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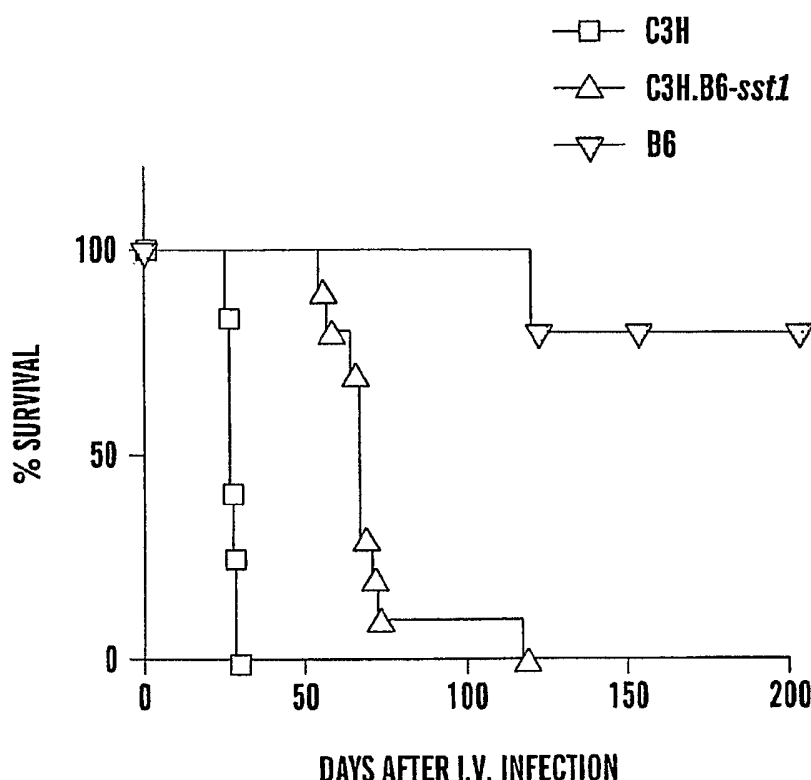
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(54) Title: METHODS FOR TREATING AND DIAGNOSING SUSCEPTIBILITY TO CYTOSOLIC PATHOGENS

(57) Abstract: The present invention is based on the identification of the *Ipr1* gene as a mediator of innate immunity to cytosolic pathogens (e.g. *Mycobacterium tuberculosis*, *Listeria monocytogene*, and viruses such as Hepatitis C virus and Epstein Barr virus). Accordingly, the invention is directed to methods for determining an individuals susceptibility to infection by these pathogens. Methods of prophylaxis and methods for treatment of infection are also provided.



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METHODS FOR TREATING AND DIAGNOSING SUSCEPTIBILITY TO CYTOSOLIC PATHOGENS

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit under 35 U.S.C. 119(e) of U.S. Provisional Patent Application Serial No. 60/657,348, filed March 1, 2005, the contents of which are herein incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

[002] Innate immunity is the human body's first line of defense against disease. Each individual responds differently to invading bacterial and viral pathogens, largely due to differences in their innate immune response. For example, it is estimated that 8 million people are infected each year with the pathogen, *Mycobacterium tuberculosis* (MTB), and over 2 million die annually¹. It is estimated that about 10% of the individuals infected actually develop tuberculosis and thus many individuals are carriers.

[003] Genetic variation within host populations is known to play a significant role in tuberculosis infection in humans and animals^{2,3}. However, the nature of genetic control of host resistance to tuberculosis remains poorly understood, and the individual genes responsible for innate immunity to the pathogen have been elusive. In susceptible individuals, progression of lung tuberculosis often leads to formation of characteristic necrotic 'cavities' that destroy significant portions of the lung. Beyond their life-threatening clinical consequences, these lesions are essential for efficient transmission of MTB via aerosol. In humans, tuberculosis is transmitted primarily via the respiratory route. Therefore, the ability to cause lung disease is considered a key aspect of the pathogen's virulence strategy. Accordingly, developing assays that can be used to look at pathogenic mechanisms that are employed by virulent pathogens, such as MTB during e.g., lung tuberculosis in susceptible individuals, is essential for developing effective prevention and treatment strategies^{8,9}.

[004] There is a need in the art to identify ways to modulate innate immunity to intracellular pathogens. It is desirable to develop treatments for a variety of infections and prophylactic therapies, particularly for those who lack adaptive immunity.

SUMMARY OF THE INVENTION

[005] The present invention is based on the identification of a gene and gene product that controls host resistance to cytosolic pathogens in mammals. We have named the murine gene *Ipr1* (intracellular pathogen resistance) because it mediates innate immunity to intracellular pathogens (e.g. *Mycobacterium tuberculosis* and *Listeria monocytogene*). The absence or downregulation of *Ipr1* gene expression leads to high susceptibility to infection. In addition, the absence or downregulation of a functional *Ipr1* gene expression leads to high susceptibility to infection. Further, expression of *Ipr1* protein induces apoptosis in infected cells, controls inflammation and thus controls the severity of infection. Accordingly, the present invention is directed to methods for determining a patient's susceptibility to infection by monitoring expression of the human homologue to *Ipr1*.

[006] The present invention is also directed to methods of prophylaxis, as well as methods for treatment of infection by inducing expression of, or administering, the *Ipr1* protein human homologue. Furthermore, it has been shown that *Ipr1* regulates TNF- α and IL-10 expression in macrophages. The presence of *Ipr1* results in an increased TNF- α production, which inhibits pathogen replication and induces cell apoptosis. The absence of *Ipr1* decreases TNF- α production and increases IL-10 production, leading to suppression of apoptosis and more rapid pathogen replication. Thus, methods of prophylaxis and treatment that combine inducing expression of or administering *Ipr1* protein with the upregulation or administration of TNF- α and the downregulation of IL-10 are contemplated.

[007] In one embodiment, a method for determining a patients susceptibility to infection by a cytosolic pathogen are provided. The methods comprise analyzing gene expression, e.g. gene expression levels, of the human homologue to *Ipr1* (SEQ ID NO:11) in a biological sample, such as, a peripheral blood sample (e.g. in monocytes and macrophages) or a tissue sample (e.g. lung, spleen, heart, liver etc). In one embodiment, a positive gene expression indicates that the individual has innate resistance to infection by cytosolic pathogens. In a preferred embodiment, a negative gene expression indicates that the patient is highly susceptible to infection. As used herein, a negative gene expression includes a lack or decrease in gene expression levels, the presence of a

mutation or polymorphism that results in the expression of a *Ipr1* protein that is non-functional (e.g. has impaired antiviral or antibacterial function), or the expression of a protein that has an altered half life. In another embodiment, the method further comprises an analysis of other genetic loci that predict susceptibility to infection by cytosolic pathogens. Other loci may be, for example, loci that predict altered acquired immunity pathways.

[008] Also encompassed in the present methods are an analysis of cytokine levels, such as, for example, TNF- α and IL-10 as predictors of innate immune function. A lowered level of TNF- α and/or an increased level of IL-10 is indicative of susceptibility to infection by cytosolic pathogens. Cytokine levels may be analyzed in conjunction with the levels of *Ipr1* gene expression for the determination of one's susceptibility to infection with a cytosolic pathogen, such as, for example, MBT.

[009] A preferred human homologue of the *Ipr1* protein is SP110b¹⁸, which is expressed from a gene localized to human chromosome 2. Accordingly, a method for determining a patient's susceptibility to infection by a cytosolic pathogen is provided that comprises obtaining a sample from a patient and analyzing gene expression of *SP110b* (SEQ ID NO:1). A positive gene expression indicates that the patient has innate resistance to infection by cytosolic pathogens, while negative gene expression indicates that the individual is highly susceptible to infection.

[0010] As used herein, the term "cytosolic pathogen" refers to any pathogen that enters the cytoplasm upon infection. These include pathogens that directly infect the cell without entering an endosomal pathway, as well as pathogens that initially enter the endosomal pathway but escape from endosomes during the course of infection.

[0011] In one embodiment, the cytosolic pathogen is a bacterial pathogen such as, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Listeria monocytogenes*, or *Brucella abortus*. In another embodiment, the cytosolic pathogen is a viral pathogen, such as Hepatitis C Virus, Epstein Barr virus, or a retrovirus. In one embodiment, the cytosolic pathogen excludes Epstein Barr virus.

[0012] Gene expression (e.g. gene expression levels) can be measured by measuring levels of mRNA (e.g. quantitative RT-PCR and gene expression array analysis). Alternatively, the gene expression levels can be measured by measuring levels of protein. As used herein, an analysis of gene expression may also include an analysis

of protein degradation. Gene expression levels are determined by comparing to levels in a wild type control group. The wild type control group (e.g. sample) should preferably consist of at least 5 individuals, more preferably, at least 20, still more preferably, at least 50 individuals. More preferably, control sets should look at additional criteria such as sex and age to reduce false positives or negatives.

[0013] In one embodiment, one can screen for mutations in a *Ipr1* human homologue gene that affect gene expression and/or protein product function by DNA sequence analysis. One can also detect mutations in a gene product by immunohistochemical methods. For example, one can use an antibody to the carboxy end of the protein to determine if a mutation results in no product or a truncated product. In another embodiment, the mutation in an *Ipr1* human homologue gene results in a non-functional or lowered function protein that while expressed at levels similar to a wild type control, functions as if it has decreased expression levels. The mutation or polymorphism may reduce the function so much so as to effectively make the the gene non-functional. Thus, in one embodiment, methods for the detection of mutations and/or polymorphisms that result in either non-functional or lowered function *Ipr1* protein are contemplated.

[0014] Mutations and/or polymorphisms in the *Ipr1* gene may also result in translational modifications that affect protein processing and degradation. As such, methods to screen for mutations and/or polymorphisms that result in modified *Ipr1* proteins are also encompassed. The presence of mutations and/or polymorphisms that result in such modified *Ipr1* protein indicate susceptibility to infection with cytosolic pathogens.

[0015] Methods for treating a patient infected with a cytosolic pathogen are also provided. The method comprises administering to said patient an effective amount of a compound or agent that upregulates expression of a human homologue of *Ipr1* (e.g. *SP110b* (SEQ ID NO:1)).

[0016] Any compound or agent that upregulates expression of a human homologue of *Ipr1* can be used in methods of the invention. In one preferred embodiment, the agent that upregulates gene expression is interferon γ , interferon α , or interferon β . Furthermore, upregulation of a human homologue of *Ipr1* can be combined with upregulation of TNF- α or downregulation of IL-10. Thus, the present invention

further comprises co-administering a compound(s) or agent(s) that upregulates TNF- α or downregulates IL-10.

[0017] The methods of the invention are useful to treat both patients that innately lack expression of the *Ipr1* human homologue (e.g. *SP110b*) as well as patients that lose expression as the result of having been chronically infected with a pathogen. For example, in tuberculosis, the expression of the *Ipr1* gene is initially upregulated upon infection with the pathogen, however, during chronic infection expression of the *Ipr1* gene is downregulated. Also encompassed in the present invention are mutations and/or polymorphisms that result in lowered or lack of expression of the *Ipr1* human homologue.

[0018] Accordingly, compounds or agents that prevent downregulation of *Ipr1* gene expression are contemplated. In addition, compounds that block inhibitors of the human homologue to Ipr1 protein, compounds that interfere with post-translational modifications of the human homologue to Ipr1 protein, and compounds that inhibit degradation of the human homologue to Ipr1 protein are also contemplated for use in treatment of infection by cytosolic pathogens. Assays to determine such compounds constitute another embodiment of the invention.

[0019] In one embodiment, a method for treating a patient infected with a cytosolic pathogen that comprises administering to said patient an effective amount of Ipr1 protein human homologue, or a vector containing the corresponding gene, is provided.

[0020] In another embodiment, a method for preventing infection of an individual by a cytosolic pathogen is provided. The method comprises administering to said patient an effective amount of a human protein homologue to Ipr1 or vector containing said gene encoding such product. Prophylactic treatment is particularly useful for treating individuals at risk of infection.

[0021] Preferably the protein to be administered is a fusion protein, designed such that entry into the cell and/or entry into the nucleus is facilitated. Fragments of the human homologue to Ipr1 may be administered in lieu of the full length protein. For example, fragments of the human homologue to Ipr1 that confer antibacterial and/or antiviral activity may be administered. Furthermore, a portion of the human homologue of *Ipr1* that is responsible for TNF- α production may be administered to the patient.

[0022] The proteins of the invention can be administered by administration of a DNA that encodes human protein homologue to *Ipr1* (e.g. contained in a viral expression vector or other vector). Also encompassed is the use of fragments of the DNA that encodes human protein homologue to *Ipr1*. Such fragments should confer antimicrobial and/or antiviral activity.

[0023] The methods of the invention are particularly useful for treating immunocompromised individuals.

BRIEF DESCRIPTION OF FIGURES

[0024] Figures 1A to 1G show that the *sst1* locus mediates innate immunity to tuberculosis. Figure 1A and Figure 1B show the survival of C3H, B6, and the C3H.B6-*sst1* (*sst1*^R) mice after i.v. (Figure 1A) or aerosol (Figure 1B) infection with MTB; Figure 1C shows MTB bacterial loads in the lungs of the *sst1* congenic mice after the aerosol infection; Figures 1D and 1E show tuberculosis lung lesions 25 days after i.v. infection (Figure 1D) and 12 weeks after aerosol infection, H&E, 40X original magnification (Figure 1E); Figure 1F shows FACS analysis of mechanism of cell death of the *sst1* congenic macrophages infected with MTB (top panels) or BCG (bottom panels) *in vitro*; Figure 1G shows multiplication of MTB (left panel) or *M. bovis* BCG (right panel) in the *sst1* congenic macrophages *in vitro* (*p<0.01, **p<0.001). Error bars represent 95% confidence intervals.

