The p.Arg63Trp polymorphism controls Vav1 functions and Foxp3 regulatory T cell development

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CD4+ regulatory T cells (Treg cells) expressing the transcription factor Foxp3 play a pivotal role in maintaining peripheral tolerance by inhibiting the expansion and function of pathogenic conventional T cells (Tconv cells). In this study, we show that a locus on rat chromosome 9 controls the size of the natural Treg cell compartment. Fine mapping of this locus with interval-specific congenic lines and association experiments using single nucleotide polymorphisms (SNPs) identified a nonsynonymous SNP in the Vav1 gene that leads to the substitution of an arginine by a tryptophan (p.Arg63Trp). This p.Arg63Trp polymorphism is associated with increased proportion and absolute numbers of Treg cells in the thymus and peripheral lymphoid organs, without impacting the size of the Tconv cell compartment. This polymorphism is also responsible for Vav1 constitutive activation, revealed by its tyrosine 174 hyperphosphorylation and increased guanine nucleotide exchange factor activity. Moreover, it induces a marked reduction in Vav1 cellular contents and a reduction of Ca2+ flux after TCR engagement. Together, our data reveal a key role for Vav1-dependent T cell antigen receptor signaling in natural Treg cell development.

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such as CD28 and cytokines, including IL-2 (Josefowicz and Rudensky, 2009). TCR signaling controls $T_{reg}$ cell and conventional T cell ($T_{conv}$) development differently. $T_{reg}$ cell development is favored under conditions of TCR signaling with high strength that promotes negative selection of $T_{conv}$ cell (Jordan et al., 2001; Hsieh et al., 2004; Carter et al., 2005). In addition, deficiency of several pleiotropic signaling molecules, including TAK1, Bcl110, CARMA1, PKC-θ, IKK-β, c-Rel, LAT, and Foxo1/Foxo3a proteins, severely impairs $T_{reg}$ cell development, whereas it only marginally affects $T_{conv}$ cell development (Koonpaew et al., 2006; Wan et al., 2006; Gupta et al., 2008; Medoff et al., 2009; Ouyang et al., 2010). However, the signaling pathways precisely involved in $T_{reg}$ cell development remain poorly characterized.

Vav1 is a key signal transducer downstream of the TCR and is mandatory for the development and activation of T cells (Turner and Billadeau, 2002; Tybulewicz, 2005). In the present study, we demonstrate a major role for Vav1 protein in natural $T_{reg}$ cell development. The analysis of the genetic factors responsible for the difference in the size of the $T_{reg}$ cell compartment between two rat strains led to the identification of a nonsynonymous polymorphism in the first exon of $Vav1$, responsible for the substitution of an arginine by a tryptophan at position 63 (p.Arg63Trp or R63W). Associated with this substitution, we observed an increased proportion and absolute numbers of $T_{reg}$ cells in peripheral lymphoid organs, which most likely result from an increased output of $T_{reg}$ cells from the thymus. The Vav1-W63 variant is constitutively active, and its cellular protein levels are markedly reduced. This is associated with a decrease in $Ca^{2+}$ mobilization under TCR activation. Thus, our study highlights the importance of Vav1 in $T_{reg}$ cell development and the role that Vav1 signaling alterations could play in susceptibility to immune diseases.

RESULTS AND DISCUSSION

Brown Norway (BN) rats have higher proportions and absolute numbers of CD4+Foxp3+ $T_{reg}$ cells than Lewis (LEW) rats

