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Correspondence and requests for materials should be addressed to V.M.B. (monica.bricelj@nrc-cnrc.gc.ca). The complete sequence of the Na^+ channel pore region has been submitted to the GenBank database under accession no. AY847740.

Ipr1 gene mediates innate immunity to tuberculosis

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An estimated eight million people are infected each year with the pathogen *Mycobacterium tuberculosis*, and more than two million die annually[1]. Yet only about 10% of those infected develop tuberculosis. Genetic variation within host populations is known to be significant in humans and animals[2,3], but the nature of genetic control of host resistance to tuberculosis remains poorly understood. Previously we mapped a new genetic locus on mouse chromosome 1, designated *sst1* (for super-susceptibility to tuberculosis 1)[4]. Here we show that this locus mediates innate immunity in *sst1* congenic mouse strains and identify a candidate gene, *Intracellular pathogen resistance 1* (*Ipr1*), within the *sst1* locus. The *Ipr1* gene is upregulated in the *sst1* resistant macrophages after activation and infection, but it is not expressed in the *sst1* susceptible macrophages. Expression of the *Ipr1* transgene in the *sst1* susceptible macrophages limits the multiplication not only of *M. tuberculosis* but also of *Listeria monocytogenes* and switches a cell death pathway of the infected macrophages from necrosis to apoptosis. Our data indicate that the *Ipr1* gene product might have a previously undocumented function in integrating signals generated by intracellular pathogens with mechanisms controlling innate immunity, cell death and pathogenesis.

It is estimated that about one-third of the human population on the planet has been infected by virulent *M. tuberculosis*[1,5]. Susceptibility to clinical tuberculosis is known to be influenced by environmental factors such as stress, malnutrition, concomitant infections (for example HIV) or senescence[6,7]. Although genetic variation within host populations is also known to affect resistance and susceptibility, individual genes responsible for innate immunity to the pathogen have been elusive. In susceptible individuals, progression of lung tuberculosis often leads to the formation of characteristic necrotic 'cavities' that destroy significant portions of the lung. Beyond their life-threatening clinical consequences, these lesions are essential for the efficient transmission of *M. tuberculosis* in aerosols. Because tuberculosis in humans is transmitted primarily by the respiratory route, the ability to cause lung disease is considered a key aspect of the pathogen's virulence strategy and ensures its evolutionary success. Therefore, understanding pathogenic mechanisms that are employed by virulent *M. tuberculosis* during lung tuberculosis in susceptible individuals is essential for developing effective prevention and treatment strategies[8,9]. However, detailed mechanistic studies of pathogenesis of lung tuberculosis and its genetic control have been limited by the fact that in mouse models of *M. tuberculosis* infection, necrotic lesions in the lungs are rarely found unless the mouse is rendered systemically immunodeficient.

C3HeB/FeJ inbred mice are extremely susceptible to virulent *M. tuberculosis* and develop a marked lung pathology, which leads to their rapid death after infection[10,11]. We generated a congenic mouse strain C3H.B6-*sst1* (*sst1*^{R}) carrying the C57BL/6J-derived resistant allele at the *sst1* locus on the C3HeB/FeJ genetic background. The survival time of the *sst1*^{R} congenic mice infected either with a high dose of intravenous *M. tuberculosis* (Fig. 1a) or with a low dose of *M. tuberculosis* by the respiratory route (Fig. 1b), relative to their *sst1*^{S} counterparts, is significantly lengthened, indicating a profound effect of the locus on anti-tuberculosis immunity. However, the shorter survival of the C3H.B6-*sst1* (*sst1*^{R}) mice, in comparison with the resistant parental strain C57BL/6J (B6), indicates that the *sst1* locus is responsible for a significant portion, but not all, of the tuberculosis resistance phenotype of the B6 mice.

The specific effect of the *sst1* locus on the progression of tuberculosis was related to a more efficient control of *M. tuberculosis* multiplication, primarily in the lungs, after both respiratory challenge by aerosol (Fig. 1c) and systemic intravenous infection (Supplementary Fig. 2a). The development of large necrotic lung lesions within 4 weeks after intravenous infection, characteristic of *sst1*^{S} mice, was prevented in the presence of the *sst1*^{R} allele (Fig. 1d). After a low-dose aerosol infection, chronic tuberculosis infection ensued, and the *sst1*^{S} mice developed encapsulated necrotic lung lesions, in some cases reaching about one-third of the lung lobe (Fig. 1e), that resembled tuberculosis cavities in human lungs. Mycobacteria were present both extracellularly, within necrotic central areas surrounded by the fibrotic capsule, and within macrophages of the granuloma wall (Supplementary Fig. 1). In the *sst1*^{R} mice, lung lesions were much smaller and contained fewer infected macrophages.

