Immune regulation and vascular inflammation in genetic hypertension

Emilie C. Viel, Catherine A. Lemarié, Karim Benkirane, Pierre Paradis, and Ernesto L. Schiffrin

Hypertension and Vascular Research Unit, Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, Quebec, Canada

Submitted 29 July 2009; accepted in final form 30 December 2009

Viel EC, Lemarié CA, Benkirane K, Paradis P, Schiffrin EL. Immune regulation and vascular inflammation in genetic hypertension. Am J Physiol Heart Circ Physiol 298: H938–H944, 2010. First published December 31, 2009; doi:10.1152/ajpheart.00707.2009.—Immune cells have been implicated in the pathogenesis of hypertension. We hypothesized that under the influence of chromosome (chr)2, T lymphocytes contribute to vascular inflammation in genetic salt-sensitive hypertension. Normotensive (Brown Norway), hypertensive (Dahl salt-sensitive), and consomic rats (SSBN2; in which chr2 has been transferred from Brown Norway to Dahl rats) were studied. Systolic blood pressure, measured by tail cuff, and aortic preproendothelin mRNA, measured by quantitative RT-PCR, were elevated in Dahl rats compared with Brown Norway rats and were reduced in SSBN2 rats compared with Dahl rats (P < 0.01). Compared with Brown Norway rats, Dahl rats exhibited increased inflammatory markers and mediators such as nuclear translocation of the aortic p65 subunit of NF-κB as well as VCAM-1, ICAM-1, chemokine (C-C motif) receptor 5, and CD4 mRNA, all of which were reduced in SSBN2 rats. Aortic CD8 mRNA was equally increased in Dahl and SSBN2 rats relative to Brown Norway rats. CD4+ T cell infiltration in the aorta of SSBN2 rats was reduced compared with Dahl rats, whereas the aortic protein expression of Foxp3 and immunosuppressors transforming growth factor (TGF)-β and IL-10, the three markers associated with the regulatory T cell lineage, were enhanced in SSBN2 rats. Activation in vitro of T cells demonstrated that CD4+CD25+ and CD8+CD25+ cells (Tregs) produce IL-10 in SSBN2 rats. Thus, increased vascular inflammatory responses and hypertension in a genetic salt-sensitive hypertensive rodent model are reduced by transfer of chr2 from a normotensive strain, and this is associated with enhanced levels of immunosuppressive mediators.

Hypertension is associated with an increase in vascular inflammatory responses, which contributes to vascular dysfunction. The cause of essential hypertension remains elusive, but genetics may influence the development of high blood pressure and inflammatory responses (12, 20). Chromosome (chr)2 contains quantitative trait loci for blood pressure that were transferred from Brown Norway to Dahl rats) were studied. Systolic blood pressure, measured by tail cuff, and aortic preproendothelin mRNA, measured by quantitative RT-PCR, were elevated in Dahl rats compared with Brown Norway rats and were reduced in SSBN2 rats compared with Dahl rats (P < 0.01). Compared with Brown Norway rats, Dahl rats exhibited increased inflammatory markers and mediators such as nuclear translocation of the aortic p65 subunit of NF-κB as well as VCAM-1, ICAM-1, chemokine (C-C motif) receptor 5, and CD4 mRNA, all of which were reduced in SSBN2 rats. Aortic CD8 mRNA was equally increased in Dahl and SSBN2 rats relative to Brown Norway rats. CD4+ T cell infiltration in the aorta of SSBN2 rats was reduced compared with Dahl rats, whereas the aortic protein expression of Foxp3 and immunosuppressors transforming growth factor (TGF)-β and IL-10, the three markers associated with the regulatory T cell lineage, were enhanced in SSBN2 rats. Activation in vitro of T cells demonstrated that CD4+CD25+ and CD8+CD25+ cells (Tregs) produce IL-10 in SSBN2 rats. Thus, increased vascular inflammatory responses and hypertension in a genetic salt-sensitive hypertensive rodent model are reduced by transfer of chr2 from a normotensive strain, and this is associated with enhanced levels of immunosuppressive mediators.