We investigated CD4+Foxp3+ $T_{reg}$ cell development in LEW and BN rats, two strains which differ strikingly in their susceptibility to immune diseases (Fournié et al., 2001). Flow cytometry analysis of CD4+Foxp3+ T cells in PBMCs, LNs, and spleen revealed that the proportion (Fig. 1 A) and absolute numbers (Fig. 1 B) of Foxp3+ cells among CD4+ T cells were significantly higher in BN than in LEW rats. Similar results were observed in double-positive (DP) CD4+CD8+ and single-positive (SP) CD4+CD8+ thymocytes (Fig. 1, C and D), suggesting that these differences were probably acquired early during thymic ontogeny. To assess whether this difference was intrinsic to hematopoietic cells or caused by extrinsic factors, hematopoietic progenitor cells from either LEW or BN bone marrow were differentiated in the same thymic (LEW × BN) F1 environment. Although similar levels of chimerism were achieved in (LEW × BN) F1 recipients regardless of the donor origin, higher percentages of CD4+ Foxp3+ donor T cells were found in PBMCs, LNs, and spleen from F1 rats receiving BN bone marrow cells (Fig. 1 E). Thus, the difference in size of the $T_{reg}$ cell compartment between LEW and BN rats is controlled by factors that are intrinsic to hematopoietic cells.

Fort1, a 117-kb locus on chromosome 9, controls the size of the natural $T_{reg}$ cell population

We previously identified a 17-cM interval on chromosome 9 (c9) that controls the size of the CD45R.ClooCD4+ population in BN and LEW strains (Subra et al., 2001). Because Foxp3+ T cells were restricted to this population (Fig. S1), we reasoned that the genetic control of the $T_{reg}$ cell population...
might also be located in this interval. Using a panel of BN congenic lines and sublines for various LEW genomic regions of c9 (BN.LEW-c9), we identified an interval of 117 kb that exerts a major effect on the proportion of CD4+Foxp3+ T cells (Fig. 2, A and B). Indeed, although the size of the Treg cell population was unchanged in BN.LEW-c9-C, -H, and -Be congenic lines when compared with BN rats, it was markedly reduced in BN.LEW-c9-Ia, -B, -Bc, and -Bf congenic lines, reaching values close to those found in LEW rats. Results were similar when CD4+Foxp3+ T cells were analyzed in the LN, spleen, or thymus (unpublished data). In contrast, this locus, named Fort1 for Foxp3+ Treg cell locus 1, did not influence the size of the conventional CD4+Foxp3+ compartment (Fig. S2). We then tested whether Fort1 had an impact on the suppressive function of the Foxp3+CD4+ T cells using an in vitro assay. Purified CD4+CD25+ T cells from BN and Bf rats did not proliferate when cultured with allogeneic T cell–depleted APCs, indicating that they are anergic (Fig. 2 C). Importantly, they suppressed the proliferation of naive CD4+ T cells from (LEW × BN) F1 rats with similar efficacy, thus showing that the in vitro suppressive potential of Treg cells is independent of the genomic origin of Fort1 (Fig. 2 D). Moreover, by using LEW.BNc9 and DA.BNc9 congenics (LEW or Dark Agouti [DA] congenic rats for BN Fort1) and BN-1L rats (BN rats congenic for LEW MHC), we further demonstrated that the control of Fort1 on the size of the Treg cell compartment was independent of the genetic background and MHC haplotype (Fig. S3). Thus, the 117-kb Fort1 region contains a gene, or a set of genes, that exerts a pivotal control on the size of the Treg cell compartment.

Vav1 R63W polymorphism is associated with the difference in the size of the Treg cell compartment
Physical and high-density marker genetic maps of the 117-kb Fort1 region were constructed using Celera and NCBI Rat Genome databases. We identified four genes within Fort1: C3 (Complement 3 precursor), Gpr108 (G protein–coupled receptor 108), Trip10 (Cdk4:24-interacting protein 4), and Vav1 (Fig. 2 B).Because we did not find any differences in messenger RNA (mRNA) expression of these four genes between LEW, BN, and BN.LEWc9-Bf CD4+ T cells (Fig. S4), we investigated differences in single nucleotide polymorphisms (SNPs) by sequencing their coding regions. The 15 SNPs identified were then genotyped in the smallest congenic line (Bf) and in four additional rat strains (DA, Piebald Virol Glaxo [PVG], Biobreeding [BB], and August Copenhagen Irish [ACI]). No polymorphism was found in the Trip10 gene, and the polymorphisms found in Gpr108 were synonymous, thus rendering improbable the involvement of these genes (Table I). Conversely, identification of two nonsynonymous SNPs in C3 and Vav1 made them strong candidates for the control of Treg cell development. Vav1 appeared to be the strongest candidate gene because it is specifically expressed in hematopoietic cells (Katzav et al., 1989), and Fort1 control is intrinsic to hematopoietic cells (Fig. 1 E), whereas C3 is mainly produced is the liver (Alper et al., 1969). A further argument for

