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IRF5 Deficiency Ameliorates Lupus but Promotes Atherosclerosis and Metabolic Dysfunction in a Mouse Model of Lupus-Associated Atherosclerosis

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Premature atherosclerosis is a severe complication of lupus and other systemic autoimmune disorders. Gain-of-function polymorphisms in IFN regulatory factor 5 (IRF5) are associated with an increased risk of developing lupus, and IRF5 deficiency in lupus mouse models ameliorates disease. However, whether IRF5 deficiency also protects against atherosclerosis development in lupus is not known. In this study, we addressed this question using the gld.apoE−/− mouse model. IRF5 deficiency markedly reduced lupus disease severity. Unexpectedly, despite the reduction in systemic immune activation, IRF5-deficient mice developed increased atherosclerosis and also exhibited metabolic dysregulation characterized by hyperlipidemia, increased adiposity, and insulin resistance. Levels of the atheroprotective cytokine IL-10 were reduced in aortae of IRF5-deficient mice, and in vitro studies demonstrated that IRF5 is required for IL-10 production downstream of TLR7 and TLR9 signaling in multiple immune cell types. Chimera studies showed that IRF5 deficiency in bone marrow–derived cells prevents lupus development and contributes in part to the increased atherosclerosis. Notably, IRF5 deficiency in non–bone marrow–derived cells also contributes to the increased atherosclerosis through the generation of hyperlipidemia and increased adiposity. Together, our results reveal a protective role for IRF5 in lupus-associated atherosclerosis that is mediated through the effects of IRF5 in both immune and nonimmune cells. These findings have implications for the proposed targeting of IRF5 in the treatment of autoimmune disease as global IRF5 inhibition may exacerbate cardiovascular disease in these patients. The Journal of Immunology, 2015, 194: 1467–1479.

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ystemic lupus erythematosus (SLE) is an autoimmune disease that is characterized by the loss of tolerance to nuclear self Ags, the production of pathogenic autoantibodies, and inflammation with damage to multiple organ systems (1). Premature atherosclerosis is a severe complication of SLE leading to appreciable morbidity and mortality from cardiovascular disease (2–4). Traditional (Framingham) risk factors contribute to atherosclerosis in SLE but do not explain the majority of the increased cardiovascular risk. After controlling for traditional risk factors, patients with SLE still have a substantial increased risk of cardiovascular disease (5). This has led to the concept that the autoimmune disease itself may be the strongest risk factor for cardiovascular disease in SLE. A number of previous studies have identified possible mechanisms by which autoimmunity contributes to premature atherosclerosis (reviewed in Refs. 2, 4, 6). For example, it has been clearly demonstrated that immune system dysregulation and activation accelerates atherosclerosis in lupus-prone mice, with increased CD4+ T cell and macrophage activation, type I IFN, and impaired apoptotic cell clearance all involved in atherosclerosis pathogenesis (7–10). Thus, the chronic inflammation associated with SLE may be one important factor contributing to premature atherosclerosis in SLE, perhaps amplifying the chronic inflammation that underlies atherosclerosis pathogenesis in the general population (2–4, 11). Premature atherosclerosis is also a feature of other systemic autoimmune diseases including rheumatoid arthritis, vasculitis, and psoriasis (12, 13).

Gain-of-function polymorphisms in the transcription factor IFN regulatory factor 5 (IRF5) are strongly associated in human genetic studies with an increased risk of developing a number of autoimmune diseases including SLE, scleroderma, Sjögren’s syndrome, rheumatoid arthritis, and inflammatory bowel disease (14, 15). IRF5 was originally identified as a transcription factor involved in the induction of type I IFNs and proinflammatory cytokines by viral infection (16, 17) and subsequently was shown to play an important role in proinflammatory cytokine production following TLR signaling (18, 19). IRF5 also participates in signaling downstream of nucleotide-binding oligomerization domain 2 and dectin-1 (20, 21) and in apoptotic pathways induced by viral infection, DNA damage, Fas ligand (FasL), or TRAIL (22–24).
IRF5 promotes Th1/Th17-type immune responses and LPS-induced M1 macrophage polarization (25, 26). IRF5 deficiency ameliorates disease in a number of murine lupus models (27–32). For these reasons, IRF5 is being considered as a potential therapeutic target in SLE and other autoimmune diseases as well as in other inflammatory conditions such as postmyocardial infarction healing (14, 33). Thus, it is necessary to understand the role of IRF5 in atherosclerosis pathogenesis in the context of SLE to predict how such proposed IRF5 inhibitor treatment for SLE might impact the premature atherosclerosis that occurs in this disease.

We previously developed the gld.apoE−/− mouse model and have used it to study the interaction between atherosclerosis and SLE (7, 34, 35). The gld mutation is a point mutation in the gene encoding murine FasL and leads to the development of a mild form of spontaneous autoimmune disease on the C57BL/6 genetic background characterized by the production of low levels of autoantibodies and mild splenomegaly, lymphopenopathy, and glomerulonephritis (36–38). Apolipoprotein E–deficient (apoE−/−) mice develop hypercholesterolemia and atherosclerosis (39). The combination of the gld mutation and apoE deficiency in gld.apoE−/− mice results in more severe autoimmunity than is seen with the gld mutation alone and more severe atherosclerosis than is seen with apoE deficiency alone (7).

In this report, we used the gld.apoE−/− mouse model to determine the role of IRF5 in lupus-associated atherosclerosis. We hypothesized that IRF5 deficiency would protect against atherosclerosis development by ameliorating lupus autoimmunity, thereby reducing the chronic inflammation associated with the autoimmune process. However, we found that despite markedly reducing lupus disease severity, IRF5 deficiency unexpectedly led to an increase in atherosclerosis. Furthermore, the studies revealed an unanticipated role for IRF5 in maintaining metabolic homeostasis in that IRF5 deficiency resulted in hyperlipidemia, insulin resistance with hyperglycemia, hepatic steatosis, and increased adiposity.