Hypertension and vascular inflammation in genetic hypertension

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Hypertension and Vascular Research Unit, Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, Quebec, Canada

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Hypertension is associated with an increase in vascular inflammatory responses, which contributes to vascular dysfunction. The cause of essential hypertension remains elusive, but genetics may influence the development of high blood pressure and inflammatory responses (12, 20). Chromosome (chr)2 contains quantitative trait loci for blood pressure that were identified in the cross between Dahl and Milan rats or Dahl and Lewis rats (23) and genes encoding for inflammatory mediators (http://genome.ucsc.edu/cgi-bin/hgNear) with biological effects on T lymphocytes, such as IL enhancer binding factor 2 (Ilf2) and IL-2, IL-6, IL-7, and IL-12. Other genes on chr2 contribute to vascular dysfunction in hypertension, such as FGF, the dual endothelin (ET)-angiotensin (ANG) receptor, the ANG type 1b receptor, and enzymes involved in phospholipase D activity (7). Thus, chr2 is an attractive target to study as it contains elements related to hypertension, vascular reactivity, and immune responses. The effect of chr2 on enhanced vascular inflammatory responses that are observed in parallel with increased blood pressure levels in hypertension has not been previously investigated.

Vascular inflammation plays an important role in the development and maintenance of ANG II- and DOCA salt-induced hypertension (6, 11, 31). Vascular inflammation is characterized by a greater expression of cell adhesion molecules (VCAM-1 and ICAM-1) by endothelial cells and by the accumulation of immune cells such as dendritic cells (DCs) and T cells in the vascular wall. Numerous inflammatory diseases engage CD4 [T helper (Th)] and CD8 [T cytotoxic (Tc)] T cells that bear the Th1/Tc1, Th2/Tc2, or Th17/Tc17 phenotype based on their cytokine profile. However, the Th1 response is the prominent response found in inflamed vessels of rodents with cardiovascular disease (28). A subset of CD4 T cells, the regulatory T (Treg) cells, regulates inflammatory responses by suppressing the functions of reactive T cells (15, 25). The balance between Th1 and Treg cells determines the degree of vascular inflammation, since Treg cells play a crucial role in vascular homeostasis. Whether vascular inflammation in hypertension depends on a reduced Treg lineage and an increase of Th1 cells, resulting in enhanced vascular inflammatory responses, remains to be established.

We hypothesized that adaptive immunity is enhanced in hypertension as a result of a reduction of Treg cell number and/or function due to a genetic predisposition with loci on chr2 in hypertensive models such as the Dahl salt-sensitive rat. We studied a consomic strain (SSBN2) that contains the genome of Dahl rats (hypertensive strain) and chr2 from Brown Norway rats (control normotensive strain) to evaluate genetic influences on inflammatory responses in hypertension.

METHODS

Animals. All experimental procedures were approved by the Animal Care Committee of the Lady Davis Institute for Medical Research, McGill University, and followed the recommendations of the Canadian Council of Animal Care.

Experiments were conducted in 12-wk-old male Brown Norway (Charles River Laboratories, St-Constant, QC, Canada), Dahl salt-sensitive (Harlan Sprague Dawley, Chicago, IL), and consomic SSBN2 (Physiogenix, Wauwatosa, WI) rats. Brown Norway, Dahl salt-sensitive, and SSBN2 rats were received at least 4 wk before the experiments and offered a normal salt diet content (0.23% sodium content, Teklab 2018, Harlan Laboratories, Indianapolis, IN) before the experiments were performed to reduce artifacts due to the different normal diet given by the suppliers. SSBN2 rats were generated by transferring chr2 from a normotensive rat strain (Brown Norway) into the genetic background of hypertensive Dahl salt-sensitive rats. Hemozygous chromosomal substitution of chr2 was confirmed by the manufacturer (5). Blood pressure was measured by the tail-cuff method using a electrophysymomanometer (model PE 300, Narco Bio-Systems, Houston, TX) and a pulse pressure sensor (model PPS-4, Astro-Med, Brossard, QC, Canada) in the late morning 1 day
before rats were killed. Anesthetized rats were killed by decapitation, and their aortas were collected. The weight of the aorta was normalized by tibia length to quantify aortic growth.

