Angiotensin II–Induced Vascular Dysfunction Depends on Interferon-γ–Driven Immune Cell Recruitment and Mutual Activation of Monocytes and NK-Cells

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Objective—Immune cells contribute to angiotensin II (ATII)–induced vascular dysfunction and inflammation. Interferon-γ (IFN-γ), an inflammatory cytokine exclusively produced by immune cells, seems to be involved in ATII-driven cardiovascular injury, but the actions and cellular source of IFN-γ remain incompletely understood.

Approach and Results—IFN-$\gamma^{-/-}$ and Tbx21$^{-/-}$ mice were partially protected from ATII-induced (1 mg/kg per day of ATII, infused subcutaneously by miniosmotic pumps) vascular endothelial and smooth muscle dysfunction, whereas mice overexpressing IFN-γ showed constitutive vascular dysfunction. Absence of T-box expressed in T cells (T-bet), the IFN-γ transcription factor encoded by Tbx21, reduced vascular superoxide and peroxynitrite formation and attenuated expression of nicotinamide adenosine dinucleotide phosphate oxidase subunits as well as inducible NO synthase, monocyte chemoattractant protein 1, and interleukin-12 in aortas of ATII-infused mice. Compared with controls, IFN-$\gamma^{-/-}$ and Tbx21$^{-/-}$ mice were characterized by reduced ATII-mediated vascular recruitment of both natural killer (NK)1.1^{+} NK-cells as the major producers of IFN-γ and CD11b^{+}Gr-1^{low} interleukin-12 secreting monocytes. Selective depletion and adoptive transfer experiments identified NK-cells as essential contributors to vascular dysfunction and showed that T-bet^{+}lysozyme M^{+} myelomonocytic cells were required for NK-cell recruitment into vascular tissue and local IFN-γ production.

Conclusions—We provide first evidence that NK-cells play an essential role in ATII-induced vascular dysfunction. In addition, we disclose the T-bet-IFN-γ pathway and mutual monocyte–NK-cell activation as potential therapeutic targets in cardiovascular disease. **(*Arterioscler Thromb Vasc Biol*. 2013;33:1313-1319.)**

Key Words: angiotensin II ■ inflammation ■ interferon-γ ■ natural killer cells ■ oxidative stress ■ vascular function

Recruitment of immune cells into the vessel wall has been recognized as an important early step in angiotensin II (ATII)–induced vascular dysfunction and arterial hypertension.[1] Interferon-γ (IFN-γ), an inflammatory cytokine exclusively produced by immune cells, mainly by natural killer (NK)-cells,[2] but also natural killer T cells,[3] T helper (Th)0 and Th1 T cells, macrophages,[4] and dendritic cells,[5] is well known to promote inflammatory reactions, such as activation of macrophages,[6] to increase chemokine and adhesion molecule expression and to foster recruitment of immune cells to inflammatory sites.[7,8] Reflecting its inflammatory activity, IFN-γ is upregulated in the spleen of hypertensive rats[9] and in the kidney of ATII-infused mice.[10] Recently, it was shown that cardiac IFN-γ expression and IFN-γ–driven tissue injury in the heart are increased by ATII[11] and that IFN-γ–receptor deficient mice are partially protected from ATII-driven cardiac damage and renal injury.[12] These observations strongly support a role of IFN-γ in ATII-driven pathology, however its cellular source and specific influence on vascular function and inflammation are incompletely defined.

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Analyzing ATII-mediated vascular inflammation and dysfunction in IFN-$\gamma^{-/-}$ mice and mice lacking the transcription factor T-box expressed in T cells (T-bet), which directs IFN-γ transcription in immune cells,[13] we identified the crucial role of the T-bet–IFN-γ axis in vascular monocyte recruitment and inflammation, reactive oxygen species (ROS) production, and vascular endothelial and smooth muscle dysfunction. Additionally, we revealed a central role for IFN-γ–producing NK-cells and the mutual activation of NK-cells and monocytes in the vasculature in ATII-induced vascular injury.

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Materials and Methods

Materials and Methods are available in the online-only Supplement. Male C57BL/6, IFN-γ$^{-/-}$, Rag-1$^{-/-}$ (all from Jackson Laboratories, Bar Harbor, Maine), as well as serum amyloid P component gene–IFN-γ,[14] Tbx21$^{-/-}$,[15] lysozyme M (LysM)$^{Cre/Cre}$,[16] ROSA26$^{iDTR/iDTR}$[17] (crossed to generate male LysM$^{Cre/wt}$ and LysM$^{Cre/wt}$/ROSA26$^{iDTR/wt}$, abbreviated LysM and LysM^{iDTR})[18] all on the C57BL/6J background were used as experimental animals. Mice were treated with ATII-loaded miniosmotic pumps (1 mg/kg per day for 7 days) or sham-treated, partially equipped with carotid catheter implants for telemetric blood pressure measurements. In selected experiments, C57BL/6 mice were depleted of NK-cells by injecting depleting anti-NK1.1 antibody, or LysM^{iDTR} mice were depleted of monocytes by diphtheria-toxin receptor–mediated cell ablation. After 7 days of ATII treatment, mice were killed, and blood and aorta were collected. Tissue was subjected to vascular relaxations studies, ROS measurements (lucigenin-enhanced chemiluminescence and fluorescence oxidative microtopography), flow cytometry analysis of inflammatory cells and cytokine production, and mRNA and protein expression analysis. Data are expressed as mean±SEM or median with box plot and whiskers (min to max).

