Introduction

The physiological demands of the arterial system in vertebrates require that arteries store energy during systole and release it during diastole, which allows the heart to work at an optimal rate and stroke volume. The central feature that facilitates this function is vessel elasticity, which is conferred upon the arterial wall by the apposition of smooth muscle cells (SMCs) and a complex weave of ECM molecules. The basic morphological plan of large arteries present in all major vertebrate classes consists of repeating concentric layers of SMCs separated by elastic fibers that form interconnected fenestrated sheets, or lamellae. These elastic lamellae are designed to function as an elastic reservoir and to distribute tension uniformly throughout the vessel wall. The number of lamellar units (generally defined as the elastic lamella and adjacent SMCs) in a vascular segment is related linearly to tensional forces within the wall (1–3), with the greatest number of elastic layers occurring in the larger, more proximal vessels that experience the highest wall stress.

Acquired elastic fiber abnormalities that alter the structure and mechanical properties of vessels are associated with aging and are observed in common vascular diseases, such as atherosclerosis, aneurysms, and hypertension. Other vascular diseases have been shown to have a genetic etiology. At least three clinical conditions have been linked to mutation or deletion of the elastin gene (Eln). Autosomal dominant cutis laxa, a primarily cutaneous condition, is the result of frameshift

Supravalvular aortic stenosis is an autosomal-dominant disease of elastin (Eln) insufficiency caused by loss-of-function mutations or gene deletion. Recently, we have modeled this disease in mice (Eln+/–) and found that Eln haploinsufficiency results in unexpected changes in cardiovascular hemodynamics and arterial wall structure. Eln+/– animals were found to be stably hypertensive from birth, with a mean arterial pressure 25–30 mmHg higher than their wild-type counterparts. The animals have only moderate cardiac hypertrophy and live a normal life span with no overt signs of degenerative vascular disease. Examination of arterial mechanical properties showed that the inner diameters of Eln+/– arteries were generally smaller than wild-type arteries at any given intravascular pressure. Because the Eln+/– mouse is hypertensive, however, the effective arterial working diameter is comparable to that of the normotensive wild-type animal. Physiological studies indicate a role for the renin-angiotensin system in maintaining the hypertensive state. The association of hypertension with elastin haploinsufficiency in humans and mice strongly suggests that elastin and other proteins of the elastic fiber should be considered as causal genes for essential hypertension.


Received for publication May 27, 2003, and accepted in revised form August 26, 2003.

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Conflict of interest: The authors have declared that no conflict of interest exists.

Nonstandard abbreviations used: smooth muscle cells (SMCs); supravalvular aortic stenosis (SVAS); Williams syndrome (WS); inner diameter (ID); outer diameter (OD); phenylephrine (PE); acetylcholine (Ach); wall cross-sectional area (WCSA); distensibility per 25 mmHg increment (D25); left ventricle (LV); systolic LV volume (LVV); diastolic LV volume (LVVd); least significant difference (LSD); mean arterial pressure (MAP); left ventricular volume indexed to body weight (LVVdI); end-systolic left ventricular volume (LVVd); renin-angiotensin system (RAS).

by the apposition of smooth muscle cells (SMCs) and a complex weave of ECM molecules. The basic morphological plan of large arteries present in all major vertebrate classes consists of repeating concentric layers of SMCs separated by elastic fibers that form interconnected fenestrated sheets, or lamellae. These elastic lamellae are designed to function as an elastic reservoir and to distribute tension uniformly throughout the vessel wall. The number of lamellar units (generally defined as the elastic lamella and adjacent SMCs) in a vascular segment is related linearly to tensional forces within the wall (1–3), with the greatest number of elastic layers occurring in the larger, more proximal vessels that experience the highest wall stress.

Acquired elastic fiber abnormalities that alter the structure and mechanical properties of vessels are associated with aging and are observed in common vascular diseases, such as atherosclerosis, aneurysms, and hypertension. Other vascular diseases have been shown to have a genetic etiology. At least three clinical conditions have been linked to mutation or deletion of the elastin gene (Eln). Autosomal dominant cutis laxa, a primarily cutaneous condition, is the result of frameshift
mutations at Eln that influence elastic fiber structure through a dominant negative effect (4, 5). Supravalvular aortic stenosis (SVAS), an autosomal-dominant disorder, is caused by intragenic deletions or by a large spectrum of mutations within the elastin gene (6–8) that result in functional haploinsufficiency through either nonsense-mediated decay of mRNA from the mutant allele or the production of a nonfunctional protein (9–11). Williams syndrome (WS), a neurodevelopmental disorder that has SVAS as a component, develops as a consequence of a microdeletion in the chromosomal region 7q11.23 encompassing, among others, the elastin gene (12, 13). Narrowing of the ascending aorta is a dominant feature of SVAS (in both the isolated and WS forms), but other arteries, including pulmonary arteries, are often affected. If not corrected, SVAS may lead to increased intracardiac pressure, myocardial hypertrophy, and heart failure.

Recently, we generated mice hemizygous for the elastin gene (Eln+/−) to study the pathogenic mechanism underlying SVAS (14, 15). Characterization of the arterial wall of these mice found that elastin haploinsufficiency results in changes in arterial wall structure, including thinner elastic lamellae and an increased number of SMC layers — changes also observed in humans with SVAS (15). To investigate how these changes influence vascular function, we evaluated a number of basic hemodynamic and mechanical properties of the elastic-conducting vessels of Eln+/− mice. The vascular effects of elastin insufficiency occur early in development and result in a cardiovascular system that has undergone a remarkable adaptation to the altered mechanical properties of the vessel wall. The animals are stably hypertensive with only mild cardiac hypertrophy and do not exhibit the hypertension-induced arterial wall hypertrophy and decreased distensibility of large elastic arteries associated with essential hypertension. The results of this study provide insight into how hemodynamic forces impact vascular development and address the mechanism of hypertension in arteriopathies associated with elastin haploinsufficiency.

Methods

Animals. Wild-type C57B1/6J mice (Eln+/+ ) and mice of matching age (5–7 months) bearing a heterozygous deletion of exon 1 in the elastin gene (Eln+/−) backcrossed for more than five generations into the C57B1/6J background (14) were used for all studies. Littermates from Eln+/− crosses were used whenever possible. All housing and surgical procedures were in accordance with institutional guidelines.

