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ORIGINAL RESEARCH ARTICLE

CD4+ T Cells Expressing Viral Proteins Induce HIV-Associated Endothelial Dysfunction and Hypertension Through Interleukin 1α–Mediated Increases in Endothelial NADPH Oxidase 1

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BACKGROUND: Although combination antiretroviral therapy has increased life expectancy in people living with HIV, it has led to a marked increase in the prevalence of hypertension, the cause of which is unknown. Despite combination antiretroviral therapy, HIV-derived proteins remain expressed and produced by CD4+ T lymphocytes in people living with HIV. However, their contribution to HIV-associated hypertension and impaired endothelium-dependent relaxation remains ill defined.

METHODS: Here, we tested the hypothesis that CD4+ T cells expressing viral proteins contribute to endothelial dysfunction and hypertension using the Tg26 mouse model of HIV that expresses 7 of the 9 HIV proteins under the long terminal repeat promoter. We used male and female mice, bone marrow transplantation (BMT), adoptive transfer of CD4+ T cells, and aorta specimen discarded from people living with HIV.

RESULTS: We reported that intact Tg26 mice and mice receiving BMT (Tg26→WT) or CD4+ T cells from Tg26 mice display impaired endothelium-dependent relaxation and hypertension. Conversely, BMT from WT mice into Tg26 mice, inhibition of T cell activation, and CD4+ T cell depletion restored endothelial function and blood pressure in Tg26 mice. Cytokine profiling revealed that Tg26 mice, Tg26→WT, and Tg26 CD4+ T cells consistently exhibit high interleukin 1α (IL-1α) levels with no significant increase in other cytokines, whereas BMT from WT mice into Tg26 mice reduced IL-1α levels. IL-1α neutralization reduced blood pressure and restored endothelial function in Tg26 mice. To investigate the role of CD4+ T cells and IL-1α in endothelial dysfunction, we developed an aorta-immune cell coculture system. Exposure of WT aortas to Tg26 CD4+ T cells impaired endothelium-dependent relaxation, which was blocked by IL-1α–neutralizing antibody. While investigating the mechanisms of endothelial dysfunction, we reported that Tg26 mice, Tg26→WT aorta exhibit high NADPH oxidase (NOX) 1 expression. IL-1α exposure increased NOX1 in human microvascular endothelial cells, and NOX1 blockade restored endothelial function in Tg26 and Tg26→WT arteries, whereas NOX1 deficiency protected against Tg26 BMT-induced impaired endothelium-dependent relaxation and hypertension. Aortas from people living with HIV exhibit high NOX1 levels, and exposure of human aorta to Tg26 T cells increased NOX1 expression.

CONCLUSIONS: We provide the first evidence that CD4+ T cells expressing HIV viral proteins induced hypertension through IL-1α–mediated increases in vascular NOX1, which impairs endothelial function in males and females.

Key Words: CD4-positive T-lymphocytes ■ endothelium ■ HIV ■ hypertension ■ interleukin-1alpha ■ oxidative stress ■ viral proteins

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Clinical Perspective

What Is New?

- Although people living with HIV (PLWH) have a high prevalence of hypertension, its cause remains unknown. Here, we identified HIV-encoded proteins, which remain in circulation in PLWH despite combination antiretroviral therapy, as key contributors to hypertension.
- Clinical studies have established a positive association between CD4+ T cell count and hypertension in PLWH; however, whether CD4+ T cells contribute to hypertension has not been established yet. Here, we demonstrated that CD4+ T cells expressing HIV-encoded proteins cause hypertension through the release of the proinflammatory cytokine interleukin-1α.
- We also found that interleukin-1α impairs endothelium-dependent relaxation through nuclear factor-κB– and caspase 1–mediated increases in endothelial NADPH oxidase 1 in rodent and human arteries.

What Are the Clinical Implications?

- If confirmed in human populations, the mechanisms identified would suggest that targeting interleukin-1α signaling or the interleukin-1 receptor could represent a novel avenue to treat hypertension in PLWH on combination antiretroviral therapy.
- Similarly targeting NADPH oxidase 1–derived reactive oxygen species production could constitute a novel approach to improve endothelium-dependent relaxation and to protect against the accelerated development of cardiovascular diseases in PLWH on combination antiretroviral therapy.

Nonstandard Abbreviations and Acronyms

BM	bone marrow
BMT	bone marrow transplantation
BP	blood pressure
cART	combination antiretroviral therapy
CRC	concentration-response curve
CVD	cardiovascular disease
hMVEC	human microvascular endothelial cell
IL	interleukin
NF-κB	nuclear factor κB
NK	natural killer
NOX	NADPH oxidase
PLWH	people living with HIV
SBP	systolic blood pressure
SNP	sodium nitroprusside
WT	wild-type

Thanks to the advent of combination antiretroviral therapy (cART), life expectancy has markedly increased in people living with HIV (PLWH).[1] However, this major clinical advance has led to a shift in the spectrum of diseases related to HIV from opportunistic AIDS-related disorders to cardiovascular disease (CVD). CVD is now the leading cause of death in PLWH,[2] and hypertension, the primary risk factor for CVD,[3] is highly prevalent in PLWH on cART.[4–6] Indeed, a recent worldwide meta-analysis indicates that 35% of PLWH on cART develop hypertension, whereas hypertension affects 30% of noninfected individuals globally.[6] Further highlighting this global problem are recent studies from the United States reporting a prevalence of hypertension reaching up to 68% of PLWH on cART.[5] However, although the epidemiological problem of HIV-associated hypertension is well defined, its cause remains largely unknown, and experimental studies investigating its underlying mechanisms are nonexistent.

PLWH on cART present with more traditional risk factors than seronegative individuals. However, the higher number of noncommunicable comorbidities has been shown to be insufficient to explain the higher prevalence of hypertension in PLWH,[4] which supports the contribution of virological or treatment-related factors to the pathophysiology. It is interesting that the prevalence of hypertension is consistently higher in cART-treated compared with cART-naive PLWH[7,8] and lower in cART-naive PLWH compared with noninfected individuals.[9] These observations imply that CD4+ T lymphocytes (T cells), which are high in cART-treated PLWH and low in cART-naive PLWH, could contribute to hypertension. Further substantiating this hypothesis is the demonstration that lower baseline CD4+ T cell counts are associated with lower baseline blood pressure (BP) levels when higher CD4+ T cell counts accompanied higher BP levels in PLWH on cART.[8] Concomitant to this positive association between CD4+ T cell counts and BP levels, experimental studies conducted in animals deficient in B and T cells elegantly established a role for T cells in hypertension, notably CD4+ T cells, and involved the production of proinflammatory cytokines.[10]

CD4+ T cells represent the largest and best-characterized reservoir of HIV in PLWH on cART.[11] HIV remains transcriptionally active[12] in CD4+ T cells, and viral protein expression persists.[13] HIV-derived proteins remain in circulation in PLWH on cART[14] and create an inflammatory environment promoting CVD.[15] Therefore, we hypothesized that CD4+ T cells expressing HIV-derived proteins induce hypertension through the release of proinflammatory cytokines in male and female mice. To test this hypothesis, we combined the use of the Tg26 mouse model of HIV with bone marrow (BM)

transplantation (BMT) and adoptive transfer of T cells and analyzed aortic specimens discarded from PLWH.

METHODS

The data, study materials, and analytical methods used in this research are accessible to other researchers for the purpose of reproducing the results or replicating the procedure. Requests can be made to the corresponding author, who manages the information. All other data that support the findings of this study are available from the corresponding author on reasonable request.

Animals

Experiments were conducted in 5-month-old littermate male and female wild-type (WT) and Tg26 mice on the C57/Bl6 background. Hemizygote Tg26 mice randomly expresses a truncated HIV-1 NL4-3 genome with a 3.1-kb deletion of *Gag* and *Pol*, thus rendering this model replication deficient.[16] All animal investigations were conducted in an American Association for the Accreditation of Laboratory Animal Care–accredited facility with studies approved by the institutional animal care and use committee (protocol 2011-0108). For euthanization procedures, mice were first anesthetized in a closed chamber with 5% isoflurane (1 L/min O_2) before decapitation with a guillotine. Isolated tissues were snap-frozen in liquid nitrogen before storage at −80 °C. Because of the abundant literature supporting that the female estrous cycle does not significantly affect microvascular endothelial function and BP,[17] the stage of the female mouse estrous cycle was not determined.

BM Transplantation

Recipient male and female WT and Tg26 mice were treated with the antibiotic enrofloxacin (0.25 mg/mL) in autoclaved reverse-osmosis water at pH 3 for a week before being irradiated with 900 rads with a gamma irradiator (X-Rad 320, precision x-ray). BM was isolated from the tibias and femurs of donor WT and Tg26 mice at 12 weeks of age. Six hours after irradiation, recipient mice were given 10 million donor cells retro-orbitally, and reconstitution was allowed for 8 weeks[18] before the conduction of the procedures described below and euthanasia as described in the previous section.

Adoptive Transfer

WT and Tg26 CD4+ T cells were isolated from splenocytes as described later and adoptively transferred (10 million cells) in T cell–deficient TCRα knockout mice (Jackson Laboratory, strain 002116) through retro-orbital injection.

