IgE Contributes to Atherosclerosis and Obesity by Affecting Macrophage Polarization, Macrophage Protein Network, and Foam Cell Formation

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OBJECTIVE: By binding to its high-affinity receptor FcεR1, IgE activates mast cells, macrophages, and other inflammatory and vascular cells. Recent studies support an essential role of IgE in cardiometabolic diseases. Plasma IgE level is an independent predictor of human coronary heart disease. Yet, a direct role of IgE and its mechanisms in cardiometabolic diseases remain incompletely understood.

APPROACH AND RESULTS: Using atherosclerosis prone Apoe−/− mice and IgE-deficient Ige−/− mice, we demonstrated that IgE deficiency reduced atherosclerosis lesion burden, lesion lipid deposition, smooth muscle cell and endothelial cell contents, chemokine MCP (monocyte chemoattractant protein)-1 expression and macrophage accumulation. IgE deficiency also reduced bodyweight gain and increased glucose and insulin sensitivities with significantly reduced plasma cholesterol, triglyceride, insulin, and inflammatory cytokines and chemokines, including IL (interleukin)-6, IFN (interferon)-γ, and MCP-1. From atherosclerotic lesions and peritoneal macrophages from Apoe−/− Ige−/− mice that consumed an atherogenic diet, we detected reduced expression of M1 macrophage markers (CD68, MCP-1, TNF [tumor necrosis factor]-α, IL-6, and iNOS [inducible nitric oxide synthase]) but increased expression of M2 macrophage markers (Arg [arginase]-1 and IL-10) and macrophage-sterol-responsive-network molecules (complement C3, lipoprotein lipase, LDLR [low-density lipoprotein receptor]-related protein 1, and TFR [transferrin]) that suppress macrophage foam cell formation. These IgE activities can be reproduced in bone marrow-derived macrophages from wild-type mice, but muted in cells from FcεR1-deficient mice, or blocked by anti-IgE antibody or complement C3 deficiency.

CONCLUSIONS: IgE deficiency protects mice from diet-induced atherosclerosis, obesity, glucose tolerance, and insulin resistance by regulating macrophage polarization, macrophage-sterol-responsive-network gene expression, and foam cell formation.

Key Words: atherosclerosis ■ gene expression ■ insulin ■ macrophages ■ obesity

IgE is an important regulator of allergic disease. It binds with high affinity to FcεR1 and with low affinity to FcεR2 (CD23). In mice and humans, FcεR1 is primarily expressed on mast cells and basophils. In addition to these granulocytes, other leukocytes such as macrophages, eosinophils, monocytes and dendritic cells, and even vascular cells also express this receptor. IgE-mediated activation of macrophages releases inflammatory molecules, such as IFN (interferon)-γ and TNF (tumor necrosis factor)-α. From our previous report and those of others, plasma IgE level associates with cardiovascular events in mice and humans and serves as an independent predictor for coronary heart disease (CHD). We reported that both IgE and FcεR1 are increased in human and mouse atherosclerotic lesions and colocalize to plaque macrophages. Deficiency in
It was thought that IgE contributes to atherogenesis. Apoe−/− mice were protected from atherosclerosis. 11 Macrophages also associate with consequent immune cell infiltration and smooth muscle cell (SMC) activation, which destabilize the atherosclerotic plaques. Foam cells secrete local inflammatory cytokines, reactive oxygen species, and matrix metalloproteinases that destabilize the atherosclerotic plaques. Foamy macrophages also associate with consequent immune cell infiltration and smooth muscle cell (SMC) activation, 11 all of which are essential to atherosclerotic lesion progression and rupture. In advanced atherosclerotic lesions, dying macrophages promote necrotic core formation and plaque complexity. In contrast, macrophages that initially infiltrate into the plaques may also exhibit an alternative macrophage (M2) phenotype and have a lower susceptibility to become foam cells. These cells display high phagocytic activity and produce anti-inflammatory IL-10. As the plaque develops, however, M2 macrophages subsequently shift to an M1 phenotype and secrete IL-1β, IL-6, and TNF-α at the advanced stage of the disease. 12,13

Patients with type-2 diabetes mellitus demonstrate increased risk of having CHD with increased overall plaque burden, high rates of multivessel disease, and death from vascular complications. 14,15 Macrophages serve as an important link between type-2 diabetes mellitus and atherosclerosis. 16,17 A major pathway of this link is the IFN-γ-regulated proatherogenic macrophage-sterol-responsive network (MSRN). This sterol-responsive network was originally identified from the studies of peritoneal macrophages isolated from the Ldlr−/− mice that consumed a high cholesterol atherogenic diet. In these mice, macrophages convert free cholesterol into cholesteryl ester that accumulates as cytosolic lipid droplets and generates foam cells by perturbing the MSRN interaction of proteins during atherogenesis. This network is also dysregulated by diet-induced obesity, predisposes macrophages to cholesterol accumulation, and promotes foam cell formation. 16,17

In this study, we demonstrated that IgE deficiency in Apoe−/− mice reduced atherosclerosis by decreasing lesion macrophage content and inflammation. These mice showed altered atherosclerotic lesion and peritoneal macrophage phenotypes and improved obesity and insulin resistance. Mechanistic studies suggested that IgE controlled macrophage polarization, targeted the MSRN proteins, and promoted macrophage cholesterol accumulation, all which depended on the expression of FcR1 and its associated protein Nhe1 (Na+/H+ exchanger 1).