### Materials and Methods

#### Mice

Irf5−/− mice backcrossed eight generations to C57BL/6 were obtained from T. Taniguchi (University of Tokyo, Tokyo, Japan) with permission from T. Mak (University of Toronto, Toronto, Ontario, Canada) (18). Irf5−/− mice were backcrossed three more generations to C57BL/6 and then intercrossed with FastR3apoE−/− mice (referred to as gld.apoE−/− in this manuscript) on the C57BL/6 background (7) to generate IRF5 heterozygous (Irf5+/−) gld.apoE−/− mice. Experimental mice were generated by breeding Irf5+/− gld.apoE−/− mice to obtain Irf5+/+, Irf5−/−, and Irf5+/− gld.apoE−/− litters. All mice used for breeding were screened for the Dock2 mutation (40, 41) and found to only have wild-type (WT) Dock2. Unless otherwise stated, we combined the data obtained from male and female littersmates, as we observed a similar qualitative effect of IRF5 deficiency in both sexes. This could not be done in the case of body weight (Fig. 3) given the intrinsic differences in body weight between the sexes. Animal protocols were approved by the Institutional Animal Care and Use Committee at Boston University.

#### Western diet administration and measurement of food consumption

Western diet containing cholesterol (0.2% total cholesterol), total fat (21% by weight; 42% kcal from fat), saturated fatty acids (>60% of total fatty acids), and high sucrose (34% by weight) was purchased from Harlan Teklad (catalog number 88137). Irf5+/− gld.apoE−/−, Irf5−/− gld.apoE−/−, and Irf5−/− gld.apoE−/− littermate mice were placed on a Western diet at 7 wk of age and weighed weekly for 12 wk. Littermate groups were separated into individual cages at the beginning of the 11th week on the Western diet, and food consumption by each individual mouse was measured over a period of 7 d to determine daily food consumption.

### Bone marrow chimera studies

At 7 wk of age, male Irf5+/− gld.apoE−/− and Irf5−/− gld.apoE−/− bone marrow recipients were x-ray irradiated with two doses of 550 rad spaced 3 h apart. Bone marrow was harvested from age-matched male Irf5+/− gld.apoE−/− and Irf5−/− gld.apoE−/− donor mice, and 5 × 105 bone marrow cells were administered to recipients via tail vein injection. Bone marrow recipients were given sulfamethoxazole-trimethoprim for 3 wk and thereafter placed on Western diet for 6–9 wk and euthanized at 16–19 wk of age.

#### PCR

The PCRs used to genotype gld, apoE−/−, the Dock2 mutant, and Irf5−/− mice were described previously (7, 27, 41).

#### Serological assays

Anti-nuclear autoantibody (ANA) titer was measured by immunofluorescence using slides coated with permeabilized human epithelial cells (HeP2-coated slides; INOVA Diagnostics, San Diego, CA). Serially diluted mouse serum was incubated on HeP2 slides, washed, and incubated with Alexa Fluor 568–conjugated goat anti-mouse IgG (1:100; Invitrogen). HeP2 slides were then washed, mounted, and visualized as described below in the Microscopy section. The ANA titer is taken as the last positive serum dilution. Serum triglyceride, total cholesterol, phospholipids, and nonesterified free fatty acids were measured by the Lipid Research Laboratory at the Metabolic Phenotyping Center, University of Cincinnati (U24 DK059630). Lipoprotein profiles were measured using fast-performance liquid chromatography by the Mouse Metabolic Phenotyping Center, University of Cincinnati (U24 DK059630). Serum leptin, glucose, insulin, and cytokine levels were measured by the Analytical Core at the Mouse Metabolic Phenotyping Center at the University of Massachusetts Medical Center. IL-10 in culture supernatant was measured by ELISA using the mouse BD OptEIA Mouse IL-10 ELISA set according to the manufacturer’s instructions (BD Biosciences, San Jose, CA).

#### Histology

Kidney sections were stained with H&E and evaluated in a blinded manner. Randomly selected areas of cortex were imaged as described below in the microscopy section. Fifty glomeruli from each animal were examined to determine the percentage of glomeruli with severe lesions (defined as glomerular crescents or areas of necrosis), mild lesions (defined as mesangial expansion), or no lesions. Crescents were identified by the presence of more than three palisading layers of cells in the extracapillary space, and fibrinoid necrosis was identified by its classic eosinophilic and acellular appearance. Mesangial expansion was identified by prominence of the mesangial matrix as compared with mesangial matrix in normal glomeruli. Liver was fixed in 4% paraformaldehyde, paraffin-embedded, cut into 8-μm sections, and stained with H&E. Severity of liver steatosis was quantified on coded samples by assessing visually the amount of lipid droplets present and scored on a scale of 0–3, with 0 being no lipid droplets and 3 being the largest amount of lipid droplets. Perigonadal fat pads were fixed in 4% formalin and stored in 1× PBS. Samples were sent to the Dana-Farber/Harvard Cancer Center Pathology Core for embedding, sectioning, and H&E staining.

#### Microscopy

Fixed and stained tissue sections were viewed using a Nikon Optiphot epifluorescent microscope and digitally photographed using an RT color spot camera (Diagnostic Instruments, Sterling Heights, MI) with Spot Advanced software version 4.0.9 (Diagnostic Instruments) except for stained aortic arches, which were viewed using an Olympus SZX16 microscope and digitally photographed using an Olympus Digital DP72 Camera (Olympus, Tokyo, Japan) with CellSens Standard software version 1.11 (Olympus). Liver and aortic root sections were imaged at original magnification ×4. Liver and adipose tissue sections were imaged at ×10. Kidney sections and ANA were imaged at ×20.

#### Analysis of atherosclerosis

For aortic root analysis, the apex of the heart was cut at a 45° angle, and the cut side of the heart was placed on the bottom of a plastic cartridge filled with OCT. Correct placement of the cut heart was carried out so that the aortic root was parallel to the bottom of the plastic cartridge. The aortic arch was cut at the point at which it joins the heart. Hearts were snap-frozen in OCT in ice-cold methylbutylene and dry ice. The 8-μm serial aortic root sections were cut, and slides were fixed in 4% paraformaldehyde. The 24–30 aortic root sections per mouse heart were stained with Oil

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Red O and hematoxylin and photographed. In a blinded manner, the average area of aortic lesions was quantified using Adobe Photoshop CS3 (Adobe Systems).

For aortic arch analysis, the aortic arch from each mouse was fixed in 4% paraformaldehyde, cut longitudinally, pinned, and stained with Oil Red O to detect lipid deposition. Aortic arches were photographed, and in a blinded manner, the area of the total aortic arch and area stained positive for Oil Red O was quantified using the Adobe Photoshop CS3 color range tool (Adobe Systems). The data are reported as average percentage of total aortic arch stained positive for Oil Red O in each experimental group.