BP Recording

In intact male and female WT and Tg26 mice, BP and heart rate were recorded by radiotelemetry. Mice were implanted with subcutaneous radiotelemeters (DSI model PA-C10, New Brighton, MN) in the carotid artery as previously described.[19] Mice were given 7 to 10 days to recover from surgery before baseline systolic BP (SBP), diastolic BP, mean arterial pressure, and heart rate were recorded for 7 days, followed by 7 days of treatment. Mice were treated with the CTLA-4 agonist abatacept (10 g/kg IP every other day for 7 days),[20] the interleukin (IL)-1α–neutralizing antibody (BioXcell, catalog No. BE0243, 400 µg/mouse IP every other day), or exogenous IL-1α (32 ng/kg per day, BioLegend, catalog No. 575004, subcutaneous Azlet mini-pump, model 1002) or submitted to CD4+ T cell depletion through intraperitoneal injections of the CD4+ T cell–neutralizing antibody (BioLegend, catalog No. 100412, 400 µg/mouse at day 1 and then boosted with 100 µg at day 7). The depletion of CD4+ T cells was confirmed with fluorescence-activated cell sorting analysis. In BMT mice, SBP was measured by tail cuff (CODA noninvasive BP system, Kent Scientific). SBP was recorded at a thermostatic temperature and in a dark environment after 4 days of acclimatization to the restraining chamber and the environment. Twenty-five SBP readings were obtained, and accepted readings were averaged over 20 minutes.

Vascular Reactivity

Excised mesenteric arteries (second order) and thoracic aortas were cleaned of perivascular adipose tissue, cut into 2-mm rings, and mounted on a DMT wire myograph system (Danish MyoTechnology, Aarhus, Denmark) as previously described.[19] Briefly, endothelial function was tested by conducting concentration-response curves (CRCs) to acetylcholine (1 nmol/L–100 µmol/L) and sodium nitroprusside (SNP; 1 nmol/L–100 µmol/L) in vessels preconstricted with serotonin (1 µmol/L, thoracic aorta) or phenylephrine (1 µmol/L, mesenteric arteries). CRCs to acetylcholine were repeated in the presence or absence of the selective NADPH oxidase (NOX) 1 inhibitor GKT771 (10 µmol/L) as previously described.[21] In addition, CRCs to acetylcholine were conducted in aortas exposed to recombinant IL-1α (70 pg/mL, R&D Systems, catalog No. 200-LA-010) in the presence or absence of the nuclear factor-κB (NF-κB) inhibitor (JSH-23 20 µmol/L) or the caspase 1 inhibitor (VX-76, 25 µmol/L).

CD3+, CD4+, and CD8+ T Cell Isolation

Pan, CD4+, and CD8+ T lymphocytes were isolated from the spleen of WT and Tg26 mice. Spleens were minced and passed through a 40–µmol/L wire strainer, and T cells were isolated with Pan, CD4+, and CD8+ magnetic isolation kits (Miltenyi Biotec, pan catalog No. 130-095-130, CD4+ catalog No. 130-104-454, CD8+ catalog No. 130-104-075). BMT cells were resuspended at 1 million cells/mL; for culture, T cells were resuspended at 125 million cells/mL. T cells were resuspended in TexMACS buffer (Miltenyi Biotec references of the kit) supplemented with 10% FBS, 0.01 mM2-mercaptoethanol, 1:100 Pen/Strep, and 1:100 amphotericin. Cells were counted, and 12 million cells were plated per well in a 24-well plate (CellStar). Media (500 µL) was added to each well and incubated at 37 °C for 3 days, after which cells and media were collected or used for the aorta-immune cell coculture experiments described below.

BM-Derived Macrophages

BM was flushed from femurs and tibias of WT and Tg26 mice with RPMI complete media[18] and filtered through a 100-µm filter. Once filtered, cells were centrifuged at 300*g* for 10 minutes and resuspended in 10 mL complete media before being plated. Macrophage differentiation was induced by 20

ng/mL of murine macrophage colony-stimulating factor (R&D Systems, catalog No. 416-ML-010) applied every other day for 6 days, after which the media was replaced and cells were used for aorta-immune cell coculture experiments.

Aorta-Immune Cell Coculture Experiments

Thoracic aortas excised from male WT mice (C57Bl/6) and cleaned of perivascular adipose tissue were exposed to WT or Tg26 T cells or macrophages that had been isolated and cultured as described previously. Aortas were placed in the upper insert of the transwell plate (Costar Transwell 0.4-µm polyester membrane, catalog No. 3470) in 200 µL of fresh unconditioned media. Vessels were exposed to immune cells overnight (16–18 hours) before being mounted on the wire myograph to perform CRCs to acetylcholine as described previously. Coculture experiments were repeated in $CD4^+$ T cells exposed to IL-1α–neutralizing antibody (1 ng/mL, BioXcell, catalog No. BE0243) 24 hours before vessel incubation and conduction of CRCs.

Human Microvascular Endothelial Cell Culture and Recombinant IL-1α Treatment

Human microvascular endothelial cells (hMVECs; LONZA, from a 50-year-old White man) were plated in 6-well plates (Costar) and allowed to reach 80% confluency. Cells were treated with recombinant human IL-1α (70 pg/mL, R&D Systems, catalog No. 200-LA-010) or vehicle for 24 hours in the presence or absence of the NF-κB inhibitor (JSH-23 20 µmol/L) or the caspase 1 inhibitor (VX-76, 25 µmol/L). After treatment, hMVECs were collected in Trizol (Thermo Fisher, Waltham, MA, catalog No. 15596026) before being used for quantitative real-time polymerase chain reaction.

Human Specimens

Discarded human aorta specimens were obtained from PLWH on cART and seronegative patients undergoing cardiac bypass surgery at Augusta University Medical Center as previously described.[22] Specimens were used for either Western blotting or placed in a 96-well plate (Costar) and incubated overnight (≈16 hours) in 300 µL of conditioned media from either WT or Tg26 $CD4^+$ T cells cultured for 3 days as described previously. At the end of the incubation period, specimens were snap-frozen for real-time quantitative polymerase chain reaction analysis.

Western Blot Analysis

Human aortas were homogenized in radioimmunoprecipitation assay buffer supplemented with protease and phosphatase inhibitors and measured for protein concentration by BCA assay (Thermo Fisher), as previously described.[22] Tissue homogenates (15 µg) were separated with SDS-PAGE and transferred to Immobilin-P poly(vinylidene fluoride) membranes. Immunoblots were probed with antibodies for NOX1 (Sigma, SAB4200097) diluted 1:500.

Real-Time Quantitative Polymerase Chain Reaction Analysis

mRNA from human and mouse aortas and from $CD3^+$/$CD4^+$/$CD8^+$ T cells was extracted with Trizol-based methods (Qiagen, Germantown, MD), and a complementary DNA library was produced with SuperScript III (Thermo Fisher). Real-time quantitative polymerase chain reaction was performed on the generated cDNA with SybrGreen (Applied Biosystems) and the primers listed in Table S1. Ct values were normalized to GAPDH within the sample (Δ Ct), which was subsequently normalized to the control group ($\Delta\ \Delta$ Ct) to generate for the relative gene expression ($2^{-\Delta\ \Delta Ct}$).

Plasma Cytokine Panel

Whole blood isolated from WT, Tg26, and BMT mice, along with blood from treated and untreated mice, was centrifugated at 10 000 rpm for 10 minutes to generate plasma for analysis. Quantification of plasma cytokines was performed with LEGENDplex Mouse Inflammation Panel (13-plex) with V-bottom Plate (BioLegend catalog No. 740446) according to manufacturer instructions. In brief, plasma and media samples were thawed completely, mixed, and centrifuged to remove particulates before use. To achieve measurement accuracy, serum samples were diluted 2-fold with assay buffer, and standards were mixed with matrix solution (BioLegend) to account for additional components in the plasma samples. Standards and samples were plated with capture beads for IL-1β, IL-6, IL-10, IL-17A, granulocyte-macrophage colony-stimulating factor, IL-23, interferon-β, IL-27, tumor necrosis factor-α, and IL-1α and incubated for 2 hours at room temperature on a plate shaker (800 rpm). After the plate was washed with wash buffer, detection antibodies were added to each well. The plate was incubated on the shaker for 1 hour at room temperature. Last, without washing, streptavidin was added and incubated for 30 minutes. Samples were acquired on Novocyte Quanteon flow cytometer (Agilent Technologies).

8-Hydroxy 2 Deoxyguanosine Quantification of Reactive Oxygen Species Levels

hMVECs treated with recombinant IL-1α (70 pg/mL for 24 hours) or vehicle were washed with PBS and submitted to genomic DNA isolation (Genomic DNA Purification Kit, Thermo Fisher, catalog No. K0512) and 8-hydroxy-2'-deoxyguanosine quantification with ELISA (Abcam, catalog No. ab201734).

Fluorescence-Activated Cell Sorting Staining, Acquisition, and Analysis

Single-cell suspensions from spleens were prepared by mechanically processing with a 100-µm cell strainer and a pestle. Red blood cells were removed by additional incubation with ACK lysis buffer (Thermo Fisher, catalog No. A1049201). Cells were washed and resuspended in staining buffer (BioLegend, catalog No. 420201). For flow cytometry staining, the following antibodies were used (all from BioLegend): FcR block (clone 93), CD8b -BV421 (clone YTS156.7.7), CD4 -BV510 (clone RM4-5), CD45R -APC-Cy7 (clone RA3-6B2), CD45.2 -FITC (clone 104), CD44 -APC (clone IM7), CD3e -BV785 (clone 145-2C11), CCR6 -PE (clone 29-2L17), CD183 -BV711 (clone S18001A), CD25 -BV605 (clone PC61), TCRgd -PerCP-Cy5.5 (clone GL3), and NK1.1-PE-Cy7 (clone PK136). Samples were acquired on a Novocyte Penteon flow cytometer (Agilent Technologies) and analyzed with FlowJo version 10.8 Software (BD Life Sciences). Cell doublets were excluded

using forward scatter time of flight (wide) versus forward scatter integral (area). Live leukocytes were gated with FSC/SSC and Zombie-NIR staining live/dead indicator. Cells were subdivided on the basis of their pattern in forward and side scatter as well as lineage markers. This allowed us to identify alpha-beta T cells ($CD3^+$), helper CD4 ($CD3^+$ and $CD4^+$), cytotoxic CD8 ($CD3^+$ and $CD8^+$), $CD25^+$ regulatory ($CD3^+CD4^+CD25^+$) T cells, B cells ($B220^+$), and natural killer (NK) cells ($NK1.1^+CD3^-$), NK T cells ($CD3^+NK1.1^+$), and gamma-delta T cells ($CD3^+CD4^-CD8^-NK1.1^-TCRgd^+$). Furthermore, using the activation marker (CD44) and chemokine receptors (CXCR3 and CCR6), we identified naive ($CD44^-$) and effector/memory ($CD44^+$) T cells, as well as Th1 (CD4 T cells $CD44^+CXCR3^+$) and Th17 (CD4 T cells $CD44^+CXCR3^+$). Fluorophore information can be found in Table S2.