[0025] Figures 2A to 2E show the identification of the *sst1* candidate gene. Figure 2A, shows the physical map of the *sst1* minimal region. (#)- number of recombination events, (M) – polymorphic markers, (C) – chromosome with distances between the markers (kb); (G) – known genes; (RC) - recombinant chromosomes containing the *sst1* resistant (R) or susceptible (S) alleles, genotypes for each marker are represented by solid (B6) and opened (C3H) boxes; Figure 2B shows analysis of the *Ifi75-rs* expression in the tuberculosis lung lesions of the *sst1* congenic mice by RACE. Figure 2C shows the domain structure of the *Ipr1* and its human homolog SP110b and location of the PCR primers. Figure 2D shows *Ipr1* and *Sp100-rs* gene expression in the lungs during MTB infection (Northern blot); Figure 2E shows *Ipr1* gene expression in *sst1*^S (S) or *sst1*^R (R) macrophages infected MTB, BCG or activated with IFN- γ *in vitro*.

[0026] Figures 3A to 3C show that the lack of the *Ipr1* gene expression in the C3HeB/FeJ substrain correlates with its extreme susceptibility to MTB infection. Figure 3A shows the survival after the intravenous infection with MTB; Figure 3B shows MTB bacterial loads three weeks after the infection (4 mice per strain, ** $p < 0.001$, error bars represent standard deviation); Figure 3C shows the analysis of the *sst1*-encoded candidate gene expression in the tuberculosis lung lesions by RT-PCR three weeks after the infection.

[0027] Figures 4A to 4F show that the expression of the *Ipr1* transgene in the *sst1^S* macrophages confers resistance to intracellular pathogens MTB and *L. monocytogenes*. Figure 4A shows RT-PCR of *Ipr1* and *Sp100-rs* in macrophages isolated from the *sst1^S* (S), *sst1^R* (R) mice, their F1 hybrids (SxR, RxS) and the *Ipr1* transgenic (Tg) mice, 1 - IFN γ -stimulated, 2 - MTB-infected. Figure 4B shows MTB bacterial loads in the *Ipr1* transgenic (Tg+/-) and control (Tg-/-) mice after infection with MTB (7 mice/strain, * $p < 0.05$); Figures 4C and 4E show the growth of MTB (Figure 4C) and *L. monocytogenes* (Figure 4E) in the *Ipr1* transgenic and control (*sst1^S*) macrophages (three experiments were performed in triplicates, * $p < 0.01$, ** $p < 0.001$, error bars represent 95% confidence interval).

[0028] Figures 5A to 5B show MTB growth in the organs of the *sst1* congenic mouse strains and bone marrow chimeras after systemic i.v. infection. Figure 5A shows MTB bacterial loads in the organs from the C3H (*sst1^S*) and the C3H.B6-*sst1* (*sst1^R*) congenic mice at 2 and 3 weeks after i.v. infection with 10^5 CFU of MTB; Figure 5B shows MTB bacterial loads in the organs of the bone marrow (BM) chimeras 2 and 3 weeks after i.v. infection with 5×10^4 CFU of MTB. Recipient mice were lethally irradiated (650 Gy twice at 4 hour interval) and reconstituted with 2×10^6 bone marrow cells (S – S, S – R, R – S, R – R = BM Donor – BM Recipient; S = *sst1^S*, R = *sst1^R*). Chimeric mice were infected 2 months after the bone marrow transplantation.

[0029] Figures 6A to 6E show the analysis of the *sst1* minimal region. Figure 6A shows the fine mapping of the *sst1* locus by progeny testing. The *sst1* allelic composition of each recombinant male (bottom row) was inferred by analysis of co-segregation of the B6-derived segment of chromosome 1 with extended survival times of its progeny after the MTB infection as follows. Parental mice were produced by 4 or 5 generations of backcross on C3HeB/FeJ (*sst1^S*) background. Males that carried

recombination within the B6-derived *sst1*-containing segment of the mouse chromosome 1 were identified by genotyping. The recombinant males were bred with C3HeB/FeJ females to generate 30-40 backcross progeny for testing. The progeny of each recombinant male were tested for susceptibility to i.v. infection with MTB and genotyped using the microsatellite markers within the *sst1* region. The survival times of the heterozygous and homozygous backcross progeny for each marker was compared using Student's t test ($p=0.001$ was used as a threshold). Each column of boxes represents a genotypic class and each row of boxes represents genotypes for an individual microsatellite marker within the *sst1* candidate region (specified on the left of each row). Solid boxes represent heterozygous (B6 and C3H-derived) and opened boxes homozygous genotype (C3H only). The number of recombinant males, which were tested per each genotypic class, is denoted under each column. The two horizontal lines drawn between D1Mit439 and D1Mit49 designate the *sst1* candidate region. The number of recombinants of each genotype that were characterized by progeny testing, is presented at the bottom of each column.

[0030] Figure 6B shows the analysis of expression of the most likely candidate genes in C3HeB/FeJ (S) and C3H.B6-*sst1* (R) using RT-PCR (left column) and RACE (right column). Total RNA was isolated from lung tuberculosis lesions (Lung) or from bone marrow-derived macrophages (BMph) infected with virulent MTB for 72 hours *in vitro*. Details of the *Ifi75-rs* analysis by RACE are presented separately in Fig. 2B. Priority chart of the *sst1*-encoded candidate genes is presented in Figure 6E (see below).

[0031] Figure 6C shows the number of copies of individual exons of the *Ifi75-rs* per genome. Genomic DNA was isolated from the C3HeB/FeJ, C3H.B6-*sst1* inbred mouse strains and *Mus caroli* and tested by real time quantitative PCR with 12 pairs of the *Ipr1* exon-specific primers using AmplifluorTM Universal Detection System. A single copy gene encoding brain-derived neurotrophic factor (*Bdnf*) was used as an internal control for normalization. The number of exons per genome was estimated by comparison of normalized values obtained for the C3HeB/FeJ and C3H.B6-*sst1* genomic DNA with the genomic DNA of *Mus caroli*, which contains a single copy of the *Ifi75* gene (Weichenhan, Mamm Genome 12:590-594, 2001). Each sample was tested in triplicate. The number of exons varied between 40 copies for exon 8 (maximal copy number) and 5 for exon 12 (minimal copy number) indicating that most of the copies of

the *Ifi75-rs* contained less than a complete set of 12 exons and there are 5 or fewer full length copies of the gene encoded within the B6-derived HSR. The number of the exons 1, 2, and 4 per genome was lower in the *sst1^S* mouse.

[0032] Figure 6D shows the heterogeneity of individual exons of the *Ifi75-rs* demonstrated by Single Strand Conformation Polymorphism (SSCP) analysis. Individual exons of the *Ifi75-rs* were amplified from the genomic DNA isolated from the C3HeB/FeJ (S) or C3H.B6-*sst1* (R) congenic mice and separated on non-denaturing acrylamide gel as described in Methods. Multiple bands were produced with each exon-specific primer pair, suggesting that individual copies of the repeated exons were non identical. There was also a clear difference between the *sst1* congenic mouse strains in genomic DNA encoding exon 1.

[0033] Figure 6E shows the priority chart of the candidate genes encoded within the *sst1* region based on their expression pattern. The list of the genes corresponding to the numbers 1-22 is presented in Table 1. The sequences of the *Sp100-rs* and *Ifi75* genes, which are encoded within the repeat region, were described previously (Weichenhan et al, 2001). To prioritize the *sst1*-encoded genes we used the following criteria: the gene is expressed in critical tissue (lung) and cell type (macrophage), expression of the gene is modulated by the MTB infection, and the gene might be differentially expressed between the *sst1* disparate animals or cells. Based on priority, all the genes within the *sst1* region were divided into 5 categories: not expressed in the lungs and macrophages (unlikely candidates, 9 genes); expressed in the lungs, not in macrophages (low priority, 3 genes); expressed in the lungs and macrophages, not induced by MTB infection (priority score 2, medium priority, 10 genes); upregulated by the MTB infection (high priority, 2 genes); differentially expressed between the *sst1* congenic macrophages (highest priority, 1 gene).

[0034] Figures 7A to 7D show tuberculosis lung lesions in the *sst1* congenic mice after aerosol infection with MTB. Figure 7A and Figure 7B show tuberculosis lesions in the lungs of the C3HeB/FeJ (C3H) and the *sst1* resistant congenic mouse strain C3H.B6-*sst1* at 6 weeks (Figure 7A) and 12 weeks (Figure 7B) after aerosol challenge with MTB. Figure 7C shows tuberculosis granuloma wall adjacent to central necrosis in the lung lesions of the C3H (*sst1^S*) mice (left panel) and lung granuloma of the C3H.B6-*sst1* (*sst1^R*) mice (right panel) 12 weeks after aerosol challenge with MTB. H&E, 200X, original magnification. Figure 7D shows auromine-rodamine staining of the acid fast

bacteria (MTB), 400X, original magnification. Central necrosis with fibrous capsule is seen in lesions of the susceptible C3H mice, with numerous extracellular bacilli seen on fluorescence microscopy within necrotic masses. Lesions of the *sst1*-resistant congenic mice showed minimal necrosis, abundant lymphocytes and foamy macrophages with scant bacilli upon fluorescence microscopy. Formation of necrotic lesions in the lungs of the *sst1*-susceptible mice was observed in three independent aerosol experiments. For each mouse strain, 3-4 animals were tested at each time point and necrotic lesions of various sizes were invariably found in the lungs of every *sst1*-susceptible mouse at 12 weeks after the aerosol challenge with MTB.

[0035] Figures 8A to 8D shows TUNEL staining of the tuberculosis lung lesions of the *sst1^R* and *sst1^S* congenic mice. Apoptotic cells in the tuberculosis lung lesions of *sst1^R* (Figure 8A and 8C) and *sst1^S* (Figure 8B and 8D) congenic mouse strains were identified using a TUNEL assay and counterstained with hematoxylin. In the *sst1^R* mice no necrosis is observed and some focal lesions include cells with TUNEL-positive apoptotic nuclei (Figure 8C, arrow). In the tuberculosis lesions of the *sst1^S* mice the TUNEL-positive staining is associated with cytoplasm and extracellular exudative material (Figure 8D, arrow), which is consistent with necrotic cell death and release of DNA fragments into the exudate. The arrows show a TUNEL-positive nucleus in the resistant mice (Figure 8C) and the TUNEL-positive extracellular exudative material in the susceptible mice (Figure 8D). Figure 8A and 8B – 100X; Figure 8C and 8D – 400X original magnification.

DETAILED DESCRIPTION OF THE INVENTION

[0036] The present invention is directed to methods for determining an individual's susceptibility to infection by cytosolic pathogens (e.g. *Mycobacterium tuberculosis*, *Listeria monocytogene*, Hepatitis virus and Epstein Barr virus) as well as to methods for prophylaxis treatment and methods for treatment of such pathogenic infections.

[0037] We have identified the *Ipr1* gene as a gene involved in innate immunity to infection by cytosolic pathogens (e.g. *Mycobacterium tuberculosis* and *Listeria monocytogene*). The lack of *Ipr1* gene expression correlates with high susceptibility to infection. Further, expression of the *Ipr1* transgene in susceptible cells inhibits

multiplication of the cytosolic pathogen as well as induces apoptosis and controls inflammation. Thus, the present invention is directed to methods for determining a patient's susceptibility to infection by monitoring expression of the human homologue to *Ipr1* protein. The present invention is also directed to methods of prophylaxis, as well as to methods for treatment of infection by inducing expression of, or administering, the *Ipr1* protein human homologue.

[0038] In one embodiment, the term "human homologue to *Ipr1*" refers to a DNA sequence that has at least about 55% homology to SEQ ID NO:11, the full length nucleotide sequence of *Ipr1*. In one embodiment, the term "human homologue to *Ipr1* protein" refers to an amino acid sequence that has 55% homology to SEQ ID NO:12, the full length amino acid sequence of *Ipr1* protein, more preferably at least about 60% homology, still more preferably, at least about 70% homology, even more preferably, at least about 75% homology, yet more preferably, at least about 80% homology, even more preferably at least about 85% homology, still more preferably, at least about 90% homology, and more preferably, at least about 95% homology. In another embodiment, one looks for a domain involved in causing innate immunity to a cytosolic pathogen. As discussed above, the homology is at least about 50% to 100% and all intervals in between (i.e., 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, etc.).

[0039] The full length nucleotide sequence of *Ipr1* (SEQ ID NO:11), the full length protein sequence of *Ipr1* protein (SEQ ID NO:12), the Sp100 domain coding sequence of *Ipr1* (SEQ ID NO:13), the Sp100 protein domain of *Ipr1* protein (SEQ ID NO:14), the SAND domain coding sequence of *Ipr1* (SEQ ID NO:15), the SAND protein domain of *Ipr1* protein (SEQ ID NO:16), the nuclear receptor binding motif coding sequence of *Ipr1* (SEQ ID NO:17), the nuclear receptor binding protein domain of *Ipr1* protein (SEQ ID NO:18), and the nuclear localization coding sequence of *Ipr1* (SEQ ID NO:19), the nuclear localization amino acid sequence of *Ipr1* protein (SEQ ID NO:20) are shown below.