Although the greatest effect of the *sst1* polymorphism on the progression of tuberculosis was observed in the lungs, bone marrow transplantation experiments showed that bone marrow-derived cells, but not lung cells, were responsible for the effect of the *sst1* locus (Supplementary Fig. 2b). It is known that T lymphocytes and macrophages are of major importance in host resistance to tuberculosis. We have found that, whereas T lymphocytes are functionally

unaffected by the *sst1* polymorphism, the *sst1* disparate macrophages show considerable differences in their ability to control *M. tuberculosis in vitro* (M.R., J. Gutierrez-Pabello, H.P., O. Jobe, B.-S.Y., L. Helming, L.K. and I.K., manuscript in preparation). The rate of *M. tuberculosis* multiplication was significantly higher in the $sst1^S$ macrophages (Fig. 1g, left panel). There was also a clear distinction in the mechanism of macrophage cell death after the infection: the $sst1^S$ macrophages showed characteristic necrosis, whereas the $sst1^R$ macrophages underwent apoptosis (Fig. 1f, upper left and upper right panels, respectively). The effect of the *sst1* locus was much more pronounced after infection of macrophages with virulent *M. tuberculosis*, because an avirulent vaccine strain of *M. bovis* bacillus Calmette–Guérin (BCG) failed to multiply in the $sst1^S$ macrophages (Fig. 1g, right panel) and to induce necrosis of them (Fig. 1f, lower left panel).

In vivo, many cells within the tuberculosis lung granulomas of the $sst1^R$ mice contained apoptotic nuclei that were positive under TdT-mediated dUTP nick end labelling (TUNEL assay) and no necrosis was observed, whereas the apoptotic nuclei were largely absent from the necrotic lesions of the $sst1^S$ mice (see Supplementary Information). Thus our studies, both *in vivo* and *in vitro*, indicated that the extreme susceptibility to virulent *M. tuberculosis* of $sst1^S$ mice is associated with necrotic death of the susceptible macrophages. Virulent *M. tuberculosis* was shown to cause necrosis of the infected epithelial cell lines[12]. This necrosis-inducing propensity is specific for virulent *M. tuberculosis* and is lost in RD1 mutants of the microbe, which also have a markedly decreased virulence[12,13]. From our studies it seems that, in addition to virulence determinants of the pathogen itself, mechanisms of host cell death depend on the host polymorphic gene(s) encoded within the *sst1* locus.

To identify the critical gene(s), we employed a positional cloning strategy (see Supplementary Information and Supplementary Fig. 3 for details). First, the *sst1* minimal region was reduced to an interval between D1Mit439 and D1Mit49 markers on mouse chromosome 1 (Supplementary Fig. 3a). This region contains a so-called HSR (for homogeneously stained region) repeat (Fig. 2a). The HSR repeat region is arguably the largest repetitive region in the mouse genome[14,15]. Its size in inbred mouse strains is estimated to be between 3.5 million and 6 million bases (refs 15, 16) and it remains unfinished by both mouse genome projects. After identifying and testing progeny of additional recombinants within this interval (Fig. 2a), we concluded that the *sst1* candidate region encompasses part of the HSR repeat region and a region of mouse chromosome 1