Results

IFN-γ Promotes ATII-Induced Vascular Inflammation and Dysfunction

ATII-induced vascular endothelial and smooth muscle dysfunction in wild-type (WT) mice was accompanied by increased aortic IFN-γ and T-bet expression (Figure 1A and 1B). IFN-γ$^{-/-}$ and Tbx21$^{-/-}$ mice (deficient in the gene encoding for T-bet) remained largely protected from ATII-induced vascular dysfunction (Figure 1C and 1D; Tables I and II in the online-only Data Supplement), whereas transgenic serum amyloid P component gene–IFN-γ mice with systemic IFN-γ overproduction showed constitutive endothelial dysfunction and nox2 and p67^{phox} upregulation in aortic lysates in the absence of ATII (Figure IA and IB in the online-only Data Supplement). Indicative of a proinflammatory and pro-oxidant role of IFN-γ in ATII-induced vascular injury expression levels and membrane translocation of the nicotinamide adenosine dinucleotide phosphate oxidase subunits Nox2 (gp91^{phox}), p22^{phox}, p67^{phox}, and p47^{phox} increased in response to ATII in WT controls but remained significantly dampened in Tbx21$^{-/-}$ mice (Figure 2A–2D; Figure IIA and IIB in the online-only Data Supplement). Likewise, ATII-infused Tbx21$^{-/-}$ mice showed less superoxide formation in aortic rings and whole blood and drastically less aortic peroxynitrite formation than controls, paralleled by blunted ATII-induced aortic inducible NO synthase (iNOS) mRNA expression (Figure 2E–2H; Figure IIC in the online-only Data Supplement). In contrast to differences in ATII-induced vascular dysfunction and except for a slight delay in blood pressure increase in Tbx21$^{-/-}$ mice, blood pressure levels evaluated by continuous telemetric recording at baseline and during 7 days of ATII infusion (at 336 hours) showed no significant difference in systolic blood pressure between Tbx21$^{-/-}$ (systolic: 121.1±1.79 versus 157.9±11.48 mm Hg) and WT mice (120.9.±1.02 versus 167.1±1.00 mm Hg; Figure IID in the online-only Data Supplement).

Depletion of NK-Cells Protects From ATII-Induced Vascular Dysfunction

ATII-induced vascular dysfunction in WT mice was marked by a drastic recruitment of NK1.1^{+}T cell receptor (TCR) β^{-} NK-cells into the aortic wall, which was virtually absent in Tbx21$^{-/-}$ and IFN-γ$^{-/-}$ mice (Figure 3A). Remarkably, depletion of NK-cells by injection of a depleting monoclonal NK1.1-specific antibody before ATII treatment significantly reduced endothelial and smooth-muscle vascular dysfunction in WT mice (Figure 3B and 3C; Table III in the online-only Data Supplement) to the level observed in IFN-γ$^{-/-}$ and Tbx21$^{-/-}$ mice. In contrast, depletion of CD4^{+} T cells by injection of a depleting monoclonal anti-CD4 antibody did not significantly

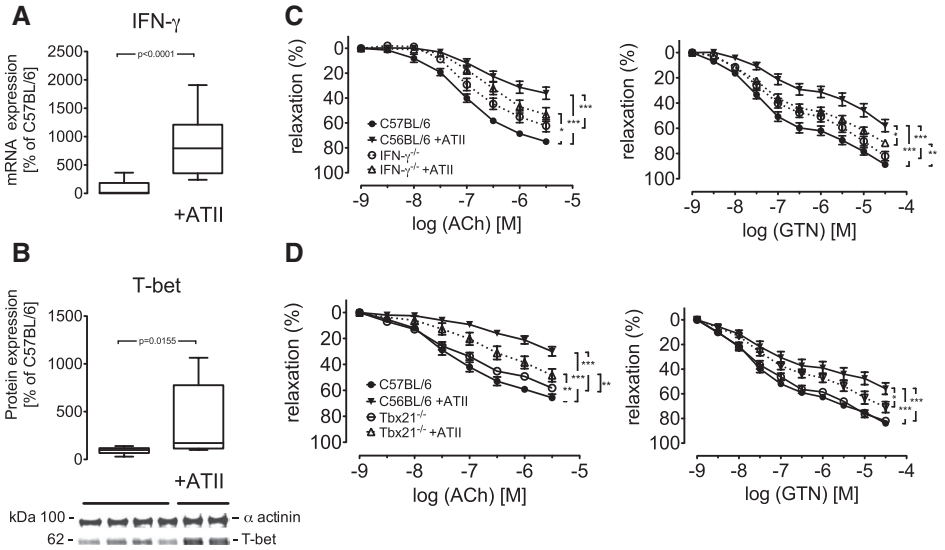

Figure 1. Angiotensin II (ATII)–induced vascular inflammation and dysfunction depends on interferon (IFN)-γ production in the vascular wall. **A**, Aortic IFN-γ mRNA expression, Mann–Whitney-test, n= 5 to 7. **B**, Aortic T-box expressed in T cells (T-bet) protein expression. **Top**, Quantification. **Bottom**, Representative Western blot (1 of 3 independent experiments shown); unpaired *t* test, n= 10 to 12. **C** and **D**, Cumulative concentration–relaxation curves of isolated aortic rings from sham-treated and ATII-treated C57BL/6, IFN-γ$^{-/-}$ mice, and Tbx21$^{-/-}$ mice in response to endothelium-dependent (acetylcholine, ACh) and endothelium-independent vasodilators (glyceryl trinitrate, GTN). One-way ANOVA of maximal relaxation, n= 6 to 11.