[0040] *Ipr1* full length nucleotide sequence

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agtggtgctgagatcacttttcatttttcttttcttgaagcctgactccccgcgggacttccaaggcagcataa
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 ttggtcactgttaaaggaatttttaaatgaagggggaagggccacatcaaaagactggaagggcggttatacg
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 aaagaaggggtgcctagcagatgaactcctgacatgatgtgctcagtcctcgtgctcgtcgcactctgtc
 tctgtctctctgtc
 tctgt
 gctctgaggtccttcgtagttgtgtgtcctcatcacaactgaccagcactgtggtacacgctcacgatgtcc
 ttgttcacagctgacttacaaatggcaaagtgcataactagmcagtaaaaaataaagctgttggtgtgtct
 gtgcgttttcaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa (SEQ ID NO 11)

[0041] *Ipr1* protein full length

MFTLTKALEKALLQHFIYMKVNIAYAINKPFFFEALRDNSFITERMYKESLEACQNLVPLSKVHNILTSI
 EQTFHPSVLLTLFSKVNLRREYPSLVAFRFRNVGYTYEENRPLTLLEDLANPAEGCSLQTLPPRPQI
 SLPSHLSSAPRVCDPRATAQPIIEILDEQSPSPRAVPLLGCIEGKTPVSSRDHQRKDKESSREMPHSPS
 GPESVVKDDSPAANDLEMAREVPCPANKKARRKKRPNWSNSKRRRQKKKPRQDEMMGVASPGHGVQEKLLA
 VSRRTLWKDDSSSTNVKEVTKTQRTMRRAQTSNSQEISKEASKTSGRKRPTARRTTQVPEKTKNDAVDFSP
 TLPVTCGKAKGTLFQEKLKQGASKKCIQNEAGDWLTVKEFLNEGGRATSKDWKGVIRCNGETLRHLEQKGLL
 FFTSKSKPQKGA (SEQ ID NO 12)

[0042] Sp100 domain coding sequence of *Ipr1*

aaagccttggaaaaggctcttctccagcatttcatatacatgaaggtgaacatcgccatcaacaag
 ccattcccccttcttcgaagcgctccgggacaattccttcatcactgagagaatgtacaaggaatctctggaa
 gcctgtcaaaatctggtccctctgtccaaagtgggtgcacaatattctcaccagtcctggagcagactttccac
 ccgtcagtgctgctgacgttgttcagcaaggtcaacctccgggaataccccagcctgggtggcaattttcaga
 agcttcagaaaacgttggttata (SEQ ID NO 13)

[0043] Sp100 protein domain of *Ipr1*

KALEKALLQHFIYMKVNIAYAINKPFFFEALRDNSFITERMYKESLEACQNLVPLSKVHNILTSLEQTFH
 PSVLLTLFSKVNLRREYPSLVAFRFRNVGY (SEQ ID NO 14)

[0044] SAND domain coding sequence of *Ipr1*

cccacactccctgtgacctgtggttaaggccaaaggactttgttccaagagaaactgaagcaaggagcctca
 aaaaagtgcattcagaatgaggcaggagattggctcactgttaaaggaatttttaaatgaagggggaagggcc
 acatcaaaagactggaagggcggttatacgttgtaacggggagacattaagacatctggagcagaaa (SEQ
 ID NO 15)

[0045] SAND protein domain of *Ipr1*

PTLPVTCGKAKGTLFQEKLKQGASKKCIQNEAGDWLTVKEFLNEGGRATSKDWKGVIRCNGETLRHLEQK
 (SEQ ID NO 16)

[0046] NRB (nuclear receptor binding) motif coding sequence of *Ipr1*

cttcagacactgctg (SEQ ID NO 17)

[0047] NRB motif of Ipr1
LQTL (SEQ ID NO 18)

[0048] NLS (nuclear localization signal) coding sequence of *Ipr1*
aagaaagcaagaagaaaaaacgtccgaactggtcaaattccaaaagaagacggcagaaaaaaagccccgt
(SEQ ID NO 19)

[0049] NLS motif Ipr1
KKARRKKRPNWSNSKRRRQKKKPR (SEQ ID NO 20)

[0050] The full length nucleotide sequence of *SP110b* (SEQ ID NO:1), the full length protein sequence of SP110b (SEQ ID NO:2), the Sp100 domain coding sequence of *SP110b* (SEQ ID NO:3), the Sp100 protein domain of SP110b protein (SEQ ID NO:4), the SAND domain coding sequence of *SP110b* (SEQ ID NO:5), the SAND protein domain of SP110b protein (SEQ ID NO:6), the nuclear receptor binding motif coding sequence of *SP110b* (SEQ ID NO:7), the nuclear receptor binding protein domain of SP110b protein (SEQ ID NO:8), the nuclear localization coding sequence of *SP110b* (SEQ ID NO:9), and the nuclear localization amino acid sequence of SP110b protein (SEQ ID NO:10) are shown below.

[0051] SP110b full length nucleotide sequence, human

gttttgctgctagcatctccctgtaactctcccaatcttgaggagtgatccctgtcccagccctggaaaag
gggcaggaacgacaaactcaaagtccaggatgttcaccatgacaagagccatggaagaggctctttttcagc
acttcatgcaccagaagctggggatcgccatgacacacaagccatttcccttctttgaaggccctcctag
acaactccatcatcactaagagaatgtacatggaatctctggaagcctgtagaaatttgatccctgtatcca
gagtgggtgcacaacatttctacccaactggagaggacttttaacctgtctcttctggtgacattgttcagtc
aaattaacctgctgaatatcccaatctggtgacgatttacagaagcttcaaagctgttggtgcttcctatg
aacggcagagcagagacacaccaatctacttgaagcccaactggcctagcagaaggaagctccctccata
ccccactggcgtgccccaccacaacccccctcaaccaagctgttcaccctgtgcgccaagagtcagtgagc
ctggaactcctccagcaaaagcgatgagatcctgagtgagtcgcccagcccatctgaccctgtcctgcctc
tccctgcatcctccaggaaggaagaagcacttcagtgaccaatgacaagttaacatccaaaatgaatgcgg
aagaagactcagaagagatgccagcctcctcactagcactgtgcaagtggccagtgacaacctgatcccc
aaataagagataaagaagaccctcaagagatgccccactctcccttgggctctatgccagagataagagata
attctccagaaccaaatagaccagaagagccccaggaggtgtccagcacaccttcagacaagaaaggaaaga
aaagaaaaagatgtatctggtcaactccaaaaggagacataagaaaaaaagcctcccaagagggacagcct
catctagacacggaatccaaaagaagctcaaaaggtggatcaggttcctcaaaagaagatgactcaactt
gtaactccacggtagagacaagggccaaaaggcgagaactgaatgtgccgaaagtgcagatcagaggaga
tcattgatggcacttcagaaatgaatgaaggaaagaggtcccagaagacgcctagtacaccacgaagggtca
cacaaggggcagcctcacctgggcatggcatccaagagaagctccaagtgggtggataaggtgactcaaagga
aagacgactcaacctggaactcagaggtcatgatgaggggtccaaaaggcaagaactaaatgtgccgaaagt
ccagatcgaaagaaaagaaaaaggagaaagatatctgttcaagctcaaaaaggagatttcagaaaaatattc
accgaagaggaaccccaaaagtgcactgtggattttcactgttcttaagctccccgtgacctgtggtgagg
cgaaagggattttatataagaagaaaatgaacacggatcctcagtgaaagtgcattcggaatgaggatggaa
cttgggttaacaccaaatagaatttgaagtgcgaaggaaaaggaaggaacgcaagaactggaaacggaatatac
gttggtgaaggaaatgacctaggagagctgctgaagagtggaacttttgcctgtcctccaagaataaatctca
agagagagttaaatagcaagtgaatttctactacctctcagtcaccatgttgacagactttccctgtctgga
ggctcaccttagagcttctgagtttccaagctctgagtcacctccacatttgggcatggcatcttcaaaaca

attaatttgcatagttaatttgggatggggaagcaaataactctaaaataaaaattaaatgaaaaagctcaa
aaaaaaaaaaaaaaaaaaaa (SEQ ID NO 1)

[0052] SP110b, full length protein, human

MFTMTRAMEEALFQHFMHQKLGIAAYAIHKPFFFEGLLDNSIITKRMYESLEACRNLI PVSRVV
HNILTQLERTFNLSLLVTLFSQINLREYPNLVTIYRSFKRVGASYERQSRDTPILLEAPTGLAEG
SSLHTPLALPPPQPPQPCSPCAPRVSEPGTSSQQSDEILSESPSPSDPVLPLPALIQEGRSTSV
TNDKLT SKMNAEEDSEEMP SLLTSTVQVASDNLI PQIRDKEDPQEMPHSPLGSMPEIRDNSPEPN
DPEEPQEV SSTPSDKKGKKRRCI WSTPKRRHKKKSLPRGTASSRHGIQKKLKRVDQVPQKKDDS
TCNSTVETRAQKARTECARKSRSEEI IDGTSEMNEGKRSQKTPSTPRRV TQGAASPGHGIQEKLO
VVDKVTQRKDDSTWNSEVMRVQKARTKARKSRSEKKEKEDI C SSSKRRFQKNIHRRGKPKSD
TVDFHCSKLPVTCGEAKGILYKKMKHGS SVKCIRNEDGTWLT PNEFEVEGKGRNAKNWKRNI RC
EGMTLGELLKSGLLLCPPRINL KRELNSK (SEQ ID NO 2)

[0053] Sp100 domain coding sequence of SP110b, human

Gaggctctttttcagcacttcatgcaccagaagctggggatcgcctatgccatacacaagccatt
tcccttcttttgaaggcctcctagacaactccatcatcactaagagaatgtacatggaatctctgg
aagcctgtagaaatttgatccctgtatccagagtgggtgcacaacattctcacccaactggagagg
acttttaacctgtctcttctggtgacattgttcagtc aaattaacctgcgtgaatatcccaatct
ggtgacgatttacagaagcttcaaactgttggtgct (SEQ ID NO 3)

[0054] Sp100 protein domain of SP110b, human

EALFQHFMHQKLGIAAYAIHKPFFFEGLLDNSIITKRMYESLEACRNLI PVSRVVHNILTQLER
TFNLSLLVTLFSQINLREYPNLVTIYRSFKRVGA (SEQ ID NO 4)

[0055] SAND domain coding sequence of SP110b, human

Tgttctaagctccccgtgacctgtggtgagggcgaaagggattttatataagaagaaaatgaaaca
cggatcctcagtgaaagtgcattcggaatgaggatggaacttggttaacaccaaatagaatttgaag
tcgaaggaaaaggaaggaacgcaaagaactggaaacggaatatacgttgtgaaggaatgacccta
ggagagctgctgaagagt (SEQ ID NO 5)

[0056] SAND protein domain of SP110b, human

CSKLPVTCGEAKGILYKKMKHGS SVKCIRNEDGTWLT PNEFEVEGKGRNAKNWKRNI RC
EGMTLGELLKS (SEQ ID NO 6)

[0057] NRB (nuclear receptor binding) motif coding sequence of SP110b,
human
ctaggagagctgctg (SEQ ID NO 7)

[0058] NRB motif of SP110b, human
LGELL (SEQ ID NO 8)

[0059] NLS (nuclear localization signal) coding sequence of SP110b, human
Aagaaaggaaagaaaagaaaagatgtatctggtcaactccaaaaggagacataagaaaaaag
cctcccaagaggggacagcctcatctagacacggaatccaaaagaagctcaaaagg (SEQ ID
NO 9)

[0060] NLS of SP110b, human
KKGKKRKRCTIWSTPKRRHKKKSLPRGTASSRHGIQKKLKR (SEQ ID NO 10)

[0061] "Homology" or "identity" or "similarity" refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology and identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When an equivalent position in the compared sequences is occupied by the same base or amino acid, then the molecules are identical at that position; when the equivalent site occupied by the same or a similar amino acid residue (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as homologous (similar) at that position. Expression as a percentage of homology/similarity or identity refers to a function of the number of identical or similar amino acids at positions shared by the compared sequences. A sequence which is "unrelated" or "non-homologous" shares less than 40% identity, though preferably less than 25% identity with a sequence of the present application.

[0062] In comparing two sequences, the absence of residues (amino acids or nucleic acids) or presence of extra residues also decreases the identity and homology/similarity. The term "homology" describes a mathematically based comparison of sequence similarities which is used to identify genes or proteins with similar functions or motifs. The nucleic acid and protein sequences of the present application may be used as a "query sequence" to perform a search against public databases to, for example, identify other family members, related sequences or homologs. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J Mol. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences homologous to nucleic acid molecules of the application. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to protein molecules of the application. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25(17):3389- 3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and BLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

[0063] As used herein, "identity" means the percentage of identical nucleotide or amino acid residues at corresponding positions in two or more sequences when the sequences are aligned to maximize sequence matching, i.e., taking into account gaps and insertions. Identity can be readily calculated by known methods, including but not limited to those described in (Computational Molecular Biology, Lesk, A. M., ea., Oxford University Press, New York, 1988; Biocomputing: Informatics and - 14 Genome Projects, Smith, D. W., ea., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988)). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research I 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Altschul, S. F. et al., J. I Molec. Biol. 215: 403-410 (1990) and Altschul et al. Nuc. Acids Res. 25: 3389-3402 (1997)). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894; Altschul, S., et al., J. Mol. Biol. 215: 403-410 (1990)). The well known Smith Waterman algorithm may also be used to determine identity.