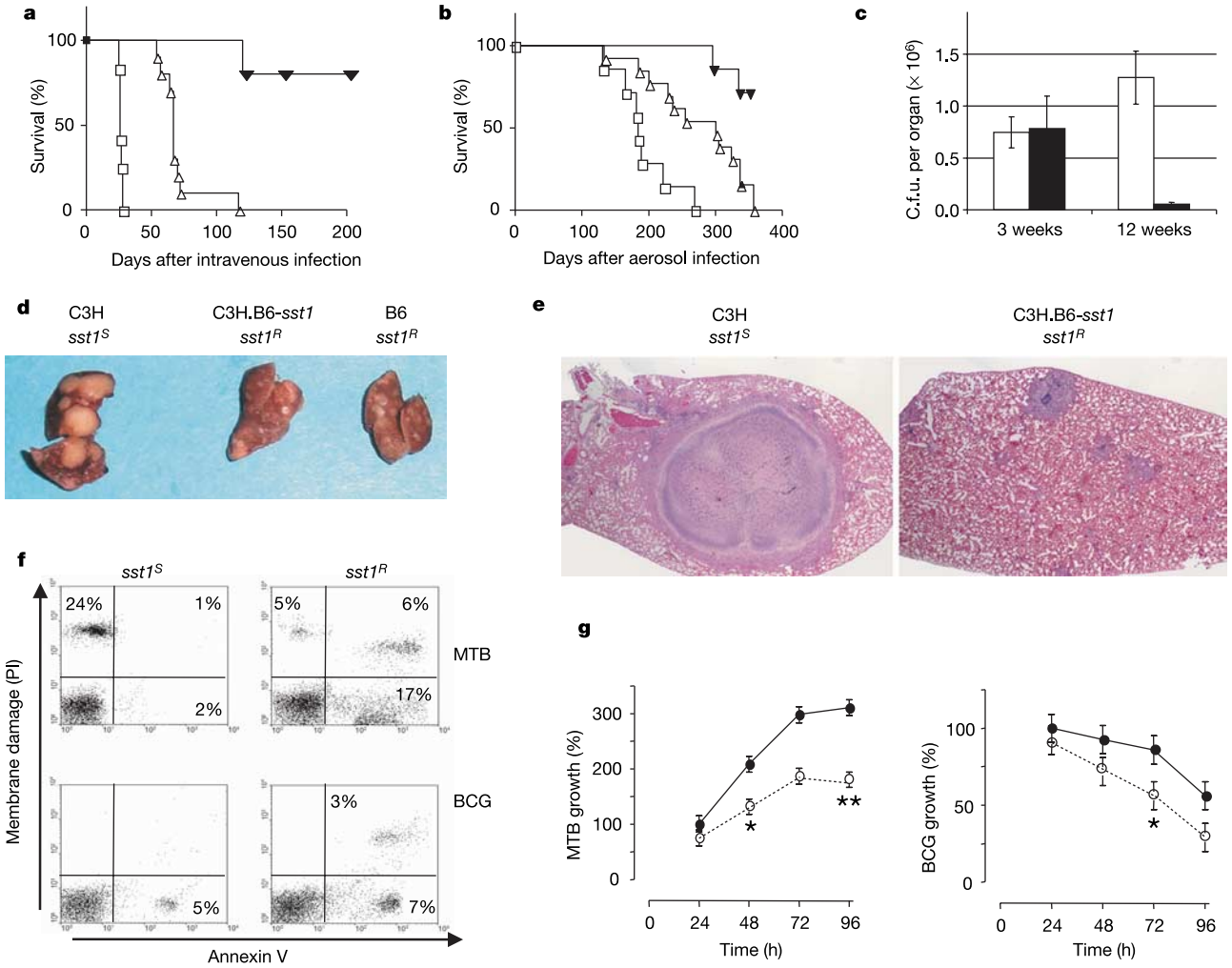

Figure 1 The *sst1* locus mediates innate immunity to tuberculosis. **a**, **b**, Survival of C3H mice (squares), B6 mice (filled triangles) and C3H.B6-*sst1* ($sst1^R$) mice (open triangles) after intravenous (**a**) or aerosol (**b**) infection with *M. tuberculosis*. **c**, *M. tuberculosis* bacterial loads in the lungs of the *sst1* congenic mice after aerosol infection. Open bars, $sst1^S$; filled bars, $sst1^R$. **d**, **e**, Tuberculosis lung lesions 25 days after intravenous infection (**d**) and 12 weeks after aerosol infection (**e**). Stain in **e**, haematoxylin/eosin; original magnification ×40. **f**, FACS analysis of the mechanism of cell death of the *sst1* congenic macrophages infected with *M. tuberculosis* (MTB; top panels) or BCG (bottom panels) *in vitro*. **g**, Multiplication of *M. tuberculosis* (left panel) or *M. bovis* BCG (right panel) in the *sst1* congenic macrophages *in vitro* (asterisk, $P < 0.01$; two asterisks, $P < 0.001$). Open circles, $sst1^S$; filled circles, $sst1^R$. Error bars show 95% confidence intervals.

immediately downstream of the repeat; that is, between the repeat region and the *NppC* gene. A total of 22 known and predicted genes are encoded within the *sst1* critical region according to the Ensembl and Celera databases of the mouse genome (Supplementary Table 1). It was impossible to reduce the *sst1* critical region further by genetic recombination. In the next step we therefore tested the expression of each of the *sst1*-encoded candidate genes in the lungs during tuberculosis infection *in vivo* and in macrophages infected with *M. tuberculosis in vitro* using reverse transcriptase-mediated polymerase chain reaction (RT–PCR) and rapid amplification of complementary DNA ends (RACE). From our studies the *Ifi75* gene seemed the most likely candidate (Supplementary Fig. 3b, e).

As shown in Fig. 2b, the 5′ RACE products of *Ifi75* in the lungs of tuberculosis-infected mice were strikingly different between the *sst1* congenic strains: a major single band was amplified from the lungs of the *sst1* resistant mice but was absent from the lungs of the *sst1* susceptible strain; instead, multiple weak products were obtained from the latter. Although some aberrant transcripts were present in the lung tissue of the $sst1^R$ animals as well, most of the *Ifi75*-related transcripts in their tuberculosis lung lesions were represented by a single isoform, which we named *Ipr1* to differentiate it from other *Ifi75*-related sequences (*Ifi75-rs*) identified by RACE and perhaps also encoded within the HSR repeat. The predicted Ipr1 protein is 92% identical to Ifi75 in *Mus caroli*. It contains an Sp100-like domain in its amino terminus, an LXXLL-type nuclear receptor binding motif (NRB), a bipartite nuclear localization signal (NLS) and a SAND domain (named after Sp100, AIRE-1, NucP41/75 and DEAF-1) at its carboxy terminus (Fig. 2c).