Renal Function

Intact male WT and Tg26 mice were placed in metabolic cages for urine collection. After 3 days of acclimatation, urine was collected for 48 hours, and 24-hour albumin (EEL119, Invitrogen) and creatinine (catalog No. 80350, Crystal Chem High Performance Assays) excretion was measured.

Statistical Analysis

All data are presented as mean±SEM. Whenever applicable, unpaired 2-sample *t* test or 1-way ANOVA followed by pairwise comparisons with multiple testing correction or 2-way ANOVA followed by the Tukey post hoc test was used to analyze the data (GraphPad Prism 7, GraphPad Software Inc, La Jolla, CA). *P* values or multiple comparison–adjusted values of $P<0.05$ were considered significant.

RESULTS

Expression of Viral Proteins in Tg26 Mice Elevates BP and Impairs Endothelium-Dependent Relaxation

To examine the role of viral proteins in the pathogenesis of HIV-associated hypertension, we used the Tg26 transgenic mouse on the C57Bl/6 background, which randomly expresses 7 of the 9 HIV viral proteins under the retroviral long terminal repeat promoter (Figure S1). Viral protein expression in Tg26 mice led to an extremely mild lipodystrophy phenotype characterized by a modest reduction in body weight, fat, and lean mass and no alterations in plasma insulin, leptin, glucose, cholesterol, and triglycerides levels. It also did not alter renal function as reflected by an absence of increases in urinary albumin, creatinine, or albumin/creatinine ratio (Table S3). Viral protein expression did not alter blood cell counts or T cell numbers (Figure S2) or promote the progression to AIDS as reflected by an absence of wasting skin disease.[23] Therefore, the Tg26 mouse provides a non-infectious model of HIV that mimics a virally repressed population, similar to what is seen in PLWH receiving antiretroviral therapy.

Using radiotelemetry and BP measurements in conscious mice, we report that expression of viral proteins in intact male and female Tg26 mice increased BP while preserving circadian rhythms (males, Figure 1A and 1B; Figure S3A and S3B; females, Figure 1D and 1E; Figure S4A and S4B). Expression in viral proteins did not affect heart rate (males, Figure 3E and 3F; females, Figure S4E and S4F). On the basis of the crucial role of the vasculature in the control of BP and the known deleterious effects of HIV on vascular function,[15] we assessed vascular reactivity in second-order mesenteric arteries. Expression of viral proteins in Tg26 mice led to a marked impairment in vasorelaxation to acetylcholine in male (Figure 1C) and female (Figure 1F) mice with no change in vasorelaxation to SNP (males, Figure S3G; females, Figure S4G), supporting a dysfunction at the endothelial cell level. While examining vascular function in conduit vessels, we reported reduced vasorelaxation to acetylcholine (males, Figure S3H; females, Figure S4H) with no impairment to SNP response (males, Figure S3I; females, Figure S4I) in aorta from male and female Tg26 mice, further supporting a role for viral proteins in the development of endothelial dysfunction.

Viral Proteins Derived From Hematopoietic Cells Increase BP and Impair Endothelial Function

HIV targets the immune system, which remains the main viral reservoir and the site of viral proteins production in PLWH on cART.[11–13] To examine whether viral proteins derived from hematopoietic cells could contribute to endothelial dysfunction and hypertension, we collected BM from WT and Tg26 mice and transplanted it in either WT or Tg26 mice following the scheme presented in Figure 1G. As a result of the absence of antibodies for viral proteins for flow cytometry, efficiency of the BMT was confirmed by measuring spleen viral protein content (Figure S5). BP measurement by tail cuff revealed that BMT from Tg26 to WT mice significantly increased SBP in male and female mice compared with WT to WT animals (males, Figure 1H; females, Figure 1I). BMT from WT to Tg26 mice abolished the hypertensive phenotype reported in male and female intact Tg26 mice and reduced BP levels to those of the WT to WT group in both sexes (males, Figure 1H; females, Figure 1I). It is remarkable that the results of the vascular reactivity studies mirrored the BP data. Indeed, BMT from Tg26 into WT mice reproduced the endothelial dysfunction reported in the mesenteric artery from intact Tg26 mice, whereas BMT from WT mice in Tg26 mice restored mesenteric artery relaxation to acetylcholine (males, Figure 1J; females, Figure 1K). BMT did not alter smooth muscle cell–dependent relaxation in either group (SNP; males, Figure S3J; females, Figure S4J). As observed in mesenteric

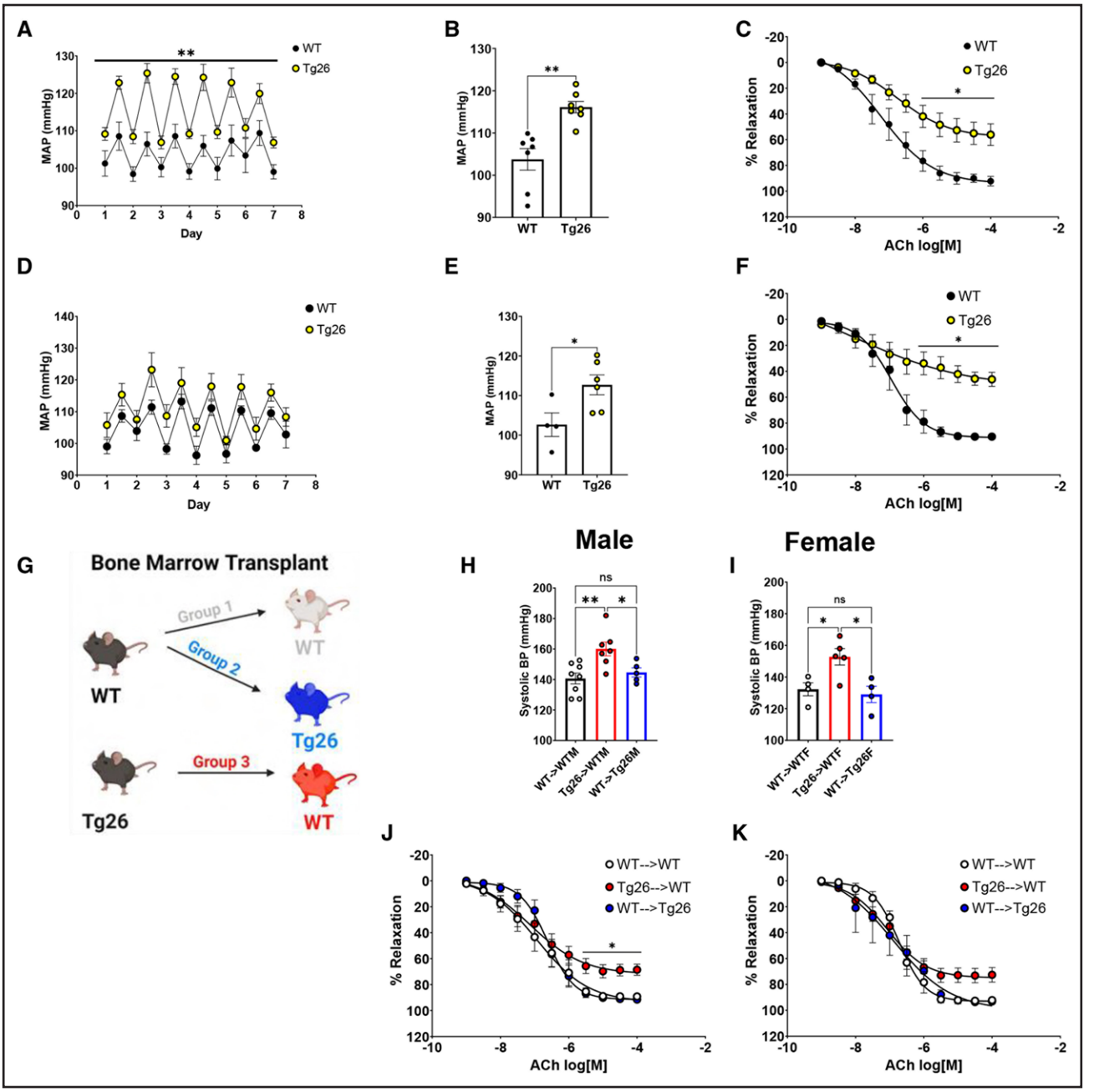

Figure 1. Expression of HIV proteins in hematopoietic cells increases BP and impairs endothelial function in resistance vessels from male and female mice.

Continuous (**A** and **D**) and 24-hour average (**B** and **E**) mean arterial pressure (MAP) measured by radiotelemetry in intact male (**A** and **B**) and female (**D** and **E**) wild-type (WT) and Tg26 mice. Mesenteric artery relaxation to acetylcholine (ACh) in intact male (**C**) and female (**F**) WT and Tg26 mice. **G**, Representation of the bone marrow transplantation (BMT) scheme used. **H** and **I**, Tail-cuff measurement of systolic blood pressure (BP) in male (**H**) and female (**I**) mice submitted to BMT. Mesenteric artery relaxation to ACh in BMT male (**J**) and female (**K**) mice. Data are presented as mean±SEM, n=4–8. **P*<0.05. ***P*<0.01.

arteries, BMT from Tg26 to WT mice impaired aorta endothelial function, whereas BMT from WT to Tg26 mice restored conduit artery response to acetylcholine without affecting SNP-mediated relaxation (males, Figure S3K and S3L; females, Figure S4K and S4L). Together, these results indicate that viral proteins derived from hematopoietic cells are the source of endothelial dysfunction and high BP in male and female Tg26 mice.