![Figure 2. Fort1, a 117-kb locus of chromosome 9, controls the proportion of CD4+Foxp3+ Treg cell and Vav1 protein expression.](image)}
excluding the involvement of the C3 nonsynonymous polymorphism is based on the haplotypic mapping conducted in six rat strains and in the BN.LEWc9-Bf congenic because the percentage of Foxp3+CD4+ T cells found in the BB rats is similar to that found in all the tested strains except the BN strain (Table I). Thus, the c.244C>T SNP in exon 1 of Vav1 is the only SNP that correlates with the Treg cell phenotype. This SNP results in the substitution of an arginine (R) by a tryptophan (W) in BN Vav1 at position 63 in the N-terminal calponin homology (CH) domain (p.Arg63Trp or R63W). Altogether, these results strongly suggest that the Vav1 R63W polymorphism plays a prominent role in the control of Foxp3+CD4+ T cells, in the absence of involvement of the three other genes present in the 117-kb interval.

### The Vav1 R63W polymorphism controls Vav1 cellular protein levels

We next analyzed the impact of the Vav1 R63W polymorphism on Vav1 protein expression. We used rat CD4+ T cells expressing the Vav1-W63 (BN rats) or the Vav1-R63 (LEW and BN.LEWc9-Bf rats) variants. Immunoblotting revealed that levels of Vav1 were markedly lower in thymic and peripheral BN CD4+ T cells (Fig. 2 E). The same difference was also observed in CD4+CD25+ (Treg cells) and in CD4+CD25- (Tconv cells; Fig. S5). To test whether the reduced Vav1 protein expression is the direct consequence of the R63W polymorphism, we performed experiments in HEK293 cells transfected with plasmids coding for human VAV1-R63 (wild type), VAV1-W63 (mutated), or the oncogenic human VAV1 (VAV1-ΔCH) that lacks the first 67 N-terminal amino acids and is known to be constitutively active. The protein levels of VAV1 were lower in HEK293 cells transfected with VAV1-W63 when compared with that of HEK293 cells transfected with VAV1-R63 (Fig. 2 F). A similar observation was made in HEK293 cells transfected with plasmids coding for rat Vav1 variants (unpublished data). The decreased VAV1 protein expression is also found in HEK293 cells transfected with VAV1-ΔCH (Fig. 2 F). Thus, these data show that the Vav1 R63W polymorphism impacts the cellular content of Vav1 and suggest that the Vav1-W63 variant might share biochemical and functional properties with the constitutively active VAV1-ΔCH.

### Vav1 R63W polymorphism influences Vav1 functions

Vav1 functions as a guanine exchange factor (GEF) for small GTPases, thereby facilitating their transition from an inactive (GDP bound) to an active (GTP bound) state. Vav1 is also an adaptor molecule that participates in protein–protein interactions. After TCR engagement, Vav1 is rapidly phosphorylated, in particular in tyrosine 174 (Tyr174), which is crucial to Vav1 activities (Aghazadeh et al., 2000; López-Lago et al., 2000). To analyze the impact of Vav1 R63W polymorphism on Vav1 functions, we first performed biochemical experiments in HEK293 cells transfected with plasmids coding for human VAV1-R63 (wild type), VAV1-W63 (mutated), or the constitutively active VAV1 (VAV1-ΔCH). Both VAV1-W63 and...
VAV1-ΔCH show increased phosphorylation on total tyrosines (Fig. 3 A) and Tyr174 (Fig. 3 B) and displayed enhanced GEF activity (Fig. 3 C) as compared with VAV1-R63. This increased GEF activity resulted in elevated activation of Rac1 (Fig. 3 D), the VAV1 preferential downstream effectors, and also in a stronger activation of RhoA (∼3-fold compared with ∼~2-fold for Rac1; Fig. 3 E). Confocal examination of transiently transfected HEK293 cells showed that VAV1-R63 did not induce major morphological changes, whereas the expression of VAV1-W63 induced lamellipodia formation and pronounced rounding of the cell body as a result of actin cytoskeleton contractility (Fig. 3 F). These morphological changes are reminiscent of what was observed for VAV1-ΔCH (Fig. 3 F; López-Lago et al., 2000). Together, these data demonstrate that the VAV1-W63 mutation leads to its constitutive activation.