[0064] As used herein, "high stringency" means the following: hybridization at 42° C in the presence of 50% formamide; a first wash at 65°C with 2 x SSC containing 1% SDS; followed by a second wash at 65°C with 0.1 x SSC.

[0065] Methods for determining a patients susceptibility to infection by a cytosolic pathogen are provided. The methods comprise determining gene expression, e.g. gene expression levels, of the human homologue to *Ipr1* (SEQ ID NO:11) in a biological sample, such as a peripheral blood sample (e.g. in monocytes and macrophages) or a tissue sample (e.g. lung, spleen, heart, liver etc). A positive gene expression indicates that the individual has innate resistance to infection by cytosolic pathogens, while negative gene expression indicates that the patient is highly susceptible to infection.

[0066] According to the present invention, a "baseline" or "control" can include a normal or negative control and/or a disease or positive control, against which a test level of gene expression can be compared. Therefore, it can be determined, based on the control or baseline level of gene expression, whether a sample to be evaluated for the human homologue of *Ipr1* gene expression has a measurable difference or substantially no difference in gene expression, as compared to the baseline level. In one aspect, the baseline control is a indicative of the level of gene expression as expected in the sample of a normal (e.g., healthy, negative control) patient. Therefore, the term "negative control" used in reference to a baseline level of gene expression typically refers to a baseline level of expression from a population of individuals which is believed to be normal (i.e., not having or developing susceptibility to infection of cytosolic pathogens). In some embodiments of the invention, it may also be useful to compare the gene expression in a test sample to a baseline that has previously been established from a patient or population of patients having decreased or lack of *Ipr1* gene expression. Such a baseline level, also referred to herein as a "positive control", refers to a level of gene expression established from one or preferably a population of individuals who had been positively diagnosed with susceptibility to infection with cytosolic pathogens.

[0067] As used herein, a negative gene expression includes a lack or decrease in gene expression levels, the presence of a mutation or polymorphism that results in the expression of a *Ipr1* protein that is non-functional (e.g. has impaired antiviral or antibacterial function), or the expression of a protein that has an altered half life (e.g. degrades faster than a control group).

[0068] In another embodiment, a preferred human homologue of the *Ipr1* protein is *SP110b*¹⁸, which is expressed from a gene localized to human chromosome 2. Accordingly, methods for determining a patient's susceptibility to infection by a cytosolic pathogen are provided that comprise determining gene expression levels of *SP110b* (SEQ ID NO:1) in a peripheral blood sample (e.g. monocyte or macrophage) or tissue sample (e.g. lung, spleen, heart, liver etc). A positive gene expression indicates that the individual has innate resistance to infection by cytosolic pathogens, while negative gene expression indicates that the patient is highly susceptible to infection.

[0069] As used herein, “tissue sample” refers to a portion, piece, part, segment, or fraction of a tissue which is obtained or removed from an intact tissue of a patient, preferably a human patient.

[0070] As used herein, a “peripheral blood sample” refers to blood drawn from a patient, preferably a human patient. The term peripheral blood sample also encompasses peripheral blood isolates, such as monocytes or macrophages.

[0071] The present invention also encompasses the use of isolates of a tissue sample in the methods of the invention. As used herein, an “isolate” of a sample refers to a material or composition (e. g., a biological material or composition) which has been separated, derived, extracted, purified or isolated from the sample and preferably is substantially free of undesirable compositions and/or impurities or contaminants associated with the sample.

Measuring levels of gene expression

[0072] Gene expression levels can be measured by any means known to those skilled in the art. In the present invention, it is generally preferred to measure mRNA. The most commonly used methods known in the art for the quantification of mRNA expression in a sample include Northern blotting and in situ hybridization (Parker & Barnes, *Methods in Molecular Biology* 106:247-283 (1999)); RNase protection assays (Hod, *Biotechniques* 13:852-854 (1992)); and reverse transcription polymerase chain reaction (RT-PCR) (Weis et al., *Trends in Genetics* 8:263-264 (1992)), U.S. Pat. No. 5,322,770, or (RT-AGLCR) as described by R. L. Marshall, et al., *PCR Methods and Applications* 4: 80-84 (1994). Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. Representative methods for sequencing-based gene expression analysis include Serial Analysis of Gene Expression (SAGE), and gene expression analysis by massively parallel signature sequencing (MPSS).

[0073] Other known amplification methods which can be utilized herein include, but are not limited to, the so-called “NASBA” or “3SR” technique described in *PNAS USA* 87: 1874-1878 (1990) and also described in *Nature* 350 (No. 6313): 91-92 (1991); Q-beta amplification as described in published European Patent Application (EPA) No. 4544610; strand displacement amplification (as described in G. T. Walker et

al., Clin. Chem. 42: 9-13 (1996)) and European Patent Application No. 684315; and target mediated amplification, as described by PCT Publication WO 9322461.

[0074] In situ hybridization visualization may also be employed, wherein a radioactively labeled antisense RNA probe is hybridized with a thin section of a tissue sample, washed, cleaved with RNase and exposed to a sensitive emulsion for autoradiography. Samples may be stained with haematoxylin to demonstrate the histological composition of the sample, and dark field imaging with a suitable light filter shows the developed emulsion. Non-radioactive labels such as digoxigenin may also be used.

[0075] One can also look for mutations such as additions, deletions, substitutions in the amino acid or nucleic acid sequence by standard means, for example, DNA sequence analysis can be used as well as assays to detect single nucleotide polymorphisms (SNPs) or single strand conformation polymorphisms (SSCP). Alternatively, immunohistochemical means can be used. In one embodiment, one can use an antibody to the carboxy portion, most preferably, the carboxy terminus of the protein. This will identify mutations resulting in truncation or lack of expression. Mutation can also include modifications to the nucleic acid in coding and non-coding portions such as methylation or ethylation can prevent expression of the gene.

Reverse Transcriptase PCR (RT-PCR)

[0076] Of the techniques listed above, the most sensitive and most flexible quantitative method is RT-PCR, which can be used to compare mRNA levels in different sample populations, with or without drug treatment, to characterize patterns of gene expression and to discriminate between closely related mRNAs. The first step is the isolation of mRNA from a target sample. The starting material is typically total RNA isolated from human tissue. Thus, RNA can be isolated from a variety of tissues, including breast, lung, colon, prostate, brain, liver, kidney, pancreas, spleen, thymus, testis, ovary, uterus, etc. The mRNA can be extracted, for example, from frozen or archived paraffin-embedded and fixed (e.g. formalin-fixed) tissue samples.

[0077] General methods for mRNA extraction are well known in the art and are disclosed in standard textbooks of molecular biology, including Ausubel et al., Current Protocols of Molecular Biology, John Wiley and Sons (1997). Methods for RNA extraction from paraffin embedded tissues are disclosed, for example, in Rupp and

Locker, Lab Invest. 56:A67 (1987), and De Andres et al., BioTechniques 18:42044 (1995). In particular, RNA isolation can be performed using a purification kit, buffer set and protease from commercial manufacturers, such as Qiagen, according to the manufacturer's instructions and Paraffin Block RNA Isolation Kit (Ambion, Inc.). Total RNA from tissue samples can be isolated using RNA Stat-60 (Tel-Test).

[0078] As RNA cannot serve as a template for PCR, the first step in gene expression profiling by RT-PCR is the reverse transcription of the RNA template into cDNA, followed by its exponential amplification in a PCR reaction. The two most commonly used reverse transcriptases are avilo myeloblastosis virus reverse transcriptase (AMV-RT) and Moloney murine leukemia virus reverse transcriptase (MMLV-RT). The reverse transcription step is typically primed using specific primers, random hexamers, or oligo-dT primers, depending on the circumstances and the goal of expression profiling. For example, extracted RNA can be reverse-transcribed using a GeneAmp RNA PCR kit (Perkin Elmer, CA, USA), following the manufacturer's instructions. The derived cDNA can then be used as a template in the subsequent PCR reaction.

[0079] Although the PCR step can use a variety of thermostable DNA-dependent DNA polymerases, it typically employs the Taq DNA polymerase, which has a 5'-3' nuclease activity but lacks a 3'-5' proofreading endonuclease activity. Thus, TaqManTM PCR typically utilizes the 5'-nuclease activity of Taq or Tth polymerase to hydrolyze a hybridization probe bound to its target amplicon, but any enzyme with equivalent 5' nuclease activity can be used.

[0080] Two oligonucleotide primers are used to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the Taq DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule

synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

[0081] TaqManTM RT-PCR can be performed using commercially available equipment, such as, for example, ABI PRISM 7700TM Sequence Detection SystemTM (Perkin-Ehner-Applied Biosystems, Foster City, CA, USA), or Lightcycler (Roche Molecular Biochemicals, Mannheim, Germany). In a preferred embodiment, the 5' nuclease procedure is run on a realtime quantitative PCR device such as the ABI PRISM 7700TM Sequence Detection SystemTM.

[0082] The system consists of a thermocycler, laser, charge-coupled device (CCD), camera and computer. The system amplifies samples in a 96-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fiber optics cables for all 96 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing the data.

[0083] 5'-Nuclease assay data are initially expressed as Ct, or the threshold cycle. As discussed above, fluorescence values are recorded during every cycle and represent the amount of product amplified to that point in the amplification reaction. The point when the fluorescent signal is first recorded as statistically significant is the threshold cycle (Ct).

[0084] To minimize errors and the effect of sample-to-sample variation, RT-PCR is usually performed using an internal standard. The ideal internal standard is expressed at a constant level among different tissues, and is unaffected by the experimental treatment. RNAs most frequently used to normalize patterns of gene expression are mRNAs for the housekeeping genes glyceraldehyde phosphate-dehydrogenase (GAPDH) and P-actin.

[0085] A more recent variation of the RT-PCR technique is the real time quantitative PCR, which measures PCR product accumulation through a dual-labeled fluorogenic probe (i.e., TaqManTM probe). Real time PCR is compatible both with quantitative competitive PCR, where internal competitor for each target sequence is used for normalization, and with quantitative comparative PCR using a normalization gene contained within the sample, or a housekeeping gene for RT-PCR. For further details see, e.g. Held et al., Genome Research 6:986-994 (1996).

[0086] The steps of a representative protocol for profiling gene expression using fixed, paraffin-embedded tissues as the RNA source, including mRNA isolation, purification, primer extension and amplification are given in various published journal articles for example: T.E. Godfrey et al., J. Molec. Diagnostics 2: 84-91, 2000; K. Specht et al., Am. J. Pathol. 158, 419.29, 2001. Briefly, a representative process starts with cutting about 10 um thick sections of paraffin-embedded tumor tissue samples. The RNA is then extracted, and protein and DNA are removed. After analysis of the RNA concentration, RNA repair and/or amplification steps may be included, if necessary, and RNA is reverse transcribed using gene specific promoters followed by RT-PCR.

[0087] According to one aspect of the present invention, PCR primers and probes are designed based upon exon and intron sequences present in the homologous *Ipr1* gene (e.g. *SP110b*) to be amplified. In this embodiment, the first step in the primer/probe design is the delineation of intron sequences within the genes. This can be done by publicly available software, such as the DNA BLAT software developed by Kent, W.J., Genome Res. 12(4):656-64 (2002), or by the BLAST software including its variations. Subsequent steps follow well established methods of PCR primer and probe design.

[0088] In order to avoid non-specific signals, it is important to mask repetitive sequences within the introns when designing the primers and probes. This can be easily accomplished by using the Repeat Masker program available on-line through the Baylor College of Medicine, which screens DNA sequences against a library of repetitive elements and returns a query sequence in which the repetitive elements are masked. The masked intron sequences can then be used to design primer and probe sequences using any commercially or otherwise publicly available primer/probe design packages, such as Primer Express (Applied Biosystems); MGB assay-by-design (Applied Biosystems); Primer3 (Steve Rozen and Helen J. Skaletsky (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Humana Press, Totowa, NJ, pp 365-386). The most important factors considered in PCR primer design include primer length, melting temperature (T_m), and G/C content, specificity, complementary primer sequences, and 3'-end sequence. In general, optimal PCR primers are generally 17-30

bases in length, and contain about 20-80%, such as, for example, about 50-60% G+C bases. T_m 's between 50 and 80 °C, e.g. about 50 to 70 °C are typically preferred.

[0089] For further guidelines for PCR primer and probe design see, e.g. Dieffenbach, C.W. et al., "General Concepts for PCR Primer Design" in: *PCR Primer, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1995, pp. 133-155; Innis and Gelfand, "Optimization of PCRs" in: *PCR Protocols, A Guide to Methods and Applications*, CRC Press, London, 1994, pp. 5-11; and Plasterer, T.N. Primerselect: Primer and probe design, *Methods Mol. Biol.* 70:520-527 (1997), the entire disclosures of which are hereby expressly incorporated by reference.