Surgical procedure and mounting of vessels on the pressure myograph. For vessel mechanical studies, animals were anesthetized by intraperitoneal injection of pentobarbital (60 mg/kg). A segment of the ascending aorta, the abdominal aorta, the left carotid artery, or the renal artery was quickly excised and placed in a physiological buffer of the following composition: 135 mM NaCl, 5 mM KCl, 1.6 mM CaCl2, 1.17 mM MgSO4, 0.44 mM KH2PO4, 2.6 mM NaHCO3, 0.34 mM Na2HPO4, 5.5 mM D-glucose, 0.025 mM EDTA, 10 mM HEPES (pH 7.4). The vessels were cleaned of adhering connective tissue and fat, then cannulated and mounted onto a pressure arteriograph (Living Systems Instrumentation Inc., Burlington, Vermont, USA, or Danish Myotechnology, Aarhus, Denmark), as described previously (16). The experiments were performed at 37°C in an organ bath filled with physiological buffer. Following a 30-minute equilibration period, the vessel was transilluminated under an inverted microscope connected to a charged-coupled device camera and to a computerized system allowing the continuous recording of the vessel diameters.

Except for vessels treated with KCN, all studies used live vessels. Recordings of vessel inner diameters (IDs) and outer diameters (ODs) were taken while increasing the intravascular (transmural) pressure from 0 to 175 mmHg by steps of 25 mmHg (5 minutes per step). Recordings were also taken while decreasing the pressure following the same steps and timing from 175 to 0 mmHg. Assessment of arterial reactivity at 75 mmHg was performed using 5 µM phenylephrine (PE) as a SMC-dependent vasoconstrictor (for 10 minutes or until maximum vasoconstriction was reached). This was followed by the addition of the endothelial cell–dependent vasodilator acetylcholine (Ach) (5 µM) that was added for 5 minutes or until maximum vasodilatation was reached. The vessel was then bathed at zero pressure in a physiological buffer containing 13 mM KCN for 45–60 minutes to poison the vessel wall cells. After verifying at 75 mmHg the nonresponse of the vessel to 5 µM PE, the intravascular pressure was raised and lowered as described above while recording the artery segment’s ID and OD. For the renal artery, the concentration of PE and Ach used was 1.5 µM. The bath medium as well as the medium filling the vessel was replaced every 15 minutes.

The thickness of the wall in the large arteries (ascending and abdominal aorta, carotid artery) precluded the localization of the inner wall edge in many transilluminated vessels at pressures lower than 100–125 mmHg. Hence, in this pressure range ID could not be measured directly. Above 125 mmHg, however, direct measurement of ID was possible because thinning of the arterial wall at high pressure allowed a clear and contrasted image of the inner wall edge. In previous studies, we showed that in both wild-type and elastin-knockout animals, vessel wall cross-sectional area (WCSA) remains relatively constant throughout the experimental pressure range. As a result, ID can be accurately calculated from OD at any given pressure and from a single measurement of WCSA (16). Thus, when necessary, ID was calculated from the measured OD at the corresponding pressure using values for WCSA calculated from the measurements of OD and ID at 175 mmHg.

Circumferential midwall strain, circumferential wall stress, and incremental elastic modulus were calculated according to the formulas given by Gibbons and
Shadwick (17). Distensibility is defined as the change in relative volume (percentage) of the lumen per pressure unit during a change in intravascular pressure, as described by Smith and Kampine (18). Expression of the distensibility is preferred to the compliance (change in absolute volume per pressure unit) because the difference in initial vessel diameter (at transmural pressure = 0 mmHg) between *Eln*+/+ and *Eln*–/– vessels may result in misleading comparisons and interpretations of the difference in the absolute volume. Because the pressure-change increment in our experiments was 25 mmHg, distensibility is expressed as the distensibility per 25 mmHg increment (D25).

**Heart weight measurements and arterial protein content.**

The hearts from 11 *Eln*+/+ and 11 *Eln*–/– 5- to 7-month-old mice were dissected, washed, and weighed (wet weight). The left ventricle and septum weights were obtained using dissected tissue. Ratios of total heart weight to body weight, as well as left ventricle plus septum weight to body weight, were then analyzed.

Vessel segments, 2 mm long, were used to determine arterial wall protein content (20 animals used). For each artery type (ascending and abdominal aorta, carotid artery), six segments from 5- to 7-month-old *Eln*+/+ and *Eln*–/– genotypes were compared. Desmosine levels were determined by radioimmunoassay (19). Hydroxyproline and total protein content were determined by amino acid analysis using standard techniques (20). Results are expressed as protein mass per vessel segment length (micrograms per millimeter plus or minus SEM).

**Blood pressure, heart rate, and physiological measurements.**

*Eln*+/+ and *Eln*–/– mice from a range of ages were used for blood pressure and heart rate measurements. The animals were anesthetized using a ketamine/xylazine (87 mg/kg and 13 mg/kg, respectively) cocktail and were then restrained on a heated holder to maintain body temperature. A Millar pressure transducer was inserted into the right carotid artery and moved to the aorta where heart rate and systolic and diastolic blood pressure were monitored.

For physiological experiments, the jugular vein of mice was catheterized with PE-10 tubing for fluid infusion, and a Millar pressure transducer was inserted into the right carotid artery for monitoring blood pressure. A series of acute mean arterial pressure responses to a bolus dose of Ang II (1 µg/kg), hexamethonium (5 mg/kg), candesartan (100 µg/kg), and saralasin (10 µg/kg) were evaluated by continuous blood pressure recording. Intravenous infusions were done over a 2.5-second period, with compounds dissolved in saline and delivered in a volume of 5–10 µl. Isotonic saline was injected as a control.

Plasma renin activity and aldosterone levels in mouse plasma were determined by the clinical laboratories at Barnes Jewish Hospital, Washington University Medical Center (St. Louis, Missouri, USA).