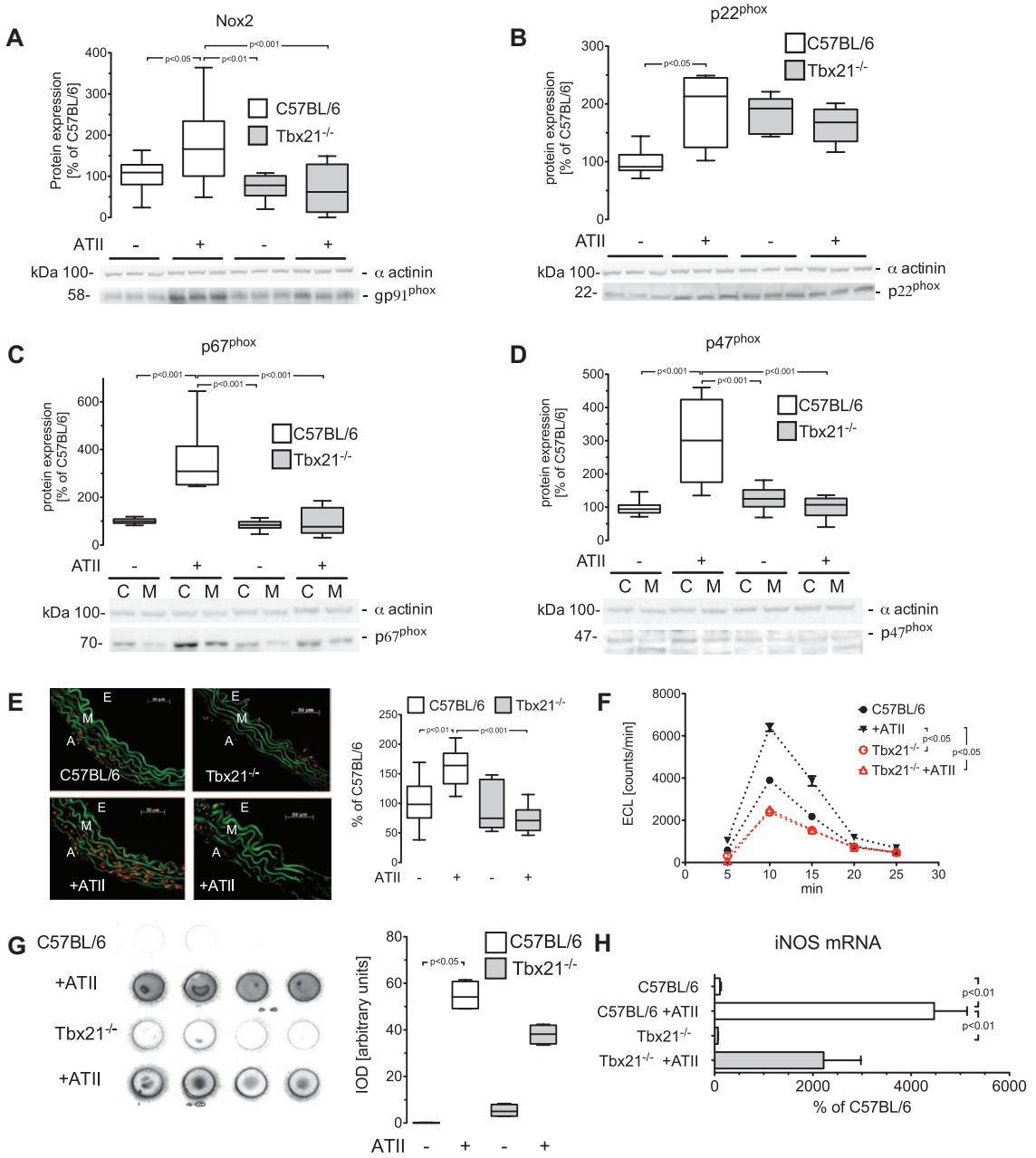

Figure 2. Attenuated upregulation of nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase expression, inducible NO synthase (iNOS) expression, and peroxynitrite formation in the aortic wall of Tbx21$^{-/-}$ mice. **A–D**, Vascular expression of the NADPH oxidase subunits Nox-2 (gp91^{phox} [**A**], p22^{phox} [**B**], p67^{phox} [**C**], and p47^{phox} [**D**] in sham-treated and angiotensin II [ATII]-treated C57BL/6 and Tbx21$^{-/-}$ mice). Protein expression was assessed by SDS-PAGE and Western blot in aortic lysates. **Top**, Quantification. **Bottom**, Representative original blots. C indicates cytosolic fraction; and M, membraneous fraction. Kruskal-Wallis test, n= 4 to 11. **E**, Superoxide formation in aortic tissue of C57BL/6 and Tbx21$^{-/-}$ mice. **Left**, Representative dihydroethidium-photomicrotopographs of aortic cryosections, superoxide formation appears in red. A indicates adventitia; E, endothelium; and M, media. **Right**, Quantification. Kruskal-Wallis test, n= 6 to 10. **F**, L-012 enhanced chemiluminescence in whole blood after phorbolester incubation (PdBU). Friedmann test, n=6**. G**, Vascular peroxynitrite formation assessed by dot blot analysis of aortic lysates using a 3-nitrotyrosine–specific antibody. **Left**, Representative blot. **Right**, Quantification. Kruskal-Wallis test n=4. **H**, mRNA expression of iNOS in aortic lysates, Kruskal-Wallis test, n=3.

influence ATII-induced vascular dysfunction (not shown). These observations indicate that NK-cells are required for the initiation of ATII-induced vascular inflammation.

