

Short Communication

Exogenous Interferon- γ Enhances Atherosclerosis in Apolipoprotein E $-/-$ Mice

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A role for interferon- γ (IFN- γ) has been implied in the atherogenic process. To determine whether exogenously administered IFN- γ exerts an effect on the development of atherosclerosis, we intraperitoneally administered either recombinant IFN- γ (100 U/g body weight) or phosphate buffered saline daily for 30 days to atherosclerosis-susceptible apolipoprotein E $-/-$ mice (16-week-old male mice, $n = 11$ per group) fed a normal diet. Atherosclerotic lesion size was quantified in the ascending aorta. The number of T lymphocytes and major histocompatibility complex (MHC) class II-positive cells within lesions were also quantified in this region. IFN- γ administration reduced serum cholesterol concentrations by 15% ($P = 0.02$). For both groups, the majority of cholesterol was present in very low density lipoproteins, which were modestly reduced in mice receiving IFN- γ . Despite the decrease in serum cholesterol concentrations, IFN- γ injections significantly increased lesion size twofold compared to controls ($119,980 \pm 18,536$ vs. $59,396 \pm 20,017 \mu\text{m}^2$; $P = 0.038$). IFN- γ also significantly increased the mean number of T lymphocytes (19 ± 4 vs. 7 ± 1 cells; $P = 0.03$) and MHC class II-positive cells (10 ± 3 vs. 3 ± 1 cells; $P = 0.04$) within lesions. These data lend further support to a pro-atherogenic role of IFN- γ . (*Am J Pathol* 2000, 157:1819–1824)

T lymphocytes are prominent components of all stages of human atherosclerosis.¹ This cell type has also been identified in atherosclerotic lesions from rabbits^{2,3} and mice.^{4,5} A functional role for T lymphocytes in atherogenesis may be inferred from their activation status and their juxtaposition to lesional macrophages.^{6,7} An active role for this cell type is further implied from the range of studies that suppress T lymphocyte function and influence development of atherosclerotic lesions in animal models of the disease.^{3,8–11}

One of the prominent cytokines secreted during activation of specific subtypes of T lymphocytes is interferon- γ (IFN- γ). Both protein and mRNA for this cytokine have been detected in atherosclerotic lesions from humans^{7,12} and mice.¹³ IFN- γ has a range of biological properties in cultured cells that could influence development of atherosclerotic lesions. These include effects on class A scavenger receptors,^{12,14,15} low density lipoprotein receptor-related protein,¹⁶ 15-lipoxygenase,¹⁷ lipoprotein oxidation,¹⁸ lipoprotein lipase,¹⁹ extracellular matrix deposition,²⁰ increased expression of vascular cellular adhesion molecule-1,²¹ and smooth muscle cell proliferation.²² This complex array of *in vitro* effects makes it difficult to predict the effects of IFN- γ on the development of atherosclerotic lesions.

The most direct evidence for a role of IFN- γ in the development of atherosclerosis is based on a study of mice deficient in IFN- γ receptors.²³ Mice with compound deficiencies of apolipoprotein E (apoE) and the IFN- γ receptor developed less atherosclerosis than mice with only an apoE deficiency.²³ In addition to the decrease in atherosclerosis, IFN- γ receptor-deficient mice developed lesions with decreased cellularity and lipid accumulation, but increased collagen deposition. There is the potential for differing responses in mice deficient for the cytokine rather than the receptor.²⁴ However, to date there have been no studies that compare the effects of these deficiencies on the development of atherosclerosis.

In the present study, we determined whether exogenous administration of IFN- γ would influence the development of atherosclerosis in apoE $-/-$ mice. We demonstrated that administration of recombinant IFN- γ increases lesion size in apoE $-/-$ mice and is accompanied by a significant increase in the number of lesion-associated T lymphocytes and major histocompatibility complex (MHC) class II-positive cells. Therefore, these

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data provide further support to the hypothesis that IFN- γ is a potent atherogenic cytokine.