Foam cell formation assay
Sera were collected from Irf5+/+ gld.apoE−/− and Irf5−/− gld.apoE−/− mice that had been on a Western diet for 12 wk. Peritoneal macrophages from C57BL/6 mice were seeded in tissue culture dishes in complete RPMI 1640 medium (10% FBS, 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin) in 12-well flat-bottom plates and allowed to rest for 4–6 h. Peritoneal macrophages were then treated with 5% FBS, 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin in complete RPMI 1640 supplemented with 7.5% conditioned medium from B16 cells transfected with Flt3L. The B16 cells were originally made by Dr. C. Yang and provided by Dr. U. Von Andrian (Harvard Medical School). The cells were used for experiments after 8 d, at which time 3 × 105 Flt3L-DCs were seeded in 96-well round-bottom plates and cultured in complete RPMI 1640 with the relevant stimuli in a total well volume of 200 μl.

To obtain B cells, spleen cells were incubated with anti–B220–biotin (BD Biosciences) for 15 min after treatment with RBC lysing buffer. The cells were washed with flow cytometry buffer (3% FBS and 2 mM EDTA in PBS), incubated with streptavidin-magnetic particles (BD Biosciences) for 30 min, and then purified using iMagnet (BD Biosciences). Four hundred thousand cells per well were seeded in 96-well flat-bottom plates.

In vitro immune cell activation
M-CSF macrophages, GM-CSF macrophages, GM-CSF DCs, Flt3L DCs, and splenic B cells from C57BL/6 WT and Irf5−/− mice were stimulated, or not, with the TLR2 ligand Pam3CSk (at 100–1000 ng/ml), the TLR3 ligand polyinosinic-polycytidylic acid (10 μg/ml), the TLR4 ligand LPS (100 ng/ml), the TLR7 ligands R848 (100 ng/ml) or R837 (0.3–1 μg/ml), and the TLR9 ligands unmethylated cytosine-guanine–rich DNA-B (CpG-B at 100 ng/ml) or CpG-A (100 ng/ml) (all from Invivogen). Supermatants were collected after 24 h, or after 5 d in the case of Flt3L DCs, and IL-10 was determined using the Adobe Photoshop CS3 color range tool.

RNA isolation
Aortic arches and the SVF pellets isolated from perigonadal fat pads were snap frozen and stored at −80°C. Upon thawing, tissue or cell pellets were immediately resuspended in QIAzol (Qiagen) and lysed using the TissueLyser (Qiagen) or homogenized by vortexing. RNA was then isolated using either the RNAeasy Fibrissy Tissue Mini Kit for aortic arch or the RNAeasy Mini Kit for SVF using试剂-free water (Qiagen). RNA yield was measured using a Nanodrop 2000 Spectrophotometer (Thermo Scientific).

Real-time PCR
RNA from the SVF of perigonadal fat and aortic arch was treated with DNase and reverse transcribed into cDNA using Thermoscript reverse transcriptase (Invitrogen). cDNA was diluted 1:100, and PCR was performed using TaqMan primers specific for murine Irf5, IL-10, IL-6, or Tnfa. Data were analyzed using the 2−ΔΔCT method and expressed as fold change (expression relative to housekeeping gene and normalized to reference sample).

Statistical analysis
For normally distributed data, groups were compared using either Student t test or ANOVA. In cases in which ANOVA was statistically significant, post hoc comparison between groups was performed by Student t test with Bonferroni correction for multiple comparisons. For data that were not normally distributed, the Mann–Whitney U test or one-way ANOVA on ranks was used. All statistics were performed using GraphPad Instat or SigmaPlot 12.5 (Systat, San Jose, CA), with p < 0.05 considered as statistically significant.

Results
Irf5 deficiency reduces autoimmune manifestations in gld. apoE−/− mice
We mated Irf5 heterozygous (Irf5+/−) gld.apoE−/− mice to generate Irf5+/− gld.apoE−/−, Irf5−/− gld.apoE−/−, and Irf5−/− gld.apoE−/− littermates and analyzed disease parameters in these experimental groups after 12 wk on a Western diet. All experiments were done with littermates to minimize any possible effects due to differences
in gut microbiota, as the microbiome has recently been shown to substantially modify both autoimmune and metabolic responses (45–48).

As in other murine lupus models, autoimmune disease in the gld.aapoE−/− model is characterized by splenomegaly, lymphadenopathy, ANA production, and kidney disease (7). Irf5+/+.gld.aapoE−/− mice had splenomegaly and lymphadenopathy (Fig. 1A, 1B), elevated serum ANA titers (Fig. 1C), and renal disease (Fig. 1D, 1E). These features of lupus autoimmunity were all markedly reduced in Irf5−/+.gld.aapoE−/− littermates. In addition, serum levels of the proinflammatory cytokines IL-12p70, IFN-γ, and TNF-α were lower in the Irf5−/+.gld.aapoE−/− mice (Fig. 1F). Notably, Irf5−/+.gld.aapoE−/− mice also exhibited markedly reduced disease manifestations, indicating that loss of even a single allele of Irf5 is sufficient to protect against the development of autoimmunity. These data demonstrate that Irf5 is required for lupus disease pathogenesis in the gld.aapoE−/− model.