Using DNA probes specific for the Sp100 and SAND domains of the *Ipr1*, we analysed the kinetics of its expression by northern hybridization in the lungs of the *sst1* congenic mouse strains during the progression of tuberculosis (Fig. 2d). Expression of *Ipr1* was detectable in the lungs of the naive $sst1^R$ mice, and its expression increased significantly 2 weeks after intravenous infection with *M. tuberculosis* and remained higher at later time points. However, expression of the Sp100- and SAND domain-containing *Ifi75-rs* in the lungs of the *sst1* susceptible C3HeB/FeJ mice remained below the level of detection by northern blot hybridization. Instead, the level of transcripts of another gene encoded within the HSR repeat

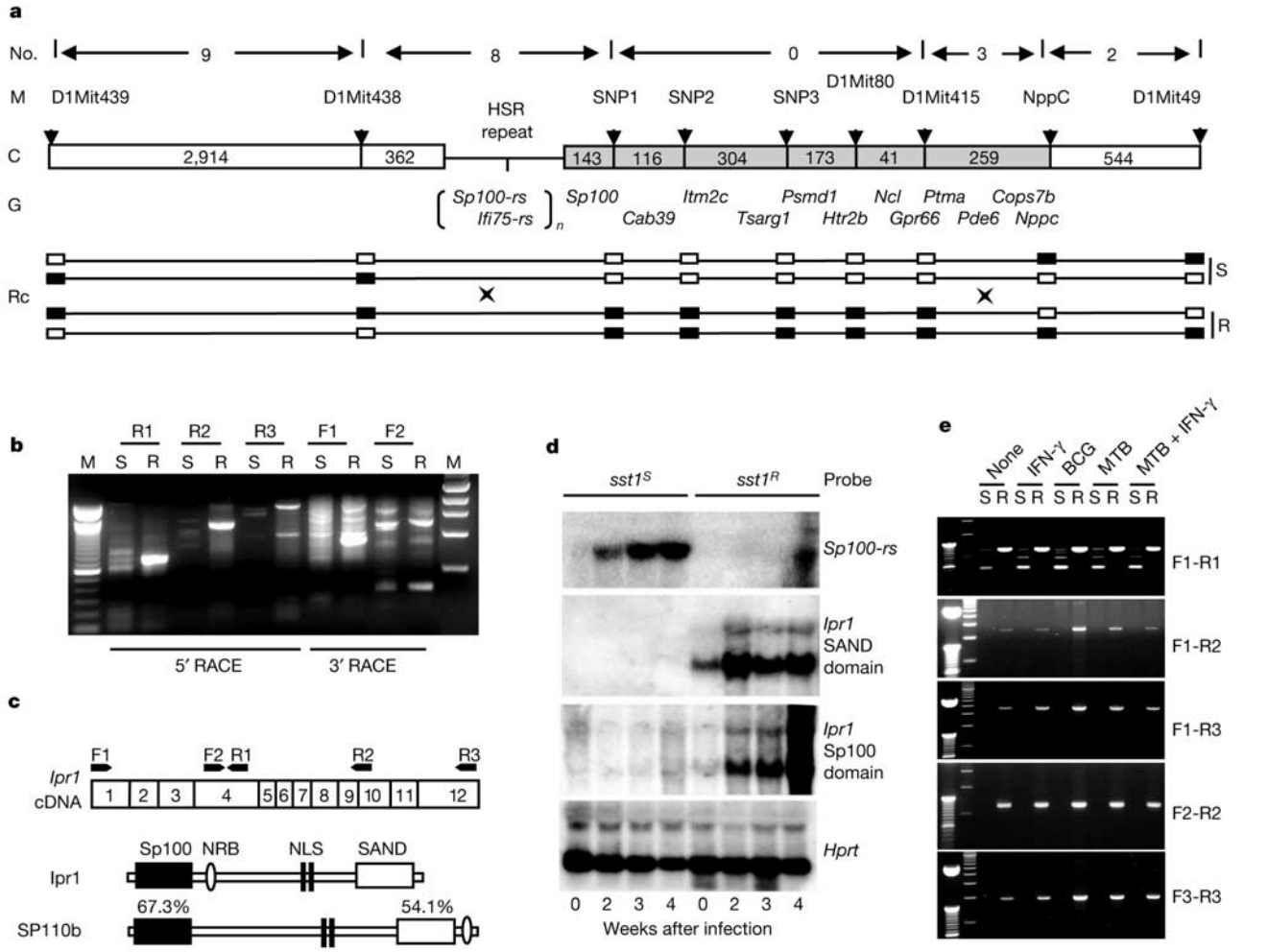

Figure 2 Identification of the *sst1* candidate gene. **a**, Physical map of the *sst1* minimal region. The top line shows the number of recombination events. M, polymorphic markers; C, chromosome with distances between the markers in kilobases; G, known genes; RC, recombinant chromosomes containing the *sst1* resistant (R) or susceptible (S) alleles. Genotypes for each marker are represented by filled (B6) and open (C3H) boxes. **b**, Analysis of the *Ifi75-rs* expression in the tuberculosis lung lesions of the *sst1* congenic mice by RACE. **c**, Domain structure of Ipr1 and its human homologue SP110b, and location of the PCR primers. **d**, Expression of *Ipr1* and *Sp100-rs* in the lungs during *M. tuberculosis* infection (northern blot). **e**, Expression of *Ipr1* in $sst1^S$ (S) or $sst1^R$ (R) macrophages infected with *M. tuberculosis* (MTB) or BCG or activated with interferon-γ (IFN-γ) *in vitro*.