Materials and Methods

Materials

Lyophilized recombinant mouse IFN- γ (catalog no. 485-MI, R&D Systems, Minneapolis, MN) was reconstituted in solution A, sterile phosphate buffered saline (PBS) containing 0.1% bovine serum albumin, to prepare a stock concentration of 50 $\mu\text{g/ml}$ (200,000 U/ml) as recommended by the manufacturer.

Animals

Twenty-two 16-week-old male apoE $^{-/-}$ mice, progeny from five breeding pairs of apoE $^{-/-}$ mice, were originally obtained from The Jackson Laboratory (Bar Harbor, ME). This strain of mouse had been back-crossed 10 times to the C57BL/6 strain. Equal numbers of siblings from the five litters were divided into two groups of 11 mice. One group of mice received daily peritoneal injections of IFN- γ (100 U/g body weight) diluted in PBS, while the second group received daily injections of solution A (to control for the presence of bovine serum albumin), also diluted in PBS. Daily injections were performed for 30 days, during which time the mice were kept on a normal laboratory diet. Mice were housed in a pathogen-free facility and exposed to a 12-hour light and dark cycle. All procedures involving animals were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Blood Collection

At the end of the injection period, mice were anesthetized by metaflane inhalation. Terminal blood samples were collected by puncture of the right ventricle. Blood was allowed to clot at room temperature for 30 minutes, then centrifuged at $1000 \times g$ for 25 minutes at 4°C. Mice were perfused with PBS via a cannula placed in the left ventricle while the perfusate drained from a severed right atrium. The hearts were separated from the aortas at the base, embedded in OCT, and frozen at -20°C .

Plasma Cholesterol and Lipoprotein Profiles

Individual serum total cholesterol concentrations were determined using a commercially available colorimetric assay (Wako Bioproducts, Richmond, VA), following a slight modification of the manufacturer's instructions to allow for quantification using a 96-well microtiter plate format. Serum samples (50 μl) from each mouse were used to determine individual lipoprotein cholesterol distributions evaluated after fractionation by size exclusion chromatography (Biological Workstation, Bio-Rad, Richmond, CA) using a Superose 6 column (Pharmacia LKB Biotechnology, Uppsala, Sweden). Fractions were col-

lected and cholesterol concentrations determined using the total cholesterol assay (Wako).

Lesion Analysis

Atherosclerotic size in the ascending aorta was determined from 4 Oil Red O-stained serial sections, cut 8 μm thick and collected 80 μm apart, starting at the region where the aortic sinus becomes the ascending aorta. Lesion area, defined by Oil Red O staining of the intima, was determined using Image-Pro software (Media Cybernetics, Silver Spring, MD) on files that were created using a Spot camera (Diagnostic Instruments, Sterling Heights, MI). The mean lesion area derived from the 4 serial sections was taken as the average lesion size for each animal.

Immunocytochemical and Histological Characterization of Atherosclerotic Lesions

Immunocytochemistry and extracellular matrix staining were performed as described previously,²⁵ on serial sections of the ascending aorta adjacent to those stained with Oil Red O. The following monoclonal antibodies were used for immunostaining: an anti-mouse Thy1.2 antibody (01011D, 7 $\mu\text{g/ml}$; PharMingen, Los Angeles, CA); and an anti-mouse MHC II antibody (LS-004-SN, 1:5 dilution; Biosource International, Camarillo, CA). An immunoperoxidase assay (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) was used to detect the primary antibody after addition of a biotinylated anti-rat IgG absorbed against mouse serum. Immunoreactivity was visualized using the chromagen 3-amino-9-ethyl carbazole (Biomedica Corp., Foster City, CA). Endogenous tissue peroxidase was removed before immunostaining by exposure of tissue to H_2O_2 . The possibility of nonspecific staining was addressed by staining serial sections as described above, but in the absence of a primary antibody. Extracellular elastin and collagen were visualized with Verhoeff's and Gomori trichrome stain, respectively.

Statistics

Data analyses were performed using SigmaStat 2.03 software (SPSS Inc., Chicago, IL). For each parameter, the mean and standard error of the mean (SEM) were calculated. Statistical analysis between PBS- and IFN- γ injected groups was by Student's *t*-test after testing that the data complied with the constraints of parametric analysis. *P* values <0.05 were considered statistically significant.