**METHODS**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>Arg-1</td>
<td>arginase-1</td>
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<tr>
<td>BMDM</td>
<td>bone marrow-derived macrophage</td>
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<tr>
<td>C3</td>
<td>complement C3</td>
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<tr>
<td>CHD</td>
<td>coronary heart disease</td>
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<td>HDL</td>
<td>high-density lipoprotein</td>
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<td>IFN</td>
<td>interferon</td>
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<td>IL</td>
<td>interleukin</td>
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<td>iNOS</td>
<td>inducible nitric oxide synthase</td>
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<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
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<tr>
<td>LPL</td>
<td>lipoprotein lipase</td>
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<tr>
<td>Lrp1</td>
<td>low-density lipoprotein receptor-related protein 1</td>
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<tr>
<td>MCP-1</td>
<td>monocyte chemoattractant protein 1</td>
</tr>
<tr>
<td>Mrc-1</td>
<td>macrophage mannose receptor 1</td>
</tr>
<tr>
<td>MSRN</td>
<td>macrophage-sterol-responsive-network</td>
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<tr>
<td>NF-κB</td>
<td>nuclear factor-κB</td>
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<tr>
<td>Nhe1</td>
<td>Na+/H+ exchanger 1</td>
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<tr>
<td>ox-LDL</td>
<td>oxidized-LDL</td>
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<td>SMC</td>
<td>smooth muscle cell</td>
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<td>TFR</td>
<td>transferrin</td>
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<td>TLR-4</td>
<td>Toll-like receptor-4</td>
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<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
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<td>WT</td>
<td>wild type</td>
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### Highlights

- IgE deficiency reduces diet-induced atherosclerosis and lesion contents of macrophages, smooth muscle cells, and microvessels in Apoe−/− mice.
- IgE deficiency reduces diet-induced bodyweight gain, glucose tolerance, and insulin resistance.
- IgE promotes macrophage polarization towards M1 phenotype.
- IgE reduces macrophage-sterol-responsive-network gene expression and promotes macrophage cholesteryl ester intracellular accumulation and foam cell formation.

**Zhang et al IgE Activity in Atherosclerosis, Obesity, and Diabetes Mellitus**
Mice

Apoe<sup>_–/–</sup> Ige<sup>_–/–</sup> and Apoe<sup>_–/–</sup> Ige<sup>_+/+</sup> mice were produced by breeding the Apoe<sup>_–/–</sup> mice (no. 002052, C57BL/6J, The Jackson Laboratory, Bar Harbor, ME) and Ige<sup>_–/–</sup> mice. 18 Nhe1<sup>_+/-</sup> mice (no. 003012, C57BL/6J, The Jackson Laboratory) were bred with Fcer1a<sup>+/–</sup> mice<sup>5</sup> to produce the Fcer1a<sup>+/–</sup>-Nhe1<sup>_+/-</sup> mice. Six-week-old Apoe<sup>_–/–</sup> Ige<sup>_–/–</sup> (C57BL/6J, N=10) and Apoe<sup>_–/–</sup> Ige<sup>_+/+</sup> (C57BL/6J, N=10) mice consumed a high cholesterol (1.25%) atherogenic diet (no. D12108C, Research Diets Inc, New Brunswick, NJ) to induce atherosclerosis. Results from this study are limited to males. It is possible that IgE activity in atherosclerosis may differ in females according to the ATVB council statement. 19 Their bodyweights and food intake were measured weekly. After 12 weeks, mice were euthanized by CO<sub>2</sub>, followed by cardiac puncture blood and peritoneal macrophage collection, heart, and aortic arch and root tissue harvest. Liver and adipose tissues, including epidymal adipose tissue, subcutaneous adipose tissue, and brown adipose tissue, were isolated and weighed. All animal procedures conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was approved by the Brigham and Women's Hospital Standing Committee on Animals (protocol no. 2016N000442).

Atherosclerotic Lesion Characterization and Immunohistological Analysis

Aortic root and arch were embedded in optimum cutting temperature and 6 µm aortic root serial sections and longitudinal aortic arch serial sections that contained all 3 branches (brachiocephalic artery, left common carotid artery, and left subclavian artery) were prepared to analyze atherosclerotic lesions as previously described. 9 We designed, executed, and reported mouse atherosclerotic lesion analysis according to the guidelines for experimental atherosclerosis studies described in the American Heart Association Statement. 20 To determine atherosclerotic lesion areas, we stained the aortic root and arch sections with oil-red O (Lesion area (intima) and oil-red O-positive area were calculated. Toluene blue (no. 89640, Sigma-Aldrich, St Louis, MO) staining detected aortic arch section mast cells as we reported previously. 21 For immunohistological analysis, serial cryostat sections were prepared and stained for Mac-3 (macrophages, 1:900, no. 553322, BD Biosciences, San Jose, CA), CD31 (endothelial cells, 1:1500, no. 553370; BD Biosciences), α-smooth muscle actin (SMC, 1:500, BD Biosciences), myosin heavy chain-11 (1:2000, no. 702544, Thermo Fisher Scientific, Waltham, MA), major histocompatibility complex class-II (1:250, no. 556999, BD Biosciences), CD4 (1:90, no. 553043, BD Biosciences), CD8 (1:100, no. 14-0081-85; eBiosciences, San Diego, CA), and MCP-1 (1-monocyte chemotactant protein 1, 1:100, no. AF-479-NA, R&D Systems, Minneapolis, MN) antibodies, followed by appropriate biotin-conjugated secondary antibodies (1:500, Vector Laboratories, Burlingame, CA) and HRP-streptavidin (DAKO, Carpinteria, CA). All images were captured using a Microscope VS120 Whole Slide Scanner (Olympus) and analyzed using the computer-assisted Image-Pro Plus software (Media Cybernetics, Bethesda, MD). For immunofluorescent staining, aortic arch sections were prepared and stained to detect M1 macrophages by rabbit anti-iNOS (1:100, no. PAI-036, Thermo Fisher Scientific) and rat anti-Mac-2 (1:100, CL8942LE, Cedarlane Laboratories, Burlington, NC), M2 macrophages by mouse anti-Arg-1 (arginase-1; 1:100, no. 678802, BioLegend, San Diego, CA) and rat anti-Mac-2 (1:100), and basophils by rat anti-CD49b (1:100, no. 14-5871-85, eBiosciences) and rabbit anti-FceR1 (1:50, no. 06-727, Millipore, Burlington, MA) antibodies, followed by Alexa Fluor 555 or 488-labeled secondary antibody detection. DAPI (no. D9542, Sigma-Aldrich) was used to stain the nuclei. All images were collected using the Olympus Fluoview FV1000 confocal laser scanning microscopy.

