Splenic autonomic denervation increases inflammatory status but does not aggravate atherosclerotic lesion development

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Kooijman S, Meurs I, van Beek I, Khedoe PP, Giezekamp A, Pike-Overzet K, Cailotto C, van der Vliet J, van Harmelen V, Boeckxstaens G, Berbée JF, Rensen PC. Splenic autonomic denervation increases inflammatory status but does not aggravate atherosclerotic lesion development. Am J Physiol Heart Circ Physiol 309: H646–H654, 2015. First published June 19, 2015; doi:10.1152/ajpheart.00787.2014.—The brain plays a prominent role in the regulation of inflammation. Immune cells are under control of the so-called cholinergic anti-inflammatory reflex, mainly acting via autonomic innervation of the spleen. Activation of this reflex inhibits the secretion of proinflammatory cytokines and may reduce the development of atherosclerosis. Therefore, the aim of this study was to evaluate the effects of selective parasympathetic (Px) and sympathetic (Sx) denervation of the spleen on inflammatory status and atherosclerotic lesion development. Female APOE*3-Leiden.CETP mice, a well-established model for human-like lipid metabolism and atherosclerosis, were fed a cholesterol-containing Western-type diet for 4 wk after which they were subdivided into three groups receiving either splenic Px, splenic Sx, or sham surgery. The mice were subsequently challenged with the same diet for an additional 15 wk. Selective Px increased leukocyte counts (i.e., dendritic cells, B cells, and T cells) in the spleen and increased gene expression of proinflammatory cytokines in the liver and peritoneal leukocytes compared with Sx and sham surgery. Both Px and Sx increased circulating proinflammatory cytokines IL-1β and IL-6. However, the increased proinflammatory status in denervated mice did not affect atherosclerotic lesion size or lesion composition. Conclusion: Predominantly selective Px of the spleen enhances the inflammatory status, which, however, does not aggravate diet-induced atherosclerotic lesion development.

atherosclerosis; inflammation; splenic denervation; transgenic mice

NEW & NOTEWORTHY

Spleenic immune cells are involved atherosclerosis development, and their inflammatory status is controlled by the anti-inflammatory reflex. Here we show that both selective sympathetic denervation and parasympathetic denervation of the spleen result in enhanced proinflammatory cytokine production but do not aggravate atherosclerosis development.

ATHEROSCLEROSIS IS A CHRONIC inflammatory disease initiated by innate and adaptive immune responses to endogenously modified structures, in particular oxidized lipoproteins, within the arterial wall (8). The autonomic nervous system may enhance innate immune responses by sympathetic activity (reviewed in Ref. 12), while it suppresses inflammation via the vagus nerve, a mechanism termed the cholinergic anti-inflammatory pathway (3, 7). In response to circulating proinflammatory cytokines, afferent vagal nerves are directly activated. Subsequent efferent vagal activity results in the release of acetylcholine, which activates the α7-nicotinic acetylcholine receptor (α7-nAChR) on resident tissue macrophages and other immune cells, thereby inhibiting the production and release of proinflammatory cytokines (e.g., TNF-α, IL-6, and IL-1β) (24). α7-nAChR is integral to the cholinergic anti-inflammatory pathway, as vagus nerve stimulation fails to inhibit TNF-α production in pharmacologically α7-nAChR-inhibited or α7-nAChR-deficient mice (18, 24). Recently, we demonstrated that hematopoietic α7-nAChR deficiency in dyslipidemic mice enhances systemic inflammation as evidenced by increased leukocytes in the blood, lymph nodes, spleen, and peritoneum (all by at least 2-fold) and increased gene expression of TNF-α in peritoneal leukocytes and spleen (15).

As the spleen contains half of the body’s monocyte population, it is not surprising that the cholinergic anti-inflammatory pathway acts mainly via the spleen. Indeed, Huston et al. (11) reported that vagus nerve stimulation fails to inhibit TNF-α production in splenectomized animals during endotoxemia, indicating an essential role for the spleen in the cholinergic anti-inflammatory pathway. Furthermore, splenectomy reduces the production of antibodies directed against oxidized LDL in apoE-deficient mice and was associated with increased atherosclerotic lesion development (16). Trauma patients who undergo splenic removal are more prone to develop coronary heart disease, in which enhanced atherosclerotic lesion development may be causal (17).