Results

Injection of IFN- γ into ApoE $^{-/-}$ Mice Reduced Serum Cholesterol Concentrations

ApoE-deficient mice develop hyperlipidemia and atherosclerosis when fed a normal laboratory diet.^{26,27} In this study, apoE $^{-/-}$ mice on a normal diet developed moderate hypercholesterolemia, the extent of which de-

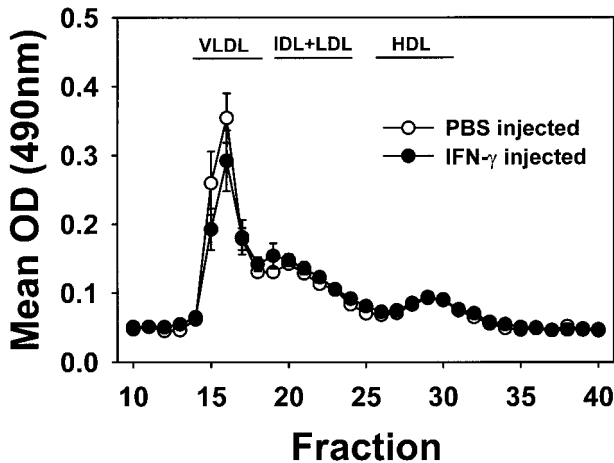


Figure 1. Serum (50 μ l) from male apoE $^{-/-}$ mice receiving daily injection of either PBS alone (open symbols) or IFN- γ (solid symbols) was resolved by size exclusion chromatography using a Superose 6 column. Total cholesterol concentrations were then determined from each fraction and expressed as mean absorbency at 490 nm. Values are represented as mean \pm SEM of 11 animals from each group. Fraction numbers correspond to VLDL; intermediate, low, and high density lipoproteins (IDL, LDL, and HDL, respectively) are indicated.

creased significantly by 15% in mice receiving IFN- γ compared to mice injected with PBS only (278 ± 11 vs. 324 ± 15 mg/dl, respectively; $P = 0.02$). Size exclusion chromatography, performed on individual serum samples, showed that for both groups, the majority of cholesterol was present in the very low density lipoprotein (VLDL) fraction, and that this fraction was modestly reduced in the group receiving IFN- γ (Figure 1).

Injection of ApoE $^{-/-}$ Mice with IFN- γ Significantly Increased Atherosclerosis, Lesion T Lymphocytes, and Lesion MHC Class II-Positive Cells

Segments of heart tissue spanning the aortic sinus and ascending aorta were embedded in OCT, serially sectioned and analyzed for atherosclerotic lesion size, and lesion number of both T-lymphocytes and MHC class II-positive cells. Daily administration of IFN- γ caused a significant twofold increase in lesion size compared to control groups ($119,980 \pm 18,536$ vs. $59,396 \pm 20,017$ μ m 2 , respectively; $P = 0.038$; Figure 2A). Accompanying this increase in lesion size, addition of exogenous IFN- γ also significantly increased the number of both T lymphocytes (19 ± 4 vs. 7 ± 1 mean cell number; $P = 0.03$; Figure 2B). and MHC class II-positive cells (10 ± 3 vs. 3 ± 1 mean cell number; $P = 0.04$; Figure 2C) in atherosclerotic lesions. A representative example of the immunostained T lymphocytes is shown in Figure 3, E and F. Based on examination of immunostained tissue sections, the bulk of lesions was composed of lipid-laden macrophages (Figure 3, C and D). Gomori trichrome staining demonstrated that there was little collagen present within the lipid-laden foam cell, and a modest accumulation within macrophages that were not so lipid-engorged (Fig-

