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Adrenalectomy stimulates the formation of initial atherosclerotic lesions: Reversal by adrenal transplantation

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ABSTRACT

Long-term changes in the secretion of immunosuppressive adrenal-derived glucocorticoid hormones influence cardiovascular disease risk. Here we determined the consequences of changes in adrenal steroid metabolism for the development of atherosclerotic lesions in mice.

Atherosclerosis-susceptible low-density-lipoprotein (LDL) receptor knockout mice were subjected to adrenalectomy (ADX) or a control (SHAM) operation and subsequently fed an atherogenic diet for 4 weeks. Atherogenic diet feeding raised plasma corticosterone levels in SHAM mice, but not adrenalectomized mice, resulting in an 83% lower ($P<0.01$) corticosterone level in adrenalectomized mice. Adrenalectomy was associated with a respectively 22% and 29% lower plasma level of cholesterol and triglycerides. In contrast, white blood cell counts were increased 2-fold ($P<0.01$) in adrenalectomized mice, which could be attributed to a significant 2.1- to 2.6-fold rise in lymphocyte ($P<0.05$) and monocyte ($P<0.05$) numbers. Probably as a result of the enhanced systemic inflammatory status, adrenalectomy was associated with a higher susceptibility for diet-induced atherosclerosis ($321 \pm 18 \times 10^3$ μm^2 for ADX vs $240 \pm 31 \times 10^3$ μm^2 for SHAM; $P<0.05$) not withstanding the lowered cholesterol levels. Restoring adrenocortical steroid secretion – but not adrenal medulla function – and the associated downstream glucocorticoid receptor signaling in adrenalectomized mice through adrenal transplantation induced a reversal of the adrenalectomy-associated rise in white blood cell numbers, plasma monocyte chemoattractant protein 1 (MCP-1) levels, and atherosclerotic lesion development (lesion size in transplanted mice: $258 \pm 34 \times 10^3$ μm^2; $P<0.05$ vs ADX).

In conclusion, our studies show that adrenal-derived steroids protect against the development of initial atherosclerotic lesions in LDL receptor knockout mice.

1. Introduction

Cholesterol-derived steroid hormones constitute an interesting class of signaling molecules as they are able to modulate the expression of genes associated with a wide variety of cellular pathways, including metabolic, inflammatory, and developmental processes. In accordance with a diverse role for steroid hormones in normal physiology, changes in plasma levels of specified steroids are associated with multiple disease pathologies. Interestingly, it also appears that optimal adrenal steroidogenesis is necessary to overcome cardiovascular disease mortality, since changes in the secretion of adrenal-derived glucocorticoids increase cardiovascular disease in man. Primary adrenal insufficiency (Addison's disease) is associated with a ~2-fold higher risk for cardiovascular mortality [1], while subjects that suffer from Cushing's syndrome, i.e. long-term glucocorticoid hypersecretion, also exhibit a higher cardiovascular risk [2,3]. Atherosclerosis, the primary underlying cause of cardiovascular diseases, is a progressive inflammatory disease that involves the accumulation of lipids in infiltrated macrophages locally within the arterial wall ultimately leading to (partial) occlusion of the vessel lumen [4].

Glucocorticoids through binding to their cognate nuclear glucocorticoid receptor (GR) modulate the expression of genes involved in metabolic processes such as hepatic gluconeogenesis and muscle fatty acid utilization [5,6]. Glucocorticoids can also markedly diminish the relative expression levels of pro-inflammatory cytokines [7] and induce cell cycle arrest and apoptosis in several types of white blood cells [8,9]. Glucocorticoids are therefore predominantly known and used clinically for their potent immunosuppressive properties. As the body's inflammatory status is a critical determinant for the initiation of atherosclerotic lesion development [10–12], in the current study, we determined the consequences of changes in adrenal glucocorticoid metabolism in an established experimental diet-induced atherosclerosis mouse model.

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2. Materials and methods

2.1. Mice

Homozygous LDL receptor knockout mice [13] and GFP transgenic mice [14] were obtained from The Jackson Laboratory, crossed back to the C57BL/6 background (≥8 generations), and bred in house at the Gorlaeus Laboratories, Leiden, The Netherlands. Animal experiments were performed in a temperature and light cycle (12 h light/12 h dark) controlled room at the Gorlaeus Laboratories of the Leiden/Amsterdam Center for Drug Research in accordance with the National Laws. All experimental protocols were approved by the Ethics Committee for Animal Experiments of Leiden University.

2.2. Adrenalectomy and transplantation studies

Adrenalectomy and adrenal transplantations were carried out essentially as previously described by Karpac et al. [15]. Postnatal day 9 adrenal glands were removed from donor GFP pups and cleaned of connective tissue but were otherwise left intact. Adrenal grafts were placed in isotonic saline on ice until transplanted; transplantation was completed within 20 min of graft preparation. ~12 week old recipient female LDL receptor knockout mice were bilaterally adrenalectomized under isoflurane inhalation anesthesia through a dorsal midline skin incision and lateral retroperitoneal incisions. Subsequently, one GFP donor adrenal per recipient was placed under the kidney capsule through a slit in the renal capsule made by tweezers. After closure of skin wounds using Michel suture clip, mice were left separated from each other overnight for efficient wound healing and were subsequently housed with 4 similarly operated mice per cage for at least 2 weeks to recover from the operation. A part of each cage surface was heated by a heating mattress. During the complete study, all mice were given 0.9% NaCl and normal water ad libitum, and were regularly and identically handled. No apparent changes in food intake or physical activity were noted between the different experimental groups. At the end of the study, no signs of endogenous adrenal regeneration were macroscopically visible in any of the adrenalectomized mice.

