Surfactant protein D is proatherogenic in mice

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Surfactant protein D (SP-D) is a member of the collectin family of collagen-like lectins (12). The protein is mainly studied in relation to pulmonary immune defense, although it is present in various mucosal surfaces (25) and in the circulation (9a, 21, 38a). SP-D is a molecule with several known functions. It is a pattern recognition molecule binding carbohydrate and lipid moieties presented on the surface of pathogenic microorganisms and apoptotic cells. The binding initiates various effector mechanisms, such as alteration of membrane permeability or activation of phagocytes, which may enable the elimination of the bound material. SP-D, moreover, shows a strong chemotactic effect on inflammatory cells, including granulocytes, and macrophages, and SP-D-deficient (Spd−/−) mice have increased levels of proinflammatory cytokines, metalloproteinases, and oxidant species in the lung (40–42).

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isolated for direct sequencing. No bands were detectable in blank reactions. Mouse thoracic aorta was frozen in liquid nitrogen after dissection, and RT-PCR was performed as previously described (26).

Western blotting and immunohistochemistry. Western blotting of tissue homogenates or lysed cell culture was performed essentially as described by Madsen et al. (25) on 8–25% (wt/vol) polyacrylamide gradient gels. Primary anti-SP-D antibody was mouse monoclonal antibody Hyb245-2 (1 μg/ml). Secondary antibodies were alkaline phosphatase-coupled rabbit anti-mouse Ig (DakoCytomation, Copenhagen, Denmark).

Cell cytopsins were acetone fixed. Formaldehyde-fixed paraffin-embedded tissue sections were mounted on slides, dried at 60°C, deparaffinized, hydrated, and processed as previously described (16). Monoclonal antibodies directed against SP-D (Hyb245-1) were produced and characterized as previously described (25). Normal human tissues and atherosclerotic tissue were from the tissue bank at the Department of Pathology, Odense University Hospital. Monoclonal anti-CD34 and anti-CD31 antibodies (DakoCytomation) were used as positive controls for endothelial cells. A biotin-streptavidin immunoperoxidase technique was used on paraffin sections. Enhanced epoCopit retrieval was obtained by microwave heat treatment of the specimen. Briefly, paraffin sections were pretreated in 10 mM Tris with 0.5 mM EGTA, pH 9.0 before microwave treatment and immunostaining. The specificity of immunostaining was verified by replacing the primary antibodies with an isotype monoclonal antibody.

Purification of human SP-D. Human SP-D was isolated from amniotic fluid by maltose-agarose (Sigma-Aldrich) affinity chromatography (Amersham Biosciences) as previously described (39).

Mouse feeding study. Homozygous Spd+/− mice (2) were backcrossed >10 times with the C57BL/6N strain. The C57BL/6N wild-type mice used in this study were obtained from Charles River Laboratories (Sulzfeld, Germany). The National Animal Ethics Committee approved the study and all procedures. The newborn mice had free access to regular chow (Altromin 1314, Brogården Aps, Gentofte, Denmark). The lighting schedule was 12 h of light and 12 h of dark. Mice were fed and given water ad libitum. A total of 67 5-wk-old wild-type and Spd+/− female mice were fed an atherogenic diet for 36 wk (TD 88051, Harlan, Kreuzelweg, Netherlands) containing 15.8% (wt/wt) fat, 1.25% (wt/wt) cholesterol, and 0.5% (wt/wt) sodium cholate as previously described (14, 29). Some mice were killed during the feeding study to follow the development of aortic lesions. One Spg+/− mouse died during the time span of the experiments because of failure to thrive. The numbers of mice used for analyses are shown in Table 1. Blood samples were collected by retroorbital puncture unless otherwise stated.

Plasma lipids. Plasma lipids were measured with a Cobas Mira instrument (Triolab A/S, Copenhagen, Denmark). Triglycerides (TG), total cholesterol (TC), LDL cholesterol (LDL-C), and HDL cholesterol (HDL-C) were measured with ABX Diagnostics reagents. All assays were performed according to the manufacturer’s directions (Triolab A/S). Plasma samples were used diluted zero- to threefold in 0.9% (wt/vol) sodium chloride.

