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HIGH FAT DIET INDUCES LUNG REMODELING IN APOE DEFICIENT MICE: AN ASSOCIATION WITH AN INCREASE IN CIRCULATORY AND LUNG INFLAMMATORY FACTORS

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Abstract

Hypercholesterolemia is increasingly considered the basis for not only cardiovascular pathologies but also several complications affecting other organs including lungs. Here, we examined the effect of hypercholesterolemia on lung integrity using a mouse model ($ApoE^{-/-}$) of high fat (HF) diet-induced atherosclerosis. A twelve-week HF diet regimen induced systemic production of TNF-α, IFN-γ, GMC-SF, RANTES, IL-1α, IL-2, and IL-12 with TNF–α as the predominant cytokine in $ApoE^{-/-}$ mice. Concomitantly, TNF-α, IFN-γ, and MIP-1α were detected in brochoalveolar lavage fluids of these mice, coinciding with lung inflammation consisting primarily of monocytes/macrophages. Such lung inflammation correlated with marked collagen deposition and an increase in matrix metalloproteinase-9 activity in $ApoE^{-/-}$ mice without mucus production. Although TGF-β1 was undetectable in brochoalveolar lavage fluid of $ApoE^{-/-}$ mice on HF diet, it displayed a much wider tissue distribution compared to that of control animals. Direct exposure of smooth muscle cellsto oxidized-LDL, in vitro, induced a time-dependent expression of TNF-α. Direct intratracheal TNF–α-administration induced a lung inflammation pattern in wild-type mice that was strikingly similar to that induced by HF diet in $ApoE^{-/-}$ mice. TNF–α administration induced expression of several factors known to be critically involved in lung remodeling including MCP-1, IL-1β, TGF–β1, adhesion molecules, collagen type-1, and TNF–α itself in the lungs of treated mice. These results suggest that hypercholesterolemia may promote chronic inflammatory conditions in lungs that are conducive to lung remodeling potentially through TNF–α-mediated processes.

Keywords

Lung remodeling; atherosclerosis; cytokines; inflammation; $ApoE^{-/-}$ mouse

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The etiology of many diseases can be linked to diet or changes in the diet. A high fat diet is increasingly regarded as a primary cause for numerous diseases including diabetes, hypertension, and cancer 1. The relationship between dietary fat and the risk for cardiovascular diseases has been extensively examined. A direct link between diets high in saturated and trans fatty acids increase LDL cholesterol levels, and the increased risk of heart disease has been well-established 2. Despite evidence for the involvement of a high fat diet in inflammatory diseases, few studies have been undertaken to examine the involvement of a high fat diet on lung inflammation. More recently, hypercholesterolemia was reported to be a potential risk factor for asthma in humans 3,4,5.

Remodeling occurs in a number of chronic lung inflammatory diseases 6, 7. Chronic inflammation is thought to initiate and perpetuate cycles of tissue injury and repair in asthma, although remodeling may also occur in parallel with inflammation. The persistence of inflammatory cells within the airways and the up-regulation of a number of inflammatory cytokines and growth factors such as tumor necrosis factor (TNF), tumor growth factor beta (TGF-β), and several members of the interleukin family, as well as adhesion molecules, participate in the onset of lung remodeling 7.

Given that high fat diet may increase the production of inflammatory factors in the circulation, and the fact that airways come into contact with circulatory elements in a continuous manner, we hypothesized that inflammatory factors may influence pathological events within the lungs. To this end, we utilized ApoE$^{-/-}$ mice, a well-established animal model of high fat diet-induced chronic inflammation, to test our hypothesis. Our results suggest that high fat diet promotes chronic inflammation in the circulation that may influence critical changes within the lungs, leading to lung remodeling and ultimately to further lung complications.

MATERIALS AND METHODS

Animals, diet, and treatment protocols

Wild type and ApoE$^{-/-}$ mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were bred in a specific-pathogen free facility at LSUHSC, New Orleans, LA, and allowed unlimited access to sterilized chow and water. Maintenance and experimental protocols were all approved by the LSUHSC Animal Care & Use Committee. C57BL/6 Six-week-old ApoE$^{-/-}$ mice received regular chow or a high-fat diet (Harlan Teklad, Madison, WI, USA) containing 21% fat by weight (0.15% cholesterol) for 12 weeks before sacrifice. Wild type mice were administered TNF–α (100 ng/mouse) intratracheally under anesthesia. Mice were sacrificed 6 or 24 h later for lung collection or brochoalveolar lavage (BAL) fluids, respectively. As a control for IL-13 production, six weeks old C57BL/6 wild type were sensitized and challenged with ovalbumin as previously described 8, 9.

Organ recovery and tissue staining

Mice were fasted, anesthetized with ketamine/xylazine (60 and 3 mg/kg, respectively), and blood was drawn for sera preparation. Animals were sacrificed and aortas or lungs were isolated as described 8–10. Lungs were fixed with 10% formalin and processed for

pathology and immunohistochemistry (IHC) analysis, immersed in *RNA Later* solution (Qiagen Inc., Valencia, CA) for RNA extraction, or subjected to brochoalveolar lavage (BAL). Formalin-fixed lungs were sectioned and subjected to hematoxylin and eosin (H&E), trichrome, or Periodic Acid-Schiff (PAS) staining using standard protocols. IHC was conducted as described previously 11 using antibodies to murine transforming growth factor-β (TGF-β) (Cell signaling Technology Inc., MA). BAL fluids were used for cytokine measurements or subjected to cyto-spin and stained with H&E for the assessment of inflammatory cells.

Perfusion-fixed aortas were dissected and prepared for either Oil-Red-O staining using standard protocol or paraffin embedding for H&E staining. Formalin-fixed hearts were incubated in 5% then 10% gelatin solutions 2 h each followed by an overnight incubation in 25% gelatin. Serial sections were obtained from aortic sinus after embedding in OCT media and stained with Oil red O as described 10.