Quantitative RT-PCR. Total RNA was isolated from the aorta without adipose tissue using TRIzol (Invitrogen, Carlsbad, CA). One microgram of RNA was used for reverse transcription with the QuantiTect reverse transcription kit (Qiagen, Mississauga, ON, Canada). PreproET-1, VCAM-1, ICAM-1, CCR5, CD4, CD8, and 40S ribosomal protein 16 (S16) cDNAs were used to determine gene expression by quantitative PCR using the QuantiTect SYBR Green PCR kit (Qiagen) in a MX3000P real-time PCR machine (Stratagene, La Jolla, CA). Primer sequences were as follows: preproET-1, forward 5′-GCTGTTGGAGGAGAAAGAC-3′ and reverse 5′-CACCAACGCTGTCCTGATC-3′; VCAM-1, forward 5′-GGGTATCTGAAGGGTGAG-3′ and reverse 5′-GCCGAGTCCTGTCCTGATC-3′; ICAM-1, forward 5′-GGATCCATCATCCATCCACAG-3′ and reverse 5′-CTCGCTTGGAGGAGAAATAC-3′; chemokine (C-C motif) receptor 5 (CCR5), forward 5′-ATCCACACCACTGGTTTGAG-3′ and reverse 5′-GTGTAGGAGGATGCTGG-3′; CD4, forward 5′-AGGACATGTGGCAGCTGACG-3′ and reverse 5′-GGCTTGATCTGAGGCTGACG-3′; CD8, forward 5′-CCGCGTCTCCCATCATTAG-3′ and reverse 5′-GGCGGATTTCTTCTGTGTTG-3′; and S16, forward 5′-TCTGGGCAAGGAGAGATTGTG-3′ and reverse 5′-CCGCGAACAATTCTTCTTGATTTC-3′. S16 was used as a reference gene.

Cell sorting. Cells were isolated from the spleen by harvesting tissue through a 100-µm filter in RPMI-1640 (Multicell, Woonsocket, RI). Splenocytes were purified by centrifugation at 4°C on a Ficoll gradient (Histopaque, Sigma-Aldrich, Oakville, ON, Canada) and washed in PBS-HEPES (20 mM). T cells were isolated from the spleen by harvesting cell suspensions through a 100-µm filter in RPMI-1640 (Multicell, Woonsocket, RI). Splenocytes were purified by centrifugation at 4°C on a Ficoll gradient (Histopaque, Sigma-Aldrich, Oakville, ON, Canada). The purity of the populations of lymphocytes was calculated as the concentration of the homogenized spleen (in cells/ml) divided by the total volume of the homogenate (in ml). The total number of T cells was calculated by total frequency (in % for 1 x 10^6 cells) x total number of splenocytes.

Western blot analysis. Proteins were extracted from frozen aortas by homogenization in lysis buffer (20 mM Tris-HCl, 5 mM EGTA, 150 mM NaCl, 20 mM glycerophosphate, 10 mM NaF, 1 mM orthovanadate, 1% Triton X-100, 0.1% Tween, 1 µg/ml pepstatin, 1 µg/ml leupeptin, 1 µg/ml aprotinin, 2 mM DTT, and 1 mM PMSF) and centrifuged at 15,700 g for 10 min at 4°C. Western blots were performed with 20 µg protein transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories) followed by an overnight incubation with the following primary antibodies (1:1,000): rat anti-Foxp3 (clone FJK-16a, Ebiosciences), rabbit anti-transforming growth factor (TGF)-β, mouse anti-OX-62 (Cell Signaling, Pickering, ON, Canada), and mouse anti-β-actin (AC-15, Sigma-Aldrich). Membranes were incubated with secondary horseradish peroxidase (HRP) antibodies (1:5,000) followed by SuperSignal West Pico chemiluminescence substrate (Pierce, Rockford, IL) and revealed with a Chemidoc (Bio-Rad Laboratories). Bands were quantified with Quantity One software (Bio-Rad Laboratories).