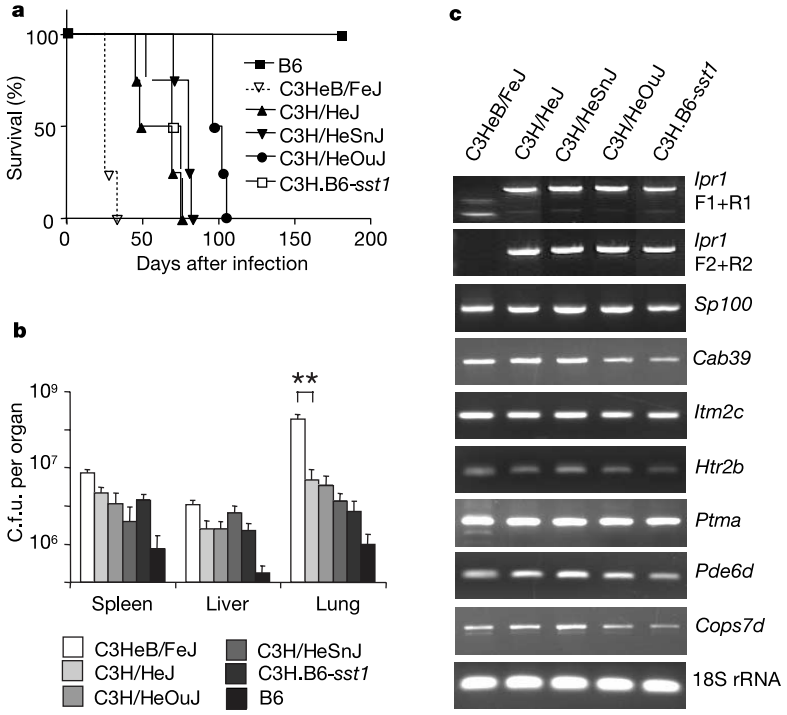

Figure 3 Lack of *Ipr1* expression in the C3HeB/FeJ substrain correlates with its extreme susceptibility to *M. tuberculosis* infection. **a**, Survival after intravenous infection with *M. tuberculosis*. **b**, *M. tuberculosis* bacterial loads 3 weeks after infection (four mice per strain; two asterisks, $P < 0.001$; error bars show standard deviations). **c**, Analysis by RT–PCR of *sst1*-encoded candidate gene expression in tuberculosis lung lesions 3 weeks after infection.

region, *Sp100-rs*, was elevated in the lungs of the *sst1*^{S} mice (Fig. 2d).

To investigate the expression of *Ipr1* in macrophages, we used five overlapping combinations of the *Ipr1*-specific PCR primers covering the full-length *Ipr1* transcript (Fig. 2e). *Ipr1* was expressed in non-activated *sst1*^{R} macrophages, and the level of its expression increased after infection with both avirulent BCG and virulent *M. tuberculosis*. No expression of the full-length transcript of *Ipr1* was seen in the *sst1*^{S} macrophages under any stimulation conditions. Macrophages isolated from the tuberculosis lung lesions of the *sst1*^{R} mice also expressed the full-length *Ipr1* transcript, whereas those from the *sst1*^{S} mice did not (data not shown). We were unable to detect additional *Ipr1*-related transcripts induced in macrophages after infection *in vivo* and *in vitro* or after stimulation with interferon-γ. Even though *Ipr1* is encoded within the HSR repeat region, our data indicate that a single major isoform of this gene might be expressed in *sst1*^{R} macrophages either before or during tuberculosis infection and that this isoform is not expressed in the *sst1*^{S} C3HeB/FeJ mice.

The C3HeB/FeJ substrain is unique among all other substrains of C3H mice in terms of its extreme susceptibility to tuberculosis[10,11]. The C3HeB/FeJ mice die abruptly within 3.5–4 weeks of infection, displaying severe lung pathology. In our experiments, the survival time of other substrains of C3H was considerably longer and was similar to that of the *sst1*^{R} congenic strain C3H.B6-*sst1* (Fig. 3a). The bacterial loads in the lungs of the C3HeB/FeJ mice at 3 weeks after infection were 50–100-fold higher than in other substrains of C3H and the *sst1*^{R} congenics (Fig. 3b), possibly indicating a unique allele at the *sst1* locus. We compared the expression of the *sst1*-encoded candidate genes in the lungs of mice of four C3H substrains and the *sst1*^{R} congenics and found that the lack of expression of *Ipr1* differentiated C3HeB/FeJ from all other substrains of C3H (Fig. 3c). Because all the C3H substrains originated from a common ancestor[17], it is likely that they are genetically identical within the *sst1* region and a unique mutation *de novo* has led to the defect of *Ipr1* expression in C3HeB/FeJ mice and is responsible for a severe defect in their resistance to tuberculosis.