Viral Proteins Derived From CD4+ T Lymphocytes Increase BP and Impair Vasorelaxation

With the knowledge that increased BP and impaired vasorelaxation are mediated by viral proteins derived from hematopoietic cells and the evidence that T cells and macrophages are the main viral reservoirs of HIV,[24,25] we

aimed to identify the immune cell subtype responsible for endothelial dysfunction and hypertension. We developed a novel system (Figure 2A) whereby WT thoracic aortas are cocultured with spleen-derived CD3+ T cells or BM-derived macrophages. Exposure of WT aortas to Tg26 CD3+ T cells impaired vasorelaxation compared with aortas exposed to WT CD3+ T cells (Figure 2B). However, exposure to BM-derived macrophages from either WT or Tg26 mice did not alter vasorelaxation (Figure 2C). Neither T cells nor macrophage exposure affected SNP-mediated relaxation (T cells, Figure S6A; macrophages, Figure S6B). Using the evidence that T cell contribution to hypertension requires costimulation,[20] we further investigated T cell contribution to the pathogenesis of HIV-associated hypertension by submitting intact WT and Tg26 mice to costimulation blockade with CTLA4-Ig (abatacept). Abatacept lowered mean arterial pressure (Figure 2D and 2E) and diastolic BP (Figure S6D) but also restored endothelial function in resistance (Figure 2F) and conduit (Figure S6I) vessels, as reflected by an absence of difference in relaxation between vessels from WT and Tg26 treated mice. Abatacept treatment did not affect smooth muscle cell–dependent relaxation (mesenteric, Figure 2G; aorta, Figure S6J). Collectively, these data provide in vitro and in vivo evidence that viral proteins derived from T cells contribute to the observed increased BP and endothelial dysfunction.

We repeated the coculture experiment (Figure 2A) with CD4+ helper and CD8+ cytotoxic T cells to narrow down the T cell subtype involved. Exposure to CD4+ (Figure 3A), but not CD8+ (Figure 3B), impaired endothelium-dependent relaxation with no change in SNP-mediated vasorelaxation (CD4+, Figure 3C; CD8+, Figure 3D). To further test the role of CD4+ T cells, we submitted intact WT and Tg26 mice to CD4+ depletion using CD4+-neutralizing antibody. CD4+ depletion, confirmed by fluorescence-activated cell sorting (Figure S7A through S7D), lowered SBP levels of Tg26 mice to those of WT mice and did not affect the BP of WT mice (Figure 3E). CD4+ depletion also restored vasorelaxation to acetylcholine as reflected by an absence of difference in relaxation between vessels from WT and Tg26 mice treated with CD4+-neutralizing antibody (Figure S3F). CD4+ depletion did not alter vascular smooth muscle cell–dependent vasorelaxation (Figure S3G). To further investigate the role of CD4+ T cells, we completed an adoptive transfer of CD4+ T cells in TCRα knockout mice, which are devoid of CD4+ and CD8+ T cells. Adoptive transfer of Tg26 CD4+ T cells, confirmed by fluorescence-activated cell sorting (Figure S7E and S7F), significantly increased SBP compared with receiving CD4+ T cells isolated from WT mice (Figure 3H). Together, these results support a role for viral protein–expressing CD4+ T cells in hypertension and endothelial dysfunction.

Viral Protein Expression Increases IL-1α Production From CD4+ T Cells

On the basis of the established role of proinflammatory cytokines in the development of hypertension[10] and in an attempt to elucidate the mechanisms by which CD4+ T cells mediate hypertension and endothelial dysfunction, we performed a cytokine panel on the plasma of intact and BMT mice. These measurements revealed that viral protein expression in intact male and female Tg26 mice led to a significant increase in circulating IL-1α levels (males, Figure 4A; females, Figure 4B) with no significant changes in other proinflammatory cytokines (males, Table 1; females, Table S4). BMT from Tg26 to WT mice increased and replacement of Tg26 BM by WTs reduced IL-1α levels (males, Figure 4C; females, Figure 4D). Again, IL-1α is the only cytokine with levels that were increased in Tg26→WT and decreased in WT→Tg26 mice (males, Table 1; females, Table S4). A cytokine panel conducted in the media of T cells in culture revealed that viral protein expression induced a more significant increase in IL-1α production in CD4+ than in CD8+ T cells (Figure 4E and 4F; Table 2). Moreover, inhibition of T cell costimulation with abatacept treatment (Figure 4G; Table 1) and CD4+ T cell depletion (Figure 4G) lowered plasma IL-1α levels in Tg26 mice, indicating that CD4+ T cells are the source of the elevation in circulating IL-1α.

To test the functional relevance of these increases in IL-1α, intact WT and Tg26 mice were treated with an IL-1α–neutralizing antibody for 7 days. IL-1α neutralization lowered Tg26 BP levels to those of WT mice (Figure 4H and 4I; Figure S8A through S8D), with no change in heart rate (Figure S8E and S9F), and restored endothelium-dependent relaxation in Tg26 resistance arteries (Figure 4J) without affecting smooth muscle cell–dependent vasorelaxation (Figure S8G). In parallel, we repeated the T cell–aorta coculture experiment in the presence of the IL-1α–neutralizing antibody. IL-1α neutralization protected aortic rings from Tg26 CD4+ T cell–induced endothelial dysfunction (Figure 4K) without affecting SNP-mediated relaxation (Figure S8H).

To further test the role of IL-1α in hypertension and endothelial dysfunction, WT mice were submitted to exogenous IL-1α treatment at the dose of 20 mg/kg per day. IL-1α infusion increased SBP (Figure 4L) and impaired endothelium-dependent vasorelaxation (Figure 4M) with no alteration in smooth muscle cell–dependent relaxation (Figure S8I). Taken together, these data support a role for CD4+ T cell–derived IL-1α in HIV viral protein–associated endothelial dysfunction and hypertension.

NOX1 Mediates Endothelial Dysfunction and Contributes to Hypertension

Excess reactive oxygen species levels, notably high NAD(P)H oxidase–derived reactive oxygen species, have

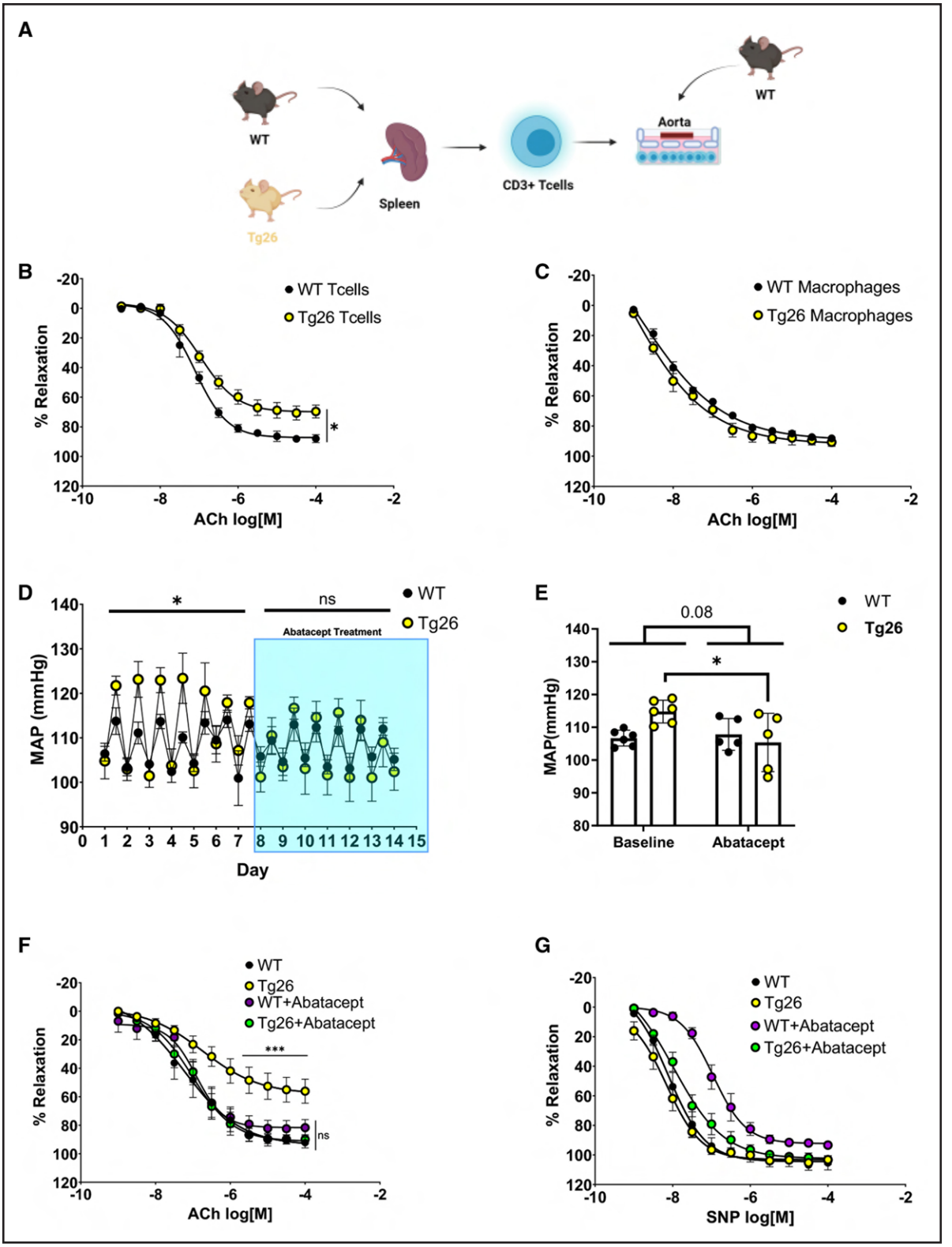

Figure 2. Expression of HIV proteins in CD3⁺ T cells induces endothelial dysfunction and hypertension.
A, Representation of the immune cell–aorta coculture system. Vascular relaxation to acetylcholine (Ach) in wild-type (WT) aortas cocultured with (**B**) pan CD3⁺ T cells or (**C**) bone marrow–derived macrophages isolated from WT or Tg26 mice. **D**, Continuous and (**E**) 24-hour mean arterial pressure (MAP) measured by radiotelemetry in WT and Tg26 mice treated with abatacept (10 mg/kg per day). Endothelium-dependent (**F**) and endothelium-independent (**G**) relaxation assessed in WT and Tg26 mice treated with abatacept. Data are presented as mean±SEM, n=4–6. *$P<0.05$.