Microarrays

[0090] Gene expression can also be identified, or confirmed using the microarray technique. Methods of preparing DNA arrays and their use are well known in the art. (See, for example U.S. Patent Nos: 6,618,679; 6,379,897; 6,664,377; 6,451,536; 5,48,257; U.S. 20030157485 and Schena et al. 1995 *Science* 20:467-470; Gerhold et al. 1999 *Trends in Biochem. Sci.* 24, 168-173; and Lennon et al. 2000 *Drug discovery Today* 5: 59-65, which are herein incorporated by reference in their entirety). Serial Analysis of Gene Expression (SAGE) can also be performed (See for example U.S. Patent Application 20030215858).

[0091] The expression of homologous genes can be measured in either fresh or paraffin-embedded tissue, using microarray technology. In this method, polynucleotide sequences of interest (including cDNAs and oligonucleotides) are plated, or arrayed, on a microchip substrate. The arrayed sequences are then hybridized with specific cDNA probes from tissues of interest. Just as in the RT-PCR method, the source of mRNA typically is total RNA isolated from the tissue to be tested, and corresponding control and reference samples. mRNA can be extracted, for example, from frozen or archived paraffin-embedded and fixed (e.g. formalin fixed) tissue samples, which are routinely prepared and preserved in everyday clinical practice.

[0092] In a specific embodiment of the microarray technique, PCR amplified inserts of cDNA clones are applied to a substrate in a dense array. Preferably at least 10,000 nucleotide sequences are applied to the substrate. The microarrayed genes, immobilized on the microchip at 10,000 elements each, are suitable for hybridization under stringent conditions.

[0093] Fluorescently labeled cDNA probes may be generated through incorporation of fluorescent nucleotides by reverse transcription of RNA extracted from tissues of interest. Labeled cDNA probes applied to the chip hybridize with specificity to each spot of DNA on the array. After stringent washing to remove non-specifically bound probes, the chip is scanned by confocal laser microscopy or by another detection method, such as a CCD camera. Quantitation of hybridization of each arrayed element allows for assessment of corresponding mRNA abundance. With dual color fluorescence, separately labeled cDNA probes generated from two sources of RNA are hybridized pairwise to the array. The relative abundance of the transcripts from the two sources corresponding to each specified gene is thus determined simultaneously. The miniaturized scale of the hybridization affords a convenient and rapid evaluation of the expression pattern for large numbers of genes. Such methods have been shown to have the sensitivity required to detect rare transcripts, which are expressed at a few copies per cell, and to reproducibly detect at least approximately two-fold differences in the expression levels (Schena et al., *Proc. Natl. Acad. Sci. USA* 93(2):106-149 (1996)).

[0094] Microarray analysis can be performed by using commercially available equipment and/or commercially available microarrays, following manufacturer's protocols, such as by using the Affymetrix GenChip technology, or Incyte's microarray technology.

[0095] Serial Analysis of Gene Expression (SAGE) Serial analysis of gene expression (SAGE) is a method that allows the simultaneous and quantitative analysis of a large number of gene transcripts, without the need of providing an individual hybridization probe for each transcript. First, a short sequence tag (about 10-14 bp) is generated that contains sufficient information to uniquely identify a transcript, provided that the tag is obtained from a unique position within each transcript. Then, many transcripts are linked together to form long serial molecules, that can be sequenced, revealing the identity of the multiple tags simultaneously. The expression pattern of any population of transcripts can be quantitatively evaluated by determining the abundance of individual tags, and identifying the gene corresponding to each tag. For more details see, e.g. Velculescu et al., *Science* 270:484-487 (1995); and Velculescu et al., *Cell* 88:243-51 (1997).

MassARRAY Technology

[0096] The MassARRAY (Sequenom, San Diego, California) technology is an automated, high-throughput method of gene expression analysis using mass spectrometry (MS) for detection. According to this method, following the isolation of RNA, reverse transcription and PCR amplification, the cDNAs are subjected to primer extension. The cDNA-derived primer extension products are purified, and dispensed on a chip array that is pre-loaded with the components needed for MALTI-TOF MS sample preparation. The various cDNAs present in the reaction are quantitated by analyzing the peak areas in the mass spectrum obtained.

Analysis of protein levels

[0097] Protein expression can also be measured as a means for determining gene expression levels. In one preferred method, the protein product of the expressed gene to be monitored is detected using an “antibody-based binding moiety” or “antibody” that specifically binds the protein product.

[0098] The term “antibody-based binding moiety” or “antibody” includes immunoglobulin molecules and immunologically active determinants of immunoglobulin molecules, e.g., molecules that contain an antigen binding site which specifically binds (immunoreacts with) to the protein to be detected. The term “antibody-based binding moiety” is intended to include whole antibodies, e.g., of any isotype (IgG, IgA, IgM, IgE, etc), and includes fragments thereof which are also specifically reactive with the protein to be detected. Antibodies can be fragmented using conventional techniques. Thus, the term includes segments of proteolytically-cleaved or recombinantly-prepared portions of an antibody molecule that are capable of selectively reacting with a certain protein. Non limiting examples of such proteolytic and/or recombinant fragments include Fab, F(ab')₂, Fab', Fv, dAbs and single chain antibodies (scFv) containing a VL and VH domain joined by a peptide linker. The scFv's may be covalently or non-covalently linked to form antibodies having two or more binding sites. Thus, “antibody-base binding moiety” includes polyclonal, monoclonal, or other purified preparations of antibodies and recombinant antibodies. The term “antibody-base binding moiety” is further intended to include humanized antibodies, bispecific antibodies, and chimeric molecules having at least one antigen binding determinant derived from an antibody molecule. In a preferred embodiment, the antibody-based binding moiety detectably labeled.

[00099] "Labeled antibody", as used herein, includes antibodies that are labeled by a detectable means and include, but are not limited to, antibodies that are enzymatically, radioactively, fluorescently, and chemiluminescently labeled. Antibodies can also be labeled with a detectable tag, such as c-Myc, HA, VSV-G, HSV, FLAG, V5, or HIS.

[00100] In the diagnostic methods of the invention that use antibody based binding moieties for the detection of gene expression levels, the level of protein present in the samples (or gene expression levels) correlates to the intensity of the signal emitted from the detectably labeled antibody.

[00101] In one preferred embodiment, the antibody-based binding moiety is detectably labeled by linking the antibody to an enzyme. The enzyme, in turn, when exposed to its substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used to detectably label the antibodies of the present invention include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Chemiluminescence is another method that can be used to detect an antibody-based binding moiety.

[00102] Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling an antibody, it is possible to detect the antibody through the use of radioimmune assays. The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography. Isotopes which are particularly useful for the purpose of the present invention are ^3H , ^{131}I , ^{35}S , ^{14}C , and preferably ^{125}I .

[00103] It is also possible to label an antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are CYE dyes, fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthalaldehyde and fluorescamine.

[00104] An antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu , or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[00105] An antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, luciferin, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[00106] In one embodiment, levels of protein are measured by contacting the sample with an antibody-based binding moiety that specifically binds to the protein, or to a fragment of. Formation of the antibody-antigen complex is then detected as a measure of the protein levels.

[00107] Means for detection of protein levels are well known to those skilled in the art. For example, levels of protein can be detected by immunoassays, such as enzyme linked immunoabsorbant assay (ELISA), radioimmunoassay (RIA), Immunoradiometric assay (IRMA), Western blotting, or immunohistochemistry. Immunoassays such as ELISA or RIA, which can be extremely rapid, are more generally preferred.

[00108] There are different forms of ELISA, which are well known to those skilled in the art. The standard techniques known in the art for ELISA are described in "Methods in Immunodiagnosis", 2nd Edition, Rose and Bigazzi, eds. John Wiley & Sons, 1980; Campbell et al., "Methods and Immunology", W. A. Benjamin, Inc., 1964; and Oellerich, M. 1984, J. Clin. Chem. Clin. Biochem., 22:895-904. Sandwich ELISA and Competitive ELISA, well known to those in the art, are also useful in methods of the invention.

[00109] Antibody arrays or protein chips can also be employed, see for example U.S. Patent Application Nos: 20030013208A1; 20020155493A1; 20030017515 and U.S. Patent Nos: 6,329,209; 6,365,418, which are herein incorporated by reference in their entirety.

Analysis of protein degradation

[00110] Also encompassed in the present invention is an analysis of protein degradation of the human homologue to Ipr1. It has been found that in some instances, the Ipr1 protein degrades faster in individuals susceptible to infection by cytosolic pathogens. Thus, methods to determine the rate of Ipr1 protein degradation in an individual are encompassed. At any given time the cellular content of a protein is believed to be regulated by a combination of its synthetic and degradation rates, with each protein having a characteristic pattern of synthesis and degradation to ensure proper cellular function. Methods for analyzing human homologue Ipr1 protein degradation are thus encompassed and are known to those of skill in the art.

[00111] Further, many amino acids, including the terminal amino acids, may be modified by natural processes, such as processing and other post-translational modifications. Known modifications include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

[00112] Such modifications are well known to those of skill in the art and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as *Proteins--Structure and Molecular Properties*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993). Many detailed reviews are available on this subject, such as by Wold, F., *Posttranslational Covalent Modification of Proteins*, B. C. Johnson, Ed., Academic Press, New York 1-12 (1983); Seifter et al. (*Meth. Enzymol.* 182: 626-646 (1990)) and Rattan et al. (*Ann. N.Y. Acad. Sci.* 663:48-62 (1992)).

[00113] Any of the above modifications may alter the degradation rate of the human homologue to *IprI*. Thus, methods to detect such alterations in a test sample, as compared to a control group, are encompassed. In analyzing protein degradation rates, it may be necessary to compare different timepoints in an individual. For example, a test biological sample may be analyzed and compared to a control group at a first time point and a second time point. An increase in the rate of protein degradation in the test sample (e.g. between the first and second time point) as compared to the control group is indicative of susceptibility to infection by cytosolic pathogens.

[00114] As discussed above, individuals having polymorphism or mutation and/or lower levels of expression of the *IprI* homolog are at greater risk for infection by a cytosolic pathogen. Identifying such individuals can permit them to take precautions to avoid infection such as staying away from infected individuals, and/or preventative chemotherapy/immunotherapy, etc. Also, identifying changes in such expression can be used to screen individuals that may be infected or at an increased risk for infection. Mutations and/or polymorphisms that reduce the function of the gene so much so as to effectively render the gene non-functional (e.g. unable to render antibacterial or antiviral functions) are encompassed. Thus, methods for analyzing such mutations or polymorphisms are encompassed.

[00115] A polymorphism is typically defined as two or more alternative sequences, or alleles, of a gene in a population. A polymorphic site is the location in the gene at which divergence in sequence occurs. Examples of the ways in which polymorphisms are manifested include restriction fragment length polymorphisms, variable number of tandem repeats, hypervariable regions, minisatellites, di- or multi-nucleotide repeats, insertion elements and nucleotide deletions, additions or substitutions. The first identified allele is usually referred to as the reference allele, or the wild type.

[00116] A patient's biological sample, such as a tissue or peripheral blood sample, may be analyzed for levels of TNF- α and IL-10 in determining susceptibility to infection by cytosolic pathogens. Methods for the analysis of TNF- α and IL-10 are known to those of skill in the art. For example, TNF- α and IL-10 antibodies or antibody fragments may be utilized in immunoassays such as ELISA, immunohisto- or immunocytochemistry or western blot. For a general review of immunoassays, see also *Methods in Cell Biology Volume 37: Antibodies in Cell Biology*, Asai, ed. Academic Press, Inc. New York (1993); and *Basic and Clinical Immunology 7th Edition*, Stites &

Terr, eds. (1991). It will be appreciated that monitoring of TNF- α and IL-10 levels can also be accomplished through other means well known to those skilled in the art which are not listed herein. For example PCR (polymerase chain reaction) techniques can be used to detect mRNA or DNA expression. Additionally, flow cytometry can be used to detect intracellular TNF- α and IL-10.

[00117] In a preferred embodiment, an ELISA is utilized. An "ELISA" is an enzyme-linked immunosorbent assay. In typical embodiments herein, an antigen (e.g., TNF- α or IL-10 present in a patient and which is optionally linked or adsorbed onto a surface) is detected through specific interaction with a labeled antibody (or antibodies) specific for the antigen. Those of skill in the art will be familiar with various variations of ELISA which are optionally utilized in embodiments herein.

[00118] Polyclonal murine antibodies to TNF are disclosed by Cerami et al. (EPO Patent Publication 0212489, Mar. 4, 1987). Rubin et al. (EPO Patent Publication 0218868, Apr. 22, 1987) discloses murine monoclonal antibodies to human TNF, the hybridomas secreting such antibodies, methods of producing such murine antibodies, and the use of such murine antibodies in immunoassay of TNF. Yone et al. (EPO Patent Publication 0288088, Oct. 26, 1988) discloses anti-TNF murine antibodies, including mAbs, and their utility in immunoassay diagnosis of pathologies.

[00119] Detection kits for IL-10 determination are available from, e.g., R & D Systems, Inc. (Minneapolis, Minn.), Endogen (Pierce Biotechnology, Inc., Rockford, Ill.), and Biosource International (Camarillo, Calif.) among others. For example, R & D Systems' Quantikine[R] HS Human IL-10 assay has found a sample serum/plasma range value of between "non-detectable" and 5.16 pg/mL (n=40, mean of detectable samples=2.00). See, kit package insert.

[00120] A method for treating a patient infected with a cytosolic pathogen is also provided. The method comprises administering to said patient an effective amount of a compound or agent that upregulates expression of a human homologue of *Ipr1* (e.g. *SP110b* (SEQ ID NO:1)).