To analyze the impact of TCR engagement on Vav1 activities, we also performed biochemical analysis on purified CD4+ T cells from BN (Vav1-W63) and BN.LEWc9-Bf (Vav1-R63) rats. In agreement with our results on HEK293 cells, we showed that Vav1 in BN CD4+ T cells exhibits an increased global phosphorylation on Vav1 tyrosines (Fig. 4 A) and Tyr174 (Fig. 4 B) as compared with Bf CD4+ T cells, both at basal state and after TCR engagement. However, the activation of Rac1 (Fig. 4 C) and RhoA (Fig. 4 D) GTPases was modest as compared with transfected HEK293 cells expressing similar levels of Vav1 protein. These data suggest that the increased Vav1-W63 activity in the CD4+ T cells might be counterbalanced by the decrease in Vav1 protein amount, thus maintaining GEF activity close to physiological levels. Using confocal microscopy, we next showed that localization and recruitment of Vav1-W63 to TCR-inducible complexes are not altered because we observed similar colocalization of Vav1 in BN and Bf CD4+ T cells (Fig. 4 E). Recently, it has been shown that many critical events involved in T cell activation, such as TCR-triggered Ca2+ flux, were mediated by a GEF-independent function of Vav1 that can act as an adaptor (Miletic et al., 2009; Saveliev et al., 2009). We therefore assessed the effect of R63W polymorphism on Ca2+ flux in thymic and peripheral CD4+ T cells. CD4+ T cells bearing Vav1-W63 (BN rats) had lower TCR-induced Ca2+ flux than those bearing Vav1-R63 (Bf and LEW rats; Fig. 4 F). Together, these data show that the Vav1 R63W polymorphism has major consequences on Vav1 biochemical properties, triggering a state of constitutive activation that leads to the imbalance between Vav1 GEF-dependent (Rac1 and RhoA activation) and -independent (Ca2+ flux) functions.

Figure 3. Biochemical analyses of VAV1 in HEK293 cells transfected with VAV1 variants reveal that VAV1-W63 is constitutively active. (A and B) Analysis of VAV1 phosphorylation in HEK293 transfected with different human VAV1 plasmids. VAV1 was immunoprecipitated and immunoblotted with anti-phosphotyrosine 4G10 mAb (A) or anti-phospho-VAV1 (Y174) antibody (B). After stripping, membranes were probed with anti-VAV1 antibody. IP, immunoprecipitation. (C) Analysis of GEF activity of VAV1 using pull-down with Rac1G15A agarose beads on serum-starved HEK293 cells transfected with different human VAV1 plasmids. (D and E) Analysis of Rac1 or RhoA activities in HEK293 cells transfected with different VAV1 variants using pull-down assay to detect relative amounts of Rac1-GTP (D) or RhoA-GTP (E). Total Rac1 and RhoA levels were used as loading controls. In each case, results show one blot of a representative experiment and a graph representing the mean ± SEM of three to four independent experiments. WB, Western blot. (F) Confocal analysis of HEK293 cells transfected with different VAV1 variants. Actin was visualized with phalloidin–Alexa Fluor 488. Representative images of three independent experiments are shown. *, P < 0.05; **, P < 0.01; ns, not significant.
might maintain Vav1 GEF activity close to normal levels but would impact Vav1 GEF-independent functions as revealed by the reduction of Ca^{2+} flux. Together with a recent study showing that Vav1 GEF activity is not required for the development of CD4^{+}CD25^{+} T cells (Saveliev et al., 2009), these observations suggest that Vav1-W63 could influence T_{reg} cell development through an effect on Vav1 GEF-independent functions.