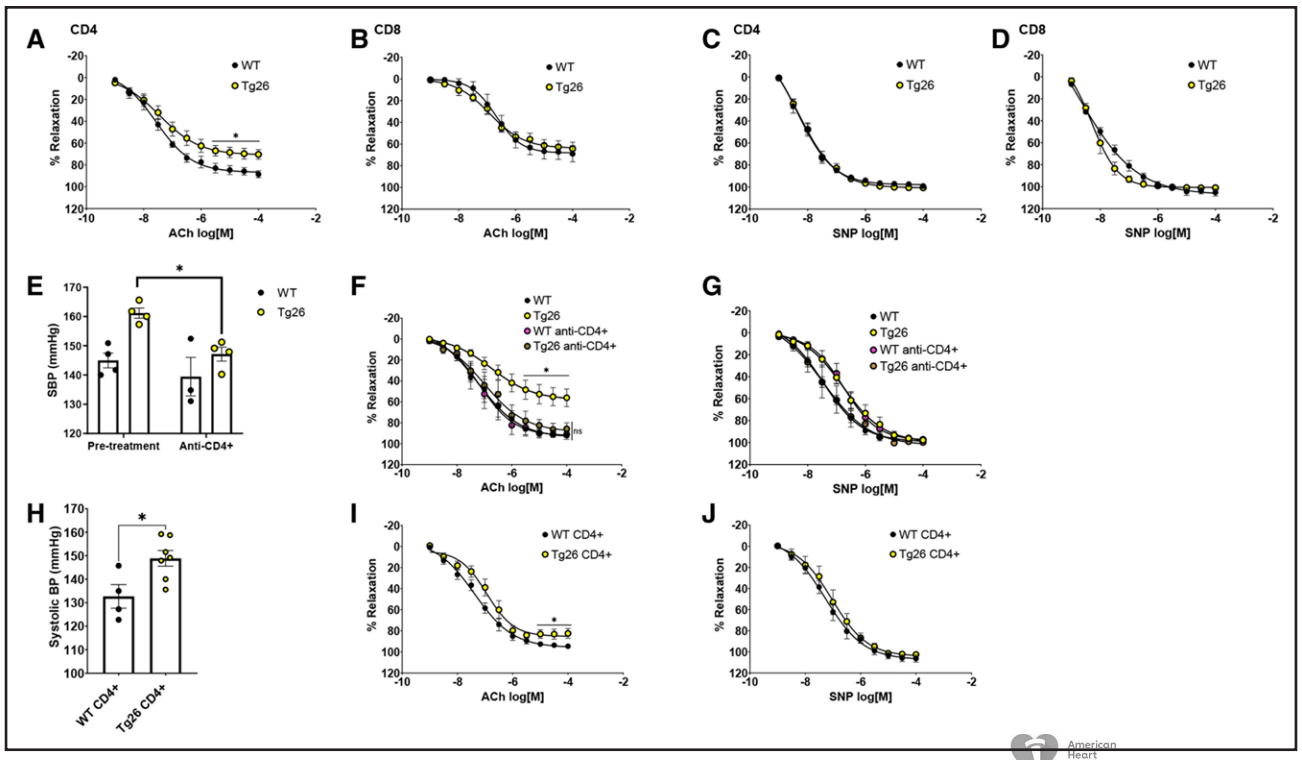

Figure 3. Expression of HIV proteins in CD4⁺, not CD8⁺, T cells mediates endothelial dysfunction and hypertension. Endothelium-dependent (**A** and **B**) and endothelium-independent (**C** and **D**) relaxation measured in wild-type (WT) aortas cocultured with either CD4⁺ T helper (**A** and **C**) or CD8⁺ cytotoxic T cells (**B** and **D**) isolated from WT and Tg26 mice. **E**, Tail-cuff measurement of systolic blood pressure (SBP) and endothelium-dependent (**F**) and endothelium-independent (**G**) relaxation measured in WT and Tg26 mice treated with anti-CD4⁺ antibody (400 µg on day 0 and 100 µg on day 7). **H**, Tail-cuff measurement of systolic blood pressure (SBP) in TCRα knockout mice receiving CD4⁺ T cells from either WT or Tg26 mice through adoptive transfer. Data are presented as mean±SEM, n=3–7. **P*<0.05.

been associated with HIV- and T cell–mediated hypertension and vascular dysfunction.[15,26] However, no causal link has been formally established. Therefore, we quantified NOX subtype transcript expression in aorta. Viral protein expression in Tg26 mice increased NOX1 with no significant increases in NOX2 or NOX4 (males, Figure 5A through 5C; females, Figure S9A through S9C). Consistent with these findings, BMT from Tg26 to WT mice increased only NOX1 in aorta, and BMT from WT to Tg26 BMT significantly lowered NOX1 expression compared with Tg26 to WT mice (males, Figure 5D through 5F; females, Figure S9D through S9F). Furthermore, exposure of WT aorta to Tg26 CD3⁺ T cells increased NOX1 only (Figure 5G through 5I). To test the translatability of these findings, we measured NOX expression in aortic biopsy specimens discarded from PLWH undergoing cardiac bypass surgery. Proteins and transcript quantification revealed that PLWH exhibit higher NOX1 levels than seronegative patients (Figure 5J through 5L). In addition, CD4⁺ depletion lowered NOX1 levels in intact mouse aortas with no significant changes in NOX2 or NOX4 levels (Figure 5M through 5O).

To test the functional relevance of these findings, CRCs to acetylcholine were repeated in mesenteric vessels from WT and Tg26 mice incubated with the selective NOX1 inhibitor GKT771.[21] GKT771 abolished the differences in vasorelaxation between WT and Tg26 male mice (Figure 5P) but also between WT to WT and Tg26 to WT BMT mice (Figure 5Q). In addition, we transplanted WT and Tg26 BM into global NOX1-deficient mice (Figure 5R). NOX1 deficiency protected against viral protein–induced hypertension and endothelial dysfunction (Figure 5S and 5T) without altering vascular smooth muscle cell–dependent relaxation (Figure 5U). Taken together, these data indicate that CD4⁺-derived viral proteins impair endothelial function and elevate BP through NOX1-dependent mechanisms.

IL-1α Increases NOX1 and Oxidative Stress in hMVECs

Our data demonstrated that viral protein–mediated endothelial dysfunction and hypertension are IL-1α and NOX1 dependent; we hypothesized that IL-1α increases endothelial NOX1 expression. To test this hypothesis, we exposed hMVECs to IL-1α. IL-1α increased endothelial NOX1 expression (Figure 6A) with no significant changes in NOX2, NOX4, or NOX5 (Figure 6B through 6D) expression. In addition, we measured 8-hydroxy-2'-deoxyguanosine levels, an index of reactive oxygen species–induced oxidative lesions,[27] and reported that IL-1α significantly increased