ATII-Induced Vascular Inflammation Depends on Mutual NK-Cell Monocyte Activation

In addition to NK-cell infiltration, ATII-induced aortic infiltration of CD11b^{+}Gr-1^{low} monocytes was significantly attenuated in Tbx21$^{-/-}$ and IFN-γ$^{-/-}$ mice (Figure 3D) and consecutively accompanied by reduced upregulation of monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1 α, and P-selectin ligand in ATII-infused Tbx21$^{-/-}$ mice (Figure 3E; Figure IIIA–IIIC in the online-only Data Supplement).

NK-cells are driven to full maturation by CD11b^{+} monocyte–derived interleukin (IL)-12.[19] Interestingly, ATII increased vascular IL-12 mRNA expression in the aortic wall

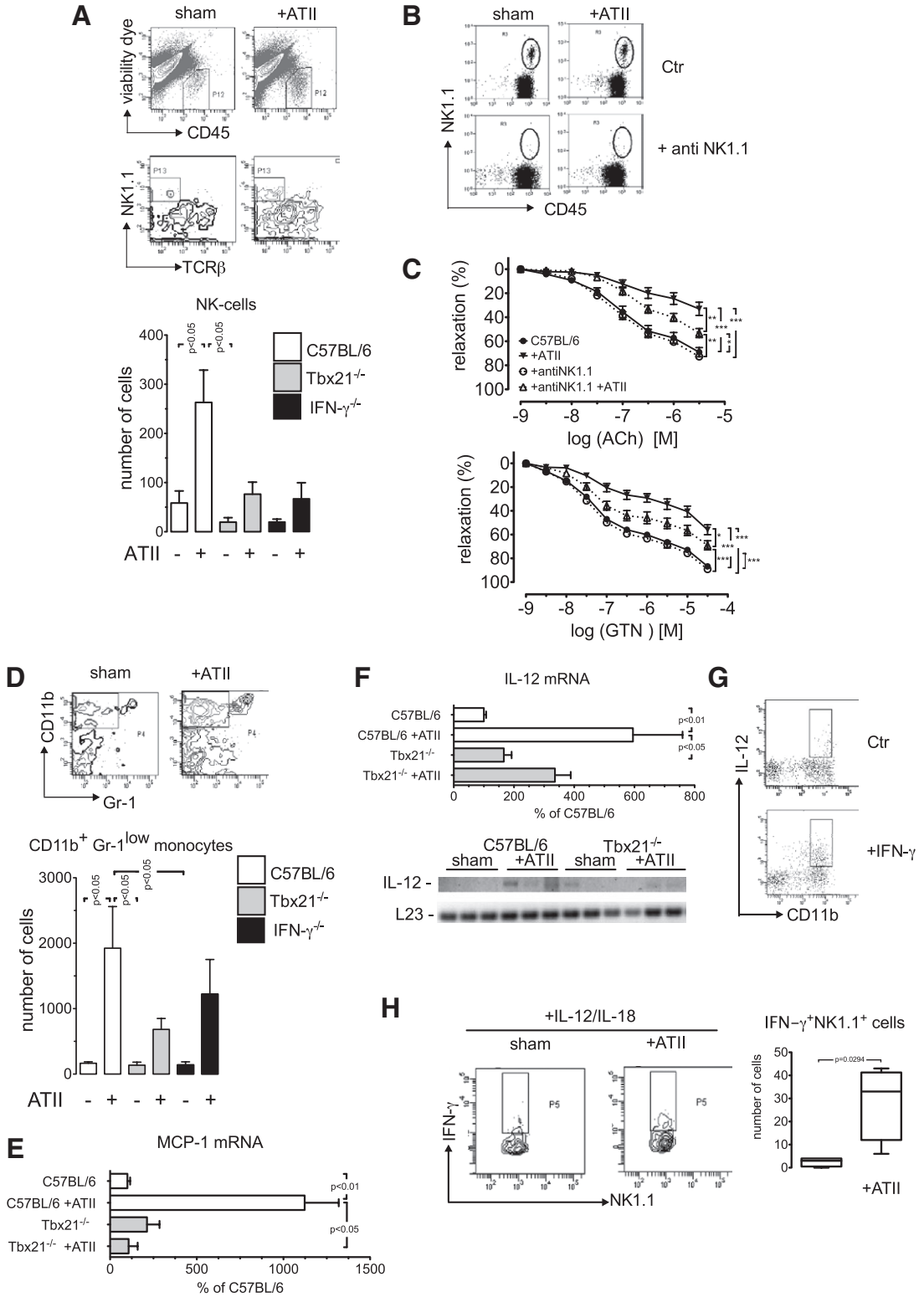

Figure 3. Depletion of NK1.1+ NK-cells attenuates angiotensin II (ATII)-induced vascular dysfunction. **A**, Flow cytometric quantification of NK-cells in aortic tissue of sham-treated and ATII-treated mice. **Top**, Representative contour plots. **Bottom**, Quantification. Kruskal-Wallis test, n= 6 to 8. **B**, Depletion of NK1.1+ NK-cells. Representative flow cytometric dot plots of venous blood. **C**, Cumulative concentration–relaxation curves of isolated aortic rings from sham-treated and ATII-treated NK1.1+-cell-depleted or sham-injected C57BL/6 mice. One-way ANOVA of maximal relaxation, n= 7 to 8. **D**, Flow cytometric quantification of CD11b+Gr-1low monocytes in aortic tissue of sham-treated and ATII-treated mice. **Top**, Representative contour plots. **Bottom**, Quantification. Kruskal-Wallis test, n= 6 to 8. **E**, Monocyte