Isolation of primary smooth muscle cells (SMCs) from mouse lung

SMCc were isolated from C57BL/6 mice as described previously 10 after adaption to lung tissue as described by Amrani et al. 12. SMCs, at passages 3–5, were grown to 80% confluency in 10% FBS-DMEM and then growth arrested for 24 h in serum-free DMEM before stimulation with 100 μg/ml oxLDL for 3 or 6 h. Cells were then collected for RNA analysis by real-time PCR.

Bioplex assay for cytokine measurement

Serum or BAL fluids were used for assessment of different cytokines using the Bio-Rad single-plex or multiplex assay kits (Bio-Rad Laboratories, Hercules, CA) as per instructions of the manufacturer and as described 9. Serum samples were processed for removal of lipids using lipoclear reagent (Iris Sample Processing, Westwood, MA) prior to testing according to the manufacturer's instructions. According to Bio-Rad Laboratories, cross-reactivity between different cytokines in the utilized multiplex system is negligible. Additionally, intra-assay and inter-assay coefficient of variation is noted to be minimal.

Reverse Transcription, PCR, and gelatin zymography

RNA was extracted from lung tissue and cDNA was generated by standard methods. Set of primers used for different genes are described in Table 1 (Supplemental Material). The specificity of the primers sets was confirmed in our previous studies 10, 13, 14. Amplification, detection, and data analysis were performed with the iCycler PCR system (Bio-Rad Laboratories). For zymography, proteins extracts were prepared by homogenizing lungs in lysis buffer (1:4 w/v) 15 and were (50 μg) immediately assayed for matrix metalloproteinases (MMP) activity with SDS-PAGE gelatin using commercial gels (Invitrogen, Carlsbad, CA) according to the manufacturer instructions.

Statistical analysis

All data are expressed as mean ± SD of values from three independent experiments having at least 6 mice per group. PRISM software (GraphPad, San Diego, CA) was used to analyze

the differences between experimental groups by one-way ANOVA. *P* values of <0.05 were considered significant.

RESULTS

Systemic production of inflammatory cytokines that have the potential of affecting lung integrity in ApoE$^{-/-}$ mice on a high fat diet

Figure 1A shows that subjecting ApoE$^{-/-}$ mice to a high fat diet for 12 weeks promoted the marked formation of atherosclerotic plaques, as expected. The plaques exhibited cholesterol-rich lipid clefts within the intimal layer containing distinct, large macrophage-derived foam cells and SMCs (Fig. 1B). Figure 1C depicts the lipid-rich nature of the atherosclerotic plaques at the aortic sinus after staining with oil-red-o. Serum cholesterol and triglyceride levels were significantly higher in ApoE$^{-/-}$ mice on a high fat diet compared to those in mice on a regular diet (data not shown), which is consistent with our previously reported data 10 as well as with many published reports (for review 16). Serum cholesterol and triglycerides were measured to evaluate the effect of high fat diet on the lipid profile of the experimental model. Figure 1D shows that the high-fat atherogenic diet significantly increased serum TG, TC, LDL-C and HDL-C levels, which is consistent with published reports 10.

An assessment of the major cytokines produced after 12 weeks of the high fat diet (Fig. 1D) reveals a significant elevation in the production of TNF-α, IFN-γ, GMC-SF, RANTES, IL-1α, IL-2, and IL-12 compared to serum levels of animals that were fed a regular diet. The anti-inflammatory cytokine IL-10 was also elevated to levels similar to those of the pro-atherogenic cytokine IL-12 in response to the high fat diet in ApoE$^{-/-}$ mice. A number of other cytokines such as MIP-1α, keratinocyte-derived cytokine (KC), IL-17, G-CSF, and IL-6 were also measured but no significantly increased levels were detected in the high fat diet-group (data not shown). Given that lungs can be exposed to systemic factors because of their significant exchange with the vasculature, and the fact that the roles of the cytokines induced by the high fat diet, in our experimental model, in lung remodeling are well-established (for review 17), we surmised that the high fat diet may promote cytokine and growth factor elevation that could create chronic inflammatory conditions conducive to lung remodeling.

The high fat diet induces production of TNF-α, IFN-γ, and MIP-1α in lungs of ApoE$^{-/-}$ mice

To test the potential effect of a high fat diet on lung homeostasis and integrity, we assessed different cytokine levels in our animal model. Figure 2 shows that varying levels of TNF-α, IFN-γ, and MIP-1α were present in the BAL fluid of high fat diet-fed ApoE$^{-/-}$ mice compared to those of mice receiving regular diet. However, no major differences were observed in the levels of GMC-SF, IL-1α, IL-12, or IL-10 between the two experimental groups (data not shown); IL-2 and RANTES were undetectable in both experimental groups (data not shown). The reasons behind the observed selective cytokine elevation in the lungs are not clear.

Deletion of the ApoE gene has been shown to promote inflammatory conditions. Accordingly, ApoE deficiency may represent a confounding factor on whether high lipid levels would directly affect lung homeostasis. To investigate whether high cholesterol levels could promote an inflammatory response in lung cells, we examined whether a direct exposure of lung SMCs to oxLDL would culminate in an induction of TNF-α expression. Fig. 2B shows that oxLDL induced a significant increase in TNF-α expression and in a time-dependent manner as assessed by real-time PCR. This result clearly suggests that lung cells can be susceptible to elevated cholesterols and that the latter may produce inflammatory conditions in the lung.