Taken together, these findings suggest that autonomic innervation of the spleen and the development of atherosclerosis may be closely interrelated. Therefore, the aim of this study was to determine the effect of selective parasympathetic denervation (Px), compared with sympathetic denervation (Sx) of...
the spleen and sham surgery, on systemic inflammation and atherosclerotic lesion development in female APOE*3-Leiden.CETP mice, a well-established mouse model for human-like lipoprotein metabolism.

MATERIALS AND METHODS

Animals. APOE*3-Leiden mice were crossbred with mice expressing human cholesteryl ester transfer protein (CETP) under control of its natural flanking regions to generate heterozygous APOE*3-Leiden.CETP mice (25). Mice were housed under standard conditions with a 12:12-h light-dark cycle and had free access to food and water. At the age of 10–12 wk, female APOE*3-Leiden.CETP mice received a Western-type diet (WTD) containing 0.1% cholesterol (wt/wt), 1% (wt/wt) corn oil, and 15% (wt/wt) cacao butter (AB Diets; Woerden, The Netherlands). After a run-in period of 4 wk, mice (n = 45) were randomized based on plasma lipid levels and body weight into three groups (n = 15 each) receiving either splenic Px, splenic Sx, or sham surgery. A schematic representation of the innervation of the spleen and the sites of denervation can be found in Fig. 1A. For all surgeries, mice were anesthetized by an intraperitoneal injection of a mixture of fentanyl citrate/fluanisone (Hypnorm; Janssen, Beerse, Belgium), midazolam (Dormicum; Roche, Mijdrecht, The Netherlands), and H2O (1:1:2, vol/vol). All animal experiments were approved by the Institutional Ethics Committee on Animal Care and Experimentation.

Selective Px of the spleen. Since parasympathetic nerves enter the spleen at both tips, these tips were sequentially exposed during surgery to allow cutting of the nerves. After a midline abdominal incision, the spleen was pulled gently towards the site of the incision, and the nerve at the tip of the spleen was cut. The connective tissue between the tip and the first hilus was also removed, as some parasympathetic input reaches the spleen via this connective tissue. Subsequently, the spleen was further pulled towards the midline to reach the lower tip of the spleen. After the nerve was followed back to the plexus, the connective tissue from this plexus back to the spleen was removed. The wound was closed with novosyn suture (B. Braun Medical, Oss, The Netherlands) (5).

Selective Sx of the spleen. A midline abdominal incision was performed along the linea alba, and the stomach was pushed up and to the right to reveal the blood vessels to and from the spleen. After the arterial branch to the stomach, a bifurcation indicates the first branching point of the arterial supply to the spleen. From this bifurcation on the arteries will split many times and end at the hilus of the spleen. Sympathetic nerves run along and around these arteries to reach the spleen. The area just before and after the bifurcation was chosen to remove the sympathetic nerves. The wound was closed with novosyn.

Fig. 1. Confirmation of splenic denervations and gating strategy. A: schematic representation of the innervation of the spleen. The sympathetic input (pink) reaches the spleen via the arteries, and the parasympathetic input (green) reaches the spleen via both tips of the spleen. The location at which denervations were performed are shown by the dashed lines. B: gating strategy for flow cytometry analysis. C: confirmation of sympathetic denervation (Sx) by measurement of tyrosine hydroxylase (TH) protein content of the spleen. As controls, TH content for the brain and (denervated) brown fat are included. Px, parasympathetic denervation.
suture (B. Braun Medical) (5). Sx was confirmed 15 wk after surgery by Western blotting for tyrosine hydroxylase (TH; anti-TH antibody; AB-112; Abcam).

Gene expression analysis in spleen, liver, and peritoneal leukocytes. After surgery, the mice were fed the WTD for another 15 wk. Subsequently, mice were killed, organs were collected, and peritoneal leukocytes were isolated by lavage of the peritoneum with ice-cold PBS. Total RNA from spleen, liver, and peritoneal leukocytes was isolated using the Nucleospin RNA II kit (Macherey-Nagel, Düren, Germany) according to manufacturer’s instructions. One microgram of total RNA was converted to cDNA with iScript cDNA Synthesis kit (Bio-Rad) and purified with Nucleospin Extract II kit (Macherey-Nagel). Real-time polymerase chain reaction (RT-PCR) was conducted on the IQ5 PCR machine (Bio-Rad) using the Sensi-mix SYBR Green RT-PCR mix (Quanta, London, UK). mRNA levels were normalized to mRNA levels of 18S (H9251), 18S cyclophilin, and acidic ribosomal phosphoprotein P0 (36B4).