Plasma cytokines. The cytokine contents in plasma from mice after 36 wk of diet was measured by means of the Mouse Th1/Th2 cytokine cytometric bead array (CBA) kit (Becton-Dickinson, Copenhagen, Denmark), using a FACSCalibur flow cytometer (Becton-Dickinson) and the corresponding CBA analysis software (Becton-Dickinson). The kit contains five populations of beads that have been coated with capture antibodies for TNF-α, INF-γ, IL-2, IL-4, and IL-5, respectively. The assay was performed according to the manufacturer’s directions. IL-4 measurements were excluded from analysis because 16 of 37 measurements fell below the detection limit. The numbers of mice used for cytokine analyses are shown in Table 1.

Aortic lesions. For aortic lesion analysis, nonfasting mice were anesthetized with 100 mg/kg pentobarbital (Den Kgl. Veterinær og Landbrohskoles apotek, Copenhagen, Denmark), exsanguinated, and perfusion fixed, and the pathoanatomy was analyzed as described previously (1). The amount of atherosclerosis in the aortic root was expressed as 1 mean plaque area/animal (mean of 3 sections) measured in a blinded fashion by one person (G. L. Sorensen).

Half-life of recombinant fragment human SP-D in Spd+/− mouse plasma. Eight 3- to 4-mo-old Spd+/− mice were included in this study (Table 1). Each mouse received one bolus of 90 μg of recombinant fragment human SP-D (rfhSP-D) in 250 μl of sterile isotonic sodium chloride solution injected in the tail vein. The fragment was a 60-kDa homotrimer of recombinant SP-D, composed of eight Gly-Xaa-Yaa repeats from the collagen region, the α-helical coiled-coil neck region, and the CRD, and was expressed in Escherichia coli as previously described (24). The endotoxin level in the rfhSP-D infusion was <0.2 EU/ml measured by the Limulus amoebocyte lysate QCL-1000 assay (BioWhittaker, Walkersville, MD). The rfhSP-D concentration was measured by ELISA technique as previously described (21), and the half-life was estimated. Plasma samples were frozen before the immunoassay was performed.

Treatment of mice with rfhSP-D. Five-week-old Spd+/− female mice were fed the atherogenic diet for 5 days. One group of mice received 9 μg rfhSP-D/280 μl sterile isotonic sodium chloride by daily tail vein infusions. The endotoxin level in the rfhSP-D infusion was <0.02 EU/ml. Another group received 9 μg human serum albumin (HSA)/280 μl sodium chloride solution daily. Plasma lipids and rfhSP-D concentrations were measured as described above (Table 1). Blood samples from untreated mice were collected by retroorbital puncture, whereas blood samples from HSA- or rfhSP-D-treated mice were collected by heart puncture in anesthetized mice. The serum albumin was prepared for human infusion under endotoxin-free conditions and kindly provided by Claus Koch (State Serum Institute, Copenhagen, Denmark). The two groups of mice were killed by anesthesia (as described above) and exsanguination.

Statistics. Differences between groups of data were evaluated by the nonparametric Mann-Whitney U-test. A value of P < 0.05 was regarded as significant. Relationships between plaque areas and mouse HDL-C were analyzed by linear regression. All statistical analyses were performed with Intercooled Stata version 7.0.