Association between pro-inflammatory cytokines in BAL fluid from high fat diet-fed ApoE$^{-/-}$ mice and lung inflammation consisted primarily of monocytes/macrophages

The roles of TNF–α, IFN–γ, and MIP–1α in lung pathologies have been reported in numerous studies (reviewed in 7). To determine the consequences of elevated cytokine production, we assessed the histopathology of lungs from the two experimental groups. H&E staining of lung sections from the high fat diet-fed ApoE$^{-/-}$ mice revealed a substantial recruitment of inflammatory cells, mostly macrophages/monocytes (Figure 3A), which was largely absent in the ApoE$^{-/-}$ mice that received a regular diet. Figure 3B confirms the macrophage/monocytic nature of the recruited inflammatory cells by subjecting BAL fluid to a cytospin followed by H&E staining. Additional support for the identity of macrophages/monocytes was obtained by immunofluorescence with antibodies to CD68 (Fig. 3B, bottom panel). Figure 3C shows a significant increase in total cells in the high fat diet-fed group compared to control animals, with macrophages/monocytes constituting the majority of the recruited cells. It is noteworthy that a small number of neutrophillic-polymorphonucleated cells (PMN) were also consistently observed, representing a statistically significant increase over those found in control animals. The number of lymphocytes was also higher in the lungs of high fat diet-fed animals but the increase was not statistically significant (Fig. 3B). Interestingly, a number of epithelial cells were found in BAL fluid, suggesting that some of these cells might have been sloughed off as a result of the inflammation (Fig. 3B). Control animals exhibited a small number of monocyte/macrophages, which is consistent with the low detected levels of inflammatory cytokines (Fig. 2).

High fat diet-induced lung inflammation is associated with tissue remodeling and activation of matrix metalloproteinase (MMP) in ApoE$^{-/-}$ mice without mucus production

The observed perivascular and peribronchial inflammatory cell recruitment in high fat diet-fed ApoE$^{-/-}$ mice is suggestive of chronic inflammation. The persistence of inflammatory factors within lungs is known to induce tissue remodeling. To this end, we determined whether high fat diet-induced inflammation was associated with changes in lung integrity. Figure 4A shows an intense trichrome staining in lungs of high fat diet-fed ApoE$^{-/-}$ mice, which is indicative of a robust matrix deposition. Further, matrix deposition was associated with a marked sub-epithelial and peri-vascular thickening (Fig. 4A). Alveolar septae were also thickened by infiltrates of chronic inflammatory cells (data not shown).

The role of TGF–β in lung remodeling is well-established in a number of respiratory pathologies 18. Interestingly, the levels of TGF–β in BAL fluids collected from the two experimental groups were undetectable as assessed by ELISA (data not shown). However, immunohistochemical analysis with antibodies to murine TGF–β of lung sections of the two groups, revealed a more pronounced and widespread TGF–β-immunoreactivity (Figure 4B). Although these results do not provide an unequivocal evidence for an increase in TGF–β in the lungs of high fat diet-fed mice, they do show a clear change in tissue distribution of the cytokine, which may be suggestive of a potential association between TGF–β and high fat died-induced lung remodeling in our experimental system. Obviously, a more extensive experimentation is required for establishing or negating the role of TGF–β in high fat died-induced lung remodeling.

A number of cytokines or growth factors including TNF–α and TGF–β trigger cell signaling culminating in expression of matrix metalloproteinases (MMP) 19. Protein extracts from the lungs of the two experimental groups show that an increase in matrix metalloproteinase (MMP) activity was present in the lungs of ApoE$^{-/-}$ mice on the high fat diet (Fig. 4C). The 85 kDa MMP-9 appeared to be the major member of its family activated in our experimental model. Interestingly, lung remodeling and increased MMP activity was not associated with mucus production, as assessed by PAS staining of lung sections (Fig. 4D). The lack of PAS-positive cells correlates with a complete absence of IL-13 production in BAL fluid of high fat diet-fed ApoE$^{-/-}$ mice (data not shown). Collectively, our results suggest that high fat diet induces a chronic inflammatory response in the lungs of ApoE$^{-/-}$ mice, creating conditions conducive for tissue remodeling.

Intratracheal TNF–α administration induces a pattern of lung inflammatory cell recruitment as well as expression of key inflammatory factors similar to that observed in high fat diet-fed ApoE$^{-/-}$ mice

As observed in Figures 1 and 2, TNF–α was the predominantly-produced cytokine both in circulation and in the lungs of ApoE$^{-/-}$ mice on a high fat diet. To determine whether TNF–α alone can induce a pattern of inflammatory cell recruitment similar to that observed in high fat diet-fed ApoE$^{-/-}$ mice, wild type mice were subjected to intra-tracheal administration of TNF–α, and were sacrificed either 6 or 24 h later. Lungs were removed for RNA preparation or subjected to BAL fluid collection, respectively. It is important to note that wild type mice were chosen for this experiment to avoid any interference by *ApoE* gene deletion or with the associated high serum lipids. Figure 5A shows that TNF–α exposure primarily induced macrophage/monocyte recruitment to the lungs, together with a small number of neutrophils. Many sloughed epithelial cells were also detected. This pathology appears to be similar to that observed in lungs of ApoE$^{-/-}$ mice that were fed a high fat diet for 12 weeks. Inflammatory cell recruitment into the airways in response to TNF–α exposure was preceded by expression of the adhesion molecules VCAM-1 and ICAM-1 as assessed by RT-PCR of RNA from lungs 6 h after treatment (Fig. 5B). TNF–α exposure also induced cytokines that are highly relevant to lung inflammation and associated remodeling including MCP-1, TGF–β1, and IL-1β (Fig. 5C) and TNF–α itself (Fig. 5D), as well as collagen type 1 (Fig. 5E). Interestingly, TNF–α treatment for 24 h did not promote a striking production of mucus as assessed by PAS staining of lung sections. These results suggest that

TNF–α may be an important player in high fat diet-induced lung inflammation and associated lung remodeling in ApoE$^{-/-}$ mice.