Glucose Tolerance Test and Insulin Tolerance Test

After 12 weeks of an atherogenic diet, both Apoe<sup>_–/–</sup> Ige<sup>_–/–</sup> and Apoe<sup>_–/–</sup> Ige<sup>_+/+</sup> mice were also used for glucose tolerance test and insulin tolerance test assays after fasting. 22 For the glucose tolerance test, mice were intraperitoneally injected with D-glucose (1 g/kg bodyweight, Sigma-Aldrich) after 16 hours of fasting. For the insulin tolerance test, mice were intraperitoneally injected with insulin (1.5 IU/kg bodyweight, Novolin) after 6 hours of fasting. Blood glucose level was measured from tail veins using a blood glucose meter (Bayer Healthcare LLC, Mishawaka, IN) at 0, 15, 30, 45, 60, 90, and 120 minutes after injection.

Plasma Insulin, Inflammatory Cytokines, and Lipoprotein Measurement

The plasma levels of insulin (no. 90080, Crystal Chem, Elk Grove Village, IL), IL-1β (no. 88-7013-22, Invitrogen, Carlsbad, CA), TNF-α (no. 88-7324-22, Invitrogen), IL-6 (no. 88-7064-88, Invitrogen), MCP-1 (no. 88-7391-88, Invitrogen), IFN-γ (no. 88-7314-88, Invitrogen), and histamine (no. RE59221, IBL, Hamburg, Germany) were assessed using the mouse ELISA kits according to manufacturers. Plasma total cholesterol, triglycerides, and HDL (high-density lipoprotein) levels were determined by enzymatic methods using reagents from Pointe Scientific (Canton, MI) according to the manufacturer. The LDLs (low-density lipoproteins) cholesterol level was determined using the Friedewald formula: Plasma LDL cholesterol concentration (mg/dL) = total cholesterol − HDL cholesterol − (triglycerides/5). 23 To measure the enzymatic activities of β-hexosaminidase, we treated plasma with 1.3 mg/mL p-nitrophenyl-N-acetyl-β-D-glucosaminide (no. N9376, Sigma-Aldrich) in 0.1 mol/L sodium citrate (pH 4.5, no. S4641, Sigma-Aldrich) for 60 minutes at 37°C. The reaction was stopped with 0.2 mol/L glycine (pH 10.7, no. G7126, Sigma-Aldrich), Absorbance at 405 nm measured the release of 4-p-nitrophenol.

Mouse plasma lipoprotein profiling was determined using high-resolution size-exclusion chromatography as previously reported. 24 Briefly, mouse plasma samples were fractionated by fast-performance liquid chromatography (Merck-HPLC System) using the Superox 6 HR 10/30 size-exclusion chromatography column (GE Healthcare, Buckinghamshire, United Kingdom). The column was equilibrated with 10 mmol/L sodium phosphate buffer, pH 7.4 containing 140 mmol/L NaCl. After equilibration, 400 µL of mouse plasma pool was applied to the column with a flow rate of 0.5 mL/min at room temperature, and 0.5 mL fractions were collected and analyzed for cholesterol and triglyceride. For each group of animals, plasma pools were prepared from a combination of 3 mice.
The elution fractions were analyzed for total cholesterol (enzymatic method, CHOD-PAP 1489232) and for triglycerides (enzymatic method, CHOD-PAP 1488872), both from Roche Diagnostics, Mannheim, Germany.

Cell Culture
Bone marrow-derived macrophages (BMDMs) were prepared from wild type (WT), Fcer1α<sup>−/−</sup>, Fcer1α<sup>−/−</sup>Nhe1<sup>−/−</sup>, and C3<sup>−/−</sup> mice. Fresh bone marrow was isolated from the femurs and tibias bone and cultured DMEM supplemented with 10% FBS and 20 ng/mL of macrophage-colony stimulating factor (PeproTech, Rocky Hill, NJ) for 7 days. Mouse peritoneal macrophages were isolated from Apoe<sup>−/−</sup>Ige<sup>−/−</sup> and Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice after consuming an atherogenic diet for 12 weeks by lavaging the peritoneal cavity with PBS containing 2% BSA. After red blood cell lysis and washed with PBS, cells were cultured in DMEM with 10% FBS for 24 hours to adhere before use. To assess the purity of peritoneal macrophages, we stained peritoneal cells with anti-CD45-PerCP-Cyanine5.5, anti-c-kit-APC, and anti-Ly6C-fluorescein isothiocyanate (1:250, no. 45-0451-82, Invitrogen), anti-CD11b-APC, and anti-F4/80-PerCP-Cyanine5.5. To detect mono-macrophages with anti-CD45-fluorescein isothiocyanate and anti-F4/80-PerCP-Cyanine5.5, we stained cells with anti-CD45-PerCP-Cyanine5.5, anti-Fc receptor-1 (1:100, no. 17-0112-82, eBiosciences) and anti-Fc recep-tor-1 (1:100, no. 143910, BioLegend), and CD200R3-PE (1:100, no. 142206, BioLegend).

Real-Time Polymerase Chain Reaction
Total RNA was isolated from the aorta or cultured cells using Trizol reagent (Invitrogen). cDNA was synthesized from total RNA reverse transcriptase (Invitrogen) according to the manufacturer. The cDNA was used as a template for real-time polymerase chain reaction (RT-PCR; CFX Connect Real-Time PCR Detection System, Bio-Rad, Hercules, CA) in the presence of iTaq Universal SYBR Green Supermix (Bio-rad). All primer sequences are listed in Table I in the online-only Data Supplement. Data were processed using the ΔΔCT method. GAPDH was used as the reference gene.