We generated transgenic mice that expressed a full-length copy of the *Ipr1* cDNA on the susceptible C3HeB/FeJ background in a macrophage-specific manner under the control of the human scavenger receptor A promoter (SR-A). Mature bone marrow-derived macrophages (BMDM), as well as resident peritoneal macrophages obtained from those mice, expressed the *Ipr1* transgene (Fig. 4a). Despite the fact that the regulation of *Ipr1* expression in the transgenic macrophages was clearly less efficient from the SR-A promoter than from the endogenous *Ipr1* promoter (Fig. 4a), when the *Ipr1* transgenic mice were infected with virulent *M. tuberculosis* we observed a statistically significant difference in the bacterial loads between the *sst1*^{S} ($Tg^{-/-}$) and the *Ipr1* transgenic ($Tg^{+/-}$) animals in the lungs (Fig. 4b). *In vitro*, the *Ipr1* transgenic macrophages also controlled the multiplication of *M. tuberculosis* more effectively (Fig. 4c) and turned on the apoptotic pathway of cell death after interaction with virulent *M. tuberculosis* (Fig. 4d, right panels). The growth of another intracellular pathogen, *L. monocytogenes*, was markedly suppressed (50–100-fold) in the *Ipr1* transgenic macrophages (Fig. 4e). As in the *M. tuberculosis* infection, necrotic death accompanied infection of the *sst1*^{S} macrophages with virulent *L. monocytogenes* (Fig. 4f, left panel), whereas the *Ipr1* transgenic macrophages showed markers of apoptotic death (Fig. 4f, right panel).

Thus the expression of a single gene, *Ipr1*, in the *sst1*^{S} macrophages restored key functions related to the pathogenesis of tuberculosis that are encoded within the *sst1* locus: greater control of multiplication of virulent *M. tuberculosis in vivo* and *in vitro* as well as an apoptotic mechanism of *M. tuberculosis*-induced macrophage cell death. Moreover, *Ipr1* mediates macrophage resistance to another intracellular pathogen, *L. monocytogenes*, indicating that the *Ipr1* product might control a common mechanism of innate resistance against several intracellular pathogens.

The closest homologue of the predicted Ipr1 protein in humans (41% identity) is SP110b (ref. 18), which localizes to a region of