2.3. Blood cell analysis

Total white blood cell counts and the distribution over different subclasses of white blood cells were routinely measured using an automated Sysmex XT-2000iV Veterinary Heamatology analyzer (Sysmex Corporation). Verification of effects on specified white blood cell subclasses was performed using flow cytometry (FACS analysis) by staining cells with appropriate antibodies (CD11b, Ly6G, CD4, CD8, CD19, all obtained form Ebioscience, Belgium). For this purpose, blood was lysed using 0.83% NH_4CL in 0.01 M Tris/HCL pH 7.2. Subsequently 300,000 cells were stained with the indicated antibodies. FACS analysis was performed on the FACSCalibur (Becton Dickinson, Mountain View, CA). Data were analyzed using Cell Quest software.

2.4. Corticosterone measurements

Blood samples for hormone analysis were drawn through tail chop between 9:00 and 10:00 AM (2- to 3 h in the light period). Levels of corticosterone were determined using a ^{125}I radio immuno assay (RIA) with a lower detection limit of 5 ng/ml, according to the manufacturer's specifications (MP Biomedials). During blood draws mice were restrained for a maximum of 30 s.

2.5. Plasma lipids

Plasma concentrations of total cholesterol and triglycerides were determined using enzymatic colorimetric assays (Roche Diagnostics). The distribution over the different lipoproteins in plasma was analyzed by fractionation of 30 μl of serum of each mouse using a Superose 6 column (3.2 mm × 300 mm, Smart-system, Pharmacia). Total cholesterol, content of the effluent was determined using enzymatic colorimetric assays (Roche Diagnostics).

2.6. Determination of plasma cytokine concentrations

Murine monocyte chemoattractant protein 1 (MCP-1) levels were assayed in plasma using a MCP-1 instant ELISA kit (eBioscience, Hatfield, UK) according to the manufacturer's instructions.

2.7. Aortic root atherosclerotic lesion analysis

To induce the development of initial atherosclerotic lesions at the aortic root, LDL receptor knockout mice were fed a semi-synthetic atherogenic diet containing 15% (w/w) coco butter, 1% (w/w) cholesterol, and 0.5% cholic acid (Diet N, Hope Farms, Woerden, NL) for 4 weeks. The arterial tree was perfused in situ with PBS (with the pressure of 100 mm Hg) for 10 min via a cannula in the left ventricular apex. The heart plus aortic root was excised and stored in 3.7% neutral-buffered formalin (Formalfixx®, Shandon Scientific Ltd., UK). The atherosclerotic lesion areas in Oil red O stained cryostat sections of the aortic root were quantified using the Leica image analysis system, consisting of a Leica DMRE microscope coupled to a video camera and Leica Qwin Imaging software (Leica Ltd., Cambridge, UK). Mean lesion area (μm^2) was calculated from 10 Oil red O-stained sections, starting at the appearance of the tricuspid valves. Macrophages in atherosclerotic lesions were detected using immunohistochemical staining with MOMA-2 antibody (rat antibody directed against murine monocytes/macrophages, Serotec, Oxford, UK). Lesion collagen content was determined using Masson's Trichrome staining. All quantifications were done blinded by computer aided morphometric analysis using the Leica image analysis system.

2.8. Adrenal transplant histology and immunohistochemical analysis for the presence of GFP and TUNEL-positive apoptotic cells

Formalin-fixed cryosections (10 μM) of adrenal transplants were prepared on a Leica CM3050-S cryostat. Cryosections were routinely stained with hematoxylin & eosin and Oil red O for neutral lipids. For immunohistochemical staining of GFP, cryostat sections were peroxidase blocked with 1% H_2O_2/methanol followed by antigen retrieval for 5 min by boiling in sodium citrate. Subsequently, slides were blocked with 5% BSA in PBS, and incubated with a primary GFP antibody (1:100 Living Colors A.V. Peptide Antibody Cat#632377) and a secondary HRP-conjugated antibody (DakoCytomation polyclonal Goat anti-Rabbit IgG HRP 1:100 1 h RT). A biotin streptavidin enhance step (AB complex (DAKO); 1:100 30 min RT) was performed, followed by counterstaining with 0.3% Methylene Green. Apoptotic cells were detected by terminal deoxynucleotidyl transferase-mediated dUTPbiotin nick-end labeling (TUNEL) with an in situ cell death detection kit (Roche). Nuclei were counterstained with 0.3% Methylene Green. Images were obtained with a Leica image analysis system, consisting of a Leica DMRE microscope coupled to a camera and Leica Qwin Imaging software (Cambridge, UK).