Histochemistry, immunohistochemistry, and immunofluorescence. Vascular fibrosis was evaluated with Sirius red staining and analyzed by an image-analysis system (Northern Eclipse 5.0, EMPIX Imaging, Mississauga, ON, Canada). Collagen density was defined as the ratio of the area stained to the total tissue area and expressed as a percentage. For immunohistochemistry, aortic rings were fixed with 4% paraformaldehyde, and paraffin-embedded sections were deparaffinized and rehydrated. Antigen was unmasked for VCAM-1 staining with 0.1% trypsin for 12 min and for ICAM-1 staining with 10 mM sodium citrate at 95°C for 20 min. Sections were stained with goat anti-VCAM-1 (1:100, clone C-19, Santa Cruz Biotechnology) or goat anti-ICAM-1 antibodies (1:100, clone M-19, Santa Cruz Biotechnology) overnight at 4°C followed by an incubation with biotinylated anti-goat antibody (Santa Cruz Biotechnology) for 30 min. Sections were then incubated with Vectastain Elite ABC Reagent (Vector Laboratories, Burlingame, CA) and NovaRed HRP substrate (Vector Laboratories) as recommended by the manufacturer and counterstained with hematoxylin.

Immunofluorescence staining for the p65 subunit of NF-κB was performed by incubating slides with mouse anti-p65 antibody (1:100, F-6, Santa Cruz Biotechnology) for 1 h at room temperature followed by an incubation with secondary rhodamine anti-mouse antibody (1:200, Invitrogen). Slides were incubated with 4′,6-diamidino-2-phenylindole and then mounted with Vectashield hard set mounting medium (Vector Laboratories). For immunochemistry, aortic rings were fixed with 4% paraformaldehyde, and paraffin-embedded sections were deparaffinized and rehydrated. Antigen was unmasked for VCAM-1 staining with 0.1% trypsin for 12 min and for ICAM-1 staining with 10 mM sodium citrate at 95°C for 20 min. Sections were stained with goat anti-VCAM-1 (1:100, clone C-19, Santa Cruz Biotechnology) or goat anti-ICAM-1 antibodies (1:100, clone M-19, Santa Cruz Biotechnology) overnight at 4°C followed by an incubation with biotinylated anti-goat antibody (Santa Cruz Biotechnology) for 30 min. Sections were then incubated with Vectastain Elite ABC Reagent (Vector Laboratories, Burlingame, CA) and NovaRed HRP substrate (Vector Laboratories) as recommended by the manufacturer and counterstained with hematoxylin.

Statistical analyses. Results are expressed as means ± SE. Statistical differences were tested by one-way ANOVA followed by the Student-Newman-Keuls post hoc test. P < 0.05 was considered statistically significant.

RESULTS

Enhanced vascular inflammation in hypertensive rats is reduced in SSBN2 rats. Chr2 replacement in Dahl salt-sensitive rats resulted in lower aortic growth, lower preproET-1, and reduced collagen deposition in SSBN2 rats (P < 0.01; Fig. 1, A–C). Blood pressure was higher in Dahl salt-sensitive rats (175 ± 3 mmHg) than in Brown Norway rats (113 ± 5 mmHg) and SSBN2 rats (150 ± 3 mmHg, P < 0.01). To evaluate the effect of chr2 on the degree of vascular inflammation, the expression levels of adhesion molecules in the aorta were measured by quantitative RT-PCR and immunohistochemistry. Expression of VCAM-1 and ICAM-1 mRNA and protein were enhanced in Dahl salt-sensitive rats, and these increases were abrogated in SSBN2 rats (P < 0.05; Fig. 2, A–C). Nuclear translocation of the p65 subunit of proinflammatory NF-κB, visualized by immunofluorescence, revealed an increase of the nuclear signal only in Dahl salt-sensitive rats (Fig. 2D).