**Cardiac output and left-ventricular volume.**

Animal preparations and image acquisition were performed by echocardiography as described previously (21). Three consecutive cardiac cycles from each of two sequentially acquired cine loops (a total of six frames per animal) were analyzed for end-diastolic and end-systolic left-ventricular volumes. End-diastolic and end-systolic frames of parasternal long-axis images were used for computer-assisted manual tracking of the endocardial border of the left ventricle (LV) chamber. Systolic and diastolic LV volumes (LVM and LVV, respectively) were determined by the disk summation method. Stroke volume (LVM – LVV) was deduced from these measurements. The values were indexed to the body weight for each animal. Five animals were used in each group.

**Immunohistochemical staining with proliferating cell nuclear antigen Ab.** Sections from *Eln*+/+ and *Eln*–/– descending aortic tissue fixed in 10% formalin and embedded in paraffin were incubated with a monoclonal antiproliferating cell nuclear antigen (anti-PCNA) Ab (1:200; BioGenex, San Ramon, California, USA). Primary Ab was visualized by an immunoperoxidase method using the TrueBlue peroxidase substrate according to the manufacturer’s instructions.

**Statistical analysis.** Comparisons of *Eln*+/+ and *Eln*–/– body weight, heart weight/body weight ratios, heart rate, systolic, diastolic, and mean systemic blood pressure, total protein, desmosine and hydroxyproline contents (as well as their ratios) in all three vessel types, and vessel diameters (OD and ID) as a function of vasoactive agent treatment were assessed using one- or two-way ANOVA, followed when necessary by Fisher’s least significant difference (LSD) test or Student’s *t* test for protein dosages for paired value comparisons.

Vessel diameter as a function of transmural pressure level (0–175 mmHg), pressure variation status (increasing or decreasing), KCN treatment (before or after), and genotype (*Eln*+/+ or *Eln*–/–) were compared using a four-way ANOVA followed when necessary by LSD tests for paired value comparisons. Vessel midwall strain, stress, incremental elasticity modulus, distensibility, and local midwall strain comparisons were assessed using the nonparametric Mann-Whitney *U* test.

Unless otherwise indicated, the results are presented as mean values plus or minus SEM, and *P* values equal to 0.05 were chosen as the threshold for statistically significant differences.

**Results**

**Animal weight, blood pressure, heart rate, and heart weight measurements.** Comparison of adult *Eln*+/+ and *Eln*–/– mice revealed a similar average body weight (approximately 32 g), with no statistically significant difference (*P* > 0.73) (Table 1). Systolic, diastolic, and mean blood pressures, however, were substantially higher at all ages in *Eln*–/– compared with *Eln*+/+ animals (Table 1). At 6 months of age, mean arterial pressure (MAP) was 96 ± 5 mmHg in *Eln*+/+ mice and 123 ± 7 mmHg in *Eln*–/– mice. This pressure difference was also evident at 7 weeks of age, with MAPs of 92 mmHg and 136 mmHg in the *Eln*+/+ and *Eln*–/– genotypes, respectively. At 15–18 months of age,
MAP remained elevated in the Eln\textsuperscript{+/–} genotype (144 ± 4 mmHg) compared with wild type (93 ± 2 mmHg). The pressure difference between the two phenotypes at 6 months of age was confirmed using a tail cuff sphygmomanometer (mean = 121 mmHg for Eln\textsuperscript{+/+}, 86 mmHg for Eln\textsuperscript{+/–}) as well as by femoral arterial catheterization (mean = 110 mmHg for Eln\textsuperscript{+/+}, 84 mmHg for Eln\textsuperscript{+/–}). The tail cuff results were from conscious mice and confirm that elevated blood pressure is not a consequence of anesthesia.

No statistically significant difference in heart rate could be detected between the two genotypes (both were in the range of 365 beats per minute in 5- to 7-month-old mice measured under anesthesia). Total heart weight to body weight ratios, as well as LV plus septum to body weight ratios, were higher (15% and 13%, respectively) in Eln\textsuperscript{+/–} than in Eln\textsuperscript{+/+} animals (Table 1).

LV volume and cardiac output. End-diastolic LV volume indexed to body weight (LVVdI) was increased in Eln\textsuperscript{+/–} mice, while end-systolic LV volume (LVVsI) was not significantly different between the Eln\textsuperscript{+/–} and control animals. Consequently, stroke volume was significantly increased and, even in the presence of slightly decreased heart rate, cardiac output indexed to body weight was significantly increased in Eln\textsuperscript{+/–} mice (Table 1).

Elastin and collagen changes in the wall of large arteries of Eln\textsuperscript{+/–} mice. We noted previously that elastic lamellae were thinner in Eln\textsuperscript{+/–} mice and that elastin mRNA levels were decreased in the arterial wall (15). To determine how these differences translate to changes in total elastin content, elastin concentration in arterial segments was determined by quantifying desmosine (a crosslinking amino acid unique to elastin) following protein hydrol-

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Eln\textsuperscript{+/+}</th>
<th>Eln\textsuperscript{+/–}</th>
<th>Difference (Eln\textsuperscript{+/–} versus Eln\textsuperscript{+/+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>32.0 ± 0.8</td>
<td>31.6 ± 1.0</td>
<td>ND</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>121 ± 6</td>
<td>165 ± 6</td>
<td>+36% \textsuperscript{A}</td>
</tr>
<tr>
<td>Diastolic</td>
<td>84 ± 5</td>
<td>102 ± 7</td>
<td>+21% \textsuperscript{A}</td>
</tr>
<tr>
<td>Mean</td>
<td>96 ± 5</td>
<td>123 ± 7</td>
<td>+28% \textsuperscript{A}</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>351 ± 27</td>
<td>379 ± 31</td>
<td>ND</td>
</tr>
<tr>
<td>Total heart weight/BW</td>
<td>0.43 ± 0.01</td>
<td>0.50 ± 0.02</td>
<td>+15% \textsuperscript{A}</td>
</tr>
<tr>
<td>(LV + septum) weight/BW</td>
<td>0.35 ± 0.01</td>
<td>0.40 ± 0.02</td>
<td>+13% \textsuperscript{A}</td>
</tr>
<tr>
<td>LVV and stroke volumes (µl/g) and cardiac output (ml/min/g) indexed to BW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVV diastolic</td>
<td>0.80 ± 0.16</td>
<td>1.07 ± 0.11</td>
<td>+34% \textsuperscript{A}</td>
</tr>
<tr>
<td>LVV systolic</td>
<td>0.23 ± 0.06</td>
<td>0.23 ± 0.04</td>
<td>ND</td>
</tr>
<tr>
<td>Stroke volume</td>
<td>0.57 ± 0.10</td>
<td>0.83 ± 0.08</td>
<td>+46% \textsuperscript{A}</td>
</tr>
<tr>
<td>Cardiac output</td>
<td>0.37 ± 0.05</td>
<td>0.47 ± 0.02</td>
<td>+27% \textsuperscript{A}</td>
</tr>
<tr>
<td>Mean wall thickness (µm) at physiological pressure of each genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascending aorta</td>
<td>88.9</td>
<td>73.1</td>
<td>–18% \textsuperscript{A}</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>76.7</td>
<td>57.5</td>
<td>–25% \textsuperscript{A}</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>52.5</td>
<td>40.5</td>
<td>–24% \textsuperscript{A}</td>
</tr>
<tr>
<td>Renal artery</td>
<td>29.9</td>
<td>30.5</td>
<td>ND</td>
</tr>
</tbody>
</table>