Western Blot
For immunoblot analysis, an equal amount of proteins extracted from BMDM of WT, Fcer1α<sup>−/−</sup>, Fcer1α<sup>−/−</sup>Nhe1<sup>−/−</sup>, and C3<sup>−/−</sup> mice were separated on SDS-PAGE, blotted, and detected with the following antibodies: rabbit anti-iNOS (1:1000, no. PA1-036, Thermo Fisher Scientific), rat anti-Mac-2 (1:1000, no. CL8942LE, Cederlane Laboratories), mouse anti-Arg-1 (1:1000, no. 678802, BioLegend), rabbit anti-complement C3 (C3; 1:1000, no. PA5-21349, Thermo Fisher Scientific), rabbit anti-pERK (1:1000, no. 4370), mouse anti-ERK antibody (1:1000, no. 9107), rat anti-pp38 (1:1000, no. 4631), mouse anti-p38 (1:1000, no. 9228), and rabbit anti-GAPDH (1:2000, no. 2118S) antibodies from Cell Signaling Technology (Danvers, MA).

Statistics
All data were expressed as mean±SEM. We used non-parametric Mann-Whitney U test followed by Bonferroni correction to compare 2-group data that did not pass the normality test. The statistical significance of weekly bodyweight was determined with a 2-way ANOVA repeated-measures; a Bonferroni post-test was used to obtain the P value for the bracketed pairwise comparison. Nonparametric Kruskal-Wallis test (1-way ANOVA on ranks) was used for all cell culture data analysis that contained multiple group comparisons and that did not pass the normality test. SPSS16 version was used for analysis, and P<0.05 were considered significant.

RESULTS
IgE Deficiency Reduces Atherosclerosis and Lesion SMC and Microvessel Contents in Apoe<sup>−/−</sup> Mice
To test a direct role of IgE in atherogenesis, we fed IgE-deficient Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice and their Apoe<sup>−/−</sup>Ige<sup>−/−</sup>
littermates an atherogenic diet for 12 weeks. IgE deficiency reduced atherosclerosis development in the whole aorta from thoracic-abdomen to aortic arch and branches (Figure 1A), aortic arch (Figure IA in the online-only Data Supplement), branches (brachiocephalic artery, left common carotid artery, and left subclavian artery; Figure IB in the online-only Data Supplement), and aortic root (Figure IC in the online-only Data Supplement), as determined by oil-red O staining. During the development of these atherosclerotic lesions, SMC and endothelial cell undergo phenotypic switching, proinflammatory activation, and abnormal proliferation and migration.25,26 IgE deficiency reduced atherosclerotic lesion intima α-smooth muscle actin- or myosin heavy chain-11-positive SMC contents (Figure 1B and 1C) and CD31-positive microvessel contents (Figure 1D) in the aortic arch, although we did not analyze those in the thoracic-abdominal aorta or in the aortic root. Reduced atherosclerosis in Apoe<sup>−/−</sup>Ige<sup>+/+</sup> mice was also associated with decreased plasma total cholesterol and total triglyceride levels, although plasma LDL and HDL levels did not differ from those in Apoe<sup>−/−</sup>Ige<sup>+/+</sup> mice (Figure 1E). Fast-performance liquid chromatography-based analysis for mouse lipoprotein profiling revealed significantly reduced plasma VLDL-C and VLDL-TG in Apoe<sup>−/−</sup>Ige<sup>+/+</sup> mice compared with those in Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice (Figure 1F and 1G). Reduced plasma VLDL can be secondary to the reduced atherosclerosis in Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice. It is still possible that IgE may directly contribute to VLDL synthesis or metabolism, a hypothesis that has not been tested in this study.

IgE Deficiency Reduces Systemic and Atherosclerotic Lesion Inflammation and Promotes M2 Macrophage Polarization

Blood-borne leukocyte infiltration into the atherosclerotic lesions, such as T cells, monocytes, and macrophages, increases lesion inflammatory cytokine expression, matrix-degrading protease expression and activation, and promotes atherogenesis. Immunostaining did not detect significant difference of aortic arch lesion CD4<sup>+</sup> and CD8<sup>+</sup> T-cell numbers between Apoe<sup>−/−</sup>Ige<sup>+/+</sup> and Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice. Immunofluorescent double staining with antibodies against basophil CD49b and FcεR1 revealed few basophils in the aortic arch intima and their numbers did not differ between Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice and those from Apoe<sup>−/−</sup>Ige<sup>+/+</sup> mice (Figure 2C). Although IgE deficiency did not affect blood macrophage contents (Figure 2D), blood Ly6C<sup>−</sup> and Ly6C<sup>+</sup> monocytes were all reduced in Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice (Figure 2E), which may contribute to reduced atherosclerotic lesion macrophage contents in these mice.27 Chemokine and inflammatory cytokines, such as MCP-1, IL-1β, TNF-α, and IFN-γ, regulate lesion macrophage accumulation and inflammation.15 Although we did not detect significant differences in plasma IL-1β or TNF-α levels between Apoe<sup>−/−</sup>Ige<sup>−/−</sup> and Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice, IgE deficiency reduced aortic arch lesion MCP-1 expression (Figure 2F) and plasma cytokines IL-6 and IFN-γ and chemokine MCP-1 in Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice compared with those from Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice (Figure 2G).

Mast cells and basophils are the primary FcεR1 expressing cells. We tested whether IgE deficiency affected lesion or peripheral mast cell and basophil contents and their activities.3 Toluidine blue staining did not detect significant difference of aortic arch intima and adventitia total (Figure IIIA in the online-only Data Supplement) and degranulated/activated mast cell numbers (Figure IIIB in the online-only Data Supplement) between Apoe<sup>−/−</sup>Ige<sup>−/−</sup> and Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice. FACS analysis detected low blood c-kit FcεR1<sup>+</sup> mast cell progenitors28 from both groups of mice, and significantly fewer mast cells in the peritoneal cavity in Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice than in Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice after consuming an atherogenic diet for 12 weeks (Figure IIIC and IIID in the online-only Data Supplement). Consistent with these observations, plasma histamine level and β-hexosaminidase activity (OD450 nm) from Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice did not differ from those from Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice (Figure IIIE and IIIF in the online-only Data Supplement), suggesting that IgE deficiency did not affect systemic or local mast cell activation and other mechanisms replaced the role of IgE in mast cell activation.29 Similarly, immunofluorescent double staining with antibodies against basophil CD49b and FcεR1 revealed few basophils in the aortic arch intima and their numbers did not differ between Apoe<sup>−/−</sup>Ige<sup>−/−</sup> and Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice (Figure IV in the online-only Data Supplement). FACS analysis using basophil activation marker CD63<sup>30</sup> and CD220R3 demonstrated that IgE deficiency significantly reduced the numbers of activated basophils in blood but not those in the peritoneal cavity (Figure IVB and IVC in the online-only Data Supplement). Therefore, mast cells and possibly basophils may contribute negligibly to reduced atherosclerosis in Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice.