chemoattractant protein-1 (MCP-1) mRNA expression in aortic lysates, Kruskal-Wallis test, n= 2 to 3. **F**, Interleukin (IL)-12 mRNA expression. **Top**, Quantification, **Bottom**, Representative gel. Kruskal-Wallis test, n= 3 to 5. **G**, IL-12 formation of CD11b+ cells with or without interferon (IFN)-γ–stimulation. Representative flow cytometry dot plot. **H**, Flow-cytometric analysis of IFN-γ–producing NK1.1+ NK-cells in aortic tissue of sham-treated and ATII-treated C57BL/6 mice. **Left**, Representative contour plots of IL-12/IL-18–stimulated aortic cells. **Right**, Quantification. *t* test (Wilcoxon-matched pairs test), n= 4 to 5.

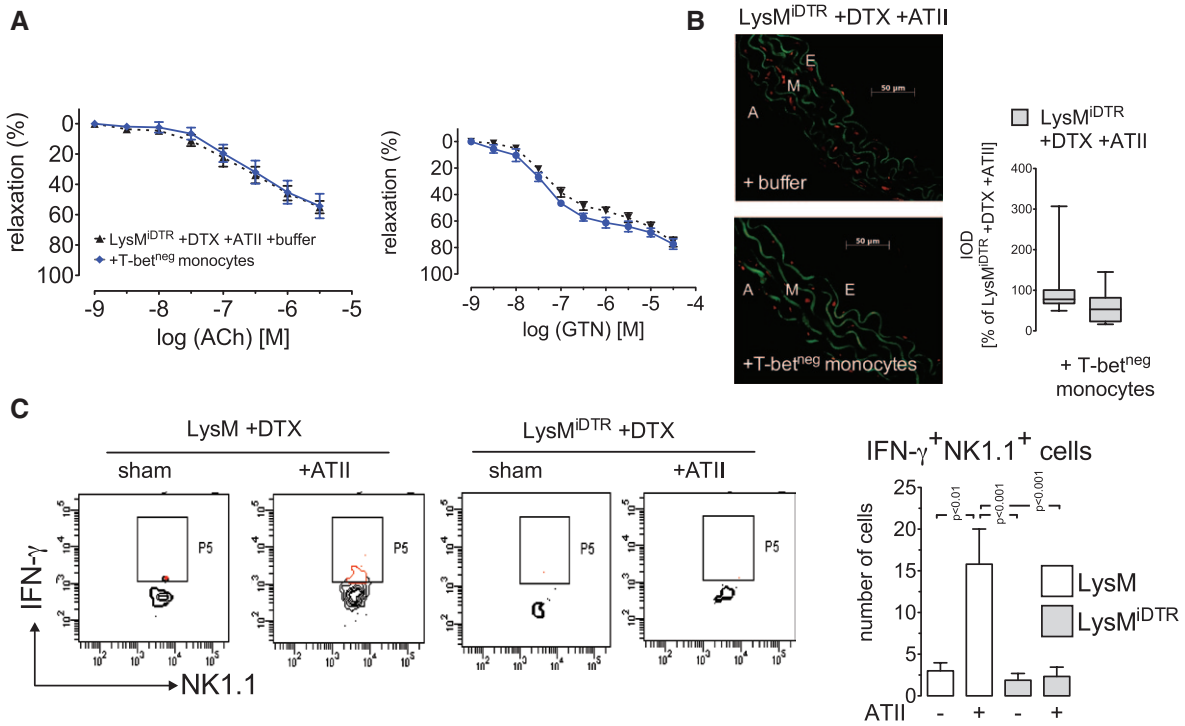

Figure 4. Vascular interferon (IFN)-γ production by NK-cells depends on the presence of T-box expressed in T cells (T-bet)$^+$ monocytes. Monocyte-depleted and angiotensin II (ATII)–infused LysM^{iDTR} were reconstituted with either wild-type or T-bet^{neg} monocytes. **A**, Cumulative concentration–relaxation curves of isolated aortic rings in response to acetylcholine (ACh) and glyceryl trinitrate (GTN). One-way ANOVA of maximal relaxation, n= 3 to 5. **B**, Superoxide formation in aortic cryosections. **Left**, Representative dihydroethidium-photomicrotopographs. Right, Quantification. *t* test, n=10. **C**, Flow-cytometric analysis of IFN-γ–producing NK1.1$^+$ NK-cells in aortic tissue of LysM and monocyte-depleted LysM^{iDTR} mice. **Left**, Representative dot plots. **Right**, Quantification. One-way ANOVA, n= 6 to 8. A indicates adventitia; E, endothelium; and M, media.