Irf5 deficiency promotes atherosclerosis and hyperlipidemia in gld.aapoE−/− mice

To determine the role of Irf5 in atherosclerosis development, we evaluated atherosclerosis lesions using Oil Red O staining. Unexpectedly, Irf5−/+.gld.aapoE−/− mice had larger lesions in the aortic root and more extensive lesions in the aortic arch than Irf5+/+.gld.aapoE−/− littermates, both indicative of more severe atherosclerosis (Fig. 2A–C). As this result was the opposite of that predicted, we evaluated atherosclerosis lesions using Oil Red O staining. Unexpectedly, Irf5−/+.gld.aapoE−/− mice had larger lesions in the aortic root and more extensive lesions in the aortic arch than Irf5+/+.gld.aapoE−/− littermates, both indicative of more severe atherosclerosis (Fig. 2A–C). As this result was the opposite of that predicted, we sought to identify possible mechanisms to explain this finding. We noted that the sera of the Irf5−/+.gld.aapoE−/− mice were more turbid than the sera of the Irf5+/+.gld.aapoE−/− littermates, suggesting the presence of hyperlipidemia (Fig. 2D). This was confirmed with serum lipid measurements demonstrating increased serum levels of triglycerides, cholesterol, nonesterified fatty acids, and phospholipids in the Irf5−/+.gld.aapoE−/− mice (Fig. 2E). Additional analysis showed increased levels of very-low-density lipoprotein and decreased levels of high-density lipoprotein in the Irf5−/+.gld.aapoE−/− mice consistent with an atherogenic profile (Fig. 2F). To further evaluate the atherogenic capacity of the sera, we performed in vitro assays of foam cell generation. Foam cells form after macrophage uptake of lipoproteins in the subendothelium of atherosclerotic lesions and are thought to play a central role in atherosclerosis pathogenesis (49, 50). Sera from the Irf5−/+.gld.aapoE−/− mice were much more effective in inducing foam cell formation from C57BL/6 WT peritoneal macrophages than sera from the Irf5+/+.gld.aapoE−/− mice (Fig. 2G). These data indicate that Irf5 deficiency promotes the development of atherosclerosis in gld.aapoE−/− mice and that this might be due, at least in part, to an increase in the degree of hyperlipidemia.

Irf5 deficiency is associated with increased adipose tissue mass and inflammation in gld.aapoE−/− mice

To determine whether Irf5 exerts other effects on metabolism, we next evaluated food intake, weight gain, and adipose tissue mass. There was no difference in food intake or weight gain between Irf5-sufficient and Irf5-deficient gld.aapoE−/− littermates (Fig. 3A, 3B). However, despite the lack of difference in total body weight, Irf5−/+.gld.aapoE−/− mice had increased amounts of perigonadal and s.c. white fat (Fig. 3C). Adipocyte size was also

![FIGURE 1](http://www.jimmunol.org/DownloadedFrom)

**FIGURE 1.** Irf5 deficiency reduces lupus disease severity. Male and female gld.aapoE−/− mice were euthanized at 19 wk of age after 12 wk on a Western diet. Spleen (A) and lymph node (B) weights from male and female Irf5+/+ (n = 27), Irf5−/− (n = 28), and Irf5−/− (n = 24) gld.aapoE−/− littermate mice were measured. (C) ANA titers in sera from Irf5+/+ (n = 13), Irf5−/− (n = 11), and Irf5−/− (n = 9) gld.aapoE−/− littermate mice were measured by immunofluorescence on Hep2 cells. Representative ANA immunofluorescence staining with sera from Irf5+/+ and Irf5−/− gld. aapoE−/− mice at a dilution of 1:100 is shown in the bottom panels at original magnification ×20. Glomerular disease score (D) and the average percentage of glomeruli per kidney that are necrotic or crescentic (E) was measured in kidney sections from Irf5+/+ (n = 15), Irf5−/− (n = 9), and Irf5−/− (n = 15) gld. aapoE−/− littermate mice. (F) Serum IL-12p70, TNF-α, IFN-γ, and IL-6 cytokine levels from Irf5+/+ (n = 9) and Irf5−/− (n = 9) gld.aapoE−/− littermate mice were measured by Luminex. Error bars represent the mean ± SEM. Two-way ANOVA (A–E) and Mann–Whitney U test (F). *p < 0.05, **p < 0.01, ***p < 0.001.
FIGURE 2. IRF5-deficient gld.aapoE−/− mice develop more severe atherosclerosis and increased hyperlipidemia. (A–F) Male and female gld.aapoE−/− mice were euthanized at 19 wk of age after 12 wk on Western diet and samples collected for analysis. (A) Serial aortic root sections from Irf5+/+ (n = 12), Irf5+/− (n = 8), and Irf5−/− (n = 14) gld.aapoE−/− littermate mice were stained with Oil Red O to detect lipid deposition in atherosclerotic lesions, and the lesion area was quantified. **Left panel,** Average aortic root lesion size. **Right panel,** Aortic root lesion size (fold change) in Irf5+/+ and Irf5−/− gld.aapoE−/− littermate mice relative to the Irf5+/+ gld.aapoE−/− littermate control. (B) Left panel, Percentage of aortic arch stained positive for Oil Red O from Irf5+/+ (n = 6), Irf5+/− (n = 6), and Irf5−/− (n = 7) gld.aapoE−/− littermate mice. **Right panel,** The y-axis represents the fold change of percentage of aortic arch stained positive for Oil Red O in Irf5+/+ and Irf5−/− gld.aapoE−/− littermate mice relative to the Irf5+/+ gld.aapoE−/− littermate control. (C) Representative images of Oil Red O–stained aortic roots from Irf5+/+, Irf5+/−, and Irf5−/− gld.aapoE−/− mice, with arrowheads designating atherosclerotic lesions. An Oil Red O–stained aortic root from a C57BL/6 mouse not on Western diet is shown as a reference. Original magnification ×4. (D) Representative images of sera from Irf5+/+ and Irf5−/− gld.aapoE−/− littermates. (E) Levels of triglycerides, total cholesterol, nonesterified fatty acids, and... (Figure legend continues)
increased (Fig. 3D). Serum leptin levels correlate with the mass of white adipose tissue (51) and were accordingly increased in the Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice (Fig. 3E). To validate these findings, nuclear magnetic resonance imaging was performed on a separate cohort of littermates and revealed a >2-fold increase in fat mass in the Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice (Fig. 3F, 3G). This was accompanied by a corresponding decrease in lean body mass in the Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice.

Obesity is frequently accompanied by adipose tissue inflammation that is thought to play a role in atherosclerosis pathogenesis (52). To determine whether the increased adiposity was associated with increased adipose tissue inflammation, we fractionated adipose tissue into adipocytes and the nonadipocyte SVF and measured macrophage infiltration and proinflammatory cytokine production in the SVF. Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice had an increased percentage of macrophages in the SVF compared with Irf5\(^{+/+}\).gld.apoE\(^{-/-}\) mice (Fig. 3H, 3I) and also increased expression of TNF-α with a trend toward an increase in IL-6 (Fig. 3J). This, IRF5 deficiency results in both increased adipose tissue mass and increased adipose tissue inflammation in gld.apoE\(^{-/-}\) mice. These effects of IRF5 deficiency on adipose tissue were not due to autoimmunity or inflammation-induced cachexia in the Irf5\(^{+/+}\).gld.apoE\(^{-/-}\) mice, as there was no difference in body weight between the experimental groups (Fig. 3B), and Irf5\(^{+/+}\).gld.apoE\(^{-/-}\) mice actually had a greater lean body mass than the Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice (Fig. 3F, 3G).