RESULTS

SP-D expression by endothelial cells. SP-D was previously localized to cells in a variety of tissues by the specific monoclonal antibody Hyb245-1 (25). This antibody also detects

Table 1. Number of mice used in experiments

<table>
<thead>
<tr>
<th>Lesion Areas</th>
<th>Plasma Lipids</th>
<th>Plasma Cytokines</th>
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<tbody>
<tr>
<td>Spd+/−</td>
<td>24 F</td>
<td>20 F</td>
</tr>
<tr>
<td>Wild type</td>
<td>22 F</td>
<td>18 F</td>
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<tr>
<td>rfhSP-D</td>
<td>16 F</td>
<td>21 F</td>
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Values are numbers of mice used for measurements in the atherosclerosis study, the recombinant fragment human surfactant protein D (rfhSP-D) half-life study, and the rfhSP-D treatment study. Horizional rows show different measurements from the same group of mice. In some studies there was not sufficient plasma for all lipid or cytokine analyses, and the number of measurements thus varies with the type of analysis. F, female; M, male; HSA, human serum albumin.
SP-D in vascular endothelial cells in all tissues examined. Hyb245-1 reacted with endothelial cells in arteries and veins in microvasculature as well as in macrovasculature and in high-endothelium venules. SP-D immunoreactivity to endothelial cells is shown for selected tissues in Fig. 1.

Primary HUAEC cell lines were established to study SP-D expression. HUAEC cytopsin preparations reacted with Hyb245-1. Staining was not detected with primary isotype control antibodies (data not shown). Immunostaining was highly granular, indicating that the intracellular localization of SP-D is vesicular. The primary endothelial cells costained for endothelial markers CD31 and CD34 (Fig. 2, A–E). In the seventh to eighth passage the endothelial cell cytopsin preparations reacted with Hyb245-1, although the immunoreactivity was significantly diminished in parallel with the endothelial cell marker CD31 (data not shown). The cultures sustained the expression of the endothelial cell marker CD34 throughout all investigated passages. The loss of SP-D protein expression was suspected to be due to cell culture senescence.

Whole arteries were isolated from human umbilical cords. RT-PCR analysis amplifying the SP-D neck/CRD region and successive sequencing of the PCR product demonstrated the synthesis of SP-D polyadenylated RNA in the vascular tissue (Fig. 2F). The corresponding fragment could also be amplified from HUAEC cultures. The SP-D mRNA expression was accelerated with increasing seeding density of the cell culture. The PCR reaction was normalized to β-actin mRNA expression (Fig. 2G). RT-PCR analysis amplifying the full-length SP-D transcript showed mRNA from HUAEC culture to be identical in size to that from the lung, and the sequence obtained from the PCR product was identical to the known sequence obtained from lung mRNA (data not shown).

Western blotting of lysates prepared from HUAEC culture demonstrated that the monoclonal anti-human SP-D antibody Hyb245-2 recognized endothelial SP-D protein. The fully reduced endothelial protein showed slightly altered mobility in the SDS-PAGE compared with the 43-kDa SP-D polypeptide monomers of SP-D protein purified from amniotic fluid, and no higher oligomeric forms were detected in the unreduced sample (Fig. 2H). A band appeared in the blot from HUAEC, suggesting proteolytic cleavage of the cellular SP-D possibly preventing oligomerization. Corresponding Hyb245-2 reactiv-

Fig. 1. Localization of surfactant protein D (SP-D) in human vascular endothelial cells. SP-D immunoreactivity in endothelial cells in a cross section of a pulmonary artery and in type II cells (A), a cross section of a vein in the small intestine (B), a longitudinal section of a vein in the lung and in Clara cells (C), high-endothelium venules in the tonsil (D), a longitudinal section of a vein in the skin (E), and a medium-sized artery with an atherosclerotic lesion (F) is shown. This tissue stems from a leg amputated because of ischemia caused by atherosclerosis. Bars = 100 μm.
ity was observed in lysates of human umbilical arterial and vein tissues (data not shown).

RT-PCR analysis amplifying the mouse SP-D neck/CRD mRNA and successive sequencing of the PCR product demonstrated the synthesis of SP-D polyadenylated RNA in the vascular tissue (Fig. 2I). The PCR reaction was normalized to mouse transferrin receptor mRNA expression. Spd expression in the aorta was detectable after 36 PCR cycles. In contrast, no expression product appeared in the heart sample after 40 PCR cycles. The same RT-PCR protocol previously demonstrated highly specific patterns of mouse SP-D expression (26). The main sites of synthesis were in tissues like lung, uterus, and salivary gland, whereas there was no detectable expression in tissues like heart and spleen.