Vascular infiltration of Th lymphocytes in hypertensive rats is reduced in SSBN2 rats. Immune cells bearing CCR5 receptors, which are expressed during immune cell infiltration, CD4 or CD8 for T cell identification, or OX-62 for DC identification...
were measured by quantitative RT-PCR of their respective mRNAs in the aortic wall. High levels of CCR5, CD4, CD8, and OX-62 expression were observed in Dahl salt-sensitive compared with Brown Norway rats (\(P < 0.05\); Fig. 3, A and B). Transfer of chr2 from Brown Norway rats resulted in reduced levels of CCR5 and CD4 mRNA in consomic SSBN2 rats (\(P < 0.05\)). OX-62 protein (identifying integrin-\(\beta\)E on DCs) and CD8 mRNA were unaffected by chr2 replacement. The release of GM-CSF and TNF-\(\alpha\) from aortic rings cultured for 48 h into the culture media was higher in SSBN2 rats than in Dahl salt-sensitive rats (\(P < 0.01\); Fig. 3C).

Regulatory T cell mediators Foxp3, TGF-\(\beta\), and IL-10 are upregulated in SSBN2 rats. Foxp3 is the transcriptional regulator that determines the activity of Treg cells (27). Two isoforms of Foxp3 were detected by Western blot analysis in the rat aorta (Fig. 4A). The elevated Foxp3a observed in Dahl salt-sensitive rats compared with Brown Norway rats (\(P < 0.001\)) was blunted in SSBN2 rats, whereas Foxp3b was increased in Dahl salt-sensitive rats and further enhanced in SSBN2 rats (\(P < 0.01\) and \(P < 0.05\), respectively; Fig. 4, B and C). TGF-\(\beta\) expression (Fig. 4, D and E) and IL-10 release (Fig. 4F), which exerts anti-inflammatory effects, were elevated only in aortic rings from SSBN2 rats (\(P < 0.05\)).

CD4\(^{+}\)CD25\(^{+}\) and CD8\(^{+}\)CD25\(^{+}\) cells are predisposed to produce the anti-inflammatory cytokine IL-10. To identify, independently of blood pressure levels, a genetic predisposition with loci on chr2 to produce the anti-inflammatory cytokine IL-10, sorted T cells were isolated and studied in culture. T cells obtained from the spleen were activated through the T cell receptor by creating a cross-link using the coated anti-CD3 antibody. IL-10 release was increased in CD4\(^{+}\)CD25\(^{+}\) and CD8\(^{+}\)CD25\(^{+}\) T cells originating from the spleen of SSBN2 rats only (\(P < 0.001\); Fig. 5, A and B) and the levels of IL-10 were 70% higher in the media of CD8\(^{+}\)CD25\(^{+}\) cells than CD4\(^{+}\)CD25\(^{+}\) T cells. FACS analysis did not reveal differences in the frequency of parent from CD4\(^{+}\)CD25\(^{+}\) and CD8\(^{+}\)CD25\(^{+}\) T cell populations between Brown Norway, Dahl salt-sensitive, and SSBN2 rats (data not shown). However, the total number of...
spleocytes was increased at similar levels in Dahl salt-sensitive and SSBN2 rats compared with Brown Norway rats \( (P < 0.001; \text{Fig. } 5C) \). The total number of CD4\(^+\)CD25\(^+\) cells in the spleen of Dahl salt-sensitive rats was higher than in the spleen of SSBN2 rats \( (P < 0.01; \text{Fig. } 5D) \), and the total number of CD8\(^+\)CD25\(^+\) cells was increased in Dahl salt-sensitive and SSBN2 rats \( (P < 0.001; \text{Fig. } 5E) \) compared with Brown Norway rats and was at similar levels between Dahl salt-sensitive and SSBN2 rats.