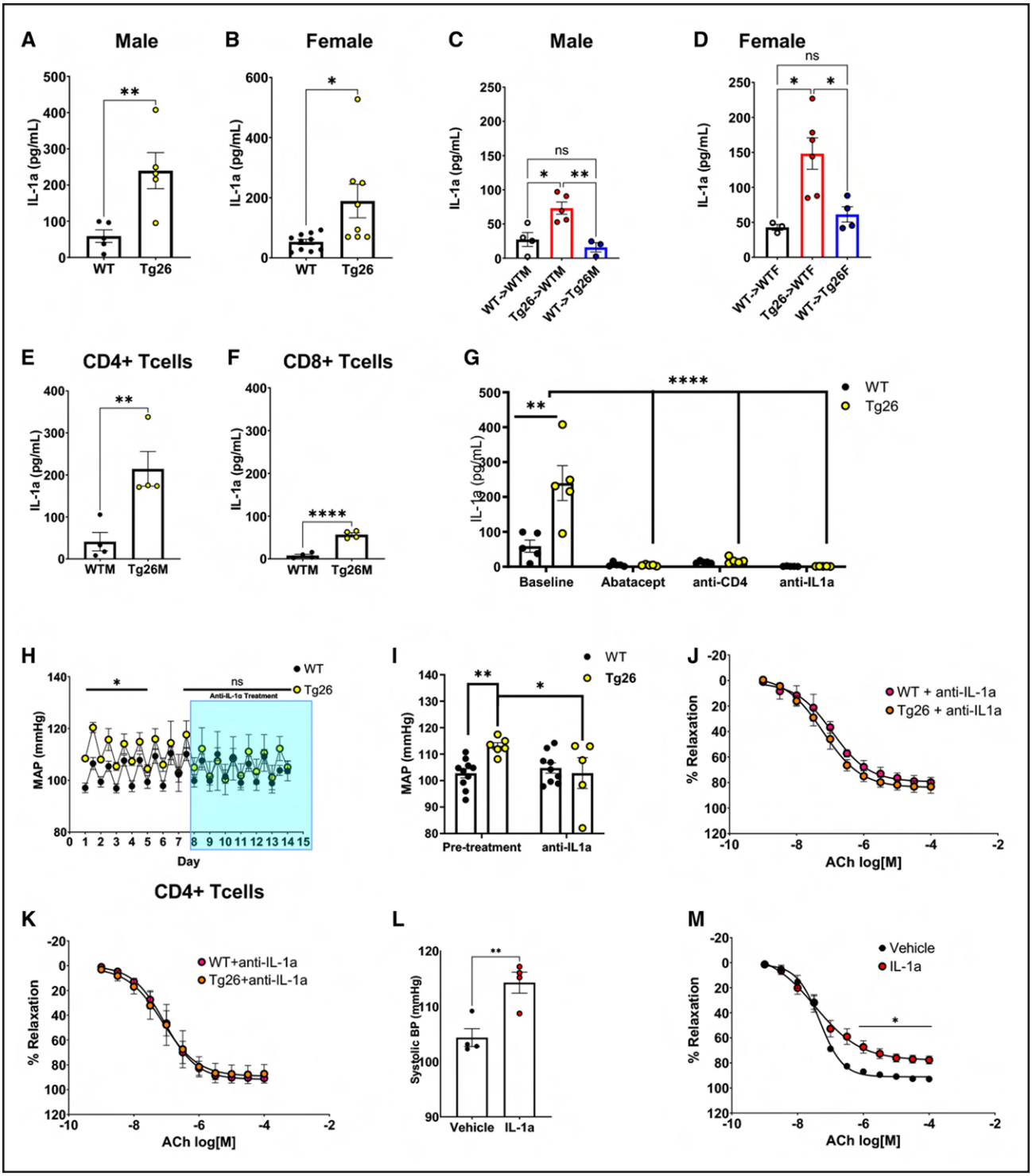

Figure 4. Expression of viral proteins in CD4⁺ T cells induces IL-1α expression, which impairs endothelial function and induces hypertension.

Plasma interleukin (IL)-1α levels in intact (male, **A**; female, **B**), bone marrow transplantation (BMT; male, **C**; female, **D**), CD4⁺ T cell media (**E**), CD8⁺ T cell media (**F**), and abatacept–, anti-CD4⁺–, and anti-IL-1α–treated mice (**G**) as measured by cytokine panel and ELISA. Continuous (**H**) and 24-hour average (**I**) mean arterial pressure (MAP) and endothelium-dependent vasorelaxation (**J**) measured in male mice treated with anti-IL-1α (20 mg/kg per day). **K**, Vascular relaxation in wild-type (WT) aortas cocultured in CD4⁺ T cells isolated from WT and Tg26 mice treated with anti–IL-1α (1 ng/mL). **L**, Systolic blood pressure (SBP) measured by tail cuff and (**M**) endothelium-dependent relaxation measured in WT mice treated with exogenous IL-1α (32 ng/kg per day) or vehicle. Data are presented as mean±SEM, n=4–8. **P*<0.05. ***P*<0.01. ****P*<0.005. *****P*<0.001.

Table 1. Inflammatory Cytokine Panel Conducted in the Plasma of Intact and BMT-, Abatacept-, and Anti–IL-1a Treated Male WT and Tg26 Mice

	Intact		Bone Marrow Transplant			Abatacept		Anti-IL-1α	
	WT male	Tg26 male	WT→WT male	Tg26→WT male	WT→Tg26 male	WT+abatacept male	Tg26+Abatacept male	WT+anti–IL-1α male	Tg26+anti–IL-1α male
IL-1β	6.7±0.3	10.0±0.7	32.2±4.8	25.1±8.2	72.0±16.0	5.6±0.7	5.6±0.4	52.9±3.6*†	44.7±5.0*†
IL-6	3.7±0.3	2.5±0.1	17.6±4.1	16.7±3.4	9.7±2.3	4.4±0.6	4.9±0.8	5.0±0.7	4.7±0.3
IL-10	56.2±5.9	64.6±2.0	65.9±11.3	63.8±4.4	85.3±5.7	9.4±1.2*†	12.0±1.7*†	154.1±7.4*†	93.0±10.1*‡
IL-17A	5.1±1.8	4.3±1.2	7.7±3.0	4.3±1.2	9.0±3.6	Not detected	Not detected	14.5±2.8	9.4±3.0
IL-23	13.7±1.0	12.3±0.8	27.3±3.2	7.4±0.2§	6.9±1.1§	45.1±3.4*	29.3±0.9*	432.5±44.7*†	356.1±63.7*†
IL-27	59.2±10.4	193.74±25.9	176.2±36.0	186.5±17.9	126.8±14.5	154.4±15.2	94.8±10.0	304±26.0*	336.7±34.1*
IL-12-p70	1.28±0.1	1.32±0.1	1.3±0.1	1.3±0.04	1.3±0.1	1.2±0.2	0.9±0.03	2.4±0.2	2.2±0.2
IFN-γ	3.1±0.1	4.1±0.2	7.0±0.8	5.8±0.4	6.4±1.0	6.2±0.6	3.5±0.5	10.8±1.3*	9.6±1.3
MCP-1	7.86±0.5	12.7±1.5	Not detected	Not detected	Not detected	8.3±0.5	9.5±0.8	14.7±1.3*	11.8±1.0*
TNFα	4.18±0.2	8.21±0.9	23.5±4.8	9.2±0.8	28.0±6.5	20.4±3.5*	16.6±3.8*	89.0±10.5*†	91.4±11.1*†

BMT indicates bone marrow transplantation; IFN-γ, interferon-γ; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TNFα, tumor necrosis factor-α; and WT, wild-type.

Data are presented as mean±SEM, n=5–8.

*P<0.05 vs WT.

†P<0.05 vs Tg26.

‡P<0.05 vs WT+anti–IL-1α.

§P<0.05 vs WT→WT.

8-hydroxy-2'-deoxyguanosine levels in hMVECs (Figure 6E), introducing IL-1α as the source of the increase in NOX1 expression and reactive oxygen species production. To further assess the interaction between IL-1α and NOX1, we repeated the CRC to acetylcholine in the vessels of the IL-1α–treated WT mice in the presence of the NOX1 inhibitor GKT771. NOX1 inhibition abolished the differences in vasorelaxation between mesenteric vessels from IL-1α–treated mice and that from vehicle-treated mice (Figure 6F). To investigate the potential mechanisms whereby IL-1α promotes endothelial NOX1 expression, we tested the role of NF-κB and caspase 1. Both NF-κB and caspase 1 inhibition protected against IL-1α–induced impaired endothelium-dependent relaxation (Figure 6G) and IL-1α–mediated increases in endothelial NOX1 (Figure 6H and 6I).

Viral Protein Expressing CD4+ T Cell Media Increases NOX1 Levels in Human Aortas

To determine whether CD4+ T cells could mediate the increase in NOX1, discarded human aortic specimens from seronegative individuals undergoing cardiac bypass

Table 2. Inflammatory Cytokine Panel Conducted in the Media of CD4+ and CD8+ T Cells in Culture Initially Isolated From Intact WT and Tg26 Mice

	CD4+ T cells		CD8+ T cells	
	WT CD4+ media	Tg26 CD4+ media	WT CD8+ media	Tg26 CD8+ media
IL-1β	32.9±0.2	32.1±0.3	3.04±0.2	30.6±0.6*
IL-6	Above limit of detection	32615.4 80.6	2.25±0.3	25 300.3±1670.7*
IL-10	115.7±2.5	128.0±7.6	Undetected	99.1±2.4
IL-23	279.04±4.3	306.7±4.2	Undetected	285.7±10.7
IL-27	194.7±2.0	214.3±2.5	Undetected	285.7±10.7
IL-12p70	8.2±0.04	8.8±0.1	0.93±0.03	8.1±0.3†
IFN-γ	279.8±88.3	4963.3±594.3†	458.0±13.5	5329.7±1218.2
MCP-1	13 106±1340.0	8467.2±526.2	Undetected	7243.02±845.5
TNFα	429.7±24.7	728.5±29.4†	35.6±3.9	590.1±56.7

IFN-γ indicates interferon-γ; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TNFα, tumor necrosis factor-α; and WT, wild-type.

Data are presented as mean±SEM, n=5.

*P<0.05 vs WT CD8+T cells.

†P<0.05 vs WT CD4+T cells.