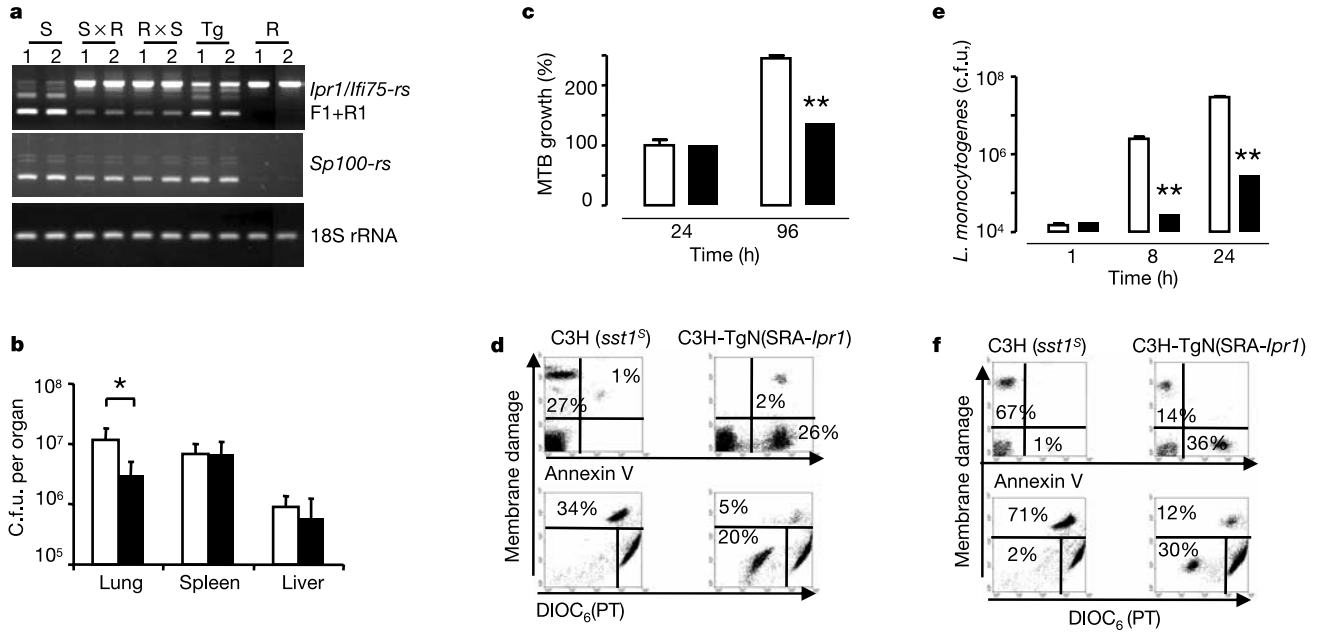

Figure 4 Expression of the *Ipr1* transgene in the *sst1* ^{S} macrophages confers resistance to intracellular pathogens *M. tuberculosis* and *L. monocytogenes*. **a**, RT–PCR of *Ipr1* and *Sp100-rs* in macrophages isolated from *sst1* ^{S} (S) and *sst1* ^{R}(R) mice, their F_1 hybrids (S×R, R×S) and *Ipr1* transgenic (Tg) mice: 1, stimulated with interferon-γ; 2, infected with *M. tuberculosis*. **b**, Bacterial loads of *M. tuberculosis* in *Ipr1* transgenic ($Tg^{+/-}$; filled columns) and control ($Tg^{-/-}$; open columns) mice after infection with *M. tuberculosis* (seven mice per strain; asterisk, $P < 0.05$). **c**, **e**, Growth of *M. tuberculosis* (MTB) (**c**) and *L. monocytogenes* (**e**) in *Ipr1* transgenic (filled columns) and control (*sst1* ^{S}; open columns) macrophages. Three experiments were performed in triplicate. Asterisk, $P < 0.01$; two asterisks, $P < 0.001$; error bars show 95% confidence intervals. **d**, **f**, FACS analysis of cell death of the *Ipr1* transgenic (right panels) and control (*sst1* ^{S}; left panels) macrophages infected with *M. tuberculosis* (**d**) and *L. monocytogenes* (**f**). Apoptotic cells are Annexin V^{+} (upper panels) and $DiOC6^{low}$ (lower panels); PT, mitochondrial membrane permeability transition. Necrotic cells are identified by membrane damage (see Methods).

human chromosome 2 syntenic with the *sst1* minimal region on mouse chromosome 1. Both Ipr1 and the human SP110 proteins contain motifs that are involved in protein–protein interactions (Sp100 domain)[19,20], chromatin binding (SAND domain)[21,22], nuclear localization signal (NLS) and the NRB motif LXXLL. Recent evidence indicates that human SP110 protein might function as a transcriptional cofactor for nuclear hormone receptors[18] and might directly bind the retinoic acid receptor[23]. Signalling through nuclear receptors such as the corticosteroid receptor, retinoic acid receptor, peroxisome-proliferator-activated receptors and vitamin D receptor is important in the control of various aspects of the macrophage life cycle, including differentiation, activation, response to pathogens and apoptosis[24]. The expression of both *Ipr1* and its human homologue *SP110* is regulated by interferons[25], additionally indicating a possible role in immunity in both species. Moreover, polymorphisms in *SP110* have been associated with susceptibility to the hepatitis C virus[26], and the SP110b protein has been shown to interact physically with viral proteins such as Epstein–Barr virus SM protein and hepatitis C virus core protein[23,27]. It is possible that the Ipr1 and SP110 proteins mediate cross-talk between nuclear receptors, interferon signalling and pathogens. Viruses, and perhaps intracellular pathogens, might have evolved mechanisms to interfere with or exploit the Ipr1/SP110 function. Taken together, these data suggest that in mammals, because no *Ipr1* homologues were found in yeasts or insects, the Ipr1-related proteins might have a previously undescribed function in integrating signals generated by intracellular pathogens or viruses with mechanisms regulating the activation, gene expression and cell death of host cells[28]. Therefore, *SP110* might be a candidate gene for testing for association with susceptibility to tuberculosis in human populations. □

Methods

Animals

C57BL/6J, C3HeB/FeJ, C3H/HeJ, C3H/HeOuJ and C3H/HeSnJ inbred mice were obtained from the Jackson Laboratory. The congenic C3H.B6-*sst1* (*sst1* ^{R}), B6.C3H-*sst1* and transgenic C3H-TgN(SRA-*Ipr1*) mouse strains were generated in our laboratory. The C3H.B6-*sst1* congenic mice were obtained by introgression of a roughly 20-centimorgan (cM) segment of B6-derived chromosome 1 with a proximal recombination breakpoint between D1Mit215 (47 cM) and D1Mit334 (49 cM) and a distal limit between D1Mit187 (64 cM) and D1Mit200 (75 cM) on the C3HeB/FeJ genetic background using ten backcrosses. The congenic interval transferred from the B6 resistant background did not include *Slc11a1* (formerly known as *Nramp1*), which is located at 39.2 cM. The *sst1* resistant congenic mouse strain C3H.B6-*sst1* therefore carries the same allele of the *Nramp1* as the parental *sst1* susceptible C3HeB/FeJ mice. The B6.C3H-*sst1* mice were obtained by transferring the *sst1* susceptible allele on the B6 genetic background using ten backcrosses. The transgenic C3H-TgN(SRA-*Ipr1*) mice were established by expressing the C57BL/6J-derived *Ipr1* gene under the control of a macrophage-specific Scavenger Receptor A (SRA) promoter[29] on the C3HeB/FeJ genetic background (see Supplementary Methods for details).