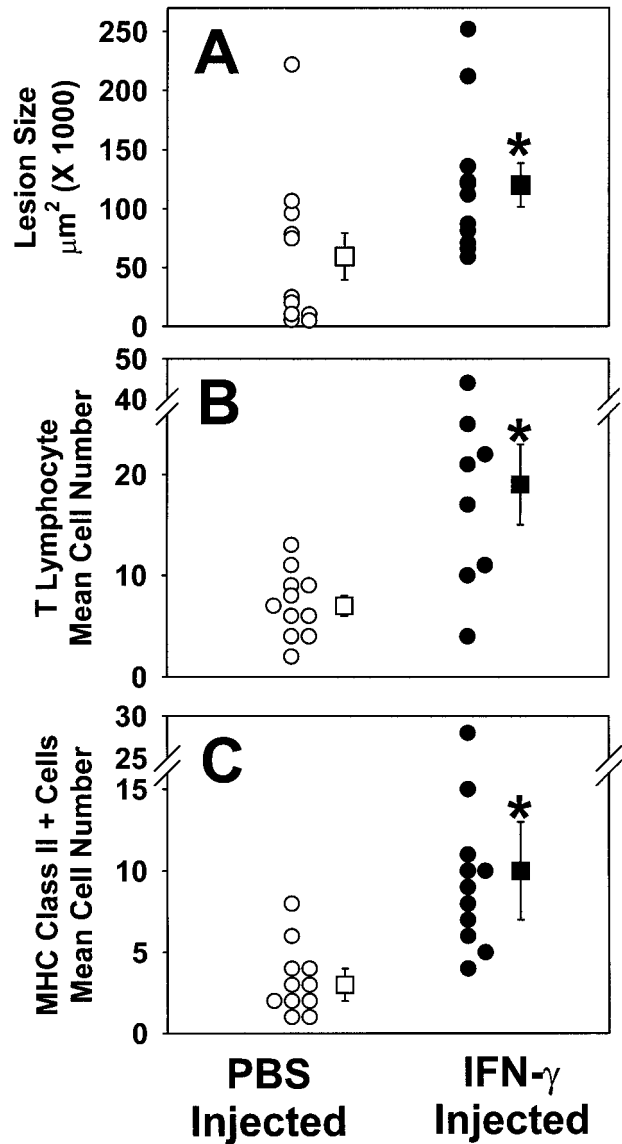
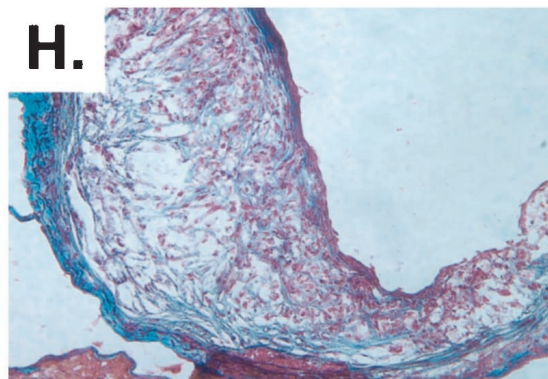
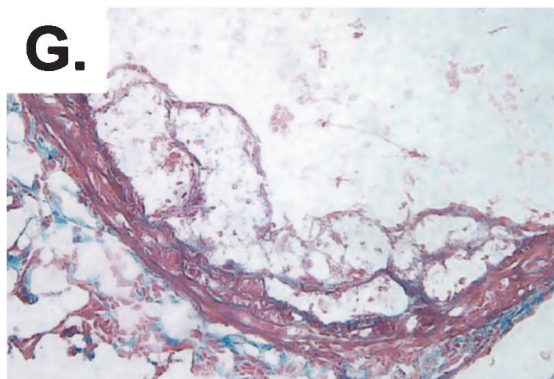
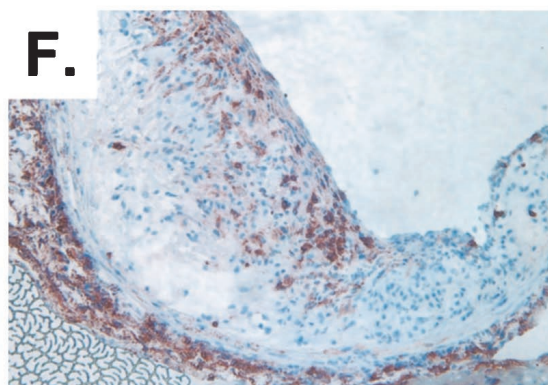
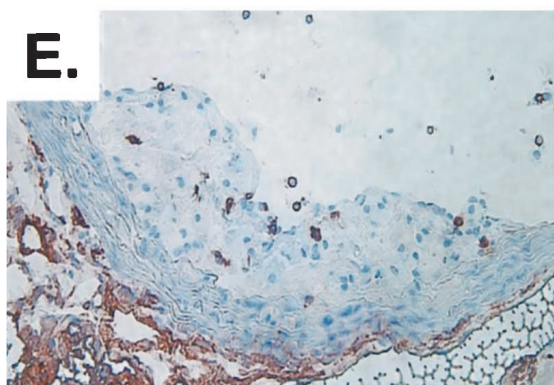