2.9. Analysis of gene expression by real-time quantitative PCR

Quantitative gene expression analysis on snap-frozen liver was performed as described [16]. In short, total RNA was isolated according to Chomczynski and Sacchi [17] and reverse transcribed using RevertAid™ reverse transcriptase. Gene expression analysis was performed using real-time SYBR Green technology (Eurogentec). Primers were validated for identical efficiencies and sequences can be provided upon request. Beta-actin, GAPDH and HPRT were used as the standard housekeeping genes. Relative gene expression numbers were calculated by subtracting the threshold cycle number (Ct) of the target gene from the average Ct of beta-actin, GAPDH, and HPRT (Ct housekeeping) and raising 2 to the power of this difference. Genes that exhibited a Ct value of >35 were considered not detectable. The average Ct of three housekeeping genes was used to exclude that changes in the relative expression were caused by variations in the expression of the separate housekeeping genes.

2.10. Data analysis

Statistical analysis was performed using Graphpad Instat software (San Diego, USA, http://www.graphpad.com/). Normality testing of the experimental groups was performed using the method Kolmogorov and Smirnov (Graphpad Instat). The significance of differences was calculated using a two-tailed Student's *t* test or one way analysis of variance where appropriate. Probability values less than 0.05 were considered significant.

3. Results

3.1. Adrenalectomy stimulates the formation of initial atherosclerotic lesions

Glucocorticoids exhibit potent anti-inflammatory and immunosuppressive properties and may thereby indirectly affect atherosclerotic lesion development susceptibility. To evaluate the effect of diminished glucocorticoid levels on atherogenesis, we subjected LDL receptor knockout mice – an established diet-induced atherosclerosis mouse model [13] – to bilateral adrenalectomy. In parallel a control group of age- and sex-matched LDL receptor knockout mice was sham operated. Two weeks after recovery of the operation on a regular chow low fat diet, both groups of mice were subsequently fed a commonly used atherogenic diet for 4 weeks to induce the development of early atherosclerotic lesions. Plasma corticosterone levels were significantly increased in SHAM-operated mice upon feeding the atherogenic diet (+78%; $P<0.05$; Fig. 1). In contrast, adrenalectomized mice showed, as anticipated, decreased plasma glucocorticoid levels (−70%; $P<0.05$), resulting in an 83% lower ($P<0.001$) plasma corticosterone level in adrenalectomized mice as compared to SHAM controls upon atherogenic diet feeding (Fig. 1).

The presence of low plasma glucocorticoid levels in adrenalectomized mice was associated with a significantly higher degree of aortic root atherosclerosis (+34%; $P<0.05$; Fig. 2). After 4 weeks of atherogenic diet feeding, SHAM-operated controls exhibited atherosclerotic lesions of $240 \pm 31 \times 10^3\ \mu m^2$, while aortic root lesion sizes were $321 \pm 18 \times 10^3\ \mu m^2$ in adrenalectomized mice (Fig. 2). In line with an the expected initial phase of lesion formation ("fatty streak lesions"), the atherosclerotic plaques in both groups of mice contained limited amounts of collagen ($3.1 \pm 0.2\%$ for ADX and $2.6 \pm 0.3\%$ for SHAM; $P>0.05$) and rather consisted of macrophage foam cells as judged from the fact that the majority of the cells within the lesions stained positive for MOMA-2 (Fig. 2).

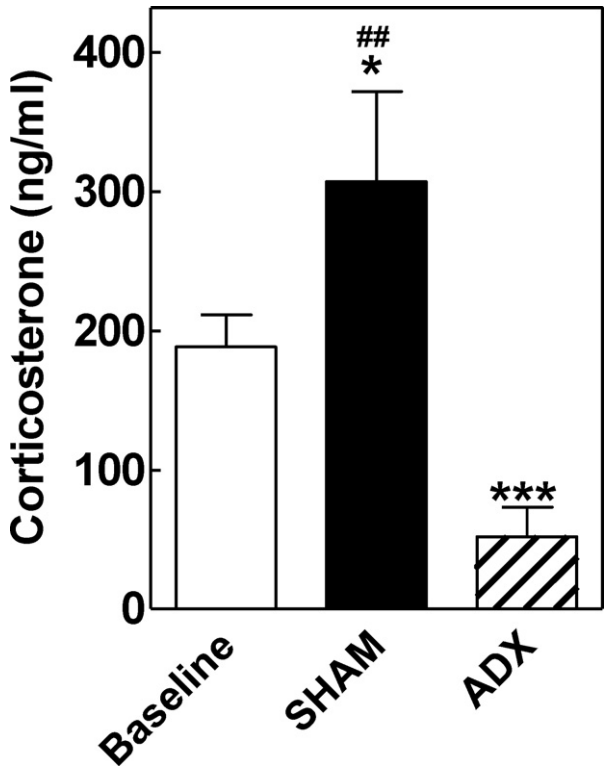

Fig. 1. Plasma corticosterone levels in overnight fasted adrenalectomized (ADX) and SHAM-operated (SHAM) LDL receptor knockout mice after 4 weeks of atherogenic diet feeding. Values represent means ± SEM of 8–10 more mice per group. $^{*}P<0.05$, $^{***}P<0.001$ vs levels in non-operated mice fed a regular chow diet (baseline). $^{\#\#}P<0.01$ vs ADX.

Previous studies in LDL receptor knockout mice have indicated that plasma total and very-low-density lipoprotein (VLDL) cholesterol levels best predict aortic root atherosclerosis [18]. As evident from Table 1, however, adrenalectomy was not associated with a rise in plasma lipid levels. Plasma total cholesterol and triglycerides levels were actually 22% ($P<0.05$) and 29% ($P<0.01$) lower in adrenalectomized mice as compared to SHAM-operated controls, which could be attributed to a 27% decrease ($P=0.018$) in the plasma level of the apoB-containing lipoproteins very-low-density lipoprotein (VLDL) and LDL (Fig. 3). This finding argues against lipid levels as a critical determinant for the extent of atherogenesis observed in the current experiment.