[00121] Any compound or agent that upregulates expression of a human homologue of *Ipr1* can be used in methods of the invention. In one preferred embodiment, the agent that upregulates gene expression is interferon γ , interferon α , or

interferon β . The portion of the human homologue of *Ipr1* that upregulates TNF- α production is particularly useful.

[00122] The methods of the invention are useful both for patients that innately lack expression of the *Ipr1* human homologue (e.g. . *SP110b*) and for patients that suffer a decrease in expression, such as those that have been chronically infected with a pathogen. For example, in tuberculosis, the expression of the *Ipr1* gene is initially upregulated upon infection with the pathogen, however, during chronic infection expression of the *Ipr1* gene is downregulated. Accordingly, compounds or agents that prevent downregulation of *Ipr1* gene expression are also useful for treating patients. In addition, compounds that block inhibitors of *Ipr1* human homologue activity are also contemplated for use in treatment of infection by cytosolic pathogens.

[00123] Compounds or agents that induce expression of a human homologue of *Ipr1* (e.g. *SP110b* (SEQ ID NO:1)) can be determined using assays well known to those skilled in the art. For example, cell based assays using reporter gene constructs containing the *Ipr1* promoter (or *Ipr1* human homologue promoter) can be used to identify compounds which modulate expression of the *Ipr1* gene and its human homologue.

[00124] The present invention also provides assays for screening for compounds or agents that prevent downregulation of the *Ipr1* gene (or its human homologue). For example, gene expression can be monitored in cells that have been infected with tuberculosis, in the presence or absence of a test compound.

[00125] The present invention also provides assays for testing of compounds or agents that upregulate *Ipr1* function or activity. These compounds or agents may activate the *Ipr1* mediated pathway. The assays involve the detection of gene expression levels of genes that are modulated by *Ipr1* (or its human homologue). For example, expression of genes that are directly regulated by *Ipr1* or genes whose gene products interact with *Ipr1* (or its human homologue).

[00126] The “compounds” or “agents” of the invention can be DNA, RNA, a small organic molecule, a natural product, protein (e.g., antibody), peptide or peptidomimetic. Compounds that induce gene expression, or that prevent downregulation of expression, can be identified, for example, by screening libraries or collections of

molecules, such as, the Chemical Repository of the National Cancer Institute, as described herein or using other suitable methods.

[00127] Another source of compounds is combinatorial libraries which can comprise many structurally distinct molecular species. Combinatorial libraries can be used to identify lead compounds or to optimize a previously identified lead. Such libraries can be manufactured by well-known methods of combinatorial chemistry and screened by suitable methods, such as the methods described herein.

[00128] The molecular pathway responsible for the effects of *Ipr1* protein on pathogenic infection is presently unknown. However, *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 nuclear receptor binding (NRB) motif LXXLL. Recent evidence suggests that human SP110 protein, may function as a nuclear hormone receptor transcriptional cofactor¹⁸ and directly bind the retinoic acid receptor²³. Signaling through nuclear receptors, such as the corticosteroid receptor, retinoic acid receptor, PPARs and vitamin D plays an important role in control of various aspects of the macrophage life cycle, including differentiation, activation, response to pathogens and apoptosis²⁴.

[00129] The present invention provides *in vivo* animal models useful for identification of members of the *Ipr1* pathway. In particular, we have generated transgenic mice that expresses a full copy of the *Ipr1* cDNA on a susceptible C3HeB/FeJ background in a macrophage specific manner under control of the human scavenger receptor A promoter (SR-A) (See Example 1). Expression of a single gene of *Ipr1* restored key functions of macrophages related to the pathogenesis of tuberculosis. This mouse model can be used in methods of differential expression analysis. We have also generated congenic mice, C3H.B6-sst1 (See Example 1) that are useful for identification of members of the *Ipr1* pathway. Identification of pathway members may lead to novel targets for "*Ipr1* activation".

[00130] The present invention further provides *in vivo* animal models for screening of compounds to treat tuberculosis infection. Detailed mechanistic studies of pathogenesis of lung tuberculosis and its genetic control have been limited by the fact that in mouse models of MTB infection, necrotic lesions in the lungs are rarely found unless the mouse is rendered systemically immunodeficient. We have discovered that non-

immunodeficient C3HeB/FeJ mice (See Figure 8) have the characteristic necrotic lesion cavities present in human lung tissue as a result of tuberculosis infection. The *sst1* susceptible congenic mouse B6.C3H-*sst1* develops encapsulated necrotic lung lesions that closely resemble tuberculosis cavities in human lungs. Thus, these mice are extremely useful in studying cytosolic infections such as MTB *in vivo*, for they are the only model currently known that resembles human infection and does not require immunosuppression. These C3H mice can be used to screen for compounds or agents that penetrate the cavity and kill the bacteria inside the cavity. Furthermore, these mice can be used to study the progression of cytosolic infections,

[00131] In addition, the C3H mice can be used to generate transgenic mice that express human *Ipr1* homologs. These mice can be used to screen for and to test compounds that modulate *Ipr1* expression or function.

[00132] A C3H.B6-*sst1* (*sst1* resistant) congenic mouse strain has been generated by transferring an approximately 20 cM chromosomal segment containing the *sst1* resistant allele on C3HeB/FeJ genetic background and as described in Example 1. This strain express the *Ipr1* gene under control of its own promoter and, therefore, the *Ipr1* gene expression is regulated in a physiologically relevant manner. Along with the *Ipr1* transgenic mice, which express the *Ipr1* gene in macrophage-specific manner, the C3H.B6-*sst1* congenic strain is useful for analysis of the *Ipr1* gene function, characterization of *Ipr1*-mediated effects on immune functions and identification of other proteins and genes, whose function is affected by the *Ipr1* gene.

[00133] We have further generated a congenic strain that does not express the *Ipr1* gene, C57BL/6J.C3H-*sst1* (susceptible) (See Example II). This strain is particularly useful for studies of *Ipr1* gene function. For example, this strain can be used for generation of transgenic mice that express human homologue of the *Ipr1* gene to study its function, as well as for screening and testing of potential activators and inhibitors of the human *Ipr1* homologue in a whole animal. In addition, these mice are more resistant to infection by cytosolic pathogens (e.g. tuberculosis) than the C3HeB/FeJ parental mice, which also do not express the *Ipr1* gene. Thus, this mouse model can be used to identify compensatory immune mechanisms that function in the absence of the *Ipr1* gene product.

[00134] The C57BL/6J.C3H-*sst1* strain is particularly useful as an animal model for chronic pulmonary tuberculosis because the mice develop tuberculosis cavities in their

lungs. Accordingly, this mouse model can be used for screening of anti-tuberculosis drugs, for testing of treatment regimens and vaccines, as well as for the development of methods of immunomodulation.

[00135] The invention also provides a method for treating a patient infected with a cytosolic pathogen comprising administering to said patient an effective amount of Ipr1 protein human homologue (e.g. SP110b protein).

[00136] Polypeptides (e.g. SP110b protein), fragments and derivatives thereof can be obtained by any suitable method. For example, polypeptides can be produced using conventional recombinant nucleic acid technology such as DNA or RNA, preferably DNA. Guidance and information concerning methods and materials for production of polypeptides using recombinant DNA technology can be found in numerous treatises and reference manuals. See, e.g., Sambrook et al, 1989, *Molecular Cloning - A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Press; Ausubel et al. (eds.), 1994, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc.; Innis et al. (eds.), 1990 *PCR Protocols*, Academic Press.

[00137] Alternatively, polypeptides (e.g. SP110b) or fragments thereof can be obtained directly by chemical synthesis, e.g., using a commercial peptide synthesizer according to vendor's instructions. Methods and materials for chemical synthesis of polypeptides are well known in the art. See, e.g., Merrifield, 1963, "Solid Phase Synthesis," *J. Am. Chem. Soc.* 83:2149 -2154.

[00138] A preformed polypeptide (e.g. SP110b) can be introduced into a cell using conventional techniques for transporting proteins into intact cells, e.g., by fusing the polypeptide to the internalization peptide sequence derived from Antennapedia (Bonfanti et al., *Cancer Res.* 57:1442-1446) or to a nuclear localization protein such as HIV tat peptide (U.S. Pat. No. 5,652,122). In some embodiments, when using SP110b polypeptide, SP 140 is concurrently introduced or co-expressed in a cell so that SP140 can recruit SP110b into nuclear bodies. See WO 02008383, which is herein incorporated by reference in its entirety.

[00139] Alternatively, the polypeptide (e.g. SP110b) can be expressed in the cell following introduction of a DNA encoding the protein (e.g. SP110b -encoding DNA), e.g. in a conventional expression vector or by a catheter or by ex vivo transplants.

[00140] In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Pat. No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

[00141] In a specific embodiment, viral vectors that contain nucleic acid sequences encoding the Ipr1 human homologues of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the Ipr1 human homologue to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg,

Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

[00142] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Another preferred viral vector is a pox virus such as a vaccinia, for example an attenuated vaccinia such as Modified Virus Ankara (MVA) or NYVAC, an avipox such as fowl pox or canary pox. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143-155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In one preferred embodiment, adenovirus vectors are used. In another embodiment, lentiviral vectors are used, such as the HIV based vectors described in U.S. Patent Nos. 6,143,520; 5,665,557; and 5,981,276, which are herein incorporated by reference.

[00143] Use of Adeno-associated virus (AAV) vectors are also contemplated (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Pat. No. 5,436,146).

[00144] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[00145] U.S. Pat. No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Pat. Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA

into cells and mammals. U.S. Pat. Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals. Such cationic lipid complexes or nanoparticles can also be used to deliver protein. The protein will preferably contain a nuclear localization sequence.

[00146] For general reviews of the methods of gene and protein therapy, see Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression*, A Laboratory Manual, Stockton Press, NY (1990).

Delivery of Therapeutics (Compound)

[00147] The compounds of the present invention are administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight and other factors known to medical practitioners. The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the art. The amount must be effective to achieve improvement including, but not limited to, improved survival rate or more rapid recovery, or improvement or elimination of symptoms and other indicators as are selected as appropriate measures by those skilled in the art.

[00148] It should be noted that it can be administered as a compound or as a pharmaceutically acceptable salt and can be administered alone or as an active ingredient in combination with pharmaceutically acceptable carriers, diluents, adjuvants and vehicles.

[00149] It is also noted that humans are treated generally longer than the mice or other experimental animals exemplified herein, which treatment has a length proportional to the length of the disease process and drug effectiveness. The doses may be single doses or multiple doses over a period of several days, but single doses are preferred.

[00150] When administering the compound of the present invention parenterally, it will generally be formulated in a unit dosage injectable form (e.g., solution, suspension, emulsion). The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

[00151] Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Non-aqueous vehicles such as cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, may also be used as solvent systems for compound compositions. Additionally, various additives which enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, e.g., parabens, chlorobutanol, phenol and sorbic acid. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the compounds.

[00152] Sterile injectable solutions can be prepared by incorporating the compounds utilized in practicing the present invention in the required amount of the appropriate solvent with various of the other ingredients, as desired.

[00153] A pharmacological formulation of the present invention can be administered to the patient in an injectable formulation containing any compatible carrier, such as various vehicles, adjuvants, additives, and diluents; or the compounds utilized in the present invention can be administered parenterally to the patient in the form of slow-release subcutaneous implants or targeted delivery systems such as monoclonal antibodies, vectored delivery, iontophoretic, polymer matrices, liposomes, and microspheres. Examples of delivery systems useful in the present invention include those

presented in U.S. Pat. Nos: 5,225,182; 5,169,383; 5,167,616; 4,959,217; 4,925,678; 4,487,603; 4,486,194; 4,447,233; 4,447, 224; 4,439,196 and 4,475,196. Other such implants, delivery systems, and modules are well known to those skilled in the art.

[00154] A pharmacological formulation of the compound utilized in the present invention can be administered orally to the patient. Conventional methods such as administering the compounds in tablets, suspensions, solutions, emulsions, capsules, powders, syrups and the like are usable. Known techniques that deliver the compound orally or intravenously and retain the biological activity are preferred.

[00155] In another embodiment, the pharmaceutically acceptable formulations comprise lipid-based formulations. Any of the known lipid-based drug delivery systems can be used in the practice of the invention. For instance, multivesicular liposomes, multilamellar liposomes and unilamellar liposomes can all be used so long as a sustained release rate of the encapsulated active compound can be established. Methods of making controlled release multivesicular liposome drug delivery systems are described in PCT Application Publication Nos: WO 9703652, WO 9513796, and WO 9423697, the contents of which are incorporated herein by reference.

[00156] The composition of the synthetic membrane vesicle is usually a combination of phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used.

[00157] Examples of lipids useful in synthetic membrane vesicle production include phosphatidylglycerols, phosphatidylcholines, phosphatidylserines, phosphatidylethanolamines, sphingolipids, cerebroside, and gangliosides, with preferable embodiments including egg phosphatidylcholine, dipalmitoylphosphatidylcholine, distearoylphosphatidyletholine, dioleoylphosphatidylcholine, dipalmitoylphosphatidylglycerol, and dioleoylphosphatidylglycerol.

[00158] In preparing lipid-based vesicles containing an active compound such variables as the efficiency of active compound encapsulation, labiality of the active compound, homogeneity and size of the resulting population of vesicles, active compound-to-lipid ratio, permeability, instability of the preparation, and pharmaceutical acceptability of the formulation should be considered.