Spd−/− mice are protected against development of atherosclerotic lesions in aortic root. C57BL/6 female mice are known to develop atherosclerotic lesions in the aortic root when kept on an atherogenic diet (14). In contrast, male mice are not susceptible to diet-induced atherosclerosis. Spd−/− female mice and, for comparison, the corresponding C57BL/6N female mice were fed the diet, and the formation of atherosclerotic lesions in the aortic root was followed. Groups of four mice were killed and evaluated after 12 and 24 wk, but none or only small foam cell lesions were found. After 36 wk the remaining mice were killed.

Fig. 2. SP-D expression in human endothelial cells. A: cross section of a contracted umbilical artery immunostained with Hyb245-1. Bar = 250 μm. B: immunostaining of human umbilical artery endothelial cell culture (HUAEC) with Hyb245-1. C–E: cytospin section of HUAEC passage #1 immunostained with anti-CD31 antibodies (C), with anti-CD34 antibodies (D), and with Hyb245-1 (E); inset in E shows an endothelial cell at increased magnification. Bars in B–E = 50 μm. F: RT-PCR analysis of SP-D mRNA expression in human tissues and cells. G: top: human (h) SP-D mRNA expression in HUAEC positively correlating seeding density (number of cells/well, diameter = 1.5 cm). Bottom: corresponding β-actin mRNA expression. H: Western blot analysis of HUAEC total protein and SP-D purified from human amnion fluid (AF) in the reduced (R) and unreduced (U) states. MW, molecular weight. I: RT-PCR analysis of mouse (m)SP-D mRNA transcription in mouse aorta compared with transferrin receptor mouse (m) (Tfr) mRNA transcription. Numbers of PCR cycles are indicated at left.
and the aortic roots examined. No structural remodeling (nonleision) in the aortic root was apparent in Spd−/− mice. Lesion areas are shown in Fig. 3. A significant difference was found between Spd−/− mice and wild-type mice (Fig. 3A), with median lesion areas in the wild-type mice exceeding the Spd−/− mice by 5.6-fold. Lesions in Spd−/− mice consisted only of foam cells (fatty streaks), whereas many of the lesions in wild-type mice were mature atherosclerotic plaques with extracellular matrix containing lipid (cholesterol clefts) and collagen (Fig. 3, B–E).

Mean and maximal atherosclerotic lesions are shown in Fig. 3, F–I, for Spd−/− mice and wild-type mice. Lung sections obtained from the same animals are shown in Fig. 3, J–M. The pulmonary phenotype of Spd−/− mice was previously described with emphysema (2, 13). The wild-type mice showed normal lung morphology, whereas the Spd−/− mice showed alveolar infiltrations with lymphocytes and foam cells. The Spd−/− mice in our experiment were thus prone to emphysema development but partly protected from atherosclerosis.

The atherogenic diet was well tolerated by both Spd−/− mice and wild-type mice, and both groups gained weight at a faster rate than on a normal chow (data not shown).

Plasma lipids are altered in Spd−/− mice. Significantly higher levels of HDL-C and TG were observed in Spd−/− female mice compared with wild-type mice (Fig. 4), measured at individual time points throughout the study or as the integrated area under the curve (AUC). No differences were observed or sustained in TC or LDL-C between the genotypes. HDL-C levels were increased on average 18% in Spd−/− mice compared with wild-type mice, comparing genotype AUC. No initial difference was detected between TG levels. The diet-induced depression of TG was less pronounced in the Spd−/− animals, with an average difference of 27% between the genotype AUCs. In addition, plasma lipids were measured in parallel experiments also using male mice and regular chow. These experiments indicated that the HDL-C fraction is most affected in female Spd−/− mice (data not shown).