**DISCUSSION**

In the present study, we demonstrate the novel findings that reduced blood pressure and vascular inflammation upon chr2...
replacement are accompanied by increased immunosuppressive molecules produced by Treg cells in the vasculature, allowing new insights to be gained on the relationship between inflammation and hypertension. We first showed that the degree of blood pressure elevation was associated with the degree of infiltration of immune cells such as Th cells, as shown by the changes in CCR5 and CD4 mRNA levels in a chr2-dependent manner. Second, the aortic wall revealed two isoforms of Foxp3, Foxp3a and Foxp3b, which are differentially modulated depending on the degree of blood pressure elevation in a chr2-dependent manner. The transcription factor Foxp3 is highly specific for the Treg lineage, which indicates that the Treg population is important in the regulation of vascular inflammation observed in this model of hypertension. Third, the vasculature of hypertensive rats had low levels of immunosuppressive mediators (TGF-β and IL-10) associated with low levels of Foxp3b. Fourth, the production of IL-10 was increased in T cells after chr2 replacement. This study thus strongly suggests that the downregulation of these anti-inflammatory pathways in Dahl salt-sensitive rats can lead to enhanced vascular inflammation in hypertension in a chr2-dependent manner. To evaluate genetics independently of environmental factors, our model was fed with a normal salt diet, as increased sodium intake induces inflammation and blood pressure elevation in the Dahl salt-sensitive rat (17). We report here, for the first time, the presence of a quantitative trait locus for blood pressure on chr2 in the genetic crosses between Brown Norway and Dahl salt-sensitive rats that is independent of salt-induced hypertension. Our findings complement another report (22) that did not observe significant differences in blood pressure between younger Dahl salt-sensitive and SSBN2 rats studied during high salt intake. Discriminating unambiguously between blood pressure and chr2-specific effects on vascular inflammatory responses in this study is difficult, which must be recognized as a limitation of the study. Tail-cuff blood pressure measurement in rodents remains a stressful technique that can affect several responses, which can be overcome by telemetry analysis. However, consomic rats exhibited a significant, albeit modest, reduction of blood pressure. We have shown drastic increases of TGF-β1 and IL-10 production, prevention of adhesion molecule expression and NF-κB activation, and greater Foxp3b expression to occur in consomic rats only, which argues against a role of blood pressure as a mechanism playing a role in our findings. These results extend previous data that has suggested that genetics and the immune system are involved in the development of cardiovascular disease (8, 10, 21) and that T lymphocytes may influence blood pressure (11).

Foxp3 is crucial for the development and function of Treg cells and is not expressed by other T cells, B cells, and natural killer cells (9). Little is known about Foxp3 expression in the vasculature of rats. However, human studies have demonstrated that the two isoforms of Foxp3 are functional and represent either the full length of the mRNA (Foxp3a) and its truncated form lacking one exon (Foxp3b) (1). FACS analyses do not allow discrimination between the two isoforms of Foxp3, which explains the lack of report on these isoforms in rats. Increased Foxp3a and Foxp3b expression in the aorta of Dahl salt-sensitive rats indicates an increase of Treg cells associated with vascular inflammation and high blood pressure, in agreement with a previous report (29) in other models. This increase of Foxp3a and Foxp3b in Dahl salt-sensitive rats could be attributed to a mechanism of protection that may limit damage induced by inflammatory responses in target tissues. The expression and activation of Foxp3 are induced by TGF-β (26), which is mostly known for its profibrotic actions during tissue repair. Here, we report that TGF-β is increased in the aorta of consomic rats only, which supports its role in the regulation of Foxp3b expression. A recent report (33) has demonstrated that TGF-β is crucial for the maintenance of a noninflamed state of the endothelium and inhibits leukocyte rolling. This agrees with the present findings that SSBN2 rats have reduced inflammatory responses. On the other hand, the Foxp3a reduction upon chr2 replacement suggests that either blood pressure or chr2-dependent mechanisms lead to Foxp3a expression in the aorta of Dahl salt-sensitive rats, independently of TGF-β. Another putative explanation that needs to be investigated further is that Foxp3a may be expressed by a specific Treg lineage that inhibits inflammation by using, for example, cell contact instead of cytokine production, as reviewed previously (30). Thus, Foxp3a and Foxp3b could be markers of different Treg cells known to inhibit reactive T cells based on the expression of inhibitory cytokines, on cytolsis, on metabolic disruption, and on inhibition of DC function.