Importantly, immunofluorescent double staining found that IgE deficiency significantly decreased atherosclerotic lesion intima iNOS-Mac-2<sup>+</sup> M1 macrophage contents (Figure 3A) but increase intima Arg-1<sup>+</sup>Mac-2<sup>+</sup> M2 macrophage contents (Figure 3B) in aortic arches. Consistent with these observations, the mRNA levels of macrophage marker CD68, M1 macrophage markers MCP-1, TNF-α, IL-6, and iNOS (inducible nitric oxide synthase) were decreased and the mRNA levels of M2 macrophage...
markers Arg-1 and IL-10 were increased (Figure 3C) in thoracic-abdominal aortas from IgE-deficient mice. We obtained the same observations from macrophages harvested from the peritoneum of Apoe<sup>−/−</sup>Ige<sup>−/−</sup> and Apoe<sup>−/−</sup>Ige<sup>+/+</sup> mice that had consumed an atherogenic diet for 12 weeks. Peritoneal macrophages from Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice showed significantly reduced CD68 and M1 markers MCP-1, TNF-α, and iNOS but increased M2 markers Arg-1 and IL-10, although the IL-6 mRNA level did not differ from that of Apoe<sup>−/−</sup>Ige<sup>+/+</sup> mice (Figure 3D).
Figure 2. IgE deficiency reduces aortic arch lesion inflammation in Apoe<sup>−/−</sup> mice after 12 wk of an atherogenic diet.

Intima major histocompatibility complex class-II (A) and Mac-3-positive macrophage contents (B) were presented as positive area vs total intima area in percentage. C and D, Fluorescence-activated cell sorter (FACS) analysis of F4/80<sup>+</sup>CD11b<sup>+</sup> macrophages in peritoneal cavity and blood. E, FACS analysis of Ly6C<sup>−</sup>CD11b<sup>+</sup> and Ly6C<sup>+</sup>CD11b<sup>+</sup> monocytes in blood. F, Intima MCP (monocyte chemoattractant protein)-1 contents were presented as positive area vs total intima area in percentage. G, Plasma IL (interleukin)-1β, TNF (tumor necrosis factor)-α, IL-6, IFN (interferon)-γ, and MCP-1 levels. n=6–13 mice per group. Representative images for A–F are shown to the left. Scales: 200 μm, insets: 70 μm. All data are mean±SEM. *P<0.05, **P<0.01, and ***P<0.001.
FACS analysis using CD11b and F4/80 antibodies verified the peritoneal macrophage purity (≈75%: Figure V in the online-only Data Supplement).

**IgE Regulates Macrophage Polarization Towards an M1 Phenotype**

Reduced M1 macrophage content and increased M2 macrophage content in thoracic-abdominal aortas and in peritoneal macrophages from Apoe<sup>−/−</sup>Ige<sup>−/−</sup> mice after mice consumed an atherogenic diet for 12 weeks (Figure 3A through 3D) could be secondary to reduced atherosclerosis in these mice (Figure 1A; Figure IA through IC in the online-only Data Supplement). Alternatively, IgE activity may directly control macrophage polarization. We previously showed that FcεR1 and TLR (Toll-like receptor)-4 formed complexes. IgE failed to induce cytokine and chemokine expression and apoptosis in macrophages from FcεR1- or TLR-4-deficient mice. We recently showed that FcεR1 also formed complexes with Nhe1. IgE failed to induce macrophage foam cell formation and activate the foam cell formation-associated PI3K-AKT-mTOR signaling pathway in cells from Apoe<sup>−/−</sup>Nhe1<sup>+/−</sup> mice, suggesting that FcεR1 did not work alone. To test a direct role of IgE on macrophage polarization towards the M1 phenotypes, we added IgE to BMDM from both WT and IgE receptor-deficient FcεR1<sup>−/−</sup> and FcεR1<sup>−/−</sup>Nhe1<sup>+/−</sup> mice under M1- and M2-polarizing conditions in the presence of lipopolysaccharide or IL-4, respectively. Such increases remained in cells from FcεR1<sup>−/−</sup> mice but muted in cells from FcεR1<sup>−/−</sup>Nhe1<sup>+/−</sup> mice, although IgE still showed weak activity in enhancing TNF-α expression (Figure 4B and 4C). We obtained the same observations in macrophages cultured in the M2-polarizing media with IL-4. IgE blocked M2 macrophage polarization in BMDM from WT mice by reducing the expression of Arg-1, IL-10, and Mrc-1 (Figure 5D). IgE lost its activity in reducing Arg-1, and Mrc-1 expression from BMDM from FcεR1<sup>−/−</sup> and FcεR1<sup>−/−</sup>Nhe1<sup>+/−</sup> mice, although IgE still showed its activity in reducing IL-10 expression from these macrophages (Figure 4E and 4F).

Consistent with the RT-PCR results from WT, FcεR1<sup>−/−</sup> and FcεR1<sup>−/−</sup>Nhe1<sup>+/−</sup> mice, immunoblot analyses showed that IgE increased lipopolysaccharide-induced iNOS expression in BMDM from WT mice. Such activity of IgE lost in cells from FcεR1<sup>−/−</sup> mice. In contrast, lipopolysaccharide did not induce iNOS expression in BMDM.
Figure 4. IgE activity in macrophages polarization.