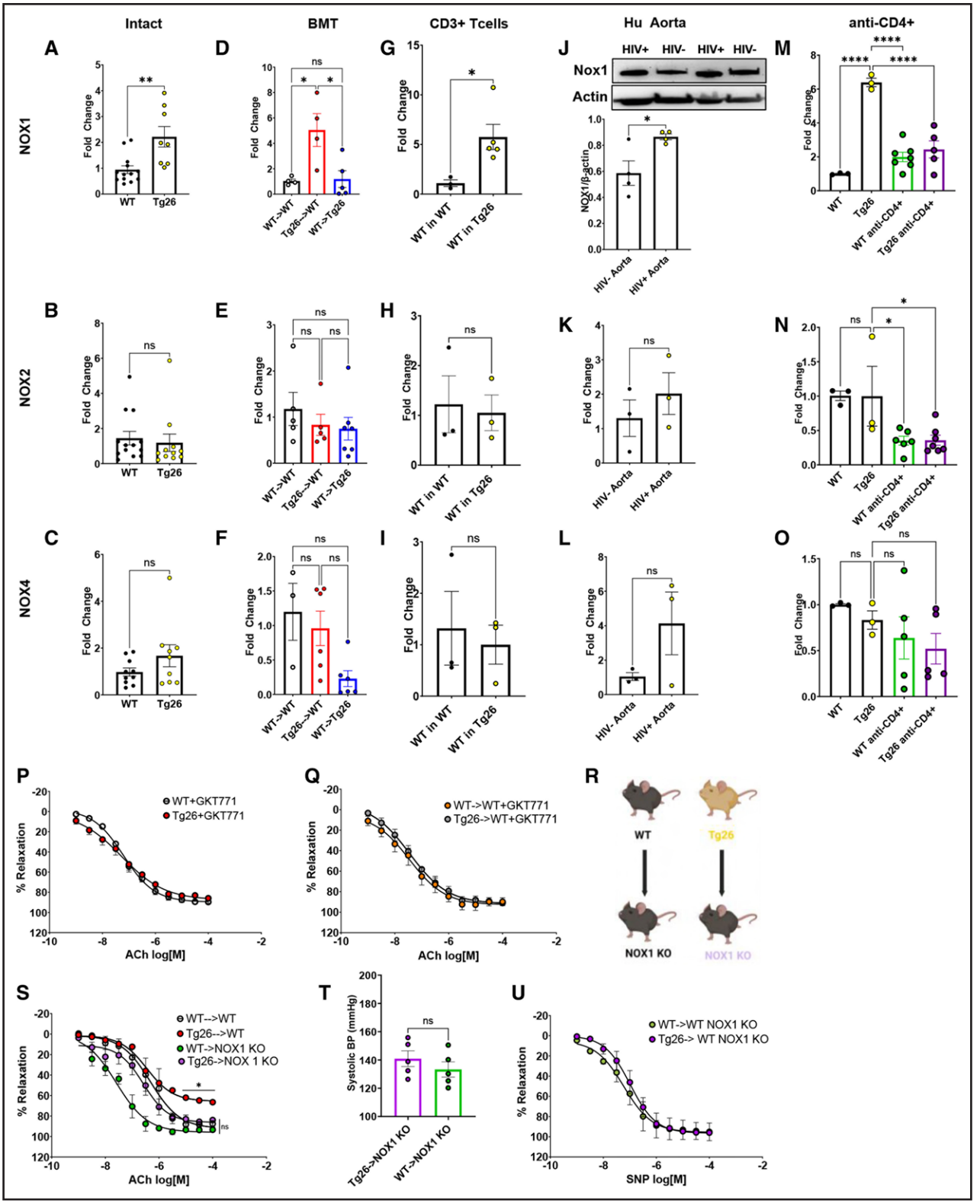

Figure 5. Expression of HIV proteins by CD4⁺ T cells increases vascular NOX1, which induces endothelial dysfunction and hypertension.

Quantitative polymerase chain reaction (qPCR) quantification of aorta NADPH oxidase (NOX) 1, NOX2, and NOX4 from intact (**A** through **C**) and bone marrow transplantation (BMT; **D** through **F**) mice, in abdominal aortas exposed to CD3⁺ T cells (**G** through **I**), and in aorta from mice treated with anti-CD4⁺ (**M** through **O**). **J**, Western blot (n=3) and (**K** and **L**) qPCR quantification of NOX1, NOX2, and NOX4 in aorta specimens discarded from HIV-positive and seronegative patients undergoing cardiac bypass surgery. Endothelium-dependent relaxation in excised second-order mesenteric arteries from intact (**P**) and BMT (**Q**) mice incubated with the selective NOX1 inhibitor GKT771. (**R**) Schematic illustrating the BMT protocol used in NOX1-deficient mice. **S** and **U**, Endothelium-dependent and endothelium-independent relaxation in (*Continued*)

Figure 5 Continued. second-order mesenteric arteries from NOX1-deficient mice receiving bone marrow from wild-type (WT) and Tg26 mice. **T**, Tail-cuff measurement of systolic blood pressure in NOX1-deficient mice receiving bone marrow from WT and Tg26 mice. Data are presented as Mean±SEM, n=3–13. ACh indicates acetylcholine. *P<0.05. **P<0.01.

surgery were exposed to WT or Tg26 CD4+ T cell–conditioned media overnight (Figure 6J). Exposure to CD4+ T cell media increased NOX1 expression and induced a trend toward an increase in NOX2 that did not reach statistical significance (Figure 6K through 6N). These results indicate that viral proteins derived from CD4+ T cells increase human vascular NOX1.

DISCUSSION

Although compelling clinical evidence has established that PLWH on cART exhibit an early onset and a high prevalence of hypertension,[4] the underlying mechanisms remain unknown because of an absence of experimental studies. In the present study, we used a mouse model of HIV to investigate the contribution of HIV-derived proteins to hypertension. Using the Tg26 mouse model expressing 7 of the 9 viral proteins, we demonstrated for the first time that CD4+ T cells expressing viral proteins secrete high levels of the proinflammatory cytokine IL-1α, which increases endothelial Nox1 expression, impairs endothelium-dependent relaxation, and elevates BP in male and female mice. Relevant to these findings are the roles of viral proteins, CD4+ T cells, IL-1α, and endothelial dysfunction in HIV-associated hypertension.

HIV-associated CVD is a complex and multifactorial pathology, involving the interplay among traditional risk factors, cART toxicity, behavioral factors, and disparities in access care in an aging population.[28] A major gap in our understanding of the cause of HIV-associated CVD, notably hypertension, is our lack of knowledge of the respective contribution of each of these factors. Here, we aimed to investigate the direct contribution of HIV-derived proteins to hypertension. This study found its rationale in the observation that the prevalence of CVD remains 50% higher in PLWH on cART after adjustment for traditional risk factors, indicating that viral infection per se, even in the presence of low viremia, is an independent risk factor for CVD.[29,30] Further evidence supporting the contribution of viral proteins is provided by the observation that viral proteins remain in circulation in virally controlled PLWH[14,31] and that cART-naive PLWH with undetectable viral load, also known as elite controllers, exhibit heightened immune activation and increased burden of subclinical CVD.[32] Together, these data indicate that HIV contributes to CVD even when naturally or pharmacologically repressed. Therefore, to test the direct role of viral proteins in HIV-associated hypertension, we used the transgenic Tg26 mouse on the C57Bl/6 background, which expresses 7 the 9 HIV-derived proteins (Env, Tat, Nef, Rev, Vif, Vpr, and Vpu) under the long terminal repeat promoter.[16,33] Because the replication-deficient proviral HIV DNA randomly integrates into the host genome and the viral transcripts are spontaneously driven by the long terminal repeat, the Tg26 mouse provides an appropriate model to study the long-term effects of HIV-derived proteins on the host.[16,33] In addition, in opposition to its counterpart on the FVBN background, the Tg26 mouse on the C57Bl/6 background does not develop HIV-associated wasting, skin diseases, and nephropathy,[34] which are AIDS-defining illnesses. This was confirmed by the demonstration that viral protein expression in Tg26 mice does not alter urinary albumin-to-creatine ratio. A complete characterization of the general and immune profile of the Tg26 mice by flow cytometry revealed no major metabolic disorders[35] or alterations in immune cell numbers, notably in CD4+ T cells. In fact, as previously reported by our group,[35] we showed that the Tg26 mouse is metabolically advantaged as reflected by a lower fat mass and an increased lean mass, which are likely attributable to a higher energy expenditure,[35] similar to that seen in PLWH.[36] Because the metabolic function is improved in the Tg26 mouse, one can reasonably rule out its contribution to the cardiovascular disorders reported. Therefore, this mouse provides an appropriate model to investigate the direct role of HIV-derived proteins without the intervention of any other confounding factors. Furthermore, this model is clinically relevant to cART-controlled HIV individuals who lack active viral replication but have continuous stress from HIV viral protein exposure.

With this mouse model, we demonstrated that expression of HIV proteins in mice induces hypertension in both males and females. Although HIV-derived proteins had been shown to contribute to endothelial dysfunction[37] and atherogenesis[23] in male animals, this study is the first to link HIV-derived proteins to hypertension and endothelial dysfunction in both sexes. Because HIV targets the immune system and remains expressed in immune reservoirs in PLWH on cART, we used BMT to increase the human relevance of the model and to restrict viral protein expression to hematopoietic cells. Using this approach, we showed that expressing viral proteins in hematopoietic cells is sufficient to cause hypertension, and removing viral proteins from hematopoietic cells is enough to restore BP. These data rule out a direct contribution of viral protein expression in cardiovascular organs and highlight the role of hematopoietic cells in HIV-associated CVD. Remarkably, these findings are consistent with the recent observation that hematopoietic stem cell transplantation cured a handful number of PLWH.[38] We then further identified CD4+ T cells as the culprit of endothelial dysfunction and hypertension. Although numerous clinical studies had established a positive correlation between CD4+ T cell count