Fig. 3. Atherosclerotic plaques in mice given atherogenic diet. A: amount of atherosclerosis after 36 wk of atherogenic diet measured in the aortic roots from SP-D-deficient (Spd−/−) and wild-type mice was significantly different (P < 10−4). Median lesion areas are indicated as horizontal bars and were 15,100 (9,700, 30,000; 95% confidence interval) and 85,200 (46,400, 115,000) μm², respectively. B–E: advanced atherosclerotic plaques in the aortic roots from wild-type mice containing foam cells (B), collagen-rich components (C), and lipid-rich components (cholesterol crystals) (D, E). Bars in B–E are 75 μm. F and G: cross sections of the aortic roots showing mean and maximal plaque areas in wild-type mice. H and I: mean and maximal plaque areas in Spd−/− mice. J and K: pulmonary sections from wild-type mice. L and M: pulmonary sections from Spd−/− mice showing infiltrating foam cells and neutrophils, respectively. Bars in F–M are 300 μm. Arrows point to a position in the slides including foam cells (B), collagen-rich components (C), and lipid-rich components (cholesterol crystals) (D, E).
HDL-C levels were negatively associated to lesion sizes in the experiment. Linear regression was applied to estimate the relationship and indicated that a drop of 50,999 µm² in lesion size was associated with a rise of 1 mM HDL-C ($P = 0.01$; $R^2 = 0.33$).

Recombinant SP-D reduces plasma lipid concentrations. Eight mice received 90 µg of rhSP-D injected intravenously. Blood samples were drawn over a period of 50 h, and ELISA technique was used to measure the retrieved rhSP-D concentrations. As predicted, the assay did not give any signal from the plasma of the untreated Spd−/− mice. The mean plasma concentrations obtained are shown in Fig. 5A. The initial mean concentration measured after 1 h was ~8 µg/ml plasma and reflected that redistribution had occurred at this time point. The declining plasma concentration corresponded to a mean half-life of 62 h.

Next, groups of Spd−/− and wild-type female mice received daily intravenous infusions of 9 µg of human recombinant rhSP-D over 5 days. Corresponding control groups of female mice received HSA. All mice received the atherogenic diet during the five treatment days. Plasma rhSP-D concentrations were measured on a daily basis in two of the rhSP-D-treated mice thereafter. rhSP-D concentrations oscillated around an average of 2,513 ng rhSP-D/ml plasma. TG and HDL-C levels were depressed by the 5 days of atherogenic diet. In contrast, LDL-C and TC were increased by the atherogenic diet during the five treatment days. Plasma rhSP-D concentrations measured on a daily basis in two of the rhSP-D-treated mice thereafter. rhSP-D concentrations oscillated around an average of 2,513 ng rhSP-D/ml plasma. TG and HDL-C levels were depressed by the 5 days of atherogenic diet. In contrast, LDL-C and TC were increased by the atherogenic diet. The relative difference in HDL-C, TC, and LDL-C levels seen between Spd−/− mice receiving rhSP-D and those receiving albumin was a lowering of the levels by 21%, 26%, and 28%, respectively (Fig. 5B, right).

Spd−/− mice on high-fat diet have decreased levels of serum TNF-α. Plasma cytokines were measured in blood collected after 36 wk of atherogenic diet (Fig. 6). Average TNF-α levels were significantly ($P = 0.0001$) decreased from 77.3 pg/ml in wild-type mice to 42.2 pg/ml in Spd−/− mice (45% difference). No significant differences were measured between Spd−/− mice and wild-type mice in INF-γ, IL-2, and IL-5 levels.

**DISCUSSION**

Monogenic causes of atherosclerosis are rare and cannot account for the prevalence of the disease. Recent studies in genetically deficient mice indicate that numerous components of both the innate and the adaptive immune system can act as modifiers of atherosclerosis (23, 38). Here we document transcription of the Spd gene in vascular endothelial cells and demonstrate that SP-D increases susceptibility to atherosclerosis in mice.