Importantly, in the current study adrenalectomy markedly stimulated total blood white blood cell counts (+104%; $P<0.01$; Fig. 4). The rise in total white blood cell numbers could be related to increases in the circulating number of CD19-positive B-lymphocytes (+114%), CD4- and CD8-expressing T-lymphocytes (+120–150%), as well as CD11b expressing Ly6G negative monocytes (+108%) as judged from the SYSMEX and FACS analyses (Fig. 4). Combined, these findings suggest that diminishing glucocorticoid levels by adrenalectomy is associated with a higher susceptibility for diet-induced atherosclerosis in LDL receptor knockout mice, probably as a result of an enhanced systemic inflammatory status.

Table 1
Body weight and plasma lipid levels of adrenalectomized (ADX) and SHAM-operated LDL receptor knockout mice fed an atherogenic diet for 4 weeks.

	SHAM ($n=10$)	ADX ($n=8$)	*P* value
Body weight (g)	23.9 ± 0.5	24.6 ± 0.3	N.S.
Plasma cholesterol (mg/dl)	4346 ± 292	3380 ± 316	<0.05
Plasma triglycerides (mg/dl)	3193 ± 209	2276 ± 82	<0.01

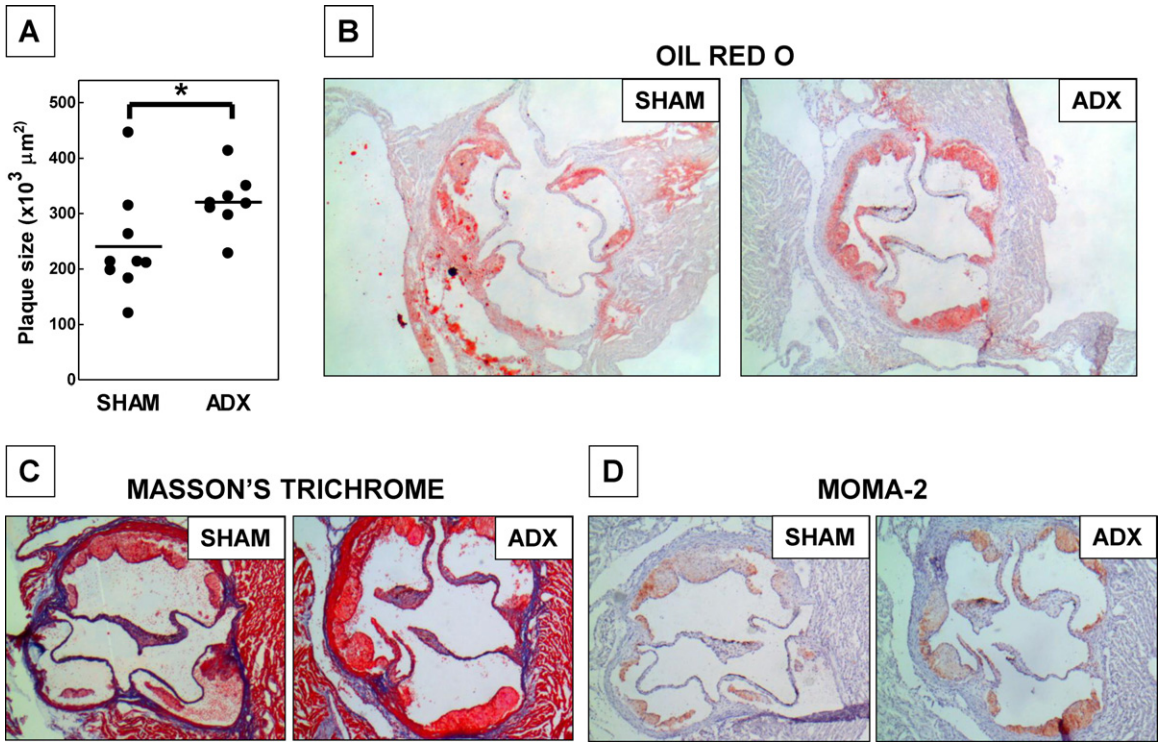

Fig. 2. Absolute atherosclerotic plaque sizes in overnight fasted adrenalectomized (ADX) and SHAM-operated (SHAM) LDL receptor knockout mice after 4 weeks of atherogenic diet feeding (A). Representative images of atherosclerotic lesions stained with Oil red O (B) and Masson's Trichrome (C) or MOMA-2 (D). *$P < 0.05$ vs SHAM.

3.2. Adrenal transplantation reverses the adrenalectomy-associated rise in inflammatory status and atherosclerosis susceptibility

Through adrenalectomy both adrenocortical steroid as well as adrenal medulla (i.e. catecholamine) function is removed. To further prove that the effect of adrenalectomy on atherosclerosis susceptibility was indeed specifically due to an impaired adrenal steroid function, we utilized the state-of-the-art technique of adrenal transplantation in our LDL receptor knockout high fat/high cholesterol diet-induced atherosclerosis mouse model.