DISCUSSION

Our present study examines a critical question as to whether a high fat diet-induced chronic inflammatory condition such as atherosclerosis influences changes within the lungs leading to important pathologies. The results of the present study raise an important question as to whether chronic inflammation in cardiovascular diseases participate in the onset of lung pathological conditions such as COPD, respiratory distress syndrome, or other inflammatory respiratory diseases in humans. Undoubtedly, this study is not sufficient to fully establish the effect of high fat diet on lung integrity, and further experimental study is required.

The direct role of TNF–α in atherogenesis is well-documented (review 20). Circulating TNF–α levels are associated with increased risks of developing atherosclerosis in humans 20, 21. TNF–α inhibition promotes a marked reduction in atherogenesis in mice fed high fat diet 22,23. IFN-γ, GMC-SF, RANTES, IL-1α, IL-2, and IL-12 were detected in the sera of high fat diet-fed animals in our study. The role of TNF–α as well as other cytokines and chemokines we detected are well documented in lung remodeling (review 24). TNF–α production has been extensively associated with lung injury both in acute as well as in chronic conditions. For example, TNF–α-deficient mice are resistant to acute lung injury in response to LPS treatment 25. Whether the circulatory TNF–α itself leaked into the lungs or it was produced by lung cells is not clear. We do know that intratracheal administration of TNF–α in wild type mice induced the expression of several inflammatory factors that are important for inflammatory cell recruitment, including the adhesion molecules ICAM-1 and VCAM-1, and the chemotactic cytokine MCP-1, IL-1β and TGF-β, TNF–α itself, as well as collagen type 1 all of which are known to contribute to lung injury and lung remodeling. The similarities between the nature of the inflammatory cells that were recruited to the lungs in high fat diet-fed ApoE$^{-/-}$ mice and in TNF–α-treated wild type mice were striking. It is noteworthy that the anti-inflammatory cytokine IL-10 was also elevated in sera of high fat diet-fed animals. Given the pathological changes elicited by high fat diet, it would be plausible to consider that the conditions in our experimental model are tilted towards a pro-inflammatory state.

Elevated TNF–α levels and lung inflammation in the high fat diet-fed mice also coincided with an increase in MMP-9 activity. MMP-9 enzymatic activity is one of the most important proteases involved in lung remodeling associated with respiratory diseases such as COPD. TNF-α is crucial for MMP-9 expression 26 and may be associated with the increased activity of MMP-9 in response to the high fat diet in ApoE$^{-/-}$ mice. Indeed, Lai et al. have recently reported that TNF-α not only increases expression of MMP-9, but it also promotes its activation in aortic vascular SMCs and in mice 27. Such increase in MMP-9 expression and activity has also been reported upon exposure to TGF-β in cultured cells 28. Tissue remodeling and expression of MMPs are closely related to TGF–β production and associated signal transduction 29. It was rather surprising that high fat diet did not induce a detectable production of TGF–β in BAL fluids of ApoE$^{-/-}$ mice but a change in its tissue distribution compared to that observed in control mice was apparent. It is difficult to attribute this

change to the observed tissue remodeling induced by high fat diet, but it is clear that the diet promoted factors that caused the change in distribution. TNF–α induced expression of TGF–β in lungs of wild type mice, which is consistent with a recent report by Brody's group 30. The reason behind the failure of high fat diet to induce production of TGF–β in ApoE$^{-/-}$ mice is not clear.

An important issue worth mentioning is the fact that ApoE$^{-/-}$ mice that are fed a regular diet exhibit serum cholesterol levels that are two to three times the levels of wild type mice. The question of why these mice did not show any signs of lung remodeling or increased cytokines in the sera is valid. A lack of such an effect could be attributed to the short treatment duration. It is noteworthy that ApoE$^{-/-}$ mice eating a regular diet can develop atherosclerotic plaques similar to those observed when given a high fat diet, but time to development is far longer than with the high fat diet. Accordingly, it is tempting to speculate that ApoE$^{-/-}$ mice could develop lung remodeling while on a regular diet, if given sufficient time. However, this hypothesis remains to be tested. Interestingly, Massaro and Massaro reported that ApoE$^{-/-}$ mice exhibited serious defects in lung function including increased lung resistance in early adulthood and high lung volume and high dynamic and static compliance in later adulthood compared with wild type mice 31. They predicted that dietary and/or genetic effects on lipid metabolism might be an upstream cause of inflammation and oxidative stress contributing to lung pathologies such as those associated with COPD. It is important to note that the role of ApoE lipoprotein in preventing and/or modulating inflammation is well-documented (review 32). For instance, ApoE protein or ApoE mimetic peptides have been shown to suppress cellular responses to LPS33, microglial activation and release of TNF-α 34 as well as modulate rapidly evolving atherosclerotic lesions in vein grafts35. Whether ApoE deficiency plays a direct role in lung remodeling observed in our study remains to be determined. However, our in vitro data though does exclude a role for ApoE in lung inflammation, it demonstrates that direct exposure of lung cells to oxidized cholesterols (oxLDL) culminates in the production of TNF-α, the primary cytokines observed in our animal model both systemically and in the lungs of high fat diet-fed animals.