Several types of Treg cells have been identified based on the production of TGF-β and IL-10, their ability to repress Th1 responses, their constitutive expression of Foxp3, and the receptors CD4*CD25+ or CD8*CD25+ that they bear (3, 4, 16). TGF-β is produced by T cells, DCs, macrophages, fibroblasts, endothelial cells, and smooth muscle cells (13). IL-10 has anti-inflammatory functions and is produced by Treg cells, natural killer cells, macrophages, and DCs (13). The present study did not identify the cell source of TGF-β, IL-10, and Foxp3. However, TGF-β and IL-10 contribute to the expansion and differentiation of Treg populations in addition to suppressing effector T cells (14). Thus, high levels of CD4 and CD8 mRNA, DC-derived peptides, and Foxp3 protein in parallel with increased anti-inflammatory cytokines in consomic rats suggest that chr2 replacement stimulates Treg cells expressing CD4*CD25+ or CD8*CD25+ and leads to a reduction of the vascular inflammation observed in Dahl salt-sensitive rats. Furthermore, TGF-β and IL-10 loci are not positioned on chr2 of the rat, which suggests that transcriptional activators or chemokines with loci on chr2 may act on other chromosomes to repress TGF-β and IL-10 production in Dahl salt-sensitive rats.

The release of GM-CSF and TNF-α from the aorta of SSBN2 rats was increased without affecting the aorta content of DCs. Peripheral blood monocytes and progenitor cells can differentiate into DCs under the influence of GM-CSF and TNF-α (19). Although we did not investigate the phenotype of DCs, GM-CSF and TNF-α stimulate the differentiation of precursor cells into the immunosuppressive phenotype of DCs (expressing CD80) (34). This DC phenotype induces naïve T cells to develop into Treg cells expressing Foxp3, which supports our findings of chr2 dependency of the changes observed.

A direct link between immune cells and hypertension has been demonstrated in several rodent models with an activated ET system (11, 18, 24). ET-1 stimulates vascular inflammation and is released by activated endothelial cells and some cells related to innate immunity such as macrophages (2, 32). Chr2
replacement lowered the aortic prepro-ET-1 elevation observed in Dahl salt-sensitive rats below that found in normotensive rats. The locus of ET-1 is found on rat chr17. Thus, the reduction of prepro-ET-1 may be attributable to a reduction in the number of immune cells, to a recovery of endothelial cell function, or to the reduction of blood pressure in a chr2-dependent manner. Our data might also reflect a negative relationship between Treg cells and ET-1 in inflammatory responses.

Limitations to our findings are the fact that blood pressure in this study was measured only with the tail-cuff technique, which has less precision than telemetric measurement. However, because of problems in generating further cohorts of SSBN2 rats by the producers of this strain, it has been impossible to confirm the tail-cuff data with telemetry. In addition, other local and circulating factors than those addressed in this report such as oxidative stress and other hormones such as the components of the renin-angiotensin-aldosterone system, hypoxia, and shear stress may account for some of our observations.

Adaptive immune responses require the sensitization of lymphocytes to specific antigens. To date, the nature of these antigens remains unknown, but the genetic predisposition of some cells such as endothelial cells, vascular smooth muscle cells, and adipocytes to express specific epitopes after gene activation or enzymatic reaction (cleavage of a key target protein) could play an important role. In addition, oxidative stress, which is increased in hypertension, could be an initiator for adaptive immune responses due to lipid peroxidation, which is highly immunogenic.

In conclusion, this study provides, for the first time, evidence of a role of Treg cells in a genetic rodent model of salt-sensitive hypertension. It shows that chr2 modulates blood pressure and vascular immune responses in genetic hypertension, although this will require further investigation. Replacement of chr2 was used to identify a novel mechanism involved in the pathogenesis of hypertension and vascular disease. We found that some immunosuppressive chemokines that drive Treg cell maturation are differentially regulated in hypertensive animals, which is highly immunogenic.

ACKNOWLEDGMENTS
The authors are grateful to A. Turgeon, M.-E. Deschenes, D. Gagné, A. Vallée, and O. Angulo for excellent technical support.

GRANTS
This work was supported by Canadian Institutes of Health Research (CIHR) Grant 37917, the Canada Research Chairs Program of the Government of Canada, and the Canada Foundation for Innovation (to E. L. Schiffrin). E. C. Viel was supported by studentships from the CHIR and from Société Québécoise d’Hypertension Artérielle, and C. A. Lemarié was supported by a fellowship from the Heart and Stroke Foundation of Canada.

DISCLOSURES
No conflicts of interest are declared by the author(s).

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