severity of the disease by determining the extent of thrombocytopenia, prolongation of the

Table 2. Fetal resorption in APS mice exposed to Ox-LDL, LDL or PBS

	aCL			HIgM		
	PBS ($n = 19$)	Ox-LDL ($n = 17$)	LDL ($n = 17$)	PBS ($n = 16$)	Ox-LDL ($n = 19$)	LDL ($n = 20$)
Live	151	38	168	202	182	185
Resorbed	73	101	88	9	10	15
Percent resorption	32·6	72·7	34·4	4·3	5·2	7·5

Statistical analysis was made using the χ^2 test. Significant differences were obtained when the APS mice infused with Ox-LDL were compared with their littermates infused with LDL ($P < 0{\cdot}0001$) or PBS ($P < 0{\cdot}0001$). No statistically significant difference was obtained when the LDL-infused APS mice were compared with their PBS-infused littermates. No statistically significant differences were noted in the groups of mice immunized with HIgM and infused with either Ox-LDL, LDL or PBS.

aPTT (as a marker for the presence of lupus anticoagulant activity) and fetal resorption rate. This model of induced APS as recently demonstrated by us [11] involves administration of anticardiolipin MoAb to naive (BALB/c) mice, following which there ensues a clinical picture similar to APS in humans. The clinical manifestations of APS in mice appear to coincide with the appearance of mouse aCL, so as to point towards the important role played by the antibodies in initiating the disease. It has been suggested that dysregulation of the idiotypic network is responsible for the induction of the model. Thus, it was presumed that active immunization of the mice with the aCL led to the production of anti-aCL (anti-idiotypes = Ab2), which in turn resulted in the elaboration of anti-anti-CL antibodies (anti-anti-idiotypes = Ab3). The latter autoantibodies resemble the original antibodies (used for immunization) in their binding properties, and may in fact precipitate the disease. Furthermore, these mice antibodies (Ab3) have been immortalized, and were able to reproduce the clinical picture of experimental APS.

This study shows that administration of Ox-LDL, compared with native LDL, results in aggravation of the clinical picture of APS in mice. Hence, it was observed that the clinical features shared by APS in humans and mice, namely thrombocytopenia, higher fetal resorption rate and prolonged aPTT, were all displayed in a more severe form. Moreover, insight into the pathogenic mechanisms underlying these results has been provided by characterization of mice aCL prior to and following infusion of Ox-LDL. We have been able to demonstrate cross-reactivity of Ox-LDL with aPL, which may provide a reasonable explanation for the aggravation of experimental APS in mice. Accordingly, Ox-LDL-depleted aPL from APS mice, infused with Ox-LDL, displayed significantly diminished cardiolipin binding on solid-phase assays. Furthermore, eluted anti-Ox-LDL from affinity-purified mouse aCL was found to bind cardiolipin.

The study exemplifies the complexity of interactions presumed to trigger the clinical picture of APS, adding another potentially significant participant, Ox-LDL, to the growing list of antigenic targets of aPL [12–14]. It is fair to speculate that the aggravation of experimentally induced APS in mice could be attributed to cross-reactivity of Ox-LDL with aPL, generating a complex which resembles the aPL-phospholipid complex, possibly associated with a prothrombotic state. Additionally, Ox-LDL could act to alter the structure of β_2-GPI, exposing a cryptic epitope which may enhance its binding to aPL and thus be responsible for the exacerbated picture seen in the APS mice.

Since Ox-LDL rather than LDL displayed the pathogenic effects, we believe that it is the alteration of the phospholipid cofactor (β_2-GPI) only by the former, possibly through the provision of a negatively charged 'medium', that was the principal contributing factor for the results obtained in the study.

This study may also have direct clinical implications, since if LDL oxidation exacerbates the clinical manifestations of APS, than anti-oxidants, interfering with this damaging process, may stand as novel treatment modalities in this selected group of patients and therefore merit further clinical investigation.

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