Serum SP-D has been regarded as a spillover from the pulmonary compartment and has been studied as a marker of several pulmonary diseases (18, 21). We have now demonstrated that SP-D is synthesized in vascular endothelial cells in both mice and humans. The mRNA expression was relatively low in both species but was inducible in cell culture. SP-D protein was located to human endothelial cells in all tissues, including atherosclerotic lesions. The endothelial cell SP-D protein was apparently proteolytically cleaved or alternatively modified compared with the purified SP-D from amniotic fluid, and no covalently linked trimeric forms were detected by Western blotting. Specific functions of a putative endothelial SP-D form may be expected and possibly could explain the apparent proinflammatory effect of SP-D in the vasculature of the mice in this study. Nevertheless, it remains to be explored whether SP-D of endothelial origin represents a physiologically active form present outside the endothelial cell and whether the putative alternative form of SP-D is restricted to umbilical endothelial cells. In this regard, serum SP-D was previously demonstrated to form higher oligomers according to individual Spd genotype variations (20). Inbred mouse strains have become a useful tool for identifying genetic factors contributing to atherosclerosis. It is possible to induce atherosclerosis in certain strains with cholesterol- and cholic acid-rich atherogenic diets. This model is nevertheless limited by incomplete penetration of the atherosclerotic phenotype (29, 30). The antiatherogenic effect of a single gene may, on the other hand, be concealed in genetically modified mouse models that develop robust atherosclerosis (36). We chose the diet-induced atherosclerosis model and observed a dramatic difference between Spd−/− mice and wild-type mice, which is comparable to the effect observed with the macrophage colony-stimulating factor-deficient apoE−/− mice (8). Lesion sizes were small in the Spd−/−
mice, which only developed foam cell lesions. In contrast, many of the wild-type mice had developed mature atherosclerosis with abundant extracellular matrix containing cholesterol clefts and collagen. The variation in lesion size in the wild-type mice corresponded to the variation observed in apoE−/− mice fed a regular chow (1).

In vivo studies previously suggested that multiple SP-D functions are mediated through the lectin domain and showed

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**A**

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Fig. 5. **A**: average concentrations of exogenous recombinant fragment human SP-D (rfhSP-D) in mouse plasma in the hours after intravenous infusion of 90 μg of rfhSP-D in Spd−/− mice. Error bars indicate SD. **B**: whisker plots of minimum, lower quartile, median, upper quartile, and maximum plasma lipid concentrations in Spd−/− female mice given atherogenic diet for 5 days together with daily infusions of either human serum albumin (HSA) or rfhSP-D. Solid circles, state-defined outliers. **Left**: lipid (HDL-C, TG, LDL-C, and TC) concentrations before the diet and treatments (blood sampling by retro-orbital puncture). **Right**: lipid concentrations after the 5-day diet and treatments (blood sampling by heart puncture in anesthetized mice). Significant difference between HSA- and rfhSP-D-treated groups: *P < 0.05, **P < 0.01.
that rhSP-D has therapeutic effects in mice (24). In our experiments, HDL-C and TG were significantly increased in Spd−/− mice and 5 days of rhSP-D treatment resulted in lowered HDL-C, LDL-C, and TC. The apparent SP-D-mediated regulation of multiple lipid fractions indicates that a general factor in lipid transport or metabolism is influenced. The effective dose of rhSP-D in the treated animals approximated 2.5 μg/ml and was considered close to physiological levels as the SP-D serum level in humans is estimated at a median value of 743 ng/ml, with the normal concentration spectrum ranging from 158 to 3,711 ng/ml (21, 38a). However, the physiological level of serum SP-D in mice with chronic inflammation is measured to approximately 100 ng/ml (9a), and differences in both structure and concentration between the rhSP-D used in the current study and serum SP-D in wild-type mice should be taken into consideration when interpreting lipid fractions affected by SP-D.