Real-time polymerase chain reaction (RT-PCR) analysis of M1 macrophage marker genes after 24 h lipopolysaccharide (LPS; 100 ng/mL) stimulation with or without IgE (50 μg/mL) in bone marrow-derived macrophage (BMDM) from wild type (WT), Fcer1α−/−, and Fcer1α−/−Nhe1+/− mice (A–C). RT-PCR analysis of M2 macrophage marker genes after 24 h IL (interleukin)-4 (10 ng/mL) stimulation with or without IgE in BMDM from WT, Fcer1α−/−, and Fcer1α−/−Nhe1+/− mice (D–F). G, Immunoblots detected the expression of iNOS (inducible nitric oxide synthase) and Mac-2 and quantification of iNOS to Mac-2 ratio after LPS stimulation or the expression of Arg (arginase)-1 and Mac-2 and quantification of Arg-1 to Mac-2 ratio after IL-4 stimulation with or without IgE in BMDM from WT, Fcer1α−/−, and Fcer1α−/−Nhe1+/− mice. Representative immunoblot images are shown to the left. RT-PCR analysis of M1 macrophage marker genes after LPS stimulation (H) and M2 macrophage marker genes after IL-4 stimulation (I) with or without IgE and anti-IgE antibody in BMDM from WT mice. Data are mean±SEM from 4 independent experiments. *P<0.05, **P<0.01, and ***P<0.001.
from *Fcer1α*−/−*Nhe1*+/− mice (Figure 4G). Similarly, IgE blocked IL-4-induced Arg-1 expression in BMDM from WT mice. IL-4 and IgE both lost their activities in affecting Arg-1 expression in BMDM from *Fcer1α*−/− and *Fcer1α*−/−*Nhe1*+/− mice (Figure 4G). To test the role of IgE on macrophage polarization further, we used 2 different concentrations of IgE neutralizing antibody to block the IgE activity on BMDM. Results showed that 50 µg/mL IgE antibody effectively blocked the IgE activity in inducing M1 macrophage polarization (Figure 4H) and reversed IgE-reduced M2 macrophage polarization (Figure 4I). Therefore, IgE may use both FcεR1 and Nhe1 to regulate macrophage polarization.

**IgE Deficiency Improves Obesity and Insulin Resistance and Increases MSRN Gene Expression**

Diabetes mellitus and obesity are significant risk factors of CHD, including increased overall plaque burden, high rates of multivessel disease, and death from vascular complications.14,15,32 Macrophages serve as an important link between these metabolic diseases and atherosclerosis.16,17,33 After 12 weeks on an atherogenic diet, *Apoe*−/−Ige−/− mice showed reduced bodyweight gain (Figure 5A) together with reduced white adipose tissue and wet liver weight (Figure 5B), compared with those of *Apoe*−/−Ige+/+ control mice, although these 2 types of mice did not show significant differences in food intake (Figure 5C). IgE deficiency also improved glucose (Figure 5D) and insulin (Figure 5E) sensitivities with significantly reduced blood insulin levels (Figure 5F). At 6 weeks of age, *Apoe*−/−Ige+/+ mice were already heavier than the *Apoe*−/−Ige−/− mice (Figure 5A). To minimize the initial weight difference, we selected mice with comparable weight and monitored their bodyweight gain under a normal laboratory diet. IgE deficiency again reduced bodyweight gain at later time points (Figure VI in the online-only Data Supplement). Therefore, IgE deficiency improved obesity and insulin sensitivity with concurrent decreases in adipose content and blood insulin levels.

Macrophage IFN-γ-regulated proatherogenic MSRN acts as an important link between metabolic diseases and atherosclerosis.16,17 MSRN genes are synergistically dysregulated in obesity, and macrophage cholesterol accumulation reduces MSRN gene expression.16 Using RT-PCR, we compared the expression of genes from this network in atherosclerotic lesion and peritoneal...
macrophages from \textit{ApoE−Ige−}\textsuperscript{−/−} and \textit{ApoE−Ige+}\textsuperscript{+/+} control mice that had consumed an atherogenic diet for 12 weeks. We found that IgE deficiency significantly increased the expression of the MSRN genes C3, Lrp1 (low-density lipoprotein-related protein 1), and LPL (lipoprotein lipase) from thoracic-abdominal aortic lesions from \textit{ApoE−Ige−}\textsuperscript{−/−} mice, compared with those from the \textit{ApoE−Ige+}\textsuperscript{+/+} control mice, although the expression of TFR (transferrin) did not differ between the groups (Figure 5G). Peritoneal macrophages yielded the same conclusion. MSRN genes C3, Lrp1, and TFR were also significantly upregulated in cells from \textit{ApoE−Ige−}\textsuperscript{−/−} mice (Figure 5H).

\textbf{IgE Reduces Macrophage IFN-γ-Regulated MSRN Gene Expression and Promotes Macrophage Foam Cell Formation}

Prior study revealed a role of obesity and diabetes mellitus in promoting atherosclerosis by dysregulating IFN-γ-regulated MSRN protein expression.\textsuperscript{17} Reduced plasma IFN-γ and increased MSRN protein expression in atherosclerotic lesions and macrophages from \textit{ApoE−Ige−}\textsuperscript{−/−} mice support a role of IgE in reducing IFN-γ-mediated macrophage MSRN protein expression and promoting foam cell formation.\textsuperscript{17} When BMDM from WT mice were stimulated with IFN-γ, IgE promoted ox-LDL uptake, and macrophage foam cell formation (Figure 6A). In contrast, when BMDM from IgE receptor-deficient \textit{Fcer1α−}\textsuperscript{−/−} \textit{Nhe1+}\textsuperscript{+/−} mice were used, IgE did not affect IFN-γ-induced macrophage foam cell formation (Figure 6B).