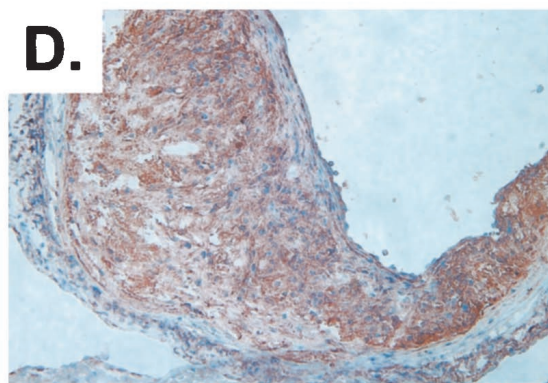
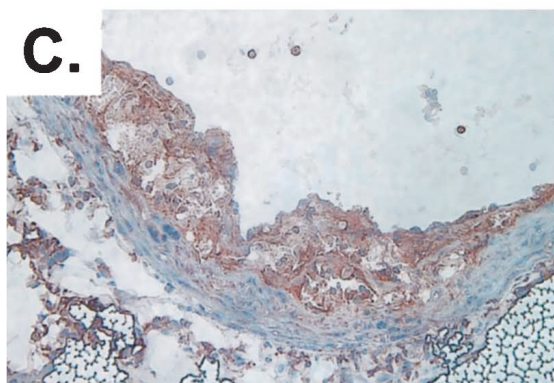
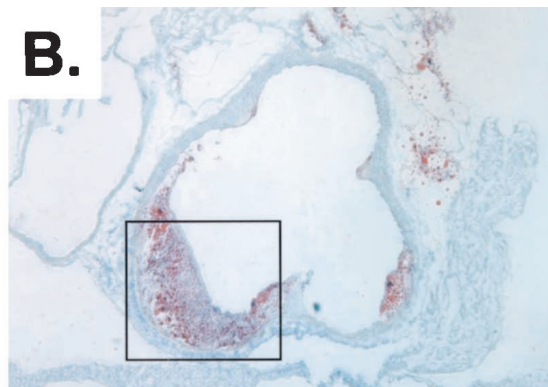
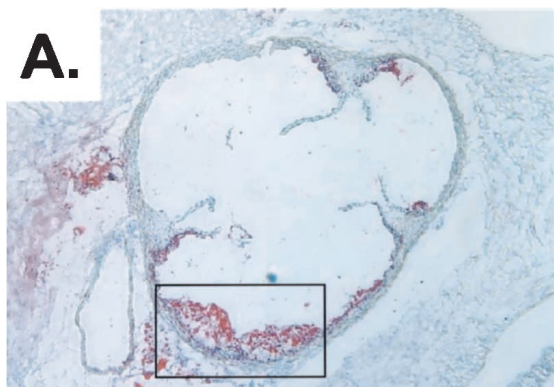