IRF5 deficiency leads to insulin resistance and hepatic steatosis in gld.apoE\(^{-/-}\) mice

As increased fat mass and adipose tissue inflammation are linked to the development of diabetes, we measured glucose levels in serum collected at the time of euthanasia. This showed that the Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice had substantially elevated nonfasting serum glucose levels, whereas those in the Irf5\(^{+/+}\).gld.apoE\(^{-/-}\) mice were normal (Fig. 4A). However, serum insulin levels were similar in the various experimental groups, suggesting that the Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice might be insulin-resistant (Fig. 4B). To test this possibility, glucose and insulin tolerance tests were performed on additional littermate cohorts, and these demonstrated that the Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice were both glucose intolerant and insulin resistant (Fig. 4C, 4D).

Hepatic steatosis can be a direct result of obesity-induced insulin resistance and is also often associated with hypertriglycerideremia and hypercholesterolemia (53, 54). Liver histology revealed marked accumulation of lipid within hepatocytes of Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice as compared with Irf5\(^{+/+}\).gld.apoE\(^{-/-}\) mice, consistent with hepatic steatosis (Fig. 4E, 4F). Taken together with the increased hyperlipidemia, these data demonstrate that IRF5 deficiency causes dysregulation of metabolic homeostasis in gld.apoE\(^{-/-}\) mice, resulting in features of metabolic syndrome.

IRF5 heterozygote gld.apoE\(^{-/-}\) mice also exhibit metabolic dysregulation

Deficiency of a single allele of Irf5 was sufficient to induce metabolic dysregulation. As compared with Irf5\(^{+/+}\).gld.apoE\(^{-/-}\) mice, Irf5\(^{+/+}\).gld.apoE\(^{-/-}\) mice had increased serum levels of triglycerides, cholesterol, and phospholipids (Fig. 2E), increased perigonadal and s.c. white fat (Fig. 2C), and were glucose intolerant and insulin resistant (Fig. 4A–D). These metabolic parameters in Irf5\(^{+/+}\).gld.apoE\(^{-/-}\) mice were affected to a very similar extent as was seen in Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice, suggesting that a certain threshold level of IRF5 is needed to protect against the development of metabolic dysregulation.

IRF5 expression in bone marrow–derived cells is required for the development of autoimmune disease

IRF5 is most highly expressed in hematopoietic cell lineages, including B lymphocytes, monocytes, DCs, and macrophages (16, 18, 26, 55, 56). However, IRF5 transcript is also detected in nonhematopoietic tissue, including muscle, liver, and preadipocytes (16, 18, 22, 57).

To gain insight into which IRF5-expressing cells are responsible for the autoimmune, atherosclerotic, and metabolic phenotypes observed, we performed bone marrow chimera studies. Seven-week-old Irf5\(^{+/+}\).gld.apoE\(^{-/-}\) and Irf5\(^{+-}\).gld.apoE\(^{-/-}\) recipient mice were irradiated and reconstituted with bone marrow from either Irf5\(^{+/+}\).gld.apoE\(^{-/-}\) or Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) age-matched mice. Mice were placed on a Western diet for 6–9 wk starting 3 wk after reconstitution, and disease parameters were evaluated at 16–19 wk of age. Statistical analysis was performed using two-way ANOVA to determine whether IRF5 expression in the donor (D) or in the recipient (R) was contributing to the disease parameter being evaluated.

Both Irf5\(^{+/+}\) and Irf5\(^{-/-}\) recipients reconstituted with Irf5\(^{+/+}\) bone marrow developed splenomegaly, lymphadenopathy, and high-titer ANAs (Fig. 5A–C). In contrast, Irf5\(^{+/+}\) and Irf5\(^{-/-}\) mice reconstituted with Irf5\(^{-/-}\) bone marrow had spleen and lymph nodes of close to normal weight and only low-level ANA titers comparable to that seen previously in Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice. IRF5 deficiency in the recipients modestly reduced spleen size but did not affect lymph node size or ANA titer. Thus, the development of autoimmune disease is primarily dependent on IRF5 expression in bone marrow–derived cells.

IRF5 expression in both bone marrow–derived and non–bone marrow–derived cells reduces atherosclerosis severity

We next measured atherosclerotic lesion size in the different bone marrow chimera groups. Irf5\(^{+/+}\) recipient mice given Irf5\(^{-/-}\) bone marrow developed substantially more atherosclerosis than Irf5\(^{+/+}\) recipient mice given Irf5\(^{-/-}\) bone marrow (Fig. 5D), confirming that IRF5 deficiency promotes atherosclerosis as previously shown (Fig. 2A–C). IRF5 deficiency in both the donor and recipient mice contributed to the increased atherosclerosis, although the more highly statistically significant contribution was seen with IRF5 deficiency in the recipients. The largest atherosclerotic lesions were seen in Irf5\(^{-/-}\) recipient mice given Irf5\(^{-/-}\) bone marrow consistent with the effects of IRF5 deficiency in bone marrow–derived and non–bone marrow–derived cells being additive. Thus, IRF5 expression in both bone marrow–derived and non–bone marrow–derived cells protects against the development of atherosclerosis in gld.apoE\(^{-/-}\) mice.