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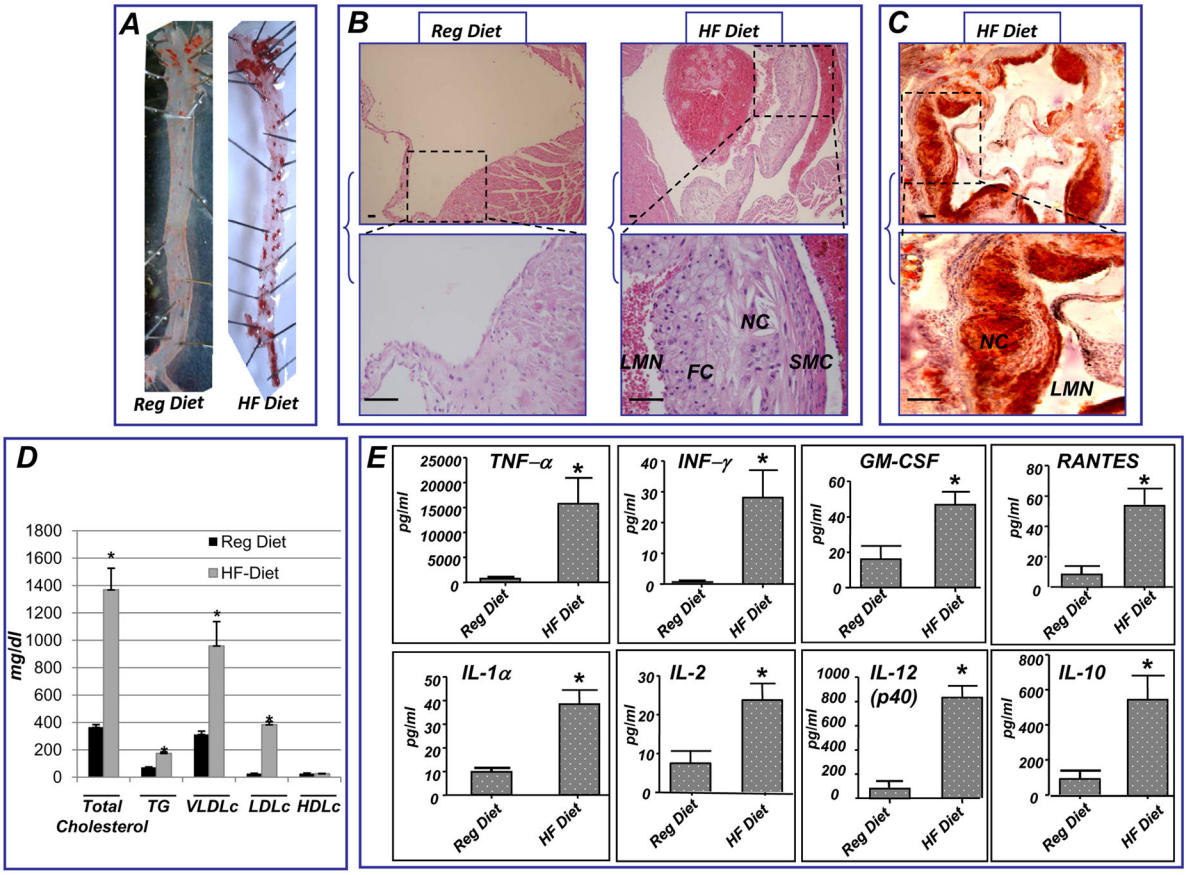

Figure 1. High fat diet induction of atherogenesis is correlated with substantial systemic inflammatory cytokines production in ApoE$^{-/-}$ mice

C57BL/6 ApoE$^{-/-}$ mice were fed either high fat (HF diet) or regular (Reg Diet) diet for 12 weeks after which mice were sacrificed. (A) Aortas were formalin (PBS)-perfused and atherosclerotic plaques were visualized by en face Oil-Red-O staining. (B) Aortic sinus sections were prepared as described in Materials and Methods, subjected to H&E staining and visualized by light microscopy; FC, foam cells; SMC, smooth muscle cells; LMN, lumen; NC, necrotic core. (C) Aortic sinus sections from high fat diet-fed mice were subjected to Oil-Red-O staining to depict lipid deposits within atherosclerotic plaques; bar: 5 μm. (D) Mice from the different experimental groups were fasted, anesthetized, and blood was drawn for sera preparation. Plasma cholesterol and LDL (mg/dl) were analyzed; n=4. * Difference from ApoE$^{-/-}$ *mice fed a regular diet for 12 weeks; P< 0.01*. (E) Sera were collected and cytokines were assessed using a multi-panel Bio-Rad Bioplex system as described 9. Data are given as means ± SD of values obtained from at least six mice per group. * Difference from ApoE$^{-/-}$ mice fed a regular diet; P< 0.01. Please note that the graphs are based on different scales.