[00159] Prior to introduction, the formulations can be sterilized, by any of the numerous available techniques of the art, such as with gamma radiation or electron beam sterilization.

[00160] When the agents are delivered to a patient, they can be administered by any suitable route, including, for example, orally (e.g., in capsules, suspensions or tablets) or by parenteral administration. Parenteral administration can include, for example, intramuscular, intravenous, intraarticular, intraarterial, intrathecal, subcutaneous, or intraperitoneal administration. The agent can also be administered orally, transdermally, topically, by inhalation (e.g., intrabronchial, intranasal, oral inhalation or intranasal drops) or rectally. Administration can be local or systemic as indicated. Agents can also be delivered using viral vectors, which are well known to those skilled in the art.

[00161] The pharmaceutically acceptable formulations can be suspended in aqueous vehicles and introduced through conventional hypodermic needles or using infusion pumps.

[00162] Preferred approaches for sustained delivery include use of a polymeric capsule, a minipump to deliver the formulation, a bioerodible implant, or implanted transgenic autologous cells (as described in U.S. Patent No. 6,214,622). Implantable infusion pump systems (such as Infusaid; see such as Zierski, J. et al, 1988; Kanoff, R.B., 1994) and osmotic pumps (sold by Alza Corporation) are available in the art. Another mode of administration is via an implantable, externally programmable infusion pump. Suitable infusion pump systems and reservoir systems are also described in U.S. Patent No. 5,368,562 by Blomquist and U.S. Patent No. 4,731,058 by Doan, developed by Pharmacia Deltec Inc.

[00163] It is to be noted that dosage values may vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the active compound and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed invention.

[00164] The amount of agent administered to the individual will depend on the characteristics of the individual, such as general health, age, sex, body weight and

tolerance to drugs as well as the degree, severity and type of rejection. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. Typically, an effective amount can range from about 0.1 mg per day to about 100 mg per day for an adult. Preferably, the dosage ranges from about 1 mg per day to about 100 mg per day.

[00165] Antibodies and antigen-binding fragments thereof, particularly human, humanized and chimeric antibodies and antigen-binding fragments can often be administered less frequently than other types of therapeutics. For example, an effective amount of such an antibody can range from about 0.01 mg/kg to about 5 or 10 mg/kg administered daily, weekly, biweekly, monthly or less frequently.

Kits

[00166] The present invention is also directed to commercial kits for the detection of *Ipr1* gene human homologue expression (e.g. *SP110b*). The kit can be in any configuration well known to those of ordinary skill in the art and is useful for performing one or more of the methods described herein for the detection of gene expression. The kits are convenient in that they supply many if not all of the essential reagents for conducting an assay for the detection of a *Ipr1* homologue (e.g. *SP110b*) in a sample. In addition, the assay is preferably performed simultaneously with a standard or multiple standards that are included in the kit, so that the results of the test can be quantitated or validated.

[00167] The kits include a means for detecting gene expression levels such as antibodies, or antibody fragments, which selectively bind to the *Ipr1* human homologue protein or DNA nucleotide primers suitable for use in methods of detecting mRNA.

[00168] In other embodiments, the assay kits may employ (but are not limited to) the following techniques: competitive and non-competitive assays, radioimmunoassay (RIA), bioluminescence and chemiluminescence assays, fluorometric assays, sandwich assays, immunoradiometric assays, dot blots, enzyme linked assays including ELISA, and immunocytochemistry. For each kit the range, sensitivity, precision, reliability, specificity and reproducibility of the assay are established by means well known to those skilled in the art. The above assay kits would further provide instructions for use.

[00169] It is understood that the foregoing detailed description and the following examples are illustrative only and are not to be taken as limitations upon the scope of the

invention. Various changes and modifications to the disclosed embodiments, which will be apparent to those skilled in the art, may be made without departing from the spirit and scope of the present invention. Further, all patents, patent applications and publications cited herein are incorporated herein by reference.

Definitions

[00170] The following definitions are provided for specific terms which are used in the following written description.

[00171] As used herein, the term “administering” to a patient includes dispensing, delivering or applying an active compound in a pharmaceutical formulation to a subject by any suitable route for delivery of the active compound to the desired location in the subject, including delivery by either the parenteral or oral route, intramuscular injection, subcutaneous/intradermal injection, intravenous injection, buccal administration, transdermal delivery and administration by the rectal, colonic, vaginal, intranasal or respiratory tract route.

[00172] As used herein, “effective amount” of an agent is an amount sufficient to achieve a desired therapeutic or pharmacological effect, such as an amount sufficient to inhibit the multiplication of a pathogen (e.g. tuberculosis). An effective amount of an agent as defined herein may vary according to factors such as the disease state, age, and weight of the subject, and the ability of the agent to elicit a desired response in the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. An effective amount is also one in which any toxic or detrimental effects of the active compound are outweighed by the therapeutically beneficial effects.

[00173] A therapeutically effective amount or dosage of an agent may range from about 0.001 to 30 mg/kg body weight, with other ranges of the invention including about 0.01 to 25 mg/kg body weight, about 0.1 to 20 mg/kg body weight, about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, and 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the infection, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of an active compound can include a single treatment or a series of treatments. In one example, a subject is treated with an agent in the range of between about 0.1 to 20 mg/kg body

weight, one time per week for between about 1 to 10 weeks, alternatively between 2 to 8 weeks, between about 3 to 7 weeks, or for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of an agent used for treatment may increase or decrease over the course of a particular treatment.

[00174] As used herein, the term “patient” or “subject” or “animal” or “host” refers to any mammal. The patient is preferably a human, but can also be a mammal in need of veterinary treatment, e.g., domestic animals (e.g., dogs, cats, and the like), farm animals (e.g., cows, sheep, fowl, pigs, horses, and the like) and laboratory animals (e.g., rats, mice, guinea pigs, and the like).

EXAMPLES

[00175] *Animals.*

[00176] C57BL/6J, C3HeB/FeJ, C3H/HeJ, C3H/HeOuJ, and C3H/HeSnJ inbred mice were obtained from The Jackson Laboratory (Bar Harbor, Maine). 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 an approximately 20 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 10 backcrosses. Importantly, the congenic interval transferred from the B6 resistant background did not include the *Slc11a1* gene (formerly known as *Nramp1*), which is located at 39.2 cM. Thus, the *sst1* resistant congenic mouse strain C3H.B6-*sst1* 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 10 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) promoter²⁹ on the C3HeB/FeJ genetic background.

[00177] *Infection of mice with MTB.*

[00178] For i.v. infection 1×10^5 of live MTB were injected via tail vein in 100 μ l of PBS. Aerosol infections were performed using aerosol apparatus manufactured by the College of Engineering Shops at the University of Wisconsin (Madison, WI). Mice were exposed to aerosol for 20 min, which resulted in the deposition of 15 to 30 CFU per

mouse. Mice were sacrificed using halothane anesthesia. Organs were homogenized in PBS containing 0.05% Tween 80 and serial 10-fold dilutions were cultured on 7H10 agar enriched with 10% OADC (Difco, MI) for 3 weeks at 37°C. Virulent *Mycobacterium tuberculosis* (MTB) Erdman strain and avirulent vaccine strain of *Mycobacterium bovis* BCG (BCG) Pasteur strain were used some studies.

[00179] *Analysis of chemokine mRNA expression in vivo*

[00180] To isolate total RNA from the organs of MTB-infected mice, organs were snap frozen in liquid nitrogen and stored at -80 °C. Frozen organs were homogenized in cold TRIZOL reagent (Invitrogen) using tissue homogenizer PRO 200 (Pro Scientific, Inc). Total RNA was isolated according to manufacturer's instructions and then treated with RNAase-free DNase and re-purified using RNeasy Mini Kit (Qiagen). Total RNA was reverse transcribed using SuperScript II, and amplified with pairs of chemokine-specific primers, which were designed using Primer 3.0 software (primer sequences are available upon request). For semi-quantitative analysis PCR was performed in the presence of ³²P-labelled dCTP (2 µCi per 40 µl reaction). QuantumRNA 18S Internal Standards kit (Ambion, TX) was used to simultaneously amplify 18S rRNA. Radiactively labelled PCR products were separated on acrylamide gel. The gels were dried and incorporated radioactivity was quantitated by PhosphorImager Storm 860 (molecular Dynamics) using ImageQuant software.

[00181] *Isolation of murine bone marrow-derived macrophages (BMDM) in vitro.*

[00182] BMDM were isolated from femurs and tibias of male C3H, C3H.B6.*sst1* and C3H.Tg (*Ipr1*) mice (6 to 8 weeks old) and depleted of RBC and neutrophils by gradient centrifugation using Nycodenz. Cells were grown in a complete culture medium 1:1 mix of Dulbecco's modified Eagle's medium (DMEM) and Ham F-12 (CellGro, MediaTech, Inc., Herndon, VA) supplemented with 10% of Fetal Calf Serum (FCS, Hyclone Logan, UT), rIL-3 at 1 ng/ml (Sigma, St. Louis, MO) and 20% L-929 fibroblast-conditioned medium as a source of macrophage colony stimulating factor in 75 cm² tissue culture flasks (Costar, OJO) for 2 days to deplete mature adherent cells and enrich for macrophage precursors. To determine MTB growth in macrophages, cells were collected and seeded onto circular 12-mm-diameter glass cover slips placed in a 60-mm-diameter Petri dish and differentiated in macrophages in media containing 20% L929-conditioned

medium for 4 - 6 days, and maintained in complete medium containing 5-10% of L929-conditioned medium. For cell death experiments and intracellular cytokine staining, cells were differentiated into macrophages in 6-wells plates and infected.

[00183] *Isolation and infection of murine bone marrow-derived macrophages (BMDM) with MTB and L. monocytogenes (LM) in vitro.*

[00184] BMDM were isolated from femurs and tibias of male C3H, C3H.B6-*sst1* and C3H-TgN(SRA-*Ipr1*) mice (6 to 8 weeks old) and infected with LM strain 10403S as previously described³⁰. Macrophage monolayers were infected at multiplicity of infection 1 MTB Erdman per 10 macrophages (MOI 1:10). After 6h the cells were washed with PBS containing 1% FCS (PBS-1% FCS). The cells were incubated in complete medium containing 10% FCS and three coverslips were removed from the culture at indicated time points and separately lysed with 0.1% Triton X-100. Serial 10-fold dilutions of cell lysates were plated on 7H10 agar containing OADC and incubated for 3 weeks at 37°C.

[00185] *Differentiation of apoptotic and necrotic pathway of macrophage cell death.*

[00186] Macrophages were infected at MOI 1:10. At indicated time points, cells were stained with 10 nM DiOC₆ (Molecular Probes) and 0.8 mM Ethidium Bromide (Sigma) for 20 min at 37°C, washed three times with PBS, fixed with 1% paraformaldehyde 20 min and washed once again with PBS. Cells were analyzed using BD FACScan flow cytometer (BD Biosciences) to differentiate between live (DiOC₆^{high}EB⁻); apoptotic (DiOC₆^{low}EB⁻) and necrotic(EB⁺) cells. For Annexin V staining cells were incubated in Annexin binding buffer (10 mM Hepes, 140 mM NaCl and 2.5 mM CaCl₂) and stained with 10 µl Annexin V-Alexa 488 (Molecular Probes) for 20 min, then counterstained with propidium iodide (PI, 1 µg/ml), washed with cold PBS twice, fixed with 1% paraformaldehyde for 30 min, and washed once with PBS. FACS analysis was used to differentiate between early apoptotic (Annexin V+ PI-), late apoptotic (Annexin V+ PI+) and necrotic (Annexin V- PI+) cells.

[00187] *Isolation and analysis of the Ipr1 cDNA.*

[00188] The *Ifi75*-specific oligonucleotide primers (F1-2 and R1-3) are presented in Fig. 2c. RACE-PCR was performed using the SMART RACE cDNA Amplification Kit (BD Clontech). The cDNAs were synthesized from the lungs of *M.*

tuberculosis–infected C3HeB/FeJ and C3H.B6-*sst1* mice. The RACE amplification products were purified using PCR purification columns (Qiagen), cloned into the plasmid vector pGEM-T (Promega) and sequenced using T7 and SP6 primers. A full length sequence of *Ipr1* was confirmed by sequencing the “end-to-end” PCR product obtained using the F1 and R3 primers.

[00189] *Statistical analysis.*

[00190] Statgraphics Plus, release 4, 1999 (Statgraphics Corp., Rockville, MD) and GraphPad Prism 3.0 (GraphPad, CA) software were used for the analysis. Comparison of bacterial loads was performed using Student's *t*-test. Results are presented as the mean $\pm$ SD. A threshold for statistical significance was $p < 0.05$. Kaplan Meier Survival curves were generated and compared using the log-rank test (GraphPad Prism). Intracellular bacterial growth and cell death were analysed by two factors ANOVA (time, genetic backgrounds and experiment). The statistical significance was tested at $p < 0.05$ as critical value using the Student-Newman-Keuls post-test to compare means between both genetic backgrounds. Data are presented as the mean $\pm$ 95% confidence interval for mean (95% CI).

[00191] *Genotyping.*

[00192] DNA was isolated from the tail tips by standard procedure³².