The link between the Vav1-W63 variant and the development of the T_{reg} cell compartment can be altered through modulations of the strength of TCR signals. For example, enrichment in T_{reg} cells was observed when TCR signaling was enhanced by the loss of the tyrosine phosphatase SHP-1, a negative regulator of TCR-mediated signaling (Carter et al., 2005). Enrichment was also observed when developing T cells were exposed to their cognate peptides with high-avidity interactions (Jordan et al., 2001; van Santen et al., 2004; Cabarrocas et al., 2006). Thus, in rats carrying the Vav1-W63 variant, the increased development of T_{reg} cells might result from Vav1-W63–dependent modifications in signaling pathways downstream of the TCR.

Given the link existing between T_{reg} cell defects and autoimmunity (Sakaguchi et al., 2008), our findings may have important implications. We recently provided evidence that Vav1 R63W variants play a central role in susceptibility to experimental autoimmune encephalomyelitis and impact the production of IFN-γ and TNF (Jagodic et al., 2009; unpublished data). Our present findings suggest that this may also be related to the effect of Vav1 variants on the T_{reg} cell compartment. Thus, genetic or acquired alterations in Vav1-dependent signaling could impact the susceptibility to immune-mediated diseases through effects on T_{reg} cell development.

**MATERIALS AND METHODS**

**Animals.** All breeding and experimental procedures were performed in accordance with European Union guidelines and were approved by the national and local ethics committee (license no. 31259; agreement no. B315558). Rats were kept under specific pathogen-free conditions. LEW, BN, and DA rats were obtained from Centre d’Élevage Janvier (Le Genest St. Isle, France). (LEW × BN) F1 rats and congenic lines were produced in our animal facility. The BN congenic lines for LEW chromosome 9 (BN, LEWc9) used in this study are summarized in Table S1 and were developed in our animal facility. The BN congenic lines for LEW chromosome 9 (BN, LEWc9) were described previously (Cautain et al., 2001; van Santen et al., 2004; Cabarrocas et al., 2006). The animals used in this study were 8–12 wk of age.

**Antibodies, cell staining, and flow cytometry.** The mAbs used for flow cytometry or purification of T cell subpopulations were as follows: W3/25 (anti–rat CD4), OX6 (anti–rat MHC class II), OX8 (anti–rat CD3), OX12 (anti–rat ß light chain), OX22 (anti–rat CD45R(C), OX39 (anti–rat CD25), R73 (anti–rat TCR-ß), V65 (anti–rat TCR-ß), 3.4.1 (anti–rat CD8eß), and 3.2.3 (anti–rat NKR-P1). The hybridomas OX6, OX7, OX8, OX12, OX22, OX39, OX40, OX95, and W3/25 were provided by D. Mason (University of Oxford, Oxford, England, UK), and the hybridomas J3319, V65, and R73 were provided by T. Hünig (University of Würzburg, Würzburg, Germany). The mAbs used for flow cytometry were fluorochrome conjugates either pre-purified in our laboratory or purchased from BD and eBioscience. The biotinylated mAbs 42-3-7 (RT1-A^a haplotype) and 163-7F3 (RT1-A^a haplotype) were.
provided by H. Kunz (University of Pittsburgh, Pittsburgh, PA). Foxp3 intracellular expression was detected using an APC-labeled anti-mouse/rat Foxp3 Staining Set from eBioscience according to their standard protocol. Data were collected on FACSCalibur or LSRII cytometers (BD) and analyzed using FlowJo or Cell Quest software package (BD).