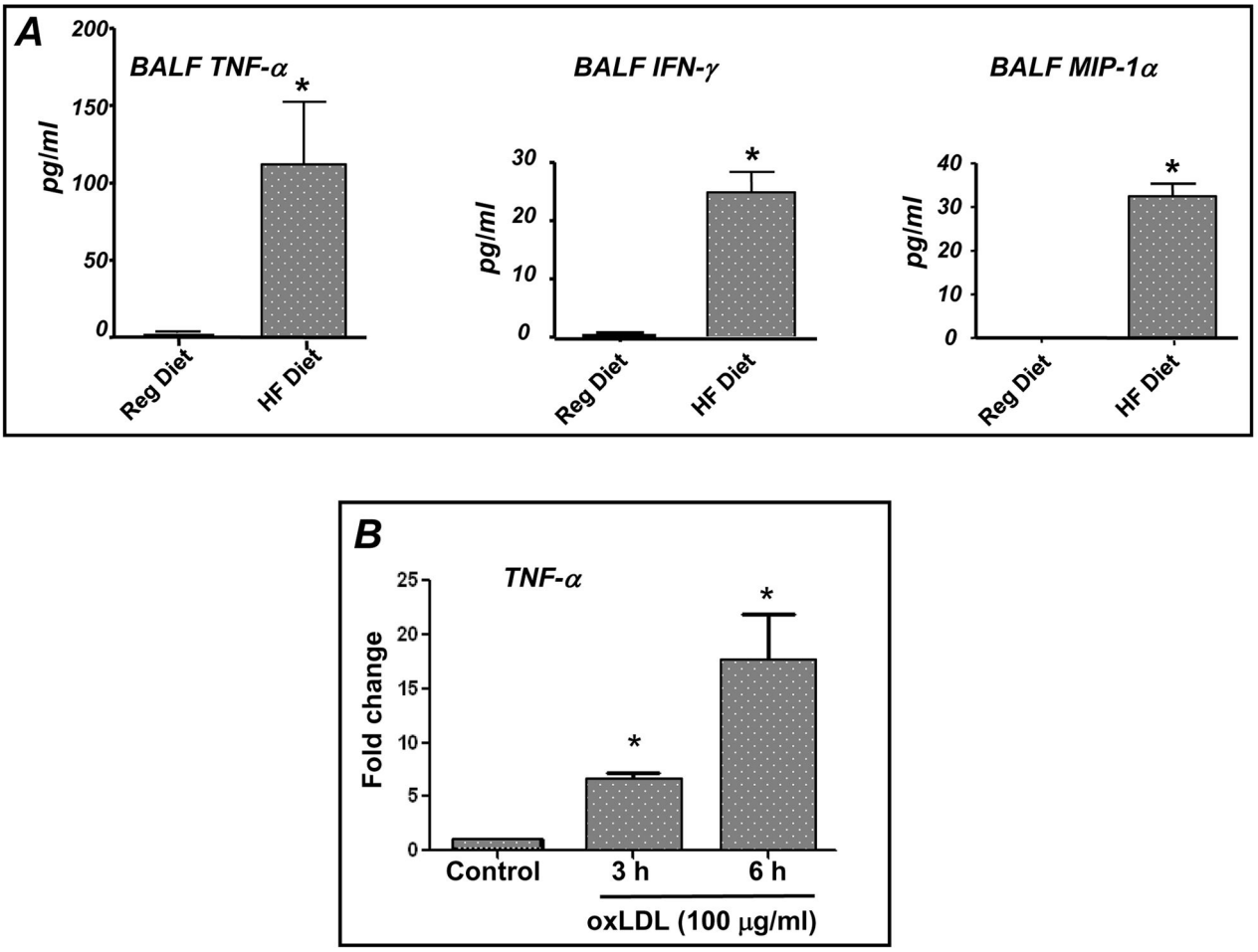

Figure 2. High fat diet induces production of TNF-α, IFN-γ, and MIP-1α in airways of ApoE$^{-/-}$ mice

(A) ApoE$^{-/-}$ mice fed either high fat (HF diet) or regular (Reg Diet) diet for 12 weeks were sacrificed then subjected to BAL. BAL fluids were collected and centrifuged. The supernatant were assessed for cytokine levels using the Bio-Rad Bioplex system. Data are given as means ± SD of values obtained from at least six mice per group. * Difference from ApoE$^{-/-}$ mice fed a regular diet; P< 0.01. Please note that the graphs are based on different scales. (B) Lung SMCs were treated with 100 μg/ml oxLDL for the indicated time intervals, after which RNA was extracted and subjected to cDNA generation. Generated cDNA was assayed by real-time PCR with primers specific to mouse TNF-α or β-actin. Data is expressed as fold increase over values from untreated cells. * Difference from untreated cells; P< 0.05.

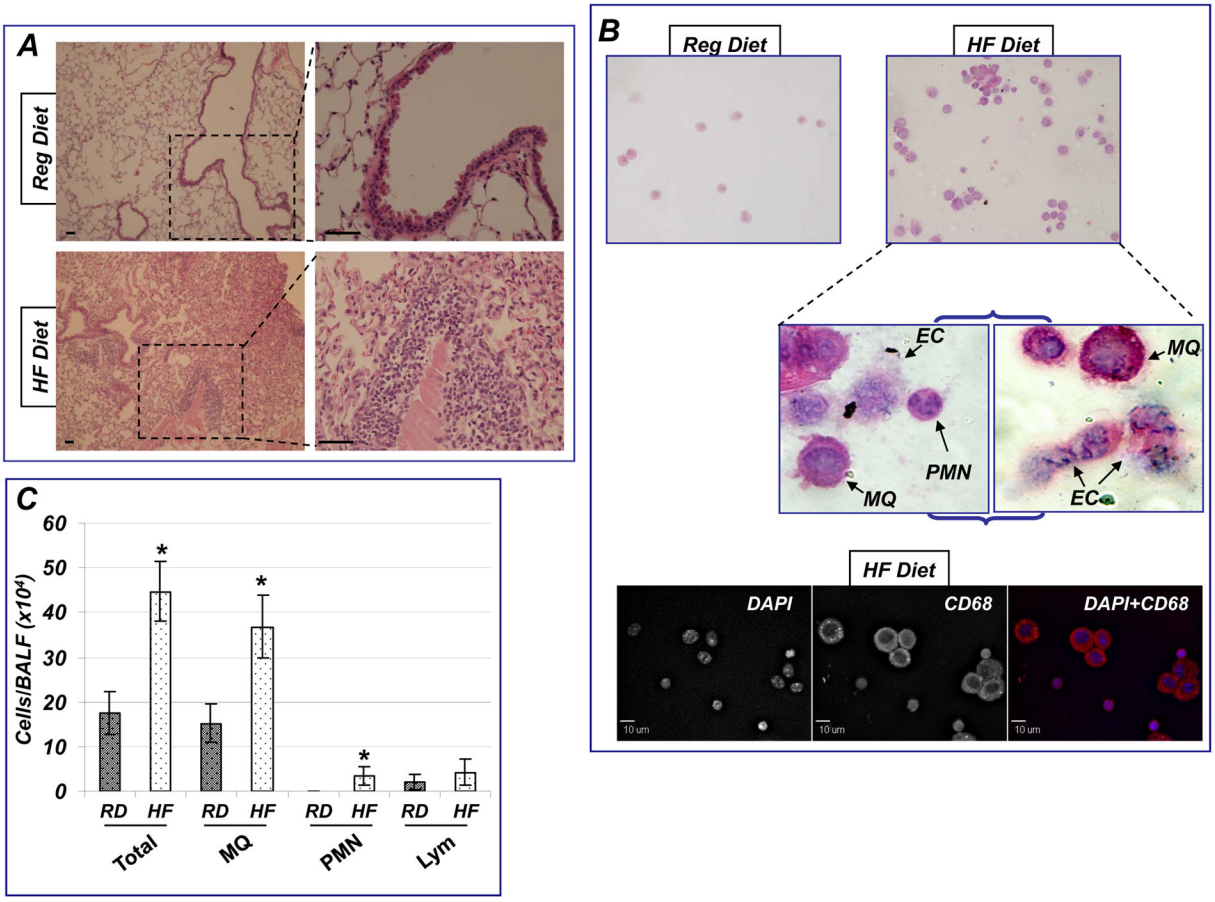

Figure 3. Association between presence of pro-inflammatory cytokines in BAL fluids from high fat diet-fed ApoE$^{-/-}$ mice and airway inflammation consisting primarily of mococytes/ macrophages