in WT, but significantly less in Tbx21$^{-/-}$ mice (Figure 3F and 3G), underlining the important role for T-bet directing IL-12 formation by CD11b$^+$ cells. Confirming the interconnection to NK-cell–derived IFN-γ in ATII-induced vascular inflammation, aortic NK1.1$^+$ NK-cells produced IFN-γ in response to IL-12/IL-18 ex vivo stimulation (Figure 3H). Because the latter suggested a role of T-bet$^+$CD11b$^+$ myelomonocytic cells in NK-cell recruitment and local activation, we depleted LysM^{iDTR} mice of monocytes and reconstituted them with Tbx21$^{-/-}$ or WT monocytes. Reconstitution of depleted LysM^{iDTR} mice with WT, but not with T-bet^{neg} monocytes,[18] re-establishes ATII-induced vascular endothelial or smooth muscle dysfunction (Figure 4A; Table IV in the online-only Data Supplement) and vascular oxidative stress (Figure 4B). In addition, depletion of LysM$^+$ cells attenuated the increase of IFN-γ$^+$NK1.1$^+$ NK-cells in response to ATII in the aortic wall (Figure 4C). Together these findings reveal a crucial role of monocytic T-bet expression in vascular NK-cell recruitment and mutual NK-cell and monocyte activation in ATII-induced vascular dysfunction.

Discussion

We show here that ATII-induced vascular dysfunction depends on vascular entry and IFN-γ production by NK-cells. Despite representing a major component of the innate immune system and playing an important role in tissue inflammation, NK-cells have surprisingly not been considered in ATII-induced vascular dysfunction earlier.

NK-cells are poised for immediate effector function and are powerful producers of various inflammatory cytokines and growth factors, such as IFN-γ, tumor necrosis factors-α, and granulocyte-macrophage colony–stimulating factor. On recruitment to inflammatory sites, NK-cells engage with monocytes in a reciprocal program of activation. Within this mutual activation, NK-cell–derived IFN-γ plays an important role in propagating the activation and maturation of monocytes into macrophages and dendritic cells that produce IL-15, IL-12, and IL-18.[20–22] In turn, IL-12 synergizes with IL-18 in stimulating IFN-γ production in NK-cells, resulting in a positive feedback loop that represents an important amplifying mechanism in the early innate inflammatory response. In accordance with this mechanism, we find that the accumulation of NK-cells and monocytes is paralleled by an increase not only of IFN-γ, but also of IL-12 in aortic tissue in response to ATII, a mechanism drastically reduced in Tbx21$^{-/-}$ mice. However, pursuant to their functional interdependency in the initiation of inflammation, neither adoptive transfer of IFN-γ competent NK-cells nor monocytes alone could re-establish ATII-induced vascular dysfunction and oxidative stress in Tbx21$^{-/-}$ mice (Figure IV in the online-only Data Supplement).

Like macrophages, NK-cells are powerful producers of the chemoattractant macrophage inflammatory protein 1 α, especially when stimulated by monocytes.[23] Additionally, MCP-1 is causally involved in monocyte recruitment via interaction with the C-chemokine receptor 2,[24,25] and monocytes are themselves the major cellular source of MCP-1 expression.[26] These

chemokines of the C-chemokine ligand family are required for effective chemoattraction, endothelial transmigration, and infiltration of myelomonocytic cells, but also lymphoid cells like NK-cells to inflamed tissue[27] and are essentially involved in the pathogenesis of atherosclerosis.[28,29] It has been demonstrated that combined knockout and inhibition of C-chemokine ligand-2 (ie, MCP-1), CX_3CR1, the receptor for fraktalkine (or CX_3CL1) and CCR5 (ie, the macrophage inflammatory protein 1 α receptor) almost completely abolishes atherosclerotic lesion formation,[30] underscoring the importance of these chemokines for atherogenesis. Notably, the expression of MCP-1/C-chemokine ligand-2 has been shown to increase dramatically in response to IFN-γ.[31] Consistent with this concept, MCP-1 and macrophage inflammatory protein 1 α, but also P-selectin ligand expression, were significantly reduced in aortic homogenates of ATII-infused $Tbx21^{-/-}$ mice as compared with ATII-infused controls,[32,33] paralleled by a reduced vascular influx of inflammatory leukocytes.

We and others previously observed that inflammatory cells of the myelomonocytic lineage promote ATII-induced vascular dysfunction mainly through phagocyte-type nicotinamide adenosine dinucleotide phosphate oxidase–driven ROS formation and inducible NO synthase activity.[18,24,34–36] Consistent with this concept, $Tbx21^{-/-}$ mice showed attenuated nicotinamid adenosin dinucleotid phosphate oxidase activity and expression as well as reduced inducible NO synthase expression and peroxynitrite formation, underscoring the importance of the T-bet/IFN-γ axis to promote ATII-induced inflammatory cell–driven oxidative stress in the vasculature. Interestingly, adoptive transfer of T-bet–deficient monocytes was insufficient to reinstate ATII-induced vascular dysfunction and oxidative stress in monocyte-depleted $LysM^{iDTR}$ mice, indicating a role of T-bet in vascular monocyte inflammatory activity. Indeed, monocytes have been shown to depend on T-bet to activate NK-cells via IL-12,[19] and when we depleted monocytes in vivo, the amount of IFN-γ–competent NK-cells in the aorta of mice challenged by ATII was drastically reduced (Figure 4). Thus, the T-bet/IFN-γ axis participates in both sides of the reciprocal monocyte NK-cell activation, initiating vascular inflammation independent from blood pressure changes, which were unaltered in $Tbx21^{-/-}$ mice. This observation is in line with previous studies on ATII-induced cardiac inflammation and cardiac damage in mice deficient in IFN-γ–signaling, revealing an unaltered blood pressure response to ATII compared with WT mice. Thus, although IFN-γ seems to be required in the initiation of vascular inflammation, it does not affect blood pressure changes, indicating that the T-bet/IFN-γ–driven proinflammatory action of ATII on the vasculature is independent of hemodynamics.