\textsuperscript{A}Significant difference between genotypes (P ≤ 0.05). ND, no significant difference. BW, body weight.

When protein levels are expressed as protein content per millimeter of vessel, total protein values were found to be significantly higher in the ascending and abdominal aortas of Eln\textsuperscript{+/+} mice than in similar segments from the Eln\textsuperscript{+/–} genotype. There is no difference in total protein content in the carotid artery from either animal group (Table 2). Within each genotype, the total protein, desmosine, and hydroxyproline content differed between vessel types (ascending aorta > abdominal aorta > carotid artery), which reflects known differences in wall mass and elastic properties of the vessel segments. Desmosine levels per millimeter of vessel were lower by 34–49% in Eln\textsuperscript{+/+} compared with Eln\textsuperscript{+/–} animals in the three vessels studied. Hydroxyproline levels per millimeter of vessel, indicative of collagen content, were found to be significantly lower in the ascending aorta (–24%) of Eln\textsuperscript{+/–} animals than in Eln\textsuperscript{+/+} mice, whereas no difference was detected between the other two vessels (Table 2).

To better understand the functional significance of differences in collagen and elastin levels, both measurements were normalized to the total protein content of the vessel wall to provide an indication of the local matrix composition. In all three vessel types, the level of elastin as a percentage of total protein (desmosine/total protein) was lower by approximately 35% in Eln\textsuperscript{+/–} compared with Eln\textsuperscript{+/+} animals. Interestingly, there was no statistically significant difference in the ratio of collagen to total protein (hydroxyproline/total protein) when the three vessel types from the two animal groups were compared, suggesting that relative collagen synthesis and accumulation was unchanged. Expression of elastin as a ratio to collagen levels (desmosine/hydroxyproline) confirms that there is more collagen relative to elastin in Eln\textsuperscript{+/+} vessels. As expected, the desmosine/hydroxyproline ratio is higher in the more

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Eln\textsuperscript{+/+}</th>
<th>Eln\textsuperscript{+/–}</th>
<th>Difference (Eln\textsuperscript{+/–} versus Eln\textsuperscript{+/+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (µg/mm ± SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascending aorta</td>
<td>67.6 ± 4.2</td>
<td>51.5 ± 2.9</td>
<td>–24% \textsuperscript{A}</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>22.3 ± 1.0</td>
<td>19.3 ± 0.6</td>
<td>–13% \textsuperscript{A}</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>13.0 ± 0.3</td>
<td>13.2 ± 0.9</td>
<td>ND</td>
</tr>
<tr>
<td>Desmosine (µg/mm ± SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascending aorta</td>
<td>0.19 ± 0.01</td>
<td>0.10 ± 0.003</td>
<td>–47% \textsuperscript{A}</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>0.05 ± 0.01</td>
<td>0.03 ± 0.001</td>
<td>–40% \textsuperscript{A}</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>0.03 ± 0.002</td>
<td>0.02 ± 0.002</td>
<td>–33% \textsuperscript{A}</td>
</tr>
<tr>
<td>Hydroxyproline (µg/mm ± SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascending aorta</td>
<td>0.99 ± 0.05</td>
<td>0.75 ± 0.06</td>
<td>–24% \textsuperscript{A}</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>0.53 ± 0.04</td>
<td>0.53 ± 0.05</td>
<td>ND</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>0.35 ± 0.02</td>
<td>0.32 ± 0.02</td>
<td>ND</td>
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</table>

\textsuperscript{A}Significant difference between genotypes calculated using two-way ANOVA (P ≤ 0.05). n = 6 animals for each measurement.
Mechanical properties of arteries. The mechanical properties of the ascending aorta, abdominal aorta, carotid artery, and renal artery of Eln+/– and Eln+/+ mice were studied by correlating changes in vessel ID and OD with alterations in transmural pressure. To determine the relative cellular and noncellular contributions to compliance of the elastic arteries, vessels from Eln+/– and Eln+/+ mice were studied before and after poisoning cells with KCN. In the three large arteries studied (ascending and abdominal aorta and carotid artery), vascular diameter changed by only a few percent following KCN treatment (data not shown). This is consistent with the findings of others showing that muscle contributes little to the mechanical properties of large arteries (22, 23). The same minimal effect of KCN on the arterial diameter is observed in the smaller renal artery.

The ODs of Eln+/– arteries were significantly smaller than Eln+/+ arteries at any given pressure over the entire range of pressure studied (Figure 1, a–d), except in the ascending aorta where no statistically significant difference could be detected at 75 and 100 mmHg (Figure 1a). In all four vessel types, the Eln+/– and Eln+/+ ODs showed the greatest divergence at high transmural pressure (125–175 mmHg) (Figure 1, a–d). Similar to ODs, the IDs of vessels in Eln+/– animals were smaller than those in Eln+/+ mice and showed the greatest divergence at high and low transmural pressure (Figure 1, e–h) (16). Wall thickness for the Eln+/– aorta and carotid artery was 20–25% less than Eln+/+ vessels at the physiological MAP of each genotype, whereas no difference between genotypes was detected in the renal artery (Table 1).