Bone marrow chimeras. (LEW × BN) F1 rats were lethally irradiated (950 rads) 1 d before bone marrow transplantation. Recipient rats were given 108 viable nucleated bone marrow cells intravenously. 14 wk after engraftment, the PBMCs, spleen, and LNs were analyzed for T cells of donor or recipient origin by using RT1-A haplotype-specific mAbs and for expression of Foxp3 in CD4+ T cells of donor origin.

Purification of T cell subsets. Rat CD4+ T cells were negatively selected from LN spleen by using a cocktail of the following mAbs: OX6, OX8, OX12, 3.2.3, and V65. After washing and incubating with anti–mouse IgG magnetic microbeads (Invitrogen), CD4+ T cells were purified by magnetic depletion. Both OX8 and W3/25 were added to the cocktails for the purification of a non–T cell fraction that included B cells and monocytes/macrophages. SP CD4+ thyomocytes T cells were negatively selected from thymus using OX8 mAb and anti-mouse IgG magnetic microbeads. The purity of CD4+ T cells was >92%. For Treg cell purification, negatively selected CD4+ T cells were stained with 341-FTTC (anti-rat CD8β), R73-APC (anti–rat TCR-β), W3/25-PE (anti–rat CD4), and OX39-PE (anti–rat CD25) and were electronically sorted using a FACSAria Il-Sorp (BD). The purity of the cells was >99%.

Proliferative responses and Treg cell suppression assays. For Treg cell suppression assays, purified (LEW × BN) F1 CD4+CD45RChigh T cells (105/well) were stimulated with irradiated allogeneic APCs (0.5 × 105/well; T cell–depleted splenocytes from DA rats) alone or in the presence of decreased numbers of FACS-sorted BN or Bf CD4+CD25+ Treg cells and cultured in 96-well plates for 72 h. Proliferation was analyzed by [3H]thymidine incorporation during the last 18 h.

Sequencing. The sequencing templates were amplified from genomic DNA, and the sequencing reactions were performed using the BigDye terminator version 3.1 (Applied Biosystems) Products were separated and recorded on an ABI 3100 (Applied Biosystems). Sequences were analyzed with Vector NTI version 3.1 (Applied Biosystems) Products were separated and recorded on an ABI 3100 (Applied Biosystems). Sequences were analyzed with Vector NTI software (InforMax). SNPs identified by comparing LEW and BN coding sequences with ABI 3100 (Applied Biosystems) were then determined by GST pull-down assay. Cells were lysed in ice-cold lysis buffer containing 10 mM Tris HCl, 1% Triton X-100, a cocktail of protease inhibitors (Complete Mini, EDTA-free; Roche), 50 mM NaF, 1 mM Na3VO4, and 1 mM DTT. For immunoprecipitation, clarified homogenates were incubated overnight at 4°C with the antibodies and a mixture of protein A/G Sepharose beads. After washes, proteins were eluted with Laemmli buffer and analyzed by SDS-PAGE followed by Western blotting on Immobilon-P membranes (Millipore) with appropriate antibodies (Vav-1 antibody, clone D7 [Santa Cruz Biotechnology, Inc.]; β-actin antibody, clone AC-15 [Sigma-Aldrich]; phosphotyrosine antibody, clone 4G10 [Millipore]; and phosphotyrosine 174 of Vav1 antibody [Santa Cruz Biotechnology, Inc.]). Immunoreactive bands were detected by chemiluminescence with the SuperSignal detection system (Thermo Fisher Scientific). Band intensities were quantified using the Scion Image software.