Consistent with the increased expression of MSRN genes in atherosclerotic lesion and macrophages from \textit{ApoE−Ige−}\textsuperscript{−/−} mice with atherosclerosis (Figure 5G and 5H), IgE enhanced the ox-LDL- and IFN-γ-induced suppression of all tested MSRN genes C3, Lrp1, TFR, and LPL in BMDM cells from WT mice (Figure 6C). IgE lost this activity when BMDM cells from \textit{ApoE−Ige−}\textsuperscript{−/−} mice were used (Figure 6D). When cells from \textit{Fcer1α−}\textsuperscript{−/−} \textit{Nhe1+}\textsuperscript{+/−} mice were used, IFN-γ and ox-LDL blunted the expression of MSRN genes C3, Lrp1, TFR, and LPL. Although we did not explore further why BMDM cells from \textit{Fcer1α−}\textsuperscript{−/−} \textit{Nhe1+}\textsuperscript{+/−} mice acted differently from those from \textit{Fcer1α−}\textsuperscript{−/−} mice, IgE showed no effect on IFN-γ and ox-LDL-suppressed TFR expression (Figure 6E). IgE antibody further confirmed the IgE activity in reducing MSRN gene expression. At 50 µg/mL, anti-IgE antibody effectively blocked IgE-induced suppression of MSRN gene expression in BMDM from WT mice (Figure 6F).

Immunoblots confirmed this IgE activity that was muted in cells from \textit{Fcer1α−} or \textit{Fcer1α−Nhe1+} mice (Figure 6G). Mechanistic studies showed that IgE enhanced ox-LDL- and IFN-γ-induced pERK and pp38 activation.\textsuperscript{5} This activity of IgE also muted in BMDM from \textit{Fcer1α−} or \textit{Fcer1α−Nhe1+} mice (Figure 6G). These observations suggest that IgE reduced MSRN gene expression by activating the MAPK signaling pathway.

Using BMDM from \textit{C3+}\textsuperscript{+/−} mice, we demonstrated a role of MSRN C3 molecule in mediating IgE activity in macrophage polarization. IgE lost its activity in increasing M1 macrophage gene expression (\textit{TNF-α}, \textit{iNOS}, and \textit{Mcp-1}) and in reducing M2 macrophage gene expression (\textit{Arg-1}, IL-10, and \textit{Mrc-1}; Figure 6H and 6I) and IgE, ox-LDL, and IFN-γ lost their activity in pERK and pp38 activation in BMDM from \textit{C3+}\textsuperscript{−/−} mice (Figure 6J).

\textbf{DISCUSSION}

Clinical and epidemiological studies link obesity and diabetes mellitus to cardiovascular diseases. Deterioration of one condition is often followed by the others.\textsuperscript{14,15,34} Patients with chronic heart failure commonly have diabetes mellitus or prediabetes mellitus,\textsuperscript{35} and similarly, patients with diabetes mellitus are prone to develop heart failure. Hyperglycemia is an important risk factor of heart failure.\textsuperscript{36} Our prior studies used the \textit{Fcer1α−}\textsuperscript{−/−} mice and indirectly suggested a role of IgE in atherosclerosis.\textsuperscript{6} Here, we used IgE-deficient mice and established a direct role of IgE in atherosclerosis, obesity, and diabetes mellitus. Mechanistic studies revealed a role of IgE in macrophage polarization and foam cell formation by regulating the expression of the IFN-γ-controlled MSRN genes.

Macrophages are among the major inflammatory cells in atherosclerotic lesions. Macrophage foam cell formation leads to atherogenesis.\textsuperscript{11} Consistent with our previous finding,\textsuperscript{2} lack of IgE decreased macrophage inflammation, including reduced atherosclerotic lesion and peritoneal cavity macrophage accumulation, as well as decreased blood Lymphocytes\textsuperscript{6} and \textit{Ly6C}\textsuperscript{−} monocytes, likely through a decrease in expression of chemokine MCP-1. A decrease of plasma IFN-γ and IL-6 levels in \textit{ApoE−Ige−}\textsuperscript{−/−} mice after mice consuming an atherogenic diet for 12 weeks suggests a role of IgE in promoting systemic inflammation. As a result, macrophages in atherosclerotic lesion from \textit{ApoE−Ige−}\textsuperscript{−/−} mice exhibited phenotypic changes from M1 to M2 phenotypes. This activity of IgE depended on the expression of macrophage Fcer1R1 and \textit{Nhe1}, likely via the activation of TLR-4 and downstream NF-κB (nuclear factor-κB) and JNK pathways as we previously described.\textsuperscript{6} Although IgE plays a central role on the activation of mast cells and basophils that are rich in Fcer1R1 expression, the number and activation status of these innate immune cells in aortic lesions did not differ between \textit{ApoE−Ige−}\textsuperscript{−/−} and \textit{ApoE−Ige+}\textsuperscript{+/+} mice. IgE deficiency decreased peritoneal mast cell and blood activated basophils but did not affect plasma histamine level and β-hexosaminidase activity. Therefore, we concluded that IgE-mediated activation of mast cells and basophils played a moderate role, but IgE actions on macrophages contributed importantly to diet-induced atherosclerosis.