In summary, we identify IFN-γ formed locally by NK-cells in the aortic wall as a critical initiator of vascular oxidative stress, inflammatory cell recruitment, and reciprocal innate immune cell activation in the vessel wall and show that NK-cell depletion largely protects from ATII-induced vascular dysfunction.

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Disclosures

None.

References

1. Harrison DG, Marvar PJ, Titze JM. Vascular inflammatory cells in hypertension. *Front Physiol*. 2012;3:128.
2. Stetson DB, Mohrs M, Reinhardt RL, Baron JL, Wang ZE, Gapin L, Kronenberg M, Locksley RM. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J Exp Med*. 2003;198:1069–1076.
3. Wang X, Mosmann T. In vivo priming of CD4 T cells that produce interleukin (IL)-2 but not IL-4 or interferon (IFN)-gamma, and can subsequently differentiate into IL-4- or IFN-gamma-secreting cells. *J Exp Med*. 2001;194:1069–1080.
4. Munder M, Mallo M, Eichmann K, Modolell M. Murine macrophages secrete interferon gamma upon combined stimulation with interleukin (IL)-12 and IL-18: a novel pathway of autocrine macrophage activation. *J Exp Med*. 1998;187:2103–2108.
5. Ohteki T, Fukao T, Suzue K, Maki C, Ito M, Nakamura M, Koyasu S. Interleukin 12-dependent interferon gamma production by CD8alpha+ lymphoid dendritic cells. *J Exp Med*. 1999;189:1981–1986.
6. Boehm U, Klamp T, Groot M, Howard JC. Cellular responses to interferon-gamma. *Annu Rev Immunol*. 1997;15:749–795.
7. Issekutz TB. Effects of six different cytokines on lymphocyte adherence to microvascular endothelium and *in vivo* lymphocyte migration in the rat. *J Immunol*. 1990;144:2140–2146.
8. Hillyer P, Mordelet E, Flynn G, Male D. Chemokines, chemokine receptors and adhesion molecules on different human endothelia: discriminating the tissue-specific functions that affect leucocyte migration. *Clin Exp Immunol*. 2003;134:431–441.
9. Shao J, Nangaku M, Miyata T, Inagi R, Yamada K, Kurokawa K, Fujita T. Imbalance of T-cell subsets in angiotensin II-infused hypertensive rats with kidney injury. *Hypertension*. 2003;42:31–38.
10. Crowley SD, Song YS, Lin EE, Griffiths R, Kim HS, Ruiz P. Lymphocyte responses exacerbate angiotensin II-dependent hypertension. *Am J Physiol Regul Integr Comp Physiol*. 2010;298:R1089–R1097.
11. Han YL, Li YL, Jia LX, Cheng JZ, Qi YF, Zhang HJ, Du J. Reciprocal interaction between macrophages and T cells stimulates IFN-γ and MCP-1 production in Ang II-induced cardiac inflammation and fibrosis. *PLoS ONE*. 2012;7:e35506.
12. Markó L, Kvakan H, Park JK, Qadri F, Spallek B, Binger KJ, Bowman EP, Kleinewietfeld M, Fokuhl V, Dechend R, Müller DN. Interferon-γ signaling inhibition ameliorates angiotensin II-induced cardiac damage. *Hypertension*. 2012;60:1430–1436.
13. Lazarevic V, Glimcher LH. T-bet in disease. *Nat Immunol*. 2011;12:597–606.
14. Toyonaga T, Hino O, Sugai S, Wakasugi S, Abe K, Shichiri M, Yamamura K. Chronic active hepatitis in transgenic mice expressing interferon-gamma in the liver. *Proc Natl Acad Sci USA*. 1994;91:614–618.
15. Szabo SJ, Sullivan BM, Stemmann C, Satoskar AR, Sleckman BP, Glimcher LH. Distinct effects of T-bet in TH1 lineage commitment and IFN-gamma production in CD4 and CD8 T cells. *Science*. 2002;295:338–342.
16. Clausen BE, Burkhardt C, Reith W, Renkawitz R, Förster I. Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res*. 1999;8:265–277.
17. Buch T, Heppner FL, Tertilt C, Heinen TJ, Kremer M, Wunderlich FT, Jung S, Waisman A. A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. *Nat Methods*. 2005;2:419–426.