Figure 2. Characteristics of lesions in the ascending aorta receiving daily injection of either PBS alone (open symbols) or IFN- γ (solid symbols) that include atherosclerotic lesion size (A), lesion T lymphocyte cell number (B), and lesion MHC class II-positive cell number (C). For all graphs, each circle represents the value for an individual mouse, square symbols positioned beside a group of circles represents the group mean, and bars represent the SEM. Segments of heart tissue spanning the aortic sinus and ascending aorta were embedded in OCT, sectioned, and stained with Oil Red O (A), a monoclonal anti-mouse Thy1.2 antibody (7 μ g/ml) (B), or a monoclonal anti-mouse MHC class II antibody (1:5 dilution; C). Each point in A represents the mean lesion size of 4 sections per mouse, collected every 80 μ m starting at the region where the aortic sinus becomes the ascending aorta. In B and C, each point represents the mean cell number associated within lesions for serial sections to those in A. Values are derived from 11 mice per group, with the exception of the IFN- γ -injected mice in B, where 8 mice per group were used. A: * $P = 0.038$. B: * $P = 0.03$. C: * $P = 0.04$.

ure 3, G and H). There was a tendency toward more areas of no lipid-laden foam cells in IFN- γ -treated mice.

Discussion

The frequent detection of IFN- γ mRNA in atherosclerotic lesions has led to speculation about its role as the medi-



ator of T lymphocyte-induced changes of the disease process. The most direct evidence for a role of this cytokine in the disease is the reduction in the size of atherosclerotic lesions that occurs in apoE $^{-/-}$ mice that are also deficient in IFN- γ receptors.²³ The lesions generated in the absence of IFN- γ receptors had marked differences in both cell and extracellular matrix composition. In this study, we looked at the effect of exogenous IFN- γ on lesion development in apoE $^{-/-}$ mice that were IFN- γ receptor-competent. In agreement with the conclusion of Gupta et al²³ that IFN- γ is a pro-atherogenic cytokine, we observed a significant increase in lesion size of mice receiving exogenous IFN- γ . However, though we noted increases in the number of T lymphocytes and MHC class II-positive cells, we did not observe any grossly observable changes in both cellular and extracellular matrix composition of the lesions. Furthermore, the increase in atherosclerosis occurred even though there was a modest reduction in plasma cholesterol concentrations attributable to a decrease in VLDL cholesterol.

Radiolabeled IFN- γ is rapidly removed from the plasma of mice following intravenous injection, with sequestration mainly in the liver and kidney.²⁸ Because absorption into the blood of solutes injected i.p. is usually rapid, it may be expected that parenterally administered IFN- γ would also be rapidly removed from the plasma compartment. However, in the present study, this apparent transient presence in plasma was sufficient to have effects on both plasma cholesterol concentrations and atherosclerosis.

The finding of IFN- γ injections decreasing plasma cholesterol are in agreement with the increases observed in IFN- γ receptor-deficient animals.²³ Since IFN- γ has no effect of low density lipoprotein receptor activity²⁹ and decreases low density lipoprotein receptor-related protein,¹⁶ the reduction in cholesterol concentrations may not be due to enhanced hepatic removal. Therefore, although this was a consistent effect, the mechanism underlying this response is not apparent. As described above, there are numerous biological effects of IFN- γ that could have direct relevance to both foam cell formation and the process of fatty streak development. Further study will be needed to determine whether the effect of IFN- γ is due to a concerted effect or whether one mechanism is dominant.

Previously, IFN- γ has been parenterally administered to demonstrate the effects on smooth muscle proliferation in rats.³⁰ Contrary to the effect we observed on atherosclerotic lesions, administration of IFN- γ to rats with carotid artery injury led to an attenuation of intimal thickening. However, subcutaneously administered IFN- γ increased arteriosclerosis in transplanted arteries in mice.³¹ In our study, the lesions formed in these young apoE $^{-/-}$ mice fed a normal diet were almost exclusively lipid-laden macrophages. Although we observed a pro-atherogenic effect under these conditions, further work

may be needed to define whether IFN- γ exerts a similar pro-atherogenic effect at other stages in the maturation of atherosclerotic lesions in which there would be a more complex cellular and biochemical composition.

Using an experimental protocol which we mimicked in our current study, Lee et al³² recently demonstrated that daily injections of interleukin-12 (IL-12) will significantly increase lesion development in apoE $^{-/-}$ mice. Given that IL-12 is a potent inducer of IFN- γ expression,³³ and that addition of exogenous IL-12³² and now IFN- γ will promote atherosclerosis in apoE $^{-/-}$ mice, IL-12 and IFN- γ may belong to a common pathway involved in promoting lesion development through induction of an inflammatory reaction.