ApoE$^{-/-}$ mice fed either high fat (HF diet) or regular (Reg Diet) diet for 12 weeks were sacrificed. Lungs were either fixed with formalin or subjected to BAL. (A) Lung sections were stained with H&E and analyzed by light microscopy; bars: 5 μm. Arrows indicate peri-bronchial and peri-vascular inflammation. BAL fluids were collected and centrifuged; cells were subjected to H&E or to immunofluorescence with antibodies to murine CD68 (B). H&E stained cells were also counted (C). Data are means ± SD of values from at least six mice per group. * Difference from ApoE$^{-/-}$ mice fed a regular diet; P< 0.01. MQ: macrophages; PMN: polymorphonucleated cells; EC: epithelial cells.

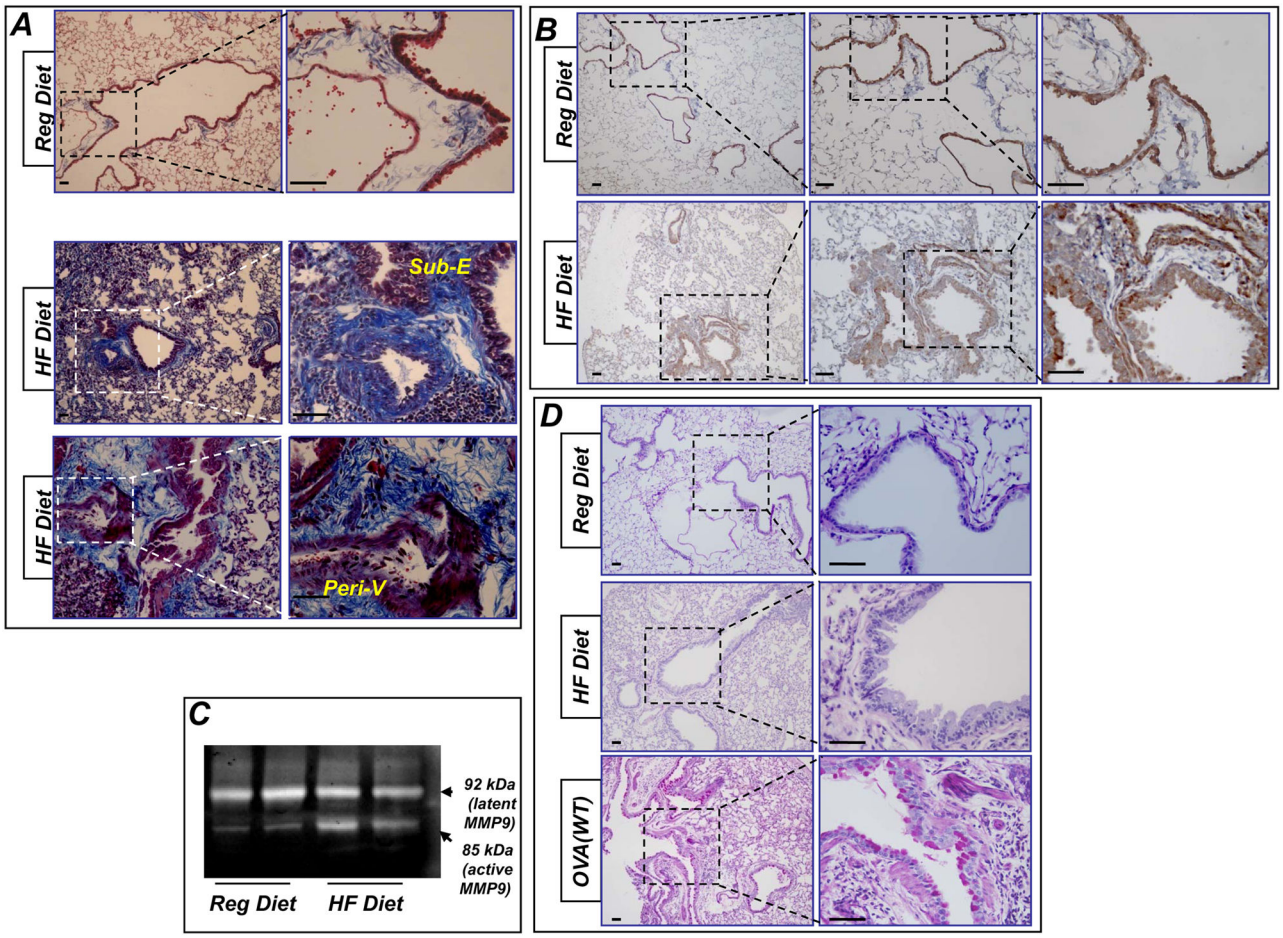

Figure 4. High fat diet-induced airway inflammation is associated with tissue remodeling and activation of matrix metalloproteinase (MMP) in ApoE$^{-/-}$ mice without an induction of mucus production

ApoE$^{-/-}$ mice fed either high fat (HF diet) or regular (Reg Diet) diet for 12 weeks were sacrificed. Lungs from these mice were then formalin-fixed for sectioning, collected for protein preparation, or subjected to BAL. Lung sections were subjected to trichrome staining (A) or immunohistochemistry with antibodies to mouse TGF–β (B) and analyzed by light microscopy; Sub-E, sub-epithelial thickening; Peri-V, peri-vascular thickening; bars: 5 μm. (C) Total protein (50 μg) prepared from the two experimental groups were subjected to zymography as described in the Methods and visualized by staining with Coomassie blue. (D) Lung sections were subjected to PAS staining. (E) BAL fluids were assessed for IL-13 using the Bio-Rad Bioplex system. Lung sections from wild type mice that were subjected to ovalbumin (OVA) sensitization and challenge as described 9 served as controls for PAS-positive staining. * Difference from ApoE$^{-/-}$ mice fed a regular diet; P< 0.01. The right panels represent higher magnifications.