Direct association between lesion size and HDL-C and the antiatherogenic properties of mouse HDL are controversial (30, 37), and the difference in HDL-C levels between Spd−/− mice and wild-type mice in our study was rather limited compared with similar studies (14, 30). Nevertheless, HDL-C was directly associated to lesion size in our study, suggesting that the HDL-C level indeed plays a role in determining the reduced lesion susceptibility in Spd−/− mice. Quantitative trait loci analyses have demonstrated that the extent of atherosclerosis and HDL-C levels are determined by the genetic background (7, 14), and Spd may be suggested as a common susceptibility gene for lesion size and HDL-C levels. Extrapolation from results obtained in mouse models to human physiology is complicated by multiple differences between the species (28, 31). Nevertheless, in a human study including 757 subjects we observed parallel inverse associations between SP-D and HDL-C resulting in a β-coefficient of −105 ng/ml SP-D per 1 mM HDL-C in healthy women (P = 0.02, R² = 0.12; G. L. Sorensen and U. Holmskov, unpublished data).

Th1, the most prevalent type of CD4+ cell, induces macrophage activation and promotes inflammation by secretion of INF-γ, IL-2, and TNF-α. However, Th2 cells suppress inflammation and dampen macrophage activity via several different anti-inflammatory cytokines like IL-4 and IL-5. In a recent study, apoE−/− mice were treated with an inhibitor of the Th1 differentiation pathway (pentoxifyllin) for 12 wk and the animals presented with reduced size of atherosclerotic lesions by 60% compared with controls (19). In the present study representative Th1 and Th2 cytokines were measured at the study end point to evaluate whether the inflammatory component of atherosclerosis was disturbed in the Spd−/− mice.

The proinflammatory Th1 cytokine TNF-α in plasma was significantly lower in Spd−/− mice compared with wild-type mice. The measured TNF-α levels were raised two- to threefold above background but did not compare to the induction measured in LPS-treated mice in thousands of picograms per milliliter. The measured difference in TNF-α levels may represent a reduced Th1 response in Spd−/− mice in accordance with studies of allergen-induced pulmonary hypersensitivity in which treatment with rhSP-D shifted the allergic response into a Th1 response (24).

TNF-α is produced primarily from adipocytes (11) and macrophages and is present in aortic fatty streak lesions (35), and the decreased level of TNF-α and the observed reduction in lesion size in the Spd−/− mice may be a consequence of reduced macrophage activity and recruitment of monocytes. The atherogenic diet leads to NF-κB induction of inflammatory cytokines (22), and TNF-α modulation in Spd−/− mice could be a direct consequence of diet sensitivity.

The consequences of an altered inflammatory marker in atherosclerosis are hard to predict. Lack of IL-6 led to an unexpected acceleration of atherosclerosis (33), whereas TNF-α has been associated with an increased risk for cardiovascular disease in clinical studies (15). In vitro studies have demonstrated both proatherogenic and antiatherogenic effects of TNF-α (6, 27, 32). TNF-α-deficient mice were not affected (35), but TNF-α-deficient apoE−/− mice had 50% reduced lesion areas (3). In contrast, lack of the TNF-α receptor p55 accelerated diet-induced atherosclerosis (34).

Pulmonary phospholipid accumulation and foam cell accumulation is present in Spd−/− mice (17). The lipid accumulation is suggested to be subjected to a local pulmonary-specific regulation (9). The mechanisms of both lipid and inflammatory disturbance in Spd−/− mouse lungs are unknown but can be reversed by the administration of recombinant SP-D (4, 5). An apparent dual behavior of SP-D (and the similar type of molecule surfactant protein A) in the regulation of inflammation was recently explained by ligand-dependent molecular orientation of the globular lectin domains of the collectins and resulting alterations in receptor binding on the surface of phagocytes leading to reverse NF-κB-signaling (10). Present investigations in our lab are focused on SP-D regulation of inflammation to elucidate further the inflammatory component of atherosclerosis.

GRANTS

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