Flow cytometry analysis. From five randomly selected animals per group, peripheral blood and spleens were processed for flow cytometry. Then, single cell suspensions were obtained by mashing the spleens. To define the effect of the selective denervations on immune cell composition, flow cytometry analyses were performed using FlowJo software (Treestar, Ashland, OR). Gating strategy is shown in Fig. 1B.

Serum measurements. Serum was isolated and stored frozen at −80°C until further analyses. The cytokines TNF-α, IL-1β, and IL-6 were determined using V-PLEX Proinflammatory Panel1 (mouse) kit (Meso Scale Discovery, Rockville, ML) according to the manufacturer’s instructions. In 50× diluted serum samples, E-selectin concentrations were measured according to the manufacturer’s instructions (DY575; R&D Systems, Minneapolis, MN).

Plasma lipid and lipoprotein analyses. Blood was collected after a 4-h fast into EDTA-containing cups by tail bleeding, and plasma was isolated by centrifugation and stored frozen at −80°C until further analyses. The concentrations of total cholesterol (TC) and triglycerides (TG) in plasma were determined using commercially available enzymatic colorimetric kits according to the manufacturer’s protocols (236691 and 1488872; Roche Molecular Biochemicals, Indianapolis, IN). The concentrations of phospholipids (PL) in plasma were determined using a commercially available enzymatic colorimetric kit (3009; Instrumentel, Delfzijl, The Netherlands). The distribution of lipids over the different lipoproteins in plasma was determined after fractionation of pooled plasma (14–15 mice per pool) by FPLC using a Superose 6 HR 10/30 column (Äkta System; Amersham Pharmacia Biotech, Piscataway, NJ).

Atherosclerosis quantification. From all mice, hearts were isolated and fixed in phosphate-buffered 4% formaldehyde, dehydrated, and embedded in paraffin. Cross sections (5 μm) were made throughout the aortic root area and stained with hematoxylin-phloxine-saffron for histological analysis. Lesions were categorized for severity according to the guidelines of the American Heart Association adapted for mice (27). Various types of lesions were discerned: no lesions, mild lesions (types 1–3), and severe lesions (types 4–5). Immunohistochemistry for determination of lesion composition was performed as described previously (10). Rat anti-mouse antibody MAC3 (1:1,000; BD Pharmingen, Breda, The Netherlands) was used to quantify macrophage area. Monoclonal mouse antibody M0851 (1:800; Dako, Heverlee, The Netherlands) against smooth muscle cell (SMC) α-actin was used to quantify SMC area. Sirius red was used to quantify collagen area. Lesion area was quantified in the aortic root starting from the appearance of open aortic valve leaflets in four subsequent sections with 50-μm intervals. In ImageJ the lesions were delineated to determine mean lesion area (in μm²) and a color threshold was set to determine the area percentage of MAC3, SMC, or collagen staining in a consistent manner across the different slides.

Statistical analysis. Data are presented as means ± SE unless indicated otherwise. To compare differences among groups one-way ANOVA with Turkey’s posttest was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA; www.graphpad.com). Differences at a P value <0.05 were considered statistically significant.

RESULTS

Female APOE*3-Leiden.CETP mice were fed a WTD during 4 wk and were randomized into three groups receiving either Px of the spleen, Sx of the spleen (Fig. 1A), or sham surgery. After surgery, the mice received WTD feeding for 15 additional wk to induce atherosclerotic lesion development. To confirm that Sx was successful and that sympathetic nerves did not regenerate, TH content of the spleen was determined (Fig. 1C), showing the absence of TH still 15 wk after Sx. Upon death of the mice, the tips of the spleen were gently exposed to confirm that the reinnervation of the parasympathetic nerves was not the case.