18. Wenzel P, Knorr M, Kossmann S, et al. Lysozyme M-positive monocytes mediate angiotensin II-induced arterial hypertension and vascular dysfunction. *Circulation*. 2011;124:1370–1381.
19. Soderquest K, Powell N, Luci C, van Rooijen N, Hidalgo A, Geissmann F, Walzer T, Lord GM, Martín-Fontecha A. Monocytes control natural killer cell differentiation to effector phenotypes. *Blood*. 2011;117:4511–4518.
20. D'Andrea A, Rengaraju M, Valiante NM, Chehimi J, Kubin M, Aste M, Chan SH, Kobayashi M, Young D, Nickbarg E. Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. *J Exp Med*. 1992;176:1387–1398.
21. Goldszmid RS, Caspar P, Rivollier A, White S, Dzutsev A, Hieny S, Kelsall B, Trinchieri G, Sher A. NK cell-derived interferon-γ orchestrates cellular dynamics and the differentiation of monocytes into dendritic cells at the site of infection. *Immunity*. 2012;36:1047–1059.
22. Welte S, Kuttruff S, Waldhauer I, Steinle A. Mutual activation of natural killer cells and monocytes mediated by NKp80-AICL interaction. *Nat Immunol*. 2006;7:1334–1342.
23. Bluman EM, Bartynski KJ, Avalos BR, Caligiuri MA. Human natural killer cells produce abundant macrophage inflammatory protein-1 alpha in response to monocyte-derived cytokines. *J Clin Invest*. 1996;97:2722–2727.
24. Bush E, Maeda N, Kuziel WA, Dawson TC, Wilcox JN, DeLeon H, Taylor WR. CC chemokine receptor 2 is required for macrophage infiltration and vascular hypertrophy in angiotensin II-induced hypertension. *Hypertension*. 2000;36:360–363.
25. Ishibashi M, Hiasa K, Zhao Q, Inoue S, Ohtani K, Kitamoto S, Tsuchihashi M, Sugaya T, Charo IF, Kura S, Tsuzuki T, Ishibashi T, Takeshita A, Egashira K. Critical role of monocyte chemoattractant protein-1 receptor CCR2 on monocytes in hypertension-induced vascular inflammation and remodeling. *Circ Res*. 2004;94:1203–1210.
26. Yoshimura T, Yuhki N, Moore SK, Appella E, Lerman MI, Leonard EJ. Human monocyte chemoattractant protein-1 (MCP-1). Full-length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. *FEBS Lett*. 1989;244:487–493.
27. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med*. 2006;354:610–621.
28. Libby P. Inflammation in atherosclerosis. *Nature*. 2002;420:868–874.
29. Zernecke A, Shagdarsuren E, Weber C. Chemokines in atherosclerosis: an update. *Arterioscler Thromb Vasc Biol*. 2008;28:1897–1908.
30. Combadière C, Potteaux S, Rodero M, Simon T, Pezard A, Esposito B, Merval R, Proudfoot A, Tedgui A, Mallat Z. Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation*. 2008;117:1649–1657.
31. Valente AJ, Xie JF, Abramova MA, Wenzel UO, Abboud HE, Graves DT. A complex element regulates IFN-gamma-stimulated monocyte chemoattractant protein-1 gene transcription. *J Immunol*. 1998;161:3719–3728.
32. Lord GM, Rao RM, Choe H, Sullivan BM, Lichtman AH, Luscinskas FW, Glimcher LH. T-bet is required for optimal proinflammatory CD4+ T-cell trafficking. *Blood*. 2005;106:3432–3439.
33. Underhill GH, Zisoulis DG, Kolli KP, Ellies LG, Marth JD, Kansas GS. A crucial role for T-bet in selectin ligand expression in T helper 1 (Th1) cells. *Blood*. 2005;106:3867–3873.
34. De Ciuceis C, Amiri F, Brassard P, Endemann DH, Touyz RM, Schiffrin EL. Reduced vascular remodeling, endothelial dysfunction, and oxidative stress in resistance arteries of angiotensin II-infused macrophage colony-stimulating factor-deficient mice: evidence for a role in inflammation in angiotensin-induced vascular injury. *Arterioscler Thromb Vasc Biol*. 2005;25:2106–2113.
35. Liu J, Yang F, Yang XP, Jankowski M, Pagano PJ. NAD(P)H oxidase mediates angiotensin II-induced vascular macrophage infiltration and medial hypertrophy. *Arterioscler Thromb Vasc Biol*. 2003;23:776–782.
36. Rey FE, Cifuentes ME, Kiarash A, Quinn MT, Pagano PJ. Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular O(2)(-) and systolic blood pressure in mice. *Circ Res*. 2001;89:408–414.

Significance

Inflammatory monocytes and macrophages are essential for angiotensin II–induced vascular dysfunction. Interferon-γ is one of the most prominent proinflammatory cytokines and seems to play a role in angiotensin II–induced cardiovascular injury and inflammation. We here show that mice deficient in interferon-γ or its transcription factor, T-bet, are largely protected from vascular oxidative stress, endothelial dysfunction, and from vascular infiltration of monocytes and NK-cells in response to angiotensin II. We uncover a hitherto unrecognized role of natural killer cells in driving angiotensin II–induced vascular inflammation and reveal that NK-cells and monocytes undergo a reciprocal pattern of activation that depends on T-bet, NK-cell–derived interferon-γ, and monocyte-derived interleukin-12 within the vascular wall. These findings could have broad implications for our understanding of atherogenesis and provide potential new therapeutic targets in the treatment of arterial hypertension and cardiovascular disease.