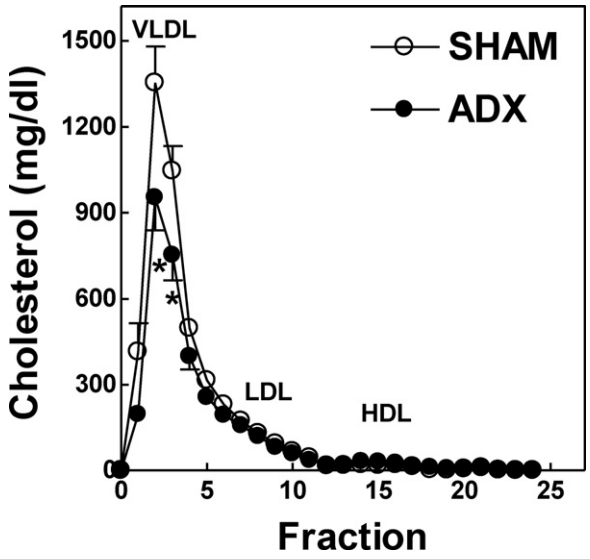

Fig. 3. Plasma lipoprotein profile in adrenalectomized (ADX) and SHAM-operated (SHAM) LDL receptor knockout mice that were fed an atherogenic diet for 4 weeks. Values represent means ± SEM of 8–10 mice per group. *$P < 0.05$ vs SHAM.

By adrenal transplantation only the adrenal steroid function is restored, since cells from the medulla rapidly die after the adrenal removal (adrenalectomy) procedure as they do not exhibit the ability to revascularize upon subsequent adrenal transplantation [19]. For efficient visualization of adrenal transplants within the recipient kidney tissue we chose to transplant adrenals from mice expressing green fluorescent protein (GFP) under the control of the ubiquitin c promoter [14] that exhibit constitutively high protein expression levels of GFP in all zones of the adrenal.

The adrenals transplanted from 9-day old GFP pups under the renal capsule of adrenalectomized LDL receptor knockout mice developed as expected, eventually consisting of a layered cortex surrounding scar tissue (i.e. non-regenerated medulla), as depicted in Fig. 5. We did not detect apoptotic TUNEL positive cells in the cortex of adrenal transplants (Fig. 5), suggesting that the adrenocortical cells within the transplants were viable and probably active. As judged from an Oil red O staining (Fig. 5), the steroid hormone-producing adrenocortical cells were filled with neutral lipids (i.e. cholesterol esters) that are needed for an optimal adrenal steroidogenesis [20,21].

In line with a restored adrenal steroidogenic function, plasma corticosterone concentrations in atherogenic diet-fed transplanted LDL receptor knockout mice were 7-fold higher ($P < 0.001$) than those in adrenalectomized mice ultimately reaching a plasma level comparable to ~50% of that observed in pair-fed SHAM-operated mice (Fig. 6). The inability to fully restore plasma corticosterone levels, which is in line with the data described in the original study of Karpac et al. [15], can be attributed to the "age" of the adrenals since the adrenals of SHAM-operated mice are ≥12 weeks older (and more evolved) than adrenal transplants.

Glucocorticoids execute their downstream actions through transcriptional modulation of gene expression by the nuclear glucocorticoid receptor. In accordance with a (partial) restoration of downstream glucocorticoid signaling in response to adrenal transplantation, we detected marked changes in the mRNA

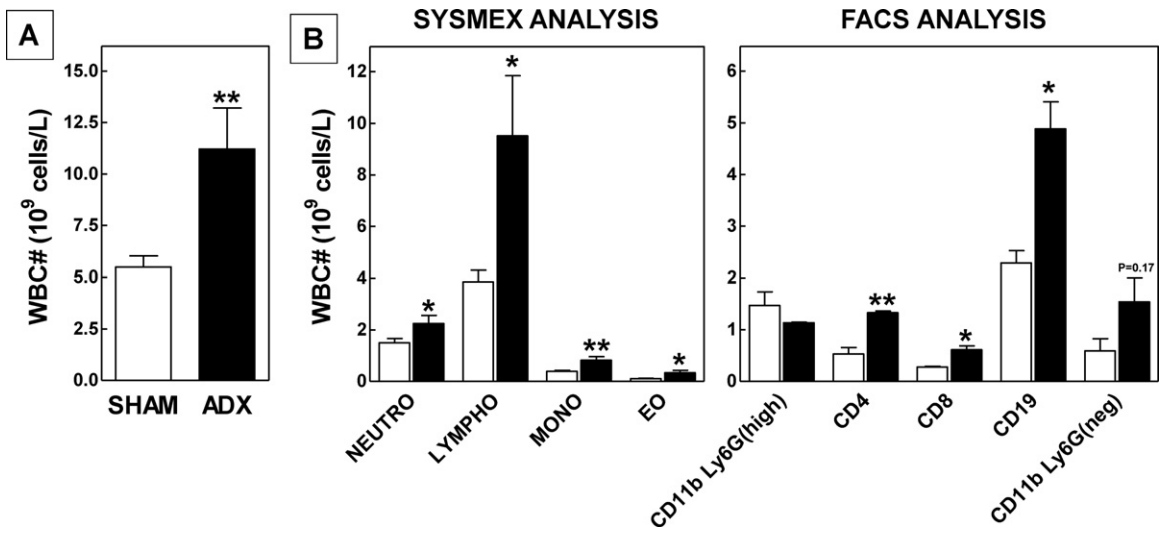

Fig. 4. The absolute white blood cell population counts in blood of overnight fasted adrenalectomized (ADX; black bars) and SHAM-operated (SHAM; white bars) LDL receptor knockout mice after 4 weeks of atherogenic diet feeding. CD11b Ly6G(high)-double positive cells represent neutrophils; CD4- and CD8-positive cells represent T-lymphocyte subclasses; CD19-positive cells represent B-lymphocytes; CD11b Ly6G(neg) cells represent monocytes. Values represent means ± SEM of 8–10 (SYSMEX) or 3–4 (FACS) mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs SHAM.