Splenic Px increases immune cell count in spleen. The spleen plays an important role in the immune system and, therefore, contains a wide range of immune cell types, including monocytes, macrophages, DCs, neutrophils, T cells, and B cells. To define the effect of the selective denervations on immune cell composition, flow cytometry analyses were performed. Total splenic immune cell count was increased (+49%; P < 0.01) in Px mice (200 ± 10 × 10⁶ cells) compared with sham-operated mice (134 ± 10 × 10⁶ cells), while Sx denervation did not affect immune cell count (156 ± 25 ± 10⁶ cells) (Fig. 2A). In a fraction of immune cells (i.e., 20 × 10⁶ cells), percentages of each cell type were analyzed using flow cytometry and multiplied with total immune cell counts (see Fig. 1B for the gating strategy). This revealed an increase in the number of various immune cell subtypes, including B cells (98 ± 7 × 10⁶ cells vs. 61 ± 5 × 10⁶ cells, P < 0.01; Fig. 2B), T cells (63 ± 4 × 10⁶ cells vs. 41 ± 4 × 10⁶ cells, P < 0.01; Fig. 2C) and DCs (10 ± 1 × 10⁶ cells vs. 7 ± 1 × 10⁶ cells, P < 0.05; Fig. 2D). Neutrophils (Fig. 2E)

Table 1. Detailed information of antibodies used for flow cytometry analysis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Conjugate</th>
<th>Clone</th>
<th>Source</th>
<th>Dilution</th>
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<tr>
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<tr>
<td>CD14</td>
<td>FITC</td>
<td>SA14-2</td>
<td>eBioscience</td>
<td>500</td>
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<tr>
<td>CD11c</td>
<td>Biotin</td>
<td>HL3</td>
<td>BD</td>
<td>200</td>
</tr>
<tr>
<td>APC</td>
<td>145-2C11</td>
<td>BD</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>CD3</td>
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<td>145-2C11</td>
<td>BD</td>
<td>400</td>
</tr>
<tr>
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<td>PerCP</td>
<td>M1/70</td>
<td>BD</td>
<td>800</td>
</tr>
<tr>
<td>Ly6G</td>
<td>E4150</td>
<td>RB6-8C5</td>
<td>eBioscience</td>
<td>800</td>
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<tr>
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<td>L3T4 G1.15</td>
<td>BD</td>
<td>1,000</td>
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<tr>
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<td>Biotin</td>
<td>53-6.7</td>
<td>BD</td>
<td>400</td>
</tr>
<tr>
<td>CD62L</td>
<td>APC</td>
<td>MEL-14</td>
<td>Biologend</td>
<td>1,600</td>
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<tr>
<td>CD25</td>
<td>PerCP</td>
<td>PC61</td>
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<td>500</td>
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<td>Second step: Sav-PE-Cy7</td>
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and monocytes/macrophages (Fig. 2F) only showed a nonsignificant increase upon Px.

As T cells are probably involved in the propagation of nerve signals towards monocytes and macrophages (19), the phenotype of the T cells (i.e., naivity or activation status of the T-helper or cytotoxic T cells) was further studied. In accordance to the general increase in various immune cells, Px increased both T-helper (TH; *P < 0.01; Fig. 2G) and cytotoxic T cells (Tcyt; +49%, *P < 0.01; Fig. 2H). Further subdivision revealed that Px increased naïve (Fig. 2I) as well as activated (Fig. 2J) TH cells and increased naïve Tcyt cells (Fig. 2K) without increasing activated Tcyt cells (Fig. 2L). Thus splenic Px resulted in an overall increase in immune cells in the spleen, while Sx did not affect immune cell count compared with sham surgery, indicating the importance of the parasympathetic nerve in regulation of the immune system.

Splenic autonomic denervation increases expression of inflammatory cytokines. During the course of the experiment, body weight gain was slightly lower in both Px and Sx mice. At the end of the experiment, body weight of sham-operated mice was 31.6 ± 1.1 g, compared with 27.3 ± 1.1 g (*P < 0.05) and 27.4 ± 1.2 g (*P < 0.05) for Px and Sx mice, respectively (Fig. 3A). Splenic weight tended to be increased in Px (Fig. 3B) and was different when expressed as percentage of the body weight gain in Px compared with Sx (Fig. 3C).