In this study, we used BMDM from WT, \textit{Fcer1α−}, and \textit{Fcer1α−Nhe1+} mice and demonstrated a role of IgE in promoting lipopolysaccharide-induced iNOS, TNF-α,
It is known that the expression of iNOS and Arg-1 and the expression of IL-10 and TNF-α use different signaling pathways. Macrophage expression of iNOS and Arg-1 uses the JNK/AP1 pathway and the protein kinase C pathway. Here, we demonstrated that the MAPK pathway activation was decreased in BMDM from both Fcer1α–/– and Fcer1α–/–Nhe1+/– mice (Figure 6G). Our earlier studies reported an interaction between FcεR1 and Mcp-1 expression and reducing IL-4-induced Arg-1, IL-10, and Mrc (macrophage mannose receptor)-1 expression in macrophages from WT. IgE activity in lipopolysaccharide- and IL-4-regulated iNOS, Mcp-1, Arg-1, and Mrc-1 expression was completely muted in cells from Fcer1α–/– mice but not in those from Fcer1α–/– mice. Yet, IgE remained effective in promoting TNF-α expression and reducing IL-10 expression in BMDM from Fcer1α–/–Nhe1+/– mice (Figure 4C and 4F). Although we currently do not have an explanation to these observations, it is known that the expression of iNOS and Arg-1 and the expression of IL-10 and TNF-α use different signaling pathways. Macrophage expression of iNOS and Arg-1 uses the JNK/AP1 pathway whereas the expression of IL-10 and TNF-α is regulated by the MyD88-mediated activation of the MAPK pathway and the protein kinase C pathway. Here, we demonstrated that the MAPK pathway activation was decreased in BMDM from both Fcer1α–/– mice Fcer1α–/–Nhe1+/– mice (Figure 6G). Our earlier studies reported an interaction between FcεR1 and Mcp-1 expression and reducing IL-4-induced Arg-1, IL-10, and Mrc (macrophage mannose receptor)-1 expression in macrophages from WT. IgE activity in lipopolysaccharide- and IL-4-regulated iNOS, Mcp-1, Arg-1, and Mrc-1 expression was completely muted in cells from Fcer1α–/– mice but not in those from Fcer1α–/– mice. Yet, IgE remained effective in promoting TNF-α expression and reducing IL-10 expression in BMDM from Fcer1α–/–Nhe1+/– mice (Figure 4C and 4F). Although we currently do not have an explanation to these observations, it is known that the expression of iNOS and Arg-1 and the expression of IL-10 and TNF-α use different signaling pathways. Macrophage expression of iNOS and Arg-1 uses the JNK/AP1 pathway whereas the expression of IL-10 and TNF-α is regulated by the MyD88-mediated activation of the MAPK pathway and the protein kinase C pathway. Here, we demonstrated that the MAPK pathway activation was decreased in BMDM from both Fcer1α–/– mice Fcer1α–/–Nhe1+/– mice (Figure 6G). Our earlier studies reported an interaction between FcεR1 and Mcp-1 expression and reducing IL-4-induced Arg-1, IL-10, and Mrc (macrophage mannose receptor)-1 expression in macrophages from WT. IgE activity in lipopolysaccharide- and IL-4-regulated iNOS, Mcp-1, Arg-1, and Mrc-1 expression was completely muted in cells from Fcer1α–/– mice but not in those from Fcer1α–/– mice. Yet, IgE remained effective in promoting TNF-α expression and reducing IL-10 expression in BMDM from Fcer1α–/–Nhe1+/– mice (Figure 4C and 4F). Although we currently do not have an explanation to these observations, it is known that the expression of iNOS and Arg-1 and the expression of IL-10 and TNF-α use different signaling pathways. Macrophage expression of iNOS and Arg-1 uses the JNK/AP1 pathway whereas the expression of IL-10 and TNF-α is regulated by the MyD88-mediated activation of the MAPK pathway and the protein kinase C pathway. Here, we demonstrated that the MAPK pathway activation was decreased in BMDM from both Fcer1α–/– mice Fcer1α–/–Nhe1+/– mice (Figure 6G). Our earlier studies reported an interaction between FcεR1
It is possible that the protein kinase C pathway remained active in macrophages from FcεR1α−/−Nhe1+/− mice. Protein kinase C activation may stimulate the activity of remaining Nhe1 in these cells41 that mediated the IgE activity,31 a hypotheses that merits further investigation. Nevertheless, in macrophages from WT mice, anti-IgE antibody effectively blocked IgE-induced TNF-α expression and IgE-suppressed IL-10 expression (Figure 4H and 4I). FcεR1 deficiency blocked IgE activity in reducing the expression of MSRN genes C3, Lrp1, TFR, and LPL and downstream signaling (pERK and pp38; Figure 6D through 6G). These results suggest that FcεR1 plays a predominant role in response to IgE. IgE regulates MSRN gene expression using FcεR1. IgE may use both FcεR1 and Nhe1 or additional receptors such as TLR-4 to regulate macrophages polarization.

Macrophage foam cell formation contributes critically to the development of advanced atherosclerotic plaques.13 Cholesteryl ester loading promotes foam cell formation and atherogenesis through the MSRN.16 Diet-induced obesity and insulin resistance produce IFN-γ from inflammatory cells to modulate the MSRN and link this network to atherogenesis.17 In this study, we revealed a direct role of IgE in controlling the macrophage expression of the MSRN genes C3, LRP-1, LPL, and TRF, as a mechanism of IgE activity in macrophage foam cell formation. Consistent with the increased expression of these MSRN genes in atherosclerotic lesions from Apoε−/−Ige−/− mice, macrophages from these mice also demonstrated increased expression of these MSRN genes. This study first linked the IgE activity to IFN-γ-regulated MSRN gene expression reduction and foam cell formation. Yet, this study did not tell whether diet-induced obesity and insulin resistance dysregulated the expression of IFN-γ-regulated MSRN genes that affected atherogenesis on the other way around. It is possible that the development of atherosclerosis increased systemic IFN-γ release and suppressed the same sets of MSRN genes, thereby promoting the development of obesity and insulin resistance, a hypothesis that has not been tested.

Together, this study revealed a detrimental role of IgE in atherosclerosis, obesity, and diabetes mellitus by polarizing macrophages towards a proinflammatory M1 phenotype and dysregulating the macrophage sterol response. These findings not only reinforce the role of IgE in atherosclerosis development but also propose a possibility that IgE links CHD to metabolic diseases via the MSRN and explains why patients with obesity and diabetes mellitus are prone to develop CHD or vice versa. Inhibition of IgE activity may become an attractive therapeutic strategy to treat atherosclerosis, obesity, and diabetes mellitus.

**REFERENCES**


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**Disclosures**

None.

Dietary cholesterol is essential to mast cell activation and associated obesity and diabetes in mice. Clin Biochem. 2004.03.008. doi: 10.1016/j.clinbiochem.2004.03.008


A macrophage sterol-responsive network linked to atherogenesis. J Clin Invest 2017;130:1785–1794. doi: 10.1172/JCI87306


March 2020
Arterioscler Thromb Vasc Biol. 2020;40:00–00. DOI: 10.1161/ATVBAHA.119.313744