Immunoblotting and immunoprecipitation. Vav1 protein expression and phosphorylation were analyzed on lysates from rat CD4+ T cells or from HEK293 cells 20 h after transfection with 2 µg of human wild-type VAV1–R63, the mutated VAV1–W63, or oncogenic VAV1–ΔCH plasmids. In some experiments, rat CD4+ T cells were stimulated with anti–TCR mAb (R73) used at 10 µg/ml and rabbit anti–mouse at 50 µg/ml for 1 min at 37°C. Total cellular proteins were extracted with ice-cold lysis buffer containing 10 mM Tris HCl, 1% Triton X-100, a cocktail of protease inhibitors (Complete Mini, EDTA-free; Roche), 50 mM NaF, 1 mM Na3VO4, and 1 mM DTT. For immunoprecipitation, clarified homogenates were incubated overnight at 4°C with the antibodies and a mixture of protein A/G Sepharose beads. After washes, proteins were eluted with Laemmli buffer and analyzed by SDS-PAGE followed by Western blotting.
(Invitrogen). HEK293 transfected with different variants of VAV1 were cultured on polyornithine-coated covergrips, fixed, and permeabilized as described above. Actin was visualized with phalloidin–Alexa Fluor 488. The preparations were analyzed by confocal microscopy using an LSM710 (Carl Zeiss) equipped with a 63× objective. Images were processed with ImageJ (National Institutes of Health).

Statistical analysis. Statistical analyses were performed by using the Instat statistical package (GraphPad Software). Data are expressed as means ± SEM. The significance of differences observed between two groups was analyzed by the Mann–Whitney test. When more than two groups were investigated simultaneously, the Kruskall-Wallis test was first performed.

Online supplemental material. Fig. S1 shows that CD4+ Fopx3+ T cells are contained within the CD45RC<sup>+</sup> subset. Fig. S2 shows that Fort I controls T<sub>reg</sub> cell numbers with no effect on the size of the T<sub>conv</sub> cell compartment. Fig. S3 shows that Fort I controls CD4+ Fopx3+ (T<sub>reg</sub>) cell numbers independently of the genetic background and the MHC. Fig. S4 shows mRNA expression of the four genes within the Fort I 117-kb region. Fig. S5 shows Vav1 expression and phosphorylation in CD25<sup>+</sup> and CD25<sup>+</sup> CD4 T cells in BN and BN.LEW<sup>c9</sup>-Bf (Bf) rats. Table S1 shows the genetic map of BN.LEW<sup>c9</sup> congenic lines and sublines used for Fort I genetic dissection. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20102191/DC1.

We thank the staff of the different platforms of our research center (Flow Cytometry, Imaging, and Animal Housing) for excellent assistance that was instrumental for the proper realization of this work. We thank Daniel Gonzalez-Dunia and Etienne Joly for critical reading of the manuscript and insightful comments.

This work was supported by Institut National de la Santé et de la Recherche Médicale, the Arthritis Fondation Courtin, Association Française contre les Myopathies, Agence Nationale de la Recherche (ANR-08-GENO-041-01), Association de Recherche sur la Sclérose en Plaques, Fondation pour la Recherche Médicale, Association de la Recherche contre le Cancer, and Région Midi-Pyrénées and Fight-MG (FP7-Health-2009-242210). A. Casemayou is supported by a grant from Fondation pour la Recherche Médicale. T. Olsson and M. Jagodic received grant support from Neuropeumis (LShf-CT-2005-018637), EURATools (LShg-CT-2005-0190115), the Swedish Research Council, and the Söderberg Foundation. The authors have no conflicting financial interests.

Author contributions: C. Colacios and A.S. Dejean designed the research, generated bone marrow chimeras, performed flow cytometry experiments, and analyzed the data. A. Casemayou and F. Gaits-lacovoni designed and conducted the biochemistry and cell signaling experiments. I. Bernard conducted the purification of T cell subsets. C. Pedros performed the suppression assays. D. Lagrange, M. Jagodic, and G. Fournié generated and characterized the congenic rats. L. Lamouroux and M. Jagodic performed the suppression assays. D. Lagrange, M. Jagodic, and G. Fournié and cell signaling experiments. I. Bernard conducted the purification of T cell subsets. D. Lagrange, M. Jagodic, and G. Fournié performed the suppression assays. D. Lagrange, M. Jagodic, and G. Fournié and cell signaling experiments. I. Bernard conducted the purification of T cell subsets.

Submitted: 15 October 2010
Accepted: 24 August 2011

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