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References

- Daugherty A, Hansson GK: Lymphocytes in atherogenesis. *Atherosclerosis*. Edited by Dean RT, Kelly D. San Diego, Academic Press, 2000 (in press)
- Hansson GK, Seifert PS, Olsson G, Bondjers G: Immunohistochemical detection of macrophages and T lymphocytes in atherosclerotic lesions of cholesterol-fed rabbits. *Arterioscler Thromb Vasc Biol* 1991, 11:745-750
- Roselaar SE, Schonfeld G, Daugherty A: Enhanced development of atherosclerosis in cholesterol-fed rabbits by suppression of cell-mediated immunity. *J Clin Invest* 1995, 96:1389-1394
- Roselaar SE, Kakkanathu PX, Daugherty A: Lymphocyte populations in atherosclerotic lesions of apoE $^{-/-}$ and LDL receptor $^{-/-}$ mice: decreasing density with disease progression. *Arterioscler Thromb Vasc Biol* 1996, 16:1013-1018
- Zhou XH, Stemme S, Hansson GK: Evidence for a local immune response in atherosclerosis: CD4(+) T cells infiltrate lesions of apolipoprotein-E-deficient mice. *Am J Pathol* 1996, 149:359-366
- van der Wal AC, Dingemans KP, Weerman MV, Das PK, Becker AE: Specialized membrane contacts between immunocompetent cells in human atherosclerotic plaques. *Cardiovasc Pathol* 1994, 3:81-85
- Hansson GK, Holm J, Jonasson L: Detection of activated T lymphocytes in the human atherosclerotic plaque. *Am J Pathol* 1989, 135:169-175
- Emeson EE, Shen ML, Bell CGH, Qureshi A: Inhibition of atherosclerosis in CD4 T-cell-ablated and nude (nu/nu) C57BL/6 hyperlipidemic mice. *Am J Pathol* 1996, 149:675-685
- Dansky HM, Charlton SA, Harper MM, Smith JD: T and B lymphocytes play a minor role in atherosclerotic plaque formation in the apolipoprotein E-deficient mouse. *Proc Natl Acad Sci USA* 1997, 94:4642-4646
- Daugherty A, Pure E, Delfel-Butteiger D, Chen S, Leferovich J, Roselaar SE, Rader DJ: The effects of total lymphocyte deficiency on the extent of atherosclerosis in apolipoprotein E $^{-/-}$ mice. *J Clin Invest* 1997, 100:1575-1580
- Elhage R, Clamens S, Reardon Alulis C, Getz GS, Fievet C, Maret A, Arnal JF, Bayard F: Loss of atheroprotective effect of estradiol in immunodeficient mice. *Endocrinology* 2000, 141:462-465
- Geng YJ, Hansson GK: Interferon- γ inhibits scavenger receptor

Figure 3. Representative sections from a region where the aortic sinus becomes the ascending aorta of PBS- (A, C, E, and G) and IFN- γ - (B, D, F and H) injected mice. A segment of heart tissue spanning the aortic sinus and ascending aorta was embedded in OCT, sectioned and stained with Oil Red O for neutral lipids (A and B); a rabbit antisera to mouse macrophages (1:3000 dilution; C and D); a monoclonal anti-mouse Thy1.2 antibody (7 μ g/ml; E and F); or Gomori trichrome (G and H). C, E, G, and D, F, H are from regions encompassed in the boxes depicted in A and B, respectively. Original magnifications, $\times 40$ (A and B) and $\times 200$ (C-H).