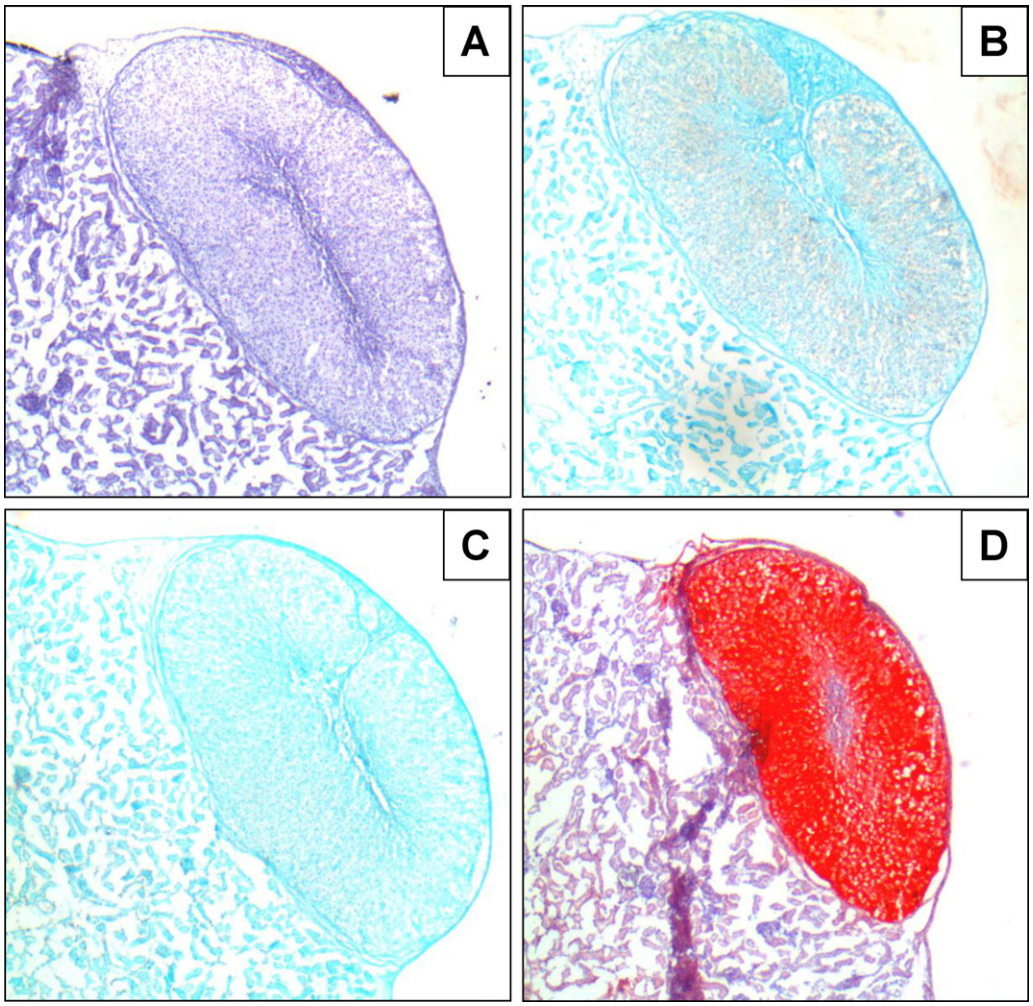

Fig. 5. Representatitive images of the hematoxylin/eosin (A), GFP (B), TUNEL apoptotic cell (C), and Oil red O neutral lipid (D) staining on sections of a GFP-positive adrenal transplant located under the renal capsule of a kidney.