phospholipids in the sera of Irf5\(^{+/+}\) (n = 18), Irf5\(^{+/+}\) (n = 15), and Irf5\(^{-/-}\) (n = 19) gld.apoE\(^{-/-}\) littermate mice. (F) Lipoprotein profiles from pooled sera of three Irf5\(^{+/+}\).gld.apoE\(^{-/-}\) and three Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice. (G) Foam cell formation assay in vitro. Peritoneal macrophages from C57BL/6 mice were treated with 5% serum from Irf5\(^{+/+}\) and Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) mice for 36 h and then stained with Oil Red O. Representative high-resolution images were taken using a ×20 objective lens and digitally magnified by ×5 (top panels) and ×15 (bottom panels). The percentage of macrophage cell area positive for Oil Red O was quantified and is shown in the right panel. Error bars represent the mean and SEM of three separate experiments using sera from three different Irf5\(^{+/+}\) and Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) littermate groups. The severity of atherosclerosis was ranked in Irf5\(^{+/+}\), Irf5\(^{-/-}\), and Irf5\(^{-/-}\).gld.apoE\(^{-/-}\) littermates housed in the same cage, and the ranks were analyzed by one-way ANOVA (A and B), two-way ANOVA (E), and Student t test (two-sided) (G), \(p < 0.05\), **p < 0.01, ***p < 0.001.
FIGURE 3. IRF5 deficiency increases adipose tissue mass and inflammation. (A) Average amount of food consumed per day by Irf5+/+ (n = 9), Irf5+/− (n = 3), and Irf5−/− (n = 7) gldapoE−/− male mice (top panel) and Irf5+/+ (n = 9), Irf5+/− (n = 7), and Irf5−/− (n = 13) gldapoE−/− female littermate mice (bottom panel). (B) Body weight over time of Irf5+/+ (n = 11), Irf5+/− (n = 15), and Irf5−/− (n = 10) gldapoE−/− male mice (top panel) and Irf5+/+ (n = 10), Irf5+/− (n = 15), and Irf5−/− (n = 10) gldapoE−/− female mice (bottom panel) on Western diet for 12 wk. (C–E) Male and female gldapoE−/− mice were euthanized at 19 wk of age after 12 wk on Western diet and samples collected for analysis. (C) Perigonadal fat and s.c. fat weights in Irf5+/+ (n = 25 to 26), Irf5+/− (n = 27), and Irf5−/− gldapoE−/− (n = 21–24) littermate mice. (D) Representative images of H&E-stained perigonadal fat pads from Irf5+/+, Irf5+/−, and Irf5−/− gldapoE−/− mice. Original magnification ×4. (E) Leptin levels in sera of Irf5+/+ (n = 4), Irf5+/− (n = 3), and Irf5−/− gldapoE−/− (n = 7) littermate mice. (F and G) Nuclear magnetic imaging studies performed on separate cohorts of Irf5+/+ (n = 4) and Irf5−/− gldapoE−/− (n = 4) female littermate mice after 12 wk on Western diet. (F) Percentage of body mass that is fat (left panel) and percentage of body mass that is lean (right panel). (G) Total fat mass (left panel) and total lean mass (right panel). (H and I) SVF of adipose tissue from Irf5+/+ (n = 7), Irf5+/− (n = 5), and Irf5−/− gldapoE−/− (n = 4) male littermate mice after 12 wk on Western diet. (H) Representative dot plots showing live cell gate (top panels) and representative histograms of F4/80+ macrophages in SVF (bottom panels). (I) Percentage of F4/80+ macrophages in SVF relative to Irf5+/+ gldapoE−/− littermate, quantitated by flow cytometry. (J) TNF- α and IL-6 expression in SVF of adipose tissue from Irf5+/+ (n = 6) and Irf5−/− gldapoE−/− (n = 5) male littermate mice after 12 wk on Western diet measured by RT-PCR. Data are expressed as the average fold change of TNF-α and IL-6 expression in SVF of adipose tissue from Irf5+/+ gldapoE−/− mice relative to the Irf5+/+ gldapoE−/− littermate control. Two-way ANOVA (C, E, and I), Student t test (two-sided) (F and G), and Mann–Whitney U test (J). Error bars represent the mean and SEM. *p < 0.05, **p < 0.01, ***p < 0.001. FSC-A, forward light scatter area; SSC-A, side scatter area.
IRF5 expression in non–bone marrow–derived cells plays the major role in reducing adipose tissue weight and the level of hyperlipidemia

IRF5 deficiency in recipient mice resulted in an increase in the amount of perigonadal and s.c. fat. This was most evident with \( \text{Irf5}^{+/+} \) recipient mice given \( \text{Irf5}^{-/-} \) bone marrow but was also seen with \( \text{Irf5}^{-/-} \) recipient mice given \( \text{Irf5}^{-/-} \) bone marrow (Fig. 5E). In contrast, IRF5 deficiency in donor mice did not result in a statistically significant increase in adipose tissue weight, although there was a strong trend toward such an increase.

Similarly, IRF5 deficiency in recipient mice resulted in an increase in the level of hyperlipidemia, with increases in triglycerides, cholesterol, nonesterified fatty acids, and phospholipids being seen (Fig. 5F). This was seen with \( \text{Irf5}^{-/-} \) recipient mice.
given either Irf5+/+ or Irf5−/− bone marrow. IRF5 deficiency in donor mice also resulted in an increase in serum cholesterol levels but did not affect the levels of triglycerides, nonesterified fatty acids, or phospholipids, suggesting the possibility that cholesterol is being regulated through pathways distinct from that of the other lipids (Fig. 5F).

Taken together, these data demonstrate that IRF5 deficiency in non–bone marrow–derived cells plays the major role in the increase in adipose tissue weight and hyperlipidemia observed in the Irf5−/−.gld.apoE−/− mice, although IRF5 deficiency in bone marrow–derived cells plays a contributory role in serum cholesterol regulation and possibly also in the regulation of adipose tissue weight. IL-10 expression is reduced in the aortae of Irf5−/−.gld.apoE−/− mice, and IRF5 is required for IL-10 production induced by TLR7 and TLR9 signaling in immune cells

The bone marrow chimera experiments demonstrated that IRF5 deficiency in both bone marrow–derived and non–bone marrow–derived cells promotes atherosclerosis development in the Irf5−/−.gld.apoE−/− mice. They further demonstrated that the increased hyperlipidemia was due largely to IRF5 deficiency in non–bone marrow–derived cells, providing a possible explanation for how IRF5 deficiency in non–bone marrow–derived cells might promote atherosclerosis. However, the chimera studies did not easily explain how IRF5 deficiency in bone marrow–derived cells might promote atherosclerosis. IL-10 is a major antiatherogenic cytokine and thought to reduce atherosclerosis severity through a number of mechanisms (58–61), with leukocytes being the predominant producers of the IL-10 (62). We found that, in addition to reducing serum levels of proinflammatory cytokines as shown in Fig. 1F, IRF5 deficiency also substantially reduced serum IL-10 levels in gld.apoE−/− mice on a Western diet for 12 wk (Fig. 6A), and thus we hypothesized that IRF5 deficiency in immune (bone marrow–derived) cells might promote atherosclerosis by reducing IL-10 levels in the atherosclerotic lesions.