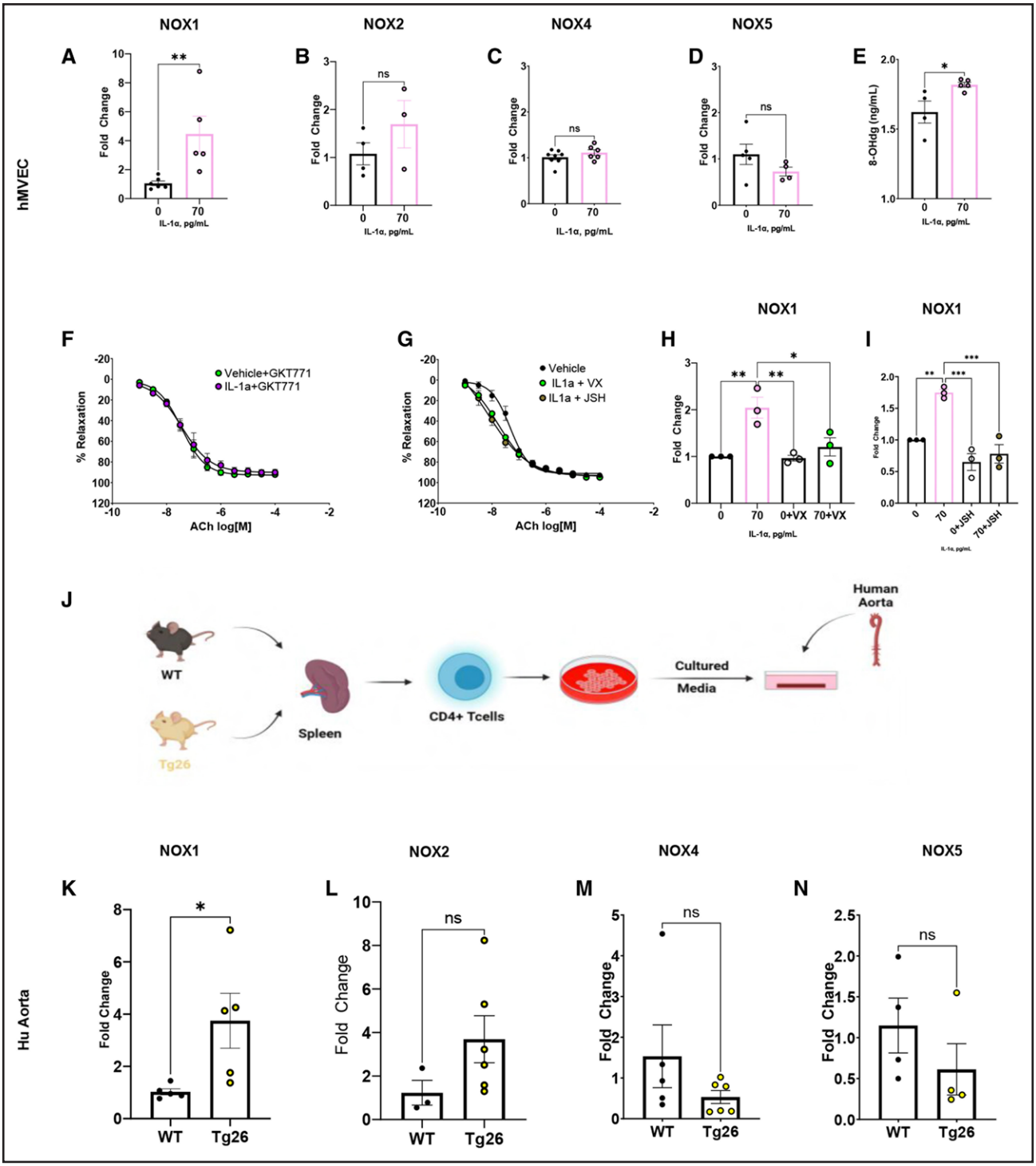

Figure 6. IL-1α and CD4⁺ T cells increase endothelial NOX1 and reactive oxygen species levels.
A through **D**, Quantitative polymerase chain reaction (qPCR) quantification of NADPH oxidase (NOX) 1, NOX2, NOX4, and NOX5 and (**E**) reactive oxygen species (8-hydroxy-2'-deoxyguanosine [8-OHdg]) measurement in human microvascular endothelial cells (hMVECs) exposed to exogenous interleukin (IL)-1α (70 pg/mL). **F**, Endothelium-dependent relaxation measured in second-order mesenteric arteries isolated from IL-1α–treated mice and incubated with GKT771. **G**, Endothelium-dependent relaxation measured in wild-type (WT) aortas exposed to exogenous IL-1α (70 pg/mL) overnight in the presence or absence of the nuclear factor-κB (NF-κB) inhibitor (JSH-23, 20 μmol/L) or caspase 1 inhibitor (VX-76, 25 μmol/L). **H** and **I**, qPCR quantification of NOX1 in hMVECs exposed to exogenous IL-1α (70 pg/mL, 24 hours) in the presence or absence of the NF-κB inhibitor (JSH-23, 20 μmol/L) or caspase 1 inhibitor (VX-76, 25 μmol/L). **J**, Schematic illustrating the protocol used to incubate human aorta specimens with CD4⁺T cell media. **K** through **N**, qPCR quantification of NOX1, NOX2, NOX4, and NOX5 in human aorta specimens exposed to WT or CD4⁺T cells. Data are presented as mean±SEM, n=3–8. ACh indicates acetylcholine. **P*<0.05. ***P*<0.01.

and BP,[7–9,39] we provided the first experimental evidence establishing a link of causality between CD4+ T cells expressing viral proteins and hypertension. These findings find their relevance in the observation that CD4+ T cells remain a reservoir for HIV and an active site of viral protein transcription in PLWH on cART.[11–14] Previous elegant work established a role for T cells, including CD4+ T cells, in the development of hypertension and end-organ damage.[10] Although that work identified T cells as an amplifying factor contributing to elevate BP in response to angiotensin II, DOCA-salt, or norepinephrine,[10] we identified CD4+ T cells expressing viral proteins as the factor initiating the elevation in BP, demonstrating for the first time that immune activation could be the cause of, not just a contributor to, hypertension and end-organ damage. Consistent with the work by Vinh et al,[20] we demonstrated that inhibition of T cell activation with the CTLA-4 agonist abatacept reduced the BP of the Tg26 mice to the levels of the WT mice, which indicates that the hypertension mediated by viral proteins depends on T cell activation. However, the mechanisms whereby viral proteins activate T cells, notably whether viral proteins could act as antigens or through autocrine or paracrine mechanisms, remain to be identified and should be the topic of future studies. Similarly, additional studies are required to identify the cells from the innate immune system acting as antigen-presenting cells.

Extensive work in seronegative individuals and mouse models has revealed a role for TH17 T cells and the proinflammatory cytokine IL-17 in hypertension.[10] Although identifying the subtype of CD4+ T cells responsible for hypertension was beyond the scope of this work, we provided compelling evidence that CD4+ T cells expressing viral proteins induced hypertension through the secretion of the proinflammatory cytokine IL-1α. Although this is the first report identifying a role for IL-1α in the development of hypertension, high IL-1α levels have been reported in PLWH[40–42] and in patients with CVD.[43] Furthermore, polymorphisms in the human *IL1A* gene have been shown to result in a high incidence of vascular malformations and a high risk for ischemic stroke.[44,45] Last, systemic IL-1α deletion reduces inflammation in instances of myocardial infarction,[46] whereas IL-1α blockade improves stroke outcomes in mice.[47] Therefore, together, these data indicate that IL-1α, in addition to IL-1β, is a significant risk factor for CVD. We extended our findings by establishing that CD4+ T cell–derived IL-1α induces hypertension through NOX1-dependent mechanisms. Consistent with the observation that NOX1 deficiency mitigates angiotensin II–mediated hypertension and vascular remodeling,[48] we demonstrated that NOX1-deficient mice are fully protected from viral protein–mediated endothelial dysfunction and hypertension. However, although the mechanisms and the cell type involved in the protective effects of NOX1 deficiency against angiotensin II–mediated hypertension have not been identified here, we introduced IL-1α as a positive regulator of endothelial NOX1 expression and a main inducer of endothelial dysfunction. We extended our findings by demonstrating that IL-1α–mediated increases in NOX1 are dependent on NF-κB, a recently identified NOX1 transcriptional regulator in endothelial cells,[49] but also on caspase 1, a key component of the inflammasome that further propagates inflammation through the activation of IL-1β and IL-18. IL-1β has also been reported to promote NOX1 expression. Although these studies provide further details on the links between IL-1α and elevated NOX1 expression, additional experiments are warranted to decipher how IL-1α increases NF-κB and caspase 1 signaling and the mechanisms by which NF-κB and caspase 1 promote NOX1 expression and activity. Although we reported no effect of IL-1α on other vascular NOX isoforms, we documented that CD4+ T cell depletion significantly reduced NOX2 expression in aortas of WT and Tg26 mice. Because NOX2 is expressed mainly in neutrophils, macrophages, and dendritic cells, it might be reasonable to speculate that CD4+ T cell depletion reduced vascular immune cell infiltration in both physiological (WT mice) and pathological (Tg26 mice) conditions. However, additional experiments are warranted to confirm this hypothesis.

In contradiction to previous in vitro reports[50] but in agreement with previous findings from our group,[37] the present study further supports the concept that HIV-associated endothelial dysfunction and vascular oxidative stress are not the consequences of the direct action of viral proteins on endothelial cells but the results of systemic inflammation induced by high IL-1α levels. We further established the human relevance of these findings by reporting that IL-1α increases NOX1 in discarded human aorta specimens and that PLWH on cART exhibit elevated vascular NOX1 levels. Together, these data suggest that targeting endothelial NOX1 may represent an avenue to reduce BP in PLWH and to present reduced vasodilatory properties as a source of BP elevations in males and females.

Although the Tg26 mouse enabled us to establish a role for viral proteins in endothelial dysfunction and hypertension, the mouse model did not allow us to identify the individual or combination of viral proteins responsible for IL-1α production by CD4+ T cells. Additional experiments would be warranted to address this limitation.

Conclusions

This study provides the first experimental evidence that expression of viral proteins in CD4+ T cells leads to the secretion of the proinflammatory cytokine IL-1α, which increases endothelial cell NOX1 levels, subsequently impairing endothelial relaxation and increasing BP. This first mechanistic study on the mechanisms of HIV introduces NOX1 inhibitors, IL-1α antibodies, and IL-1

receptor blockade as potential therapeutic avenues for the growing prevalence of hypertension in PLWH.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S4
Figures S1–S9

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