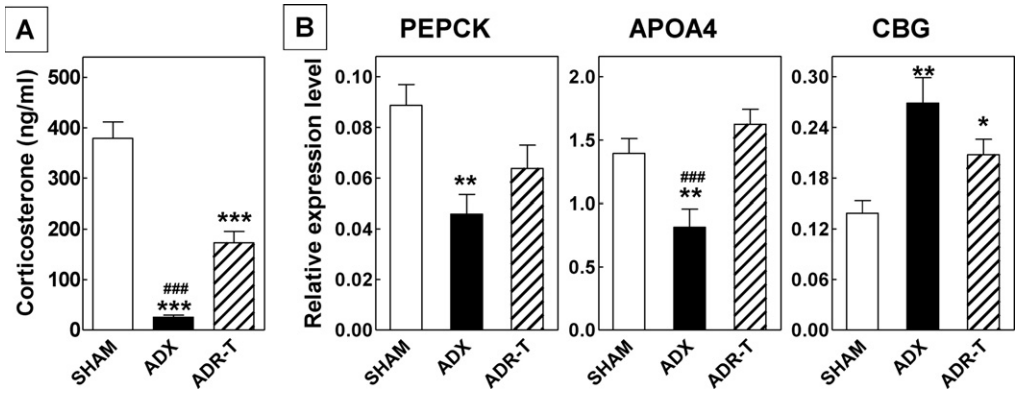

Fig. 6. Plasma corticosterone levels (A) and hepatic relative mRNA expression levels (B) in ad libitum fed SHAM-operated (SHAM; white bars), adrenalectomized (ADX; black bars), and adrenal transplanted (ADR-T; hatched bars) LDL receptor knockout mice after 4 weeks of atherogenic diet feeding. Values represent means ± SEM of 7–8 mice per group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs SHAM. $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs ADR-T.

expression of glucocorticoid responsive genes in livers of adrenal transplanted mice as compared to those subjected to adrenalectomy alone (Fig. 6). Adrenalectomy was associated with a respective 48% and 42% decrease ($P<0.01$ for both) in the relative expression level of glucocorticoid-stimulated genes phosphoenolpyruvate carboxykinase (PEPCK) and apolipoprotein A4 (APOA4). Adrenal transplantation restored the expression level of PEPCK to 72% of SHAM controls (42% reversal of the ADX effect), while APOA4 expression was fully normalized upon adrenal transplantation to 116% of SHAM control values ($P<0.001$ ADR-T vs ADX). As previously observed by Feldman et al. [22], adrenalectomy increased the hepatic expression level of the glucocorticoid carrier protein CBG (+94%; $P<0.01$). Hepatic CBG expression levels decreased again upon adrenal transplantation, but remained significantly higher in livers of adrenal transplanted mice than in SHAM controls (+48% in ADR-T vs SHAM; $P<0.05$), indicative of a partial restoration of the glucocorticoid-induced negative feedback on the hepatic CBG expression level (Fig. 6).

Restoring the adrenal steroid function through adrenal transplantation decreased white blood cell counts in adrenalectomized mice to levels observed in SHAM-operated mice ($P<0.05$ for ADR-T vs SHAM; Fig. 7). Similarly, plasma levels of the pro-atherogenic pro-inflammatory monocyte chemoattractant protein-1 (MCP-1) were initially higher in adrenalectomized mice (116 ± 11 pg/ml in ADX vs 67 ± 15 pg/ml in SHAM; $P<0.05$) and returned to basal upon adrenal transplantation (61 ± 12 pg/ml; $P<0.05$ vs ADX; Fig. 7). Combined, these findings suggest that adrenal transplantation is able to fully reverse the systemic pro-inflammatory state