Calculations of distensibility (D25) indicate that large arteries in Eln+/– mice undergo greater dilation at transmural pressures below 100 mmHg than do arteries from Eln+/+ mice. The opposite occurs at high transmural pressures (above 100 mmHg), where Eln+/+ animals show higher distensibility than arteries from Eln+/– mice (Figure 2, a–c). Wall stress, in contrast, does not differ between Eln+/– and Eln+/+ vessels at any given pressure over the tested pressure range (0–175 mmHg). At the physiological MAP of 100 mmHg in Eln+/– and 125 mmHg in Eln+/+ animals, however, wall stress is found to be significantly higher in Eln+/– than in Eln+/+ arteries in all three vessel types.

The arterial midwall strain was lower in Eln+/+ compared with Eln+/– mice in the ascending and abdominal aorta between 25–50 and 125 mmHg. Interestingly, no difference in midwall strain could be detected at any pressure in the carotid artery in a comparison of the two animal groups. In all three vessel types and in both genotypes, a similar maximum midwall strain close to 1 was measured at 175 mmHg, corresponding to a doubling of the diameter seen at ambient pressure. When comparisons were made at physiological MAP for the two animal groups, however, the arterial midwall strain in Eln+/– mice was lower than the midwall strain in Eln+/+ vessels. This is true for all three types of vessels. At similar midwall strain, the arterial wall stress is generally lower in vessels from Eln+/– mice than in the Eln+/+ arteries, with the possible exception of the carotid artery where the differences are small. When the data are analyzed in terms of incremental elastic modulus indicative of the vessel wall stiffness, there are no differences between animal groups in the pressure range of 0–125 mmHg. At higher pressures where collagen...
formed several types of pharmacological experiments with anesthetized mice. Both wild-type and Eln+/– mice displayed a similar increase in blood pressure in response to a maximal pressor dose of Ang II (Figure 4a). Blocking ganglionic transmission with hexamethonium, an inhibitor of nicotinic channels, had little effect on MAP of wild-type or Eln+/– mice (data not shown), suggesting that increased vascular tone is not resulting from neurotransmitter signaling. We found, however, that infusion of the Ang I receptor antagonists candesartan and saralasin into the Eln+/– mice caused a dramatic decrease in blood pressure (Figure 4b), reaching MAP levels slightly below the baseline of wild-type animals but still higher than the MAP of wild-type animals treated with candesartan. In wild-type mice, saralasin had a slight and transient agonist effect but had no lasting influence on MAP.

Measurement of plasma renin activity found a greater than twofold increase in Eln+/– mice compared with controls (22.5 ± 16.9 ng/ml/h for Eln+/+ compared with 55.9 ± 16 ng/ml/h of Eln+/– animals). Interestingly, aldosterone levels were equivalent for both genotypes.
Figure 4
Alteration in MAP after intravenous injection of Ang II or Ang I receptor antagonists. (a) Maximal change in MAP in response to a bolus dose (1 µg/kg i.v.; \( n = 8 \) for \( Eln^{+/–} \), \( n = 3 \) for \( Eln^{+/–} \)) of Ang II. (b) Blood pressure change in response to infusion of saralasin (10 µg/kg; \( n = 2 \) for each phenotype) and candesartan (100 µg/kg; \( n = 3 \) for \( Eln^{+/–} \), \( n = 5 \) for \( Eln^{+/–} \)). Error bars show SEM. *Significant difference (\( P \leq 0.05 \), t test) between treated and control vessels within each genotype.

(272.4 ± 86 pg/ml for \( Eln^{+/–} \), \( n = 7 \), and 307.0 ± 140 pg/ml for \( Eln^{+/–} \), \( n = 6 \)).

No PCNA-positive cells were detected in the media of either \( Eln^{+/–} \) or \( Eln^{+/–} \)-6-month-old mice (data not shown). PCNA-positive cells, however, could be readily identified in vascular tissue of newborn mice, which served as a positive control for PCNA staining (data not shown).

Discussion
The high blood pressure associated with the \( Eln^{+/–} \) genotype is consistent with systemic hypertension that is a frequent complication of SVAS and WS, diseases of elastin haploinsufficiency in humans (24–26). MAP is 30–40% higher in \( Eln^{+/–} \) mice than control animals, a trait that shows complete penetrance in the \( Eln^{+/–} \) genotype. In humans, the incidence of hypertension is less than the complete penetrance we observe in mice. In WS, for example, approximately 80% of affected individuals have clinically apparent SVAS (27) and 40–60% have documented arterial hypertension (24–26, 28). For reasons not yet understood, the incidence of hypertension in isolated SVAS is much lower. Of the two forms of SVAS, the hemizygosity of the elastin gene in WS is genetically most like the \( Eln^{+/–} \) mouse.

The association of hypertension with elastin haploinsufficiency strongly suggests that vessel wall proteins, particularly elastin, should be considered as causal genes for essential hypertension. Any factor that reduces elastin protein concentration or alters vessel compliance during a critical window of vessel wall formation could have a modifying effect on the progression of, or susceptibility to, hypertension or other vascular diseases. This could include mutations within any of the other genes that participate in elastic fiber assembly or perhaps polymorphisms within the elastin gene itself. Secondary factors could also impact elastin deposition and vascular function. There is evidence, for example, that people who had low birth weight tend to have higher blood pressure in later life. The authors of these studies argue that fetuses whose growth is impaired synthesize less elastin in the aorta and large arteries and that this deficiency leads to changes that could predispose an individual to higher blood pressure (29, 30). There is also evidence that maternal and postnatal vitamin D ingestion lowers aortic elastin content (vitamin D is known to downregulate elastin production in cultured cells) and alters vascular compliance similar to that which is seen in our mice (31). Taken together, these studies suggest that environmental or nutritional factors could impact directly vascular development or might act as modifiers on phenotypes involving elastin gene mutations or polymorphisms.