- expression and foam cell formation in human monocyte-derived macrophages. *J Clin Invest* 1992, 89:1322–1330
13. Zhou XH, Paulsson G, Stemme S, Hansson GK: Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice. *J Clin Invest* 1998, 101:1717–1725
 14. Fong LG, Fong TAT, Cooper AD: Inhibition of mouse macrophage degradation of acetyl low density lipoprotein by interferon- γ . *J Biol Chem* 1990, 265:11751–11760
 15. Cornicelli JA, Butteiger D, Rateri DL, Welch K, Daugherty A: Interleukin-4 augments acetylated LDL induced cholesterol esterification in macrophages. *J Lipid Res* 2000, 41:376–383
 16. LaMarre J, Wolf BB, Kittler ELW, Quesenberry PJ, Gonias SL: Regulation of macrophage α_2 -macroglobulin receptor/low density lipoprotein receptor-related protein by lipopolysaccharide and interferon- γ . *J Clin Invest* 1993, 91:1219–1224
 17. Conrad DJ, Kuhn H, Mulkins M, Highland E, Sigal E: Specific inflammatory cytokines regulate the expression of human monocyte 15-lipoxygenase. *Proc Natl Acad Sci USA* 1992, 89:217–221
 18. Christen S, Thomas SR, Garner B, Stocker R: Inhibition by interferon- γ of human mononuclear cell-mediated low density lipoprotein oxidation: participation of tryptophan metabolism along the kynurenine pathway. *J Clin Invest* 1994, 93:2149–2158
 19. Jonasson L, Hansson GK, Bondjers G, Noe L, Etienne J: Interferon- α inhibits lipoprotein lipase in human monocyte-derived macrophages. *Biochim Biophys Acta* 1990, 1053:43–48
 20. Sempowski GD, Derdak S, Phipps RP: Interleukin-4 and interferon- α discordantly regulate collagen biosynthesis by functionally distinct lung fibroblast subsets. *J Cell Physiol* 1996, 167:290–296
 21. Li H, Cybulsky MI, Gimbrone MA, Libby P: Inducible expression of vascular cell adhesion molecule-1 by vascular smooth muscle cells in vitro and within rabbit atheroma. *Am J Pathol* 1993, 143:1551–1559
 22. Hansson GK, Jonasson L, Holm J, Clowes MM, Clowes AW: Gamma-interferon regulates vascular smooth muscle proliferation and Ia antigen expression in vivo and in vitro. *Circ Res* 1988, 63:712–719
 23. Gupta S, Pablo AM, Jiang XC, Wang N, Tall AR, Schindler C: IFN- γ potentiates atherosclerosis in apoE knock-out mice. *J Clin Invest* 1997, 99:2752–2761
 24. Cantin E, Tanamachi B, Openshaw H, Mann J, Clarke K: Gamma interferon (IFN- γ) receptor null-mutant mice are more susceptible to herpes simplex virus type 1 infection than IFN-gamma ligand null-mutant mice. *J Virol* 1999, 73:5196–5200
 25. Daugherty A, Rateri DL: Presence of LDL receptor-related protein/ α_2 -macroglobulin receptors in macrophages of atherosclerotic lesions from cholesterol-fed New Zealand and heterozygous Watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb Vasc Biol* 1994, 14:2017–2024
 26. Plump AS, Smith JD, Hayek T, Aaltosetala K, Walsh A, Verstuyft JG, Rubin EM, Breslow JL: Severe hypercholesterolemia and atherosclerosis in apolipoprotein-E-deficient mice created by homologous recombination in ES cells. *Cell* 1992, 71:343–353
 27. Zhang SH, Reddick RL, Piedrahita JA, Maeda N: Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science* 1992, 258:468–471
 28. Gonias SL, Pizzo SV, Hoffman M: Clearance and distribution of recombinant murine gamma-interferon in mice. *Cancer Res* 1988, 48:2021–2024
 29. Stopeck AT, Nicholson AC, Mancini FP, Hajjar DP: Cytokine regulation of low density lipoprotein receptor gene transcription in HepG2 cells. *J Biol Chem* 1993, 268:17489–17494
 30. Hansson GK, Holm J: Interferon- γ inhibits arterial stenosis after injury. *Circulation* 1991, 84:1266–1272
 31. Tellides G, Tereb DA, Kirkiles-Smith NC, Kim RW, Wilson JH, Schechner JS, Lorber MI, Pober JS: Interferon- γ elicits arteriosclerosis in the absence of leukocytes. *Nature* 2000, 403:207–211
 32. Lee TS, Yen HC, Pan CC, Chau LY: The role of interleukin 12 in the development of atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* 1999, 19:734–742
 33. Gazzinelli RT, Hieny S, Wynn TA, Wolf S, Sher A: Interleukin 12 is required for the T-lymphocyte-independent induction of interferon- γ by an intracellular parasite and induces resistance in T-cell-deficient hosts. *Proc Natl Acad Sci USA* 1993, 90:6115–6119