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weight for Px (0.52 ± 0.02%; P < 0.001) and Sx (0.47 ± 0.02%; P < 0.05) compared with sham (0.41 ± 0.01%; Fig. 3C). Gene expression analyses revealed that Px only caused a trend towards an increase of the inflammatory cytokines IL-1β and IL-6 within the spleen (Fig. 3D). Further analysis of other organs showed no difference in liver weight when expressed as percentage of the body weight (Fig. 3E). Interestingly, Px increased gene expression of IL-6 in the liver (+80%; P < 0.01; Fig. 3F) and increased gene expression of TNF-α, IL-1β, and IL-6 in isolated peritoneal leukocytes, which reached significance for IL-1β (3.3-fold; P < 0.05; Fig. 3G). Next, we determined the effect of Px on white blood cell count in peripheral blood and further analyzed subsets by flow cytometry. Px tended to increase total immune cell count albeit significance was not reached (+41%; P = 0.18; Fig. 4A). Subdivision of leukocytes into B cells (Fig. 4B), T cells (Fig. 4C), DCs (Fig. 4D), neutrophils (Fig. 4E), or monocytes (Fig. 4F) did not reveal differences. However, as the number of immune cells per se does not reflect activity of these cells, we measured serum levels of TNF-α (Fig. 4G), IL-1β (Fig. 4H), and IL-6 (Fig. 4I) in serum. While TNF-α levels remained unaffected, both IL-1β and IL-6 serum concentrations were increased by Px. Interestingly, in contrast to our gene expression in liver, spleen, and peritoneal leukocytes, also Sx increased inflammatory status as IL-1β and IL-6 levels compared with sham-operated mice.

Splenic autonomic denervation does not affect atherosclerotic lesion development. Since inflammation can influence lipid metabolism (22), we next evaluated whether selective splenic denervations had an effect on lipid metabolism. Plasma TC, PL, and TG were assessed at 2, 4, 6, and 15 wk after surgery. No differences in plasma lipid concentrations were...
found between the Px, Sx and sham-operated mice at wk 2, 4, 6 (not shown), and 15 wk (Fig. 4J). Likewise, the distribution of cholesterol over the various lipoproteins did not differ between Px, Sx, or sham control mice (Fig. 4K). Serum E-selectin as marker for vascular inflammation, was increased in Px as well as in Sx compared with sham, suggesting that immune cell infiltration into atherosclerotic lesions might be enhanced by the selective denervations (Fig. 4L).

To study the effect of splenic denervation on atherosclerosis development, mice were killed after 15 wk after surgery, and atherosclerotic lesion size and lesion severity were determined in the valve area of the aortic root. Both Px and Sx did neither affect atherosclerotic lesion size (Fig. 5, A and B) nor lesion severity, when classified as mild (type 1–3) and severe (type 4–5) lesions (Fig. 5C). However, also no significant differences were observed in lesion composition between Px, Sx, and sham-operated mice, with respect to the relative area of smooth muscle cells (SMC; α-actin staining; Fig. 5D), collagen (Sirius red staining; Fig. 5E), and macrophages (MAC3 staining; Fig. 5F).

**DISCUSSION**

In the current study, we tested the hypothesis that the brain plays a prominent role in modulating the activity of immune cells and may therefore affect atherosclerosis development. We determined the effect of selective splenic Px and Sx on the inflammatory status and examined the potential consequences for plasma lipids and the development of atherosclerosis in APOE*3-Leiden.CETP mice. We showed that predominantly
splenic Px increased the inflammatory state of the body as indicated by increased leukocyte counts within the spleen and increased proinflammatory cytokine expression. However, splenic Px as well as Sx did not affect atherosclerotic lesion development.

Interestingly, we found increased circulating levels of the proinflammatory cytokines IL-1β and IL-6 on both Px and Sx. Classically, the parasympathetic and sympathetic nerve systems act in opposite direction to facilitate control over physiological responses to maintain homeostasis. However, for the spleen, it has been suggested that both systems in fact act together to restrain inflammation by projection of the vagus nerve also onto the sympathetic splenic nerve (18, 23). Previous studies even suggested absence of direct parasympathetic innervation of the spleen, as neither choline acetyltransferase nor vesicular acetylcholine transporter producing nerve endings could be detected within the spleen (2, 18). However, the absence of the classical vagus transmitter acetylcholine is not sufficient proof for the absence of direct input from the vagus. In previous studies we identified neuronal connections between the spleen and both the intermedio lateral column of the spinal cord (IML) and the dorsal motor nucleus of the vagus nerve (DMV) by retrograde tracing using pseudorabies virus and cholera toxin-b (5, 6), suggesting sympathetic as well as parasympathetic neuronal connectivity. Surgical ablation of the nerves along the splenic arteries (similar to Sx) resulted in undetectable retrograde tracer in the IML and absence of TH in the spleen. In contrast, surgical ablation of the nerve branches at the splenic ends (similar to Px) resulted in a loss of detectability of the tracers in the DMV and can therefore most