In a previous study, we noted thinner elastic lamellae and decreased elastin mRNA levels in the arterial wall of \( Eln^{+/–} \) mice (15). Quantitation of desmosine levels as an index of elastin protein confirmed what was predicted by these earlier findings, namely, that elastin protein is significantly reduced in the \( Eln^{+/–} \) animals. When normalized to total protein, our findings show that elastin levels are approximately 35% lower in the ascending, abdominal, and carotid arteries from \( Eln^{+/–} \) mice. The ratio of collagen to total protein was identical in both genotypes, indicating that relative collagen synthesis and accumulation is unchanged.

The decreased elastin to collagen ratio suggests that arteries in the \( Eln^{+/–} \) mouse should be stiffer than their wild-type counterparts. This was confirmed through mechanical studies that documented a difference in vessel distensibility between the two genotypes (Figures 1 and 2). In our initial studies, we reported that the aorta from \( Eln^{+/–} \) and \( Eln^{+/–} \) mice had similar extensibilities at a presumed physiologic pressure of 100 mmHg (15). The dramatic difference in blood pressure during a critical window of vessel wall formation could have a modifying effect on the progression of, or susceptibility to, hypertension or other vascular diseases. This could include mutations within any of the other genes that participate in elastic fiber assembly or perhaps polymorphisms within the elastin gene itself. Secondary factors could also impact elastin deposition and vascular function. There is evidence, for example, that people who had low birth weight tend to have higher blood pressure in later life. The authors of these studies argue that fetuses whose growth is impaired synthesize less elastin in the aorta and large arteries and that this deficiency leads to changes that could predispose an individual to higher blood pressure (29, 30). There is also evidence that maternal and postnatal vitamin D ingestion lowers aortic elastin content (vitamin D is known to downregulate elastin production in cultured cells) and alters vascular compliance similar to that which is seen in our mice (31). Taken together, these studies suggest that environmental or nutritional factors could impact directly vascular development or might act as modifiers on phenotypes involving elastin gene mutations or polymorphisms.

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between the two genotypes, however, alters our interpretation of the earlier physiological findings. At their higher physiological pressure (and above), Eln+/– vessels are stiffer and have a higher circumferential wall stress, circumferential wall strain, and incremental elasticity modulus than vessels from Eln+/+ animals. At lower pressures, however, Eln+/– vessels are more elastic and show greater dilation than do arteries from wild-type mice. Similar changes in vessel compliance have been reported in humans with WS. Using noninvasive ultrasound, Salaymeh and Banerjee found that children with WS have a stiffer aorta and a less-compliant systemic arterial bed (28). Interestingly, a similar study found that the compliance of the carotid artery is not modified in WS, even though increased intima-media thickness and lower arterial stiffness were consistent features (32). Despite the structural alterations in the Eln+/– vessel wall, no significant change in the functional potential of the vascular cells was detected. Vessels from Eln+/– mice responded appropriately and to the same extent as Eln+/+ vessels to both vasodilators and vasoconstrictors.

A major difference between the human pathology associated with SVAS and that seen in Eln+/– mice is that humans, but not mice, develop severe localized aortic occlusion due to subendothelial SMC proliferation. A possible explanation for this difference may relate to the higher vascular wall stress in humans compared with mice, due to their larger size. Higher circumferential wall stress could make vessels more prone to pressure-related damage, leading to stenosis. It is important to note that at their higher physiological pressures, Eln+/– vessels are working close to their maximum strain, suggesting that these animals may be more prone to develop hypertensive cardiovascular pathologies when stressed, since their vessels have a lower potential for distension if the blood pressure increases.

The smaller ID of the large elastic arteries coupled with increased arterial stiffness and elevated cardiac output is predicted to be disadvantageous to cardiac function. Under normal circumstances, this should lead to cardiac hypertrophy, circulatory dysfunction, and possibly death. When we analyzed the hearts of 6-month-old Eln+/– mice, we observed that the total heart weight as well as the LV weight was increased by 15% and 13%, respectively, over wild-type controls. It is interesting that aldosterone levels persisted at each end point. The relatively constant tension per lamellar unit suggests that the proper number of lamellar units are organized (43, 44). The smaller ID of the large elastic arteries coupled with increased arterial stiffness and elevated cardiac output is predicted to be disadvantageous to cardiac function. Under normal circumstances, this should lead to cardiac hypertrophy, circulatory dysfunction, and possibly death. When we analyzed the hearts of 6-month-old Eln+/– mice, we observed that the total heart weight as well as the LV weight was increased by 15% and 13%, respectively, over wild-type controls. It is interesting that aldosterone levels persisted at each end point. The relatively constant tension per lamellar unit suggests that the proper number of lamellar units are organized (43, 44). The smaller ID of the large elastic arteries coupled with increased arterial stiffness and elevated cardiac output is predicted to be disadvantageous to cardiac function. Under normal circumstances, this should lead to cardiac hypertrophy, circulatory dysfunction, and possibly death. When we analyzed the hearts of 6-month-old Eln+/– mice, we observed that the total heart weight as well as the LV weight was increased by 15% and 13%, respectively, over wild-type controls. It is interesting that aldosterone levels persisted at each end point. The relatively constant tension per lamellar unit suggests that the proper number of lamellar units are organized (43, 44). The smaller ID of the large elastic arteries coupled with increased arterial stiffness and elevated cardiac output is predicted to be disadvantageous to cardiac function. Under normal circumstances, this should lead to cardiac hypertrophy, circulatory dysfunction, and possibly death. When we analyzed the hearts of 6-month-old Eln+/– mice, we observed that the total heart weight as well as the LV weight was increased by 15% and 13%, respectively, over wild-type controls. It is interesting that aldosterone levels persisted at each end point. The relatively constant tension per lamellar unit suggests that the proper number of lamellar units are organized (43, 44).
and their uniformity of composition, regardless of species, indicate that the proportion of collagen, elastin, and SMCs in the media is optimal for the stresses to which the aorta is subjected (1). This is why the increased number of elastic lamellae in the arterial wall of Eln+/– mice is unique.