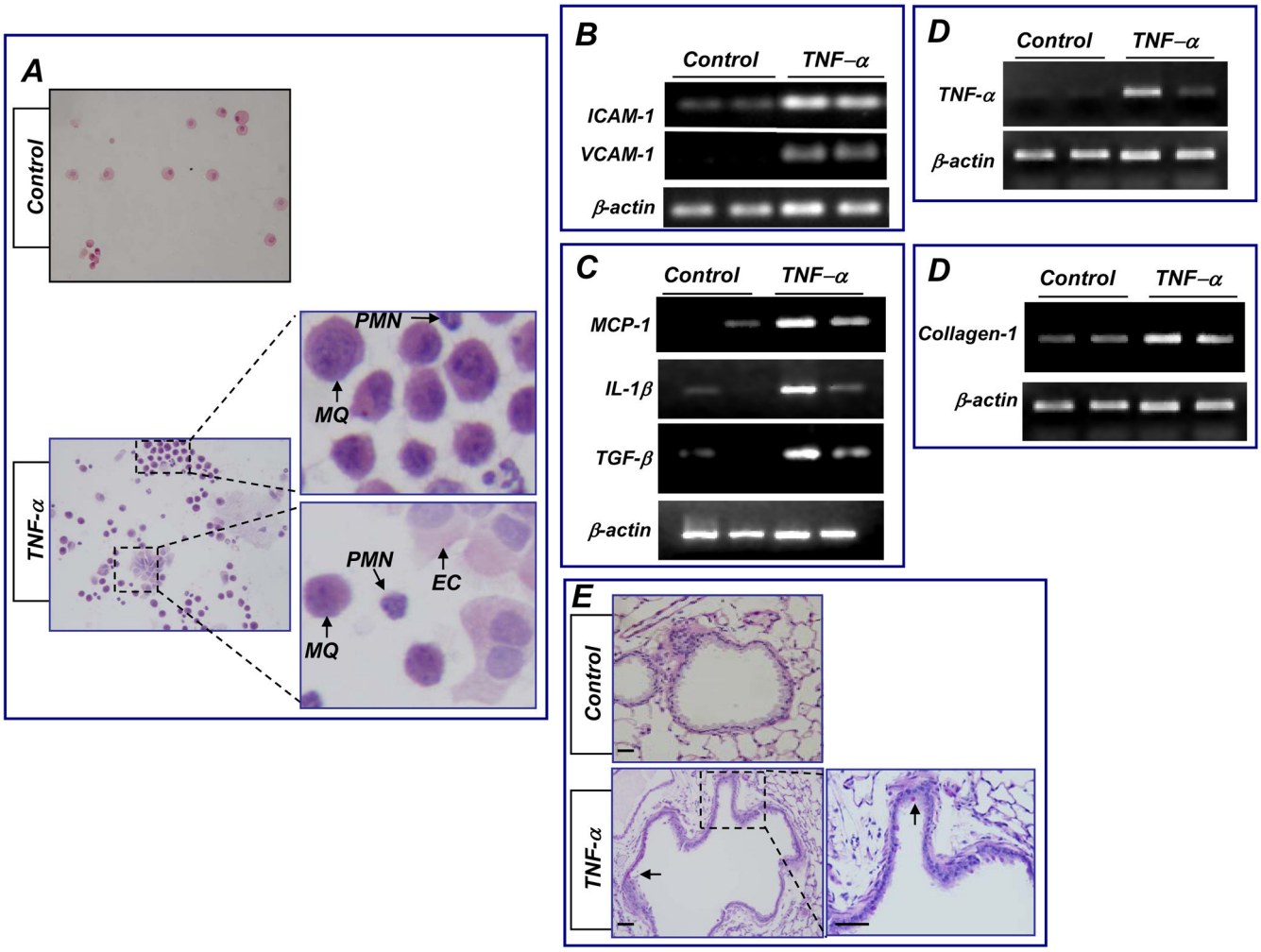

Figure 5. Intra-tracheal administration of TNF–α induces a pattern of airway inflammatory cell recruitment similar to that observed in high fat diet-fed ApoE$^{-/-}$ mice as well as expression of key inflammatory factors

C57BL/6 wild type mice were subjected to intranasal administration of recombinant mouse TNF–α (0.5 μg/mouse) in saline or saline alone, which were then sacrificed 6 or 24 h later. (A) twenty four hours after treatment, BAL fluids were collected and centrifuged; cells were then differentially stained and visualized by light microscopy; MQ: macrophages; PMN: polymorphonucleated cells; EC: epithelial cells. Six hour after treatment, whole lungs were collected and subjected to RNA extraction followed by cDNA generation. Prepared cDNA was subjected to RT-PCR for the adhesion molecules ICAM-1 and VCAM-1 (B), for the inflammatory factors MCP-1, IL-1-β, TGF-β1 (C), for TNF–α (D), or for collagen type 1 (E) using their respective specific primers; β –actin was used as an internal control. (F) Formalin-fixed lung sections from control or TNF–α-treated mice were subjected to PAS staining; arrows indicate PAS-positive epithelial cells.