Fig. 5. Effect of splenic denervation on atherosclerotic lesion size and composition. At 15 wk after Px, Sx, or sham surgery, hearts were isolated and cross sections (5 μm) with 50-μm intervals throughout the aortic root area starting from the appearance of open aortic valve leaflets were used for atherosclerosis measurements. A: sections were stained with hematoxylin-phloxine-saffron for histological analysis and representative images are shown. B: atherosclerotic mean lesion area (in μm²) was quantified in 4 subsequent cross sections. C: same 4 sections per mouse were categorized according to lesion severity. D: lesion composition was determined by immunohistochemistry in 4 subsequent cross sections using α-actin for smooth muscle cells (SMC; D), Sirius red staining for collagen content (E), and MAC3 for macrophages (F). Values represent means ± SE of 15 mice per group.
likely be allocated to the parasympathetic nerve system. In addition, Px severely diminished (~70%) LPS-induced antibody production, clearly indicating functional involvement of these nerve branches in the control of the immune response (5). In contrast, Sx did not affect the production of specific antibodies. While absence of sympathetic input 15 wk after Sx was confirmed by measuring TH content, confirmation of Px was limited to visual inspection due to the lack of specific markers for these neurons. However, consistent with the notion that vagal activation suppresses the immune response (24), we found that Px enhances the number of leukocytes and increases expression of proinflammatory cytokines. It may seem contradictory that Px results in diminished LPS-induced antibody production (5), while here we report enhanced inflammatory responses. However, one should consider the dual challenge for the brain during inflammation, namely to contain the inflammation by for example reducing cytokine production and subsequently to induce memory within the immune system to prevent new infections by inducing antibody production.

Despite that Px increased the number of immune cells in the spleen in the current study, no differences in splenic TNF-α gene expression or circulating TNF-α levels were found. Possibly stronger inflammatory stimuli are required to attenuate TNF-α production by spleen macrophages via stimulation of the vagus nerve, as has been shown for LPS-induced endotoxemia (18). Compatibile with the notion that TNF-α is crucially involved in the pathogenesis and progression of atherosclerosis (4, 14), Px and Sx did not aggravate WTD-induced atherosclerotic lesion development and did not affect lesion composition in APOE*3-Leiden.CETP mice. Similarly, we previously showed that hematopoietic α7-nAChR deficiency in ApoE−/− mice does increase inflammatory status of the body and enhances platelet reactivity but does not aggravate atherosclerosis as lesion size and plaque composition remained unaffected (15). In contrast, Johansson et al. (13) recently reported an increase in atherosclerotic lesion development upon hematopoietic α7-nAChR deficiency in Ldrl−/− mice, indicating that the genetic and environmental context are important to determine the outcome of disrupted anti-inflammatory reflexes. Despite conflicting outcomes, interfering with inflammatory reflexes might be an interesting target in the prevention of atherosclerosis. Several animal studies report beneficial effects of low-dose β-blockers on atherogenesis (20, 21), mainly via reducing of inflammatory responses rather than changes in lipid metabolism. Also in humans, the use of metoprolol slows progression of intima-media thickness (9, 26).

While the role of the autonomic splenic nerves in human physiology is unclear, splenectomy in trauma patients has been associated with frequency of ischemic coronary diseases, probably explained by increased plasma lipids (17). Rodent studies confirmed the role of the spleen in lipid metabolism as splenicized rats showed reduced HDL cholesterol and increased plasma TGs. Complete removal of the spleen in apoE-deficient mice increased plaque development, although the underlying mechanism remained elusive (16). In the current study, no differences in plasma lipids were found upon splenic denervations, suggesting that regulation of lipid metabolism via the spleen is probably not mediated via innervation of the splenic nerves, which may explain why atherosclerosis development was not aggravated in this study. Interestingly, splenectomized trauma patients do display increased infection rates and have increased leukocyte counts (1), corresponding with the data presented in the current study; however, the exact contribution of an isolated increased inflammatory status without effects on plasma lipid levels to atherosclerosis development is unclear.

In conclusion, selective disruption of mainly the splenic parasympathetic nerve increases splenic immune cell counts and the systemic inflammatory status but does not contribute to atherosclerotic lesion development.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


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