Many studies in mature organisms have shown that the response of fully developed blood vessels to hemodynamic stress is clearly different from what we have documented in Eln+/– mice. In spontaneous or essential hypertension in humans and in experimental hypertension in animals (33, 34), vessel walls become thickened through cellular maturation and increased matrix deposition, but there is no change in lamellar number (38, 39, 42, 45). The reason that fetal and mature vascular wall cells respond differently to hemodynamic stress may reflect the effects of the extensive matrix found in older vessels. Because there is more elastin in the mature vessel wall, the ECM plays a greater role in accommodating wall stress than in earlier developmental stages. Hence, the most efficient adaptive mechanism for a mature vessel to use to deal with changes in pressure is that of altering the amount of the load-bearing ECM. In elastin insufficiency, however, SMCs cannot make sufficient elastin, and the increased number of smooth muscle layers (i.e., lamellar units) may be an attempt by the cells to normalize wall stress. We know that the increase in lamellar number is established at the time of birth (15) and that elevated blood pressure can be documented in neonatal Eln+/– mice as early as pressure measurements can be obtained, suggesting that alterations in Eln+/– vessel wall structure and hemodynamics occur early in formation of the arterial wall. The changes found in the Eln+/– arterial wall suggest that presumptive vascular SMCs are capable of altering vessel wall structure by sensing and responding to wall stress and that mechanical forces play an important role in determining lamellar number. Elucidation of cellular mechanisms for sensing mechanical signals will have important implications for understanding vascular development generally as well as furthering our understanding of vascular pathology in elastin-related human genetic diseases such as SVAS, WS, and hypertension in general.

Acknowledgments
We thank Douglas Taylor (University of Utah), Gail Maher, Carla J. Weinheimer, and Michael Courtouis (Washington University) for technical assistance. This work was funded by postdoctoral fellowship grants from the Fondation pour la Recherche Médicale (France) and the American Heart Association—Missouri affiliate to G. Faury; by grants from the NIH to R.P. Mecham and D.P. Kelly (HL-61001), and to W.A. Boyle (GM-55849); and from the Association Française contre les Myopathies (France) and the European Union (Fifth framework programme—research project “Towards the maintenance of tissue elasticity for healthy aging” [TELASTAR], contract no. QLK6-CT-2001-00332) to G. Faury. Funds were also provided by a grant from the National Marfan Foundation to R.P. Mecham.

### Supplemental Table 1

Ratios of elastin (desmosine) and collagen (hydroxyproline) to total protein concentrations in arterial segments from \( Eln^{+/+} \) and \( Eln^{+-/-} \) mice.

<table>
<thead>
<tr>
<th>Genotype:</th>
<th>( Eln^{+/+} )</th>
<th>( Eln^{+-/-} )</th>
<th>Difference (( Eln^{+-/-} ) versus ( Eln^{+/+} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desmosine/total protein (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascending aorta</td>
<td>0.28</td>
<td>0.19</td>
<td>-35(^A)</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>0.22</td>
<td>0.16</td>
<td>-37(^A)</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>0.23</td>
<td>0.15</td>
<td>-35(^A)</td>
</tr>
<tr>
<td>Hydroxyproline/total protein (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascending aorta</td>
<td>1.46</td>
<td>1.46</td>
<td>ND</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>2.38</td>
<td>2.75</td>
<td>ND</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>2.69</td>
<td>2.42</td>
<td>ND</td>
</tr>
<tr>
<td>Desmosine/hydroxyproline (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascending aorta</td>
<td>19.74</td>
<td>13.44</td>
<td>-32(^A)</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>10.63</td>
<td>5.70</td>
<td>-46(^A)</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>9.11</td>
<td>6.48</td>
<td>-29(^A)</td>
</tr>
</tbody>
</table>

\(^A\)Significant difference between genotypes calculated using two-way ANOVA, \( P \leq 0.05 \). ND, no significant difference. \( n = 6 \) animals for each measurement.
Decreased elastin in vessel walls puts the pressure on

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Mice haploinsufficient for elastin develop structural changes in vessel walls similar to those seen in patients with mutations in the elastin gene. A new study (see the related article beginning on page 1419) demonstrates that due to mechanical changes in the vessel wall, these animals exhibit increased mean arterial pressures. The results evoke the possibility that alterations in elastin may contribute to the development of essential hypertension in patients.

Investigators have understood that the arrangement of the ECM is critical in the formation of organ structures during development and in the remodeling of tissues after injury. However, the ECM is perceived to play a passive role in dynamic pathological processes such as heart failure and hypertension. This misconception largely results from difficulties in interpreting the pathology seen in human diseased tissue without an understanding of the full phenotypic course of the pathologic process over time. The use of transgenic animal studies now allows us to introduce gain of function and loss of function mutations so as to evaluate the direct role of ECM molecules in disease pathogenesis. There are multiple examples of dominant mutations in structural ECM molecules that lead to abnormal tissue formation and disease phenotypes (1). In this issue of the JCI, Faury and colleagues present an interesting example of a dominant mutation in an ECM protein that results in developmental structural changes that ultimately cause the animals to acquire hypertension in their adult life (2).

**Elastin and vessel formation**

Elastin constitutes 50% of the dry weight of the aorta. During vessel development, elastin synthesized by smooth muscle cells forms elastic fibers that are arranged into concentric rings of elastic lamellae around the arterial lumen. Each elastic lamella alternates with a ring of smooth muscle, forming a lamellar unit. The elastic lamellae allow an artery to comply with the increased hemodynamic stress of cardiac systole and maintain sufficient blood pressure during diastole (Figure 1) (3).

Variations in the lamellar subunits (defined as the elastic lamellae and adjacent smooth muscle cells) determine the distribution and magnitude of the tensile strength of the vessel. Hence, the greatest number of lamellar units is found in the larger, more proximal vessels that experience higher wall stress, indicating a linear relationship between the number of lamellar units and tensional force within the vessel wall (4, 5).

**Elastin mutations in disease**

Three clinical conditions have been linked to a mutation or deletion of the elastin gene, *ELN*. Autosomal dominant cutis laxa, a primarily cutaneous condition, is the result of frameshift mutations in *ELN* that influence elastic fiber structure through a dominant-negative effect (6). Several years ago, investigators identified mutations in *ELN* in patients with supravalvular aortic stenosis (SVAS). SVAS is an autosomal dominant disorder caused by intragenic deletions or by a large spectrum of mutations within the elastin gene (7, 8). These result in functional haploinsufficiency through either nonsense-mediated decay of mRNA from the mutant allele or the production of a nonfunctional protein (9, 10). Narrowing of the ascending aorta is a dominant feature of SVAS, but other arteries, including pulmonary arteries, are often also affected. If not corrected, SVAS may lead to cardiac hypertrophy and heart failure (11). Finally, Williams syndrome, a neurodevelopmental disorder that has SVAS as a component, develops as a consequence of submicroscopic deletions within chromosomal subunit 7q11.23 involving the whole of the *ELN* gene (12).