We measured IL-10 expression in the aortic arches of Irf5+/+, Irf5−/−, and Irf5−/−.gld.apoE−/− mice by RT-PCR and found that IL-10 expression levels were markedly reduced in the Irf5−/−.gld.apoE−/− mice (Fig. 6B). To determine whether IRF5 can regulate IL-10 at the cellular level, we stimulated a number of immune cell types with TLR2 and TLR4 ligands, finding that IL-10 production was largely IRF5 dependent (Fig. 6C–G). TLR2 and TLR4 ligands also induced IL-10 production in these cell types, but this was IRF5 independent.
IRF5 deficiency results in a more severe metabolic phenotype despite markedly reducing lupus autoimmunity and systemic inflammation, results in more severe atherosclerosis. In addition, IRF5 deficiency in the aortae of IRF5-deficient mice relative to the IRF5+/+ mice after 12 wk on Western diet. Data are expressed as the average fold change of IL-10 expression in IRF5+/+ and IRF5−/− gld.apoE−/− mice relative to the IRF5−/− gld.apoE−/− littermate control. Bone marrow–derived M-CSF macrophages (MΦ) (C), GM-CSF MΦ (D), GM-CSF DCs (E), Flt3L DCs (F), and splenic B cells (G) from C57BL/6 WT and IRF5−/− mice were stimulated, or not stimulated (media), with the TLR2 ligand Pam3Cys (100–1000 ng/ml), the TLR3 ligand polyinosinic-polycytidylic acid (10 µg/ml), the TLR4 ligand LPS (100 ng/ml), the TLR7 ligands R848B (100 ng/ml) or R837 (0.3–1 µg/ml), and the TLR9 ligands CpG-B (100 ng/ml) or CpG-A (100 ng/ml). Supernatants were collected after 24 h, or after 5 d in the case of Flt3 DCs, and IL-10 was measured by ELISA. Data represent the mean and SEM of three to six independent experiments for each cell type. Mann–Whitney U test for all except (B), which was one-way ANOVA. *p < 0.05, **p < 0.01.

Thus, IRF5 is required for IL-10 production downstream of TLR7 and TLR9 in multiple cell types. This is particularly intriguing in the context of atherosclerosis, as both TLR7 and TLR9 deficiency have recently been shown to promote atherosclerosis in mouse models (63, 64).

Discussion

Premature atherosclerosis is a severe complication of SLE leading to appreciable morbidity and mortality from cardiovascular disease (2, 3). Gain-of-function polymorphisms in IRF5 are strongly associated with an increased risk of developing SLE, and IRF5 inhibition is being considered as a therapeutic approach in SLE (14). However, the role of IRF5 in atherosclerosis pathogenesis is not known. We hypothesized that IRF5 deficiency would reduce atherosclerosis severity in the context of lupus for two main reasons. First, the inflammation due to the active autoimmune process is thought to contribute to the premature atherosclerosis in SLE (2), and previous studies in other lupus mouse models suggested that IRF5 deficiency would reduce the active autoimmune process (27–32). Secondly, atherosclerosis itself, independently of systemic autoimmune disease, is driven by a Th1-skewed chronic inflammatory process in which M1-type macrophages play a dominant role (11), and IRF5 has been shown to promote both Th1 responses and M1-type macrophage development (25, 26). However, we unexpectedly found that IRF5 deficiency in the gld.apoE−/− model, despite markedly reducing lupus autoimmunity and systemic inflammation, results in more severe atherosclerosis. In addition, IRF5 deficiency results in a more severe metabolic phenotype characterized by increased hyperlipidemia, insulin resistance with hyperglycemia, hepatic steatosis, and increased adiposity. An additional surprising observation was that these effects on atherosclerosis and metabolism were driven not only by IRF5 deficiency in immune cells but also by IRF5 deficiency in non-immune cells.

The protective role of IRF5 in atherosclerosis seen in the gld.apoE−/− model may be mediated at least in part through TLRs. Although TLR2 and TLR4 promote atherosclerosis, TLR3, TLR7, and TLR9 appear to protect against atherosclerosis development. Deficiency of TLR2 or TLR4 in mouse atherosclerosis models results in a reduction in atherosclerosis lesion size (65, 66), whereas deficiency of TLR3, TLR7, or TLR9 results in an increase in atherosclerosis lesion size (63, 64, 67). The reasons for the differential effects of the cell-surface TLRs, TLR2 and TLR4, and the nucleic acid–sensing TLRs, TLR3, TLR7, and TLR9, on atherosclerosis development remain incompletely understood, although the nature of both the TLR agonist and the responding cell type are likely to play a role (68). IRF5 participates in signaling cascades downstream of TLR3, TLR4, TLR5, TLR7, and TLR9 (18, 19, 41, 69, 70), and thus, the increased atherosclerosis we observed in the IRF5-deficient gld.apoE−/− mice could be a result of altered functional outcomes following TLR3, TLR7, or TLR9 activation, leading to a loss of the atheroprotective effect normally mediated by these nucleic acid–sensing TLRs. Extracellular endogenous RNA and DNA are present within atherosclerotic lesions (63, 71). Apoptotic cell death is an important feature of atherosclerotic plaques and may provide the endogenous RNA or DNA ligands required for activation of TLR7 and TLR9 (71, 72). Such apoptotic cell death may exert beneficial effects in early atherosclerosis by the induction of the antiatherogenic cytokines IL-10 and TGF-β but detrimental effects in advanced atherosclerosis through necrotic cell accumulation and the induction of proinflammatory responses (72).