Infection of mice with *M. tuberculosis*

For intravenous infection, 10^5 live *M. tuberculosis* were injected into the tail vein in 100 μl of PBS. Aerosol infections were performed with aerosol apparatus manufactured by the College of Engineering Shops at the University of Wisconsin, Madison. Mice were exposed to aerosol for 20 min, which resulted in the deposition of 15–30 colony-forming units (c.f.u.) per mouse. Mice were killed by halothane anaesthesia. Organs were homogenized in PBS containing 0.05% Tween 80, and serial tenfold dilutions were cultured on 7H10 agar enriched with 10% oleic acid/albumin/dextrose/catalase (OADC; Difco) for 3 weeks at 37 °C.

Isolation and infection of murine BMDM *in vitro*

BMDM were isolated from femurs and tibias of male C3H, C3H.B6-*sst1* and C3H-TgN(SRA-*Ipr1*) mice (6–8 weeks old) and were infected with *L. monocytogenes* strain 10403S as described previously[30]. Macrophage monolayers were infected at a multiplicity of infection of 1 *M. tuberculosis* Erdman per 10 macrophages (m.o.i. 1:10). After 6 h the cells were washed with PBS containing 1% FCS (PBS/1% FCS). The cells were incubated in complete medium containing 10% FCS; three coverslips were removed from the culture at indicated time points, and cells on each coverslip were lysed separately with 0.1% Triton X-100. Serial tenfold dilutions of cell lysates were plated on 7H10 agar containing OADC and incubated for 3 weeks at 37 °C.

Differentiation of apoptotic and necrotic pathways

Macrophages were infected at an m.o.i. of 1:10. At indicated time points, cells were stained with 10 nM 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$; Molecular Probes) and 0.8 mM ethidium bromide (EB; Sigma) for 20 min at 37 °C, washed three times with PBS, fixed for 20 min with 1% paraformaldehyde and washed once more with PBS. Cells were analysed with a BD FACScan flow cytometer (BD Biosciences) to differentiate between live ($DiOC_6^{high}$ EB^-), apoptotic ($DiOC_6^{low}$ EB^-) and necrotic (EB^+) cells. For Annexin V staining, cells were incubated in Annexin binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$) and stained for 20 min with 10 μl of Annexin V-Alexa 488 (Molecular Probes), then counterstained with propidium iodide (PI, 1 μg ml^{-1}), washed twice with cold PBS, fixed with 1% paraformaldehyde for 30 min and washed once with PBS. Fluorescence-activated cell sorting (FACS) analysis was used to differentiate between early apoptotic (Annexin V^+ PI^-), late apoptotic (Annexin V^+ PI^+) and necrotic (Annexin V^- PI^+) cells.

Isolation and analysis of the *Ipr1* cDNA

The *Ifi75*-specific oligonucleotide primers (F1-2 and R1-3) are shown in Supplementary Methods and in Fig. 2c. RACE-PCR was performed with the SMART RACE cDNA Amplification Kit (BD Clontech). The cDNAs were synthesized from the lungs of C3HeB/FeJ and C3H.B6-*sst1* mice infected with *M. tuberculosis*. The RACE amplification products were purified with PCR purification columns (Qiagen), cloned into the plasmid vector pGEM-T (Promega) and sequenced with T7 and SP6 primers. A full-length sequence of *Ipr1* was confirmed by sequencing the 'end-to-end' PCR product obtained with the F1 and R3 primers.

Statistical analysis

Statgraphics Plus, release 4, 1999 (Statgraphics Corp.) and GraphPad Prizm 3.0 (GraphPad) software were used for the analysis. Comparison of bacterial loads was performed with Student's *t*-test. Results are presented as means ± s.d. The threshold for statistical significance was $P < 0.05$. Kaplan–Meier survival curves were generated and compared by using the log-rank test (GraphPad Prizm). Intracellular bacterial growth and cell death were analysed by two-factor analysis of variance (time, genetic backgrounds and experiment). The statistical significance was tested with $P < 0.05$ as the critical value with the Student–Newman–Keuls post-test to compare means between both genetic backgrounds. Data are presented as means ± 95% confidence intervals for the mean.

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IRF-7 is the master regulator of type-I interferon-dependent immune responses

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The type-I interferon (IFN-α/β) response is critical to immunity against viruses and can be triggered in many cell types by cytosolic detection of viral infection, or in differentiated plasmacytoid dendritic cells by the Toll-like receptor 9 (TLR9) subfamily, which generates signals via the adaptor MyD88 to elicit robust IFN induction[1–4]. Using mice deficient in the *Irf7* gene (*Irf7*$^{-/-}$ mice), we show that the transcription factor IRF-7 is essential for the induction of IFN-α/β genes via the virus-activated, MyD88-independent pathway and the TLR-activated, MyD88-dependent pathway. Viral induction of MyD88-independent IFN-α/β genes is severely impaired in *Irf7*$^{-/-}$ fibroblasts. Consistently, *Irf7*$^{-/-}$ mice are more vulnerable than *Myd88*$^{-/-}$ mice to viral infection, and this correlates with a marked decrease in serum IFN levels, indicating the importance of the IRF-7-dependent induction of systemic IFN responses for innate antiviral immunity. Furthermore, robust induction of IFN production by activation of the TLR9 subfamily in plasmacytoid dendritic cells is entirely dependent on IRF-7, and this MyD88–