**Alterations in elastin content change arterial wall structure**

To directly investigate the consequence of elastin mutations on vessel formation, a mouse with a complete loss of function in the *Ehn* gene was generated (13). The elastin-null mice died of obstructive arterial disease due to subendothelial cell proliferation and reorganization of smooth muscle. These changes occurred in isolated organ cultures of arteries and were not subject to hemodynamic stress (13).

The characterization of mice haploinsufficient for elastin (*Ehn–/–*) revealed a role for elastin in the formation of vessel wall structure (14). The arteries of *Ehn–/–* mice exhibited thinner elastic lamellae and an increased number of smooth muscle cell layers. Most interestingly, these identical changes have been observed in the arteries of patients with SVAS (14).

In the present report, Faury et al. (2) meticulously examined the mechanical...
properties of the arteries of the Eln+/– mice compared with normal Eln+/+ mice, correlating changes in inner and outer vessel diameter with alterations in transmural pressure. The Eln+/– animals were stably hypertensive with mild cardiac hypertrophy and did not exhibit the hypertension-induced arterial wall hypertrophy and decreased distensibility of large elastic arteries associated with essential hypertension (2). The mean arterial pressure (MAP) of Eln+/– mice could be reduced with angiotensin II inhibitors, and renin levels were two-fold greater than in Eln+/+ mice, suggesting that the renin-angiotensin system plays a role in maintaining the high blood pressure of the Eln+/– mice (2).

**Overview and future questions**

Vessels of patients with essential hypertension exhibit decreased arterial compliance and increased vascular resistance with an increase in vascular tone (15). Hypertensive patients maintain the decreased compliance at the same pressures as normotensive patients, implying that functional and/or structural changes other than pressure-mediated stretching of arteries contribute toward reducing arterial compliance (16, 17). The discovery of an alteration in vessel compliance in the Eln+/– mice along with increased MAP suggests that vessel elastin in patients with hypertension should be examined. Mutations in the elastin gene could ultimately be a cause of hypertension. The mechanism by which a change in elastin content leads to alterations in cell signaling and subsequent structural changes in the vessel wall remains to be determined. Matrix molecules bind to integrin receptors, and any alteration in the structural components of the matrix could consequentially alter signaling through the integrin receptors. Additionally, it will be interesting to determine whether elastin loss leads to structural changes in other organs of the Eln+/– mice in response to increased mechanical stress. For example, the lungs from the Eln+/– mice would presum-
ably have diminished elastin content. Since the rodent lung continues to develop until two months after birth, one wonders whether \textit{Eln} \textsuperscript{-/-} lungs undergo structural reorganization when exposed to postnatal transmural pressure and what the consequences of such reorganization might be on pulmonary function.


The origin of \textit{FOXP3}-expressing CD4\textsuperscript{+} regulatory T cells: thymus or periphery

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Naturally arising CD4\textsuperscript{+} regulatory T cells, which engage in the maintenance of immunologic self-tolerance, specifically express \textit{FOXP3}, which encodes a transcription-repressor protein. Genetic defects in \textit{FOXP3} cause IPEX, an X-linked autoimmune/inflammatory syndrome. With \textit{FOXP3} as a specific marker for regulatory CD4\textsuperscript{+} T cells in humans, it is now possible to determine their origin and developmental pathway (see the related article beginning on page 1437).


The immune system discriminates between self and non-self, maintaining immunologic self-tolerance (i.e., unresponsiveness to self-constituents). It is known that potentially hazardous self-reactive T and B cells are clonally delet ed at immature stages of their development or inactivated upon encounter with self-antigens in the periphery. There is now accumulating evidence that, in addition to these passive mechanisms of self-tolerance, a population of CD4\textsuperscript{+} T cells, called regulatory T cells (T\textsubscript{R} cells), engage in the maintenance of peripheral self-tolerance by actively suppressing the activation and expansion of self-reactive T cells (1–3). The majority, if not all, of such naturally occurring CD4\textsuperscript{+} T\textsubscript{R} cells constitutively express CD25 (IL-2 receptor \alpha chain) in the physiologic state. Indeed, removal of CD25\textsuperscript{+} CD4\textsuperscript{+} T cells, which constitute 5–10% of CD4\textsuperscript{+} T cells in rodents and humans, leads to spontaneous development of various autoimmune diseases in otherwise normal mice (4). The removal of CD25\textsuperscript{+} CD4\textsuperscript{+} T\textsubscript{R} cells also triggers excessive or misdirected immune responses to microbial antigens, causing immunopathology, such as inflammatory bowel disease (IBD), due to hyper-reaction of the remaining T cells to commensal bacteria in the intestine (3).

\textbf{FOXP3: master control gene for the development and function of natural CD4\textsuperscript{+} T\textsubscript{R} cells}

There is now evidence not only for the presence of CD25\textsuperscript{+} CD4\textsuperscript{+} T\textsubscript{R} cells in humans but also for their essential roles in controlling autoimmunity, immunopathology, and allergy in human diseases (5). This is best illustrated by IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome), a rare monogenic disease of male children that is accompanied by autoimmune disease (such as type 1 diabetes), IBD, and severe allergy similar to those produced in mice by depletion of CD25\textsuperscript{+} CD4\textsuperscript{+} T\textsubscript{R} cells (6). The causative gene, \textit{FOXp3} (\textit{Foxp3} in mice), which encodes a transcription repressor (7–10), is specifically expressed in CD25\textsuperscript{+} CD4\textsuperscript{+} T\textsubscript{R} cells in the thymus and periphery (11–13). Forced expression of the \textit{Foxp3} gene can convert murine naive T cells to T\textsubscript{R} cells that phenotypically and functionally resemble naturally arising CD25\textsuperscript{+} CD4\textsuperscript{+} T\textsubscript{R} cells.