Indeed, we found that production of IL-10 induced by TLR7 and TLR9 activation is markedly reduced in the absence of IRF5 in multiple immune cell types and that IL-10 expression is reduced in the aortae of IRF5-deficient gld.apoE−/− mice. In contrast, we found no effect of IRF5 deficiency on IL-10 production induced by TLR2 and TLR4 activation, and a previous study found that IRF5 deficiency actually led to an increase in IL-10 production in

FIGURE 6. IRF5 is required for IL-10 production induced by TLR7 and TLR9 signaling in immune cells. (A) Serum IL-10 cytokine levels from Irf5+/+ (n = 9) and Irf5−/− (n = 9) gld.apoE−/− littermate mice were measured by Luminex. (B) IL-10 gene expression in aortic arches of male and female Irf5+/+ (n = 7 to 8), Irf5−/− (n = 6 to 7), and Irf5+/+ gld.apoE−/− (n = 8) mice after 12 wk on Western diet. Data are expressed as the average fold change of IL-10 expression in Irf5−/− and Irf5+/+ gld.apoE−/− mice relative to the Irf5−/− gld.apoE−/− littermate control. Bone marrow–derived M-CSF macrophages (MΦ) (C), GM-CSF MΦ (D), GM-CSF DCs (E), Flt3L DCs (F), and splenic B cells (G) from C57BL/6 WT and IRF5−/− mice were stimulated, or not stimulated (media), with the TLR2 ligand Pam3Cys (100–1000 ng/ml), the TLR3 ligand polyinosinic-polycytidylic acid (10 µg/ml), the TLR4 ligand LPS (100 ng/ml), the TLR7 ligands R848B (100 ng/ml) or R837 (0.3–1 µg/ml), and the TLR9 ligands CpG-B (100 ng/ml) or CpG-A (100 ng/ml). Supernatants were collected after 24 h, or after 5 d in the case of Flt3 DCs, and IL-10 was measured by ELISA. Data represent the mean and SEM of three to six independent experiments for each cell type. Mann–Whitney U test for all except (B), which was one-way ANOVA. *p < 0.05, **p < 0.01.
M1-type macrophages following TLR4 activation (26). Thus, IRF5 is required for IL-10 production preferentially downstream of TLR7 and TLR9 activation, and this may be one mechanism by which IRF5 deficiency in immune cells promotes atherosclerosis, as IL-10 is the major anti-inflammatory cytokine responsible for protection against atherosclerosis (58–60).

An additional mechanism by which IRF5 deficiency promotes atherosclerosis in our model is through the induction of hyperlipidemia. IRF5 deficiency resulted in a marked increase in serum very low-density lipoprotein levels and a reduction in serum high-density lipoprotein, a serum lipid profile that would be expected to be proatherogenic (73, 74). Accordingly, sera from IRF5-deficient gld.apoE−/− mice induced the formation of foam cells from macrophages to a substantially greater extent than sera from IRF5-sufficient gld.apoE−/− mice. The increase in hyperlipidemia was due predominantly to IRF5 deficiency in non–bone marrow–derived cells, demonstrating that the serum lipid alterations were largely independent of lupus disease severity, as the lupus phenotype was dependent on IRF5 expression in bone marrow–derived cells. Further work will be needed to identify the non–immune cell type in which IRF5 acts to regulate these metabolic effects. The three major metabolically active tissues regulating serum lipid levels are liver, adipose tissue, and the intestine, whereas skeletal muscle is more important for maintaining glucose homeostasis (75, 76). In the initial IRF5 reports, it was shown that IRF5 is expressed in skeletal muscle but not in liver (16, 18). A subsequent study demonstrated IRF5 expression in the liver, although it was not determined whether IRF5 was expressed in hepatocytes or in nonhepatocyte cell populations in the liver, such as Kupffer cells and sinusoidal epithelial cells (22). IRF5 is expressed only at a very low level in purified isolated adipocytes from C57BL/6 mice on a regular chow diet (57); however, we have found that IRF5 expression is strongly upregulated in purified adipocytes from C57BL/6 mice on a high-fat diet (data not shown). This is consistent with published studies showing increased IRF5 expression in unFractionated adipose tissue from mice on a high-fat diet (77). Very little is known about the function of IRF5 in skeletal myocytes, hepatocytes, adipocytes, or the lipid-regulating cells of the intestine.

Fas deficiency reduces adipose tissue weight and adipocyte size without affecting total body weight in C57BL/6 mice fed a high-fat diet (78). Fas deficiency also protects against the deterioration of glucose homeostasis induced by a high-fat diet, protects against hepatic steatosis, and reduces adipose tissue inflammation (78). gld.apoE−/− mice are deficient in functional FasL and are thus unable to signal through Fas. This likely contributes to the reduced fat mass, small adipocyte size, and normal glucose homeostasis observed in the IRF5-sufficient gld.apoE−/− mice on a Western diet. These protective effects of FasL deficiency were not evident in gld.apoE−/− mice deficient in IRF5, which exhibited increased fat mass, enlarged adipocytes, reduced lean body mass, and impaired glucose tolerance. Fasl and TRAIL have opposing effects on metabolism in vivo with Fas/FasL deficiency, resulting in a favorable metabolic phenotype (as detailed above), and TRAIL deficiency, resulting in an unfavorable metabolic phenotype with hypercholesterolemia, adipocyte hypertrophy, and impaired glucose tolerance (79). As Fasl signaling is absent in gld.apoE−/− mice, the effects of TRAIL signaling may be more apparent. As IRF5 participates in both Fasl- and TRAIL-induced signaling in terms of apoptosis induction (22, 23), it is conceivable that IRF5 also participates in Fasl- and TRAIL-induced signaling in terms of metabolic regulation. If that is the case, then IRF5 deficiency may have particularly marked metabolic effects in gld.apoE−/− mice. Further studies will be needed to determine how IRF5 deficiency impacts metabolism in mice with intact Fas/FasL signaling.

Previous studies have shown that IRF5 is required for disease development in a number of different mouse lupus models, although interpretation of these studies was complicated by the possible presence of the Dock2 mutation in the IRF5 knockout mouse line used to generate the experimental mice (40, 41). In this study, we have confirmed an important role for IRF5 in the pathogenesis of the lupus phenotype in a mouse model and have further demonstrated for the first time, to our knowledge, that IRF5 expression in bone marrow–derived cells is necessary and sufficient to promote this phenotype. It will be important in future studies to identify the specific IRF5-expressing immune cell type(s) responsible for promoting lupus pathogenesis and preventing atherosclerosis development because, if they are different, it would be preferable to target IRF5 inhibition only to the cell type driving lupus pathogenesis. Cell-specific inhibition in vivo may be feasible as recently shown for IRF5 in macrophages (33).

In summary, we found that despite markedly reducing lupus disease severity, IRF5 deficiency led to an increase in atherosclerosis as well as metabolic dysregulation characterized by increased hyperlipidemia, insulin resistance, hepatic steatosis, and increased adiposity. This was due to distinct roles of IRF5 in both immune and nonimmune cells. These findings have implications for the treatment of autoimmune diseases such as lupus in which IRF5 inhibition is being considered as a therapeutic approach, as global IRF5 inhibition could potentially exacerbate cardiovascular disease in these patients.

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Disclosures
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