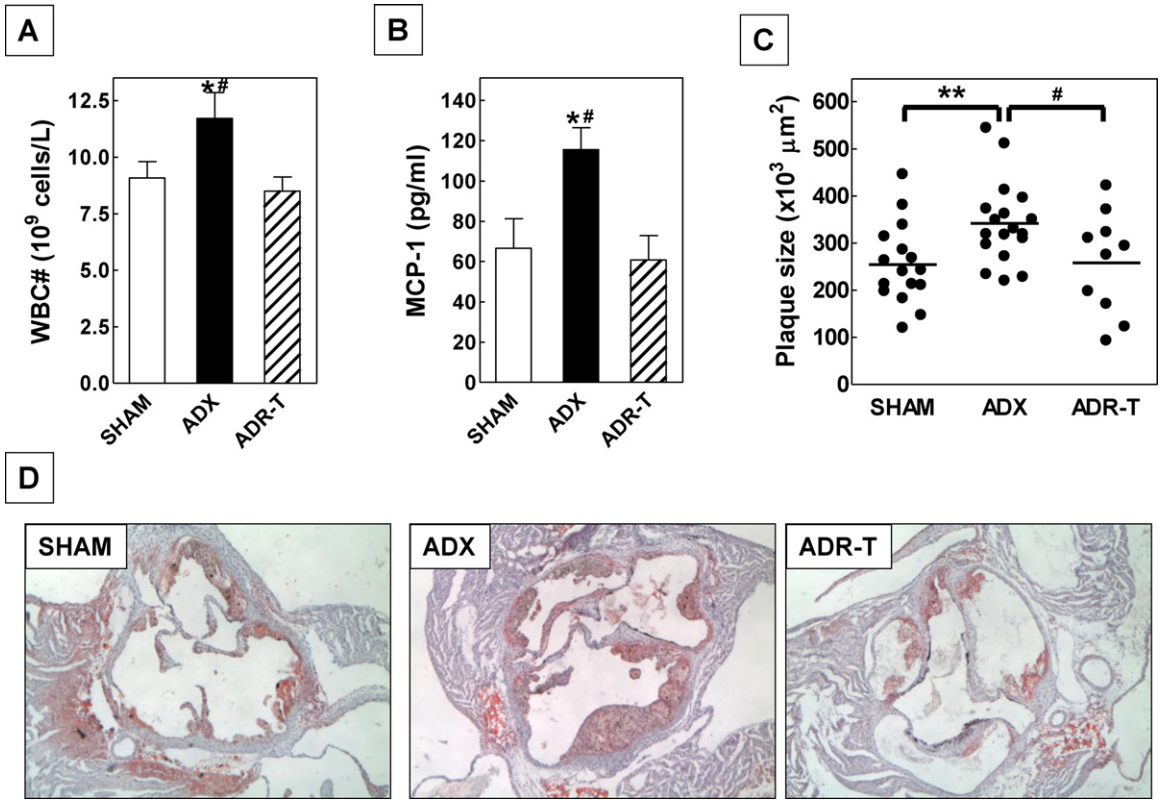

Fig. 7. White blood cell counts (A), plasma MCP-1 levels (B), and atherosclerotic plaque size (C) in ad libitum fed SHAM-operated (SHAM; white bars), adrenalectomized (ADX; black bars), and adrenal transplanted (ADR-T; hatched bars) LDL receptor knockout mice after 4 weeks of atherogenic diet feeding. White blood cell and MCP-1 values represent means ± SEM of 7–8 mice per group. (D) Representative images of atherosclerotic lesions stained with Oil red O. $^{*}P<0.05$, $^{**}P<0.01$ vs SHAM. $^{\#}P<0.05$ vs ADR-T.

associated with adrenalectomy. Importantly, atherosclerotic lesion size in adrenal transplanted mice was also virtually identical to that observed in SHAM-operated mice ($258 \pm 34 \times 10^3$ μm^2; $P < 0.05$ vs ADX; Fig. 7), which further supports the notion that the enhanced inflammatory status is the causal factor for the stimulated atherosclerotic lesion development associated with adrenalectomy.

4. Discussion

Low plasma levels of adrenal-derived glucocorticoids (i.e. cortisol), as observed in Addison's disease patients, are associated with enhanced cardiovascular disease mortality [1], suggesting that an optimal adrenal steroidogenesis rate is a perquisite to overcome cardiovascular disease-associated death in man. Here we show that low glucocorticoid levels as a result of adrenalectomy are also associated with a higher atherogenic susceptibility in LDL receptor knockout mice, an established diet-induced atherosclerosis mouse model, while specifically restoring the adrenal steroid function through adrenal transplantation is able to normalize atherosclerosis susceptibility. The observed increase in lesion formation in adrenalectomized mice coincided with a marked increase in the numbers of essentially all types of white blood cells, which can be attributed to a diminished inhibitory (immunosuppressive) action of glucocorticoids on the proliferation of these cells. In contrast, probably as a result of a reduced glucocorticoid-mediated stimulation of hepatic VLDL production [23–25], plasma cholesterol levels of pro-atherogenic apolipoprotein B-containing lipoproteins (VLDL/LDL) were lower in mice subjected to adrenalectomy. Of note, plasma total cholesterol levels in adrenalectomized mice (3380 ± 316 mg/dl) were still much higher than the ~300 mg/dl concentration that has been suggested to be obligatory to induce atherosclerotic lesion development in mice [26]. In accordance with leukocyte infiltration into the vessel wall and cholesterol-driven macrophage foam cell formation as hallmarks in the pathogenesis of atherosclerosis, a clear association exists between both white blood cell counts [27–29] as well as total cholesterol levels [30] and the risk for atherosclerotic coronary heart disease in man. It is generally assumed that plasma VLDL-cholesterol levels best predict atherosclerosis susceptibility in LDL receptor knockout mice [18]. However, based upon the atherogenic diet-induced increase in plasma corticosterone levels observed in SHAM-operated mice, we anticipate that the adrenals secrete relatively high levels of glucocorticoids as an obligatory protective anti-inflammatory response to overcome systemic inflammation. In accordance with an important role of glucocorticoids in the inhibition of systemic inflammation, adrenal glucocorticoid insufficiency and adrenalectomy have previously been associated with an enhanced susceptibility for endotoxemia and the associated mortality in mice [31–33]. Absence of this immunosuppressive response in adrenalectomized mice will thus result in an enhanced (pathological) systemic inflammatory status, which eventually leads to a higher susceptibility for atherosclerosis albeit a relatively lower pro-atherogenic cholesterol trigger.

In addition to glucocorticoids synthesized in the zona fasciculata, the adrenals produce other steroids such as mineralocorticoids (i.e. aldosterone) in the zona glomerulosa and androgens (i.e. testosterone) in the zona reticularis that may theoretically also impact on atherosclerosis susceptibility. Plasma aldosterone levels are undetectable in adrenalectomized mice [34] and are rapidly restored upon whole adrenal transplantation [15]. Inhibition of the renal–angiotensin–aldosterone axis however does not affect the development of early atherosclerotic lesions in LDL receptor knockout mice [35], which argues against a crucial role for aldosterone in the observed changes in atherosclerosis susceptibility detected in the current study. Previous studies by Shimizu et al. [36] have shown that adrenalectomy does not affect plasma levels of testosterone – a steroid predominantly produced by the gonads – which also eliminates a role for testosterone in the effects observed in the current study.

In conclusion, we are the first to show that adrenalectomy stimulates the atherogenic diet-induced formation of initial atherosclerotic lesions in LDL receptor knockout mice. Furthermore, our studies demonstrate that restoring adrenocortical steroid secretion through adrenal transplantation can fully reverse the enhanced atherosclerosis susceptibility in adrenalectomized mice, which indicates that adrenal-derived steroids protect against atherosclerotic lesion development in LDL receptor knockout mice.

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