Genotyping for simple sequence length polymorphism markers polymorphic between C57BL and C3H³³ was performed by PCR with pairs of oligonucleotide primers (Mouse MapPairs) obtained from Research Genetics (Huntsville, AL). The PCR mix contained 1X GeneAmp PCR Buffer II, 2.5 mM MgCl₂, 200 μ M of dNTP, 0.4 units of AmpliTaq Gold DNA polymerase (all obtained from Perkin–Elmer), 0.22 μ M of forward and reverse primers, and 1X Rediload dye (Research Genetics). Five microliters of a 20-fold dilution of the stock mouse tail DNA in sterile deionized water was used per reaction. PCRs were performed in 384-well plates (Robbins Scientific, Mountain View, CA) in a final volume of 11 μ l using an Thermocycler PTC-100 (MJ Research, Watertown, MA) with a heated lid as follows: 95°C for 10 min, followed by 35 cycles of 94°C for 20 sec, 50°C for 30 sec, 72°C for 40 sec, and a final step at 60°C for 10 min. Four microliters of reaction was loaded directly on 4% MetaPhor agarose gel (FMC) and run in 1X TBE buffer (90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3) at 10 V/cm with cooling

and recirculation for 30–45 min. The bands were visualized with ethidium bromide staining.

[00193] *Gene sequence resources.*

[00194] A total of 22 genes in the *sst1* critical region were defined according to Ensembl (www.ensembl.org/Mus_musculus/) and Celera (www.celeradiscoverysystem.com) databases of mouse genome including those identified by experiments or predicted from the genome sequences (Figure 7). Since the cDNA sequences annotated in both genome databases might have differences due to genetic polymorphisms among different inbred mouse strains and also due to alternative splicing, to design primers for RT-PCR analysis, we retrieved all related cDNA sequences for each gene from the Unigene database (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene) at National Center for Biotechnology Information (NCBI) and designed several pairs of oligonucleotide primers for each gene based on the conserved sequence obtained by multiple sequence alignment of all related cDNA sequences.

[00195] *RNA Isolation and Northern Analysis.*

[00196] RNA was isolated from total lungs with Trizol reagent (Invitrogen) followed by DNase treatment and clean-up with RNeasy columns (Qiagen). Northern blotting was carried out using NorthernMax™ kit (Ambion). Briefly, total RNA from each sample was electrophoresed on a formaldehyde agarose gel and transferred to a nylon membrane. DNA fragments amplified by PCR and purified from agarose gel were labeled with [α -³²P]dCTP using Prime-a-Gene Labeling System (Promega) to generate DNA probes. ULTRAhyb™ (Ambion) was used for hybridization according to the instructions of the manufacturer.

[00197] *RT-PCR and quantitative PCR.*

[00198] RNA (2 μ g) was reverse transcribed by random decamers and RETROscript™ kit (Ambion), and one twenty-fifth of each product was PCR amplified. Copy number of each individual exon of the *Ipr1/Ifi75* genes in genomic DNA was quantified by real time PCR with Amplifluor™ Universal Detection System (Intergen). Each sample was set up in triplicate, and a single-copy gene encoding brain-derived neurotrophic factor (*Bdnf*) was used as an internal control.

[00199] *Cloning of the Ipr1 cDNA.*

[00200] Oligonucleotide primers were prepared based on a large and most conserved region presented in most *Ifi75*-related cDNA and EST sequences in the GenBank database. The location of primers used for RACE and PCR amplification are shown in Fig. 2C and their sequences are listed below: F1 (5'- ATA ACT TCG GGT CCA GAC TGG GCT GTC AGG -3') (SEQ ID NO:21), R1 (5'- GAT CTC AAT GAT TGG CTG TGC GGT TGC TC -3') (SEQ ID NO:22), F2 (5'- AGA GCA ACC GCA CAG CCA ATC ATT GAG A -3') (SEQ ID NO:23), R2 (5'- CAC AGG TCA CAG GGA GTG TGG GAG AGA AAT C -3') (SEQ ID NO:24), R3 (5'- CAA ACG CAC AGA GAC ACC AAC AGC TTT A -3') (SEQ ID NO:25). RACE-PCR (rapid amplification of cDNA ends) was performed to isolate both 5' and 3' cDNA sequences by using the SMART RACE cDNA Amplification Kit (BD Clontech). Briefly, cDNAs with adaptor ends were synthesized from lungs of *M. tuberculosis*-infected C3HeB/FeJ and congenic C3H.B6-*sst1* mice. 5'- and 3'- RACE PCR were performed with R1 and F2 primers, respectively, in combination with the adaptor primer (BD Clontech). The RACE amplification products were purified using PCR purification columns (Qiagen), cloned into the plasmid vector pGEM-T (Promega) and sequenced by using T7 (5'- TAA TAC GAC TCA CTA TAG GG -3') (SEQ ID NO:26) and SP6 (5'- CGC CAA GCT ATT TAG GTG ACA -3') (SEQ ID NO:27) primers. After RACE PCR, a full length cDNA of *Ipr1* from infected lungs of congenic strain C3H.B6-*sst1*, 1911 bp in length, was then constructed. The full length sequence was further confirmed by sequencing the product of "end-to-end" PCR using F1 and R3 primers.

[00201] *Generation of transgenic mouse.*

[00202] The *XhoI-EcoRI* DNA fragment of chimeric human Scavenger Receptor A (SRA) silencer-enhancer-promoter was derived from plasmid pAL1 which was a generous gift of Dr Christopher Glass³⁴. A cDNA fragment containing 140-bp of the 5' untranslated region (UTR) and the complete opening reading frame (ORF) of *Ipr1* was generated by PCR with primer F18 (5'- TTG AAT TCG GGA CTT CCA AGG CAG CAT A -3' (SEQ ID NO:28), *EcoRI* site underlined) and R18 (5'- GGG CTA CTA GGC ACC CTT CTT TTG AGG TTT A -3' (SEQ ID NO:29), two stop codons underlined). For the macrophage-specific expression of *Ipr1*, the *Ipr1* cDNA was cloned into the *EcoRI* and *SmaI* (blunt end) sites 3' of the SRA promoter and 5' of the simian virus 40

polyadenylation sites (SV40pA). The 7323-bp SRA-*Ipr1*-SV40pA transgene was isolated by digestion with *SaII* and *NotI* and injected into fertilized C3HeB/FeJ oocytes. Offspring of the injection was genotyped by two runs of PCR with primer SRF1 (5'- GCT AAG ATG CTG GGT TGC TTC TC -3') (SEQ ID NO:30) and SRR1 (5'- GAC TTC TGT CTC CTG GTC TCT GTT G -3') (SEQ ID NO:31) or primer F9 (5'- AGA CAT TAA GAC ATC TGG AGC AGA AAG -3') (SEQ ID NO:32) and SV40R2 (5'- GAT GGC ATT TCT TCT GAG CA -3) (SEQ ID NO:33). Transgene-bearing founder mice were backcrossed to the C3HeB/FeJ mice. The transcription of the transgene was confirmed by RT-PCR with primers F1 (5'- ATA ACT TCG GGT CCA GAC TGG GCT GTC AGG -3') (SEQ ID NO:21) and R1 and total RNA prepared from peritoneal macrophages or bone marrow-derived macrophages. Homozygote transgenic mice were generated by intercrossing transgene-positive animals and selection for the transgene homozygotes using quantitative PCR.

[00203] *SSCP analysis.*

[00204] Genomic DNA was isolated from the tail tips as previously described³². SSCP analysis was performed using the primers specific for each of the 12 exons of *Ipr1* gene, labeled with [α -³²P]dCTP and separated on SequaGel MD (National diagnostics) according to the manufacturer's recommendations. Briefly, 1 μ L of PCR product was added per 10 μ L of stop solution, the sample was heated to 95°C for 5 min, cooled on ice and separated on a 0.6 $\times$ gel at a constant power of 7 Watts for 15 hours.

[00205] *Infection of mice with MTB.*

[00206] For i.v. infection bacteria were diluted by PBS containing 0.05% Tween 80. Mice were infected via tail vein with 1×10^5 live bacilli in 100 μ L. Aerosol infections were performed using aerosol apparatus developed by Dr. Don Smith and colleagues {Wiegshauss, 1970 #30} and manufactured by the College of Engineering Shops at the University of Wisconsin (Madison, WI). The bacteria were diluted to 1×10^6 CFU in 1 ml. The nebulizer compartment of an airborne-infection apparatus was filled with 75 ml of bacteria suspension and mice were exposed to aerosol for 20 min, followed by 40 min of circulation of clean air. A total of 15 ml of the bacterial suspension was delivered. This resulted in the deposition of 15 to 30 CFU per mouse. The initial deposit was determined in each experiment by plating lung homogenates of 6 mice 24 hours after infection. For determination of the bacterial loads in organs CFU were counted. Mice

were sacrificed using halothane anesthesia. Organs were homogenized in PBS containing 0.05% Tween 80 and serial 10-fold dilutions were plated on 7H10 agar enriched with 10% OADC (Difco, MI). Plates were incubated at 37°C and colonies were enumerated after 21 to 28 days.

[00207] *Histopathology.*

[00208] Lungs of infected mice were inflated with 10% buffered formaldehyde for more than 24 hours and embedded in paraffin, sectioned and stained with hematoxylin and eosin according to standard procedure at the Harvard rodent histopathology core facility. Histopathological sections were prepared from 3-4 animals per group at each time point. Microphotographs were taken of representative fields. Evaluation of pathologic changes was performed on coded slides by a histopathologist (LK).

[00209] *Auramine/Rhodamine staining.*

[00210] Identification of acid fast bacilli was performed on formalin-fixed tissue sections using auramine/rhodamine dye (0.1% Auramine O, 0.01% Rhodamine B (Sigma, MO) in H₂O) for 20 min at room temperature in the dark, followed by destaining with 3% HCl in 70% EtOH and counterstained in Mayer's Hematoxylin (VWR).

[00211] *Generation of radiation bone marrow chimeras*

[00212] Bone marrow cells (BMC) were flushed with cold DMEM medium (Gibco BRL) supplemented with 1% of Fetal Bovine Serum (HyClone) and antibiotics (penicillin/streptomycin, 100 U/ml and 100 µg/ml respectively, GibcoBRL) from the femurs and tibias of donor mice. A single-cell suspension was prepared by gently passing the cells by syringe through the needle 22G (Becton Dickinson, Franklin Lakes, NJ). The cells were washed by DMEM/1% FBS and nucleated cells counted. Recipient animals were irradiated twice with a dose of 6 Gy at a 4 h interval. Recipient mice were reconstituted with 2×10^6 allogeneic BMC 2 h after second irradiation.

[00213] *Bacterial strains*

[00214] *M. tuberculosis* (Erdman strain; Trudeau Institute, Saranac Lake, N.Y.) was passed through mice, grown in Dubos oleic albumin complex-enriched Middlebrook 7H9 liquid medium (Difco) containing 0.05% Tween 80 in roller bottles, pelleted by centrifugation (800 g for 10 min), and resuspended in PBS containing 0.05% Tween 80 (PBS-Tween 80). Glycerol was added to a final concentration of 10%, and the bacterial

suspension was aliquoted, frozen and stored at -80°C. Viable bacterial counts were determined by plating serial 10-fold dilutions on Middlebrook 7H10 (Difco) agar plates. An avirulent vaccine strain of *Mycobacterium bovis* BCG (Pasteur) was used in our studies and was grown and stored as described above for MTB.

[00215] *Neutralization of TNF α and IL-10 with specific antibodies.*

[00216] To block TNF α and IL-10, anti-TNF α (2.5 μ g/ml) Clone MP6-XT3 (Rat IgG1), anti-IL-10 (2.0 μ g/ml) Clone JES5-16E3 (Rat IgG2b) were added before and during *M. tuberculosis* infection. Rat IgG1 Clone R3-34 and IgG2b A95-1 were used as isotype controls for Anti-TNF α and anti-IL10 respectively. Blockade antibodies and isotype controls were purchased from BD Pharmingen, CA.

[00217] *Measurement of cytokine concentration in the macrophage culture medium.*

[00218] Cytokine (IL-10, TNF α , IL-12, IL-6, IL-1 α , IL-1 β , IP-10, KC, MCP-1, MIP1 α) concentrations were measured in 25 μ l of culture supernatant using Linco-plex array (LINCO Research, MO) and Luminex 100 instrument following manufacturer's instructions.

[00219] *Caspase 8 activation and blockade*

[00220] To detect active Caspase 8, cell cultures were stained for PI as described and washed at room temperature in calcium and Magnesium free PBS, 10 mM HEPES, 2 mM EDTA, pH 7.2, then re suspended in 500 μ l of assay buffer and incubated with 5mM solution of Caspase 8 substrate (IETD-R110, Molecular Probes, OR) for 60 minutes at 37°C in 5% CO₂, protected from light and mixed the twice during incubation to minimize cell settling. Samples were washed once with assay buffer and once with PBS, fixed with 2% PFA and analysed by FACS.

[00221] To block Caspase 8 cells were incubated with a cell-permeable irreversible specific peptide inhibitor of caspase 8 (z-IETD-fmk, Calbiochem, CA) using 5 μ M concentration of inhibitor dissolved by sonication in anhydrous DMSO. The cultures were incubated with the inhibitor 2 h before and during infection. Control incubations were performed using the same concentrations of DMSO.

[00222] *Ipr1 gene mediates innate immunity to tuberculosis*

[00223] C3HeB/FeJ inbred mice are extremely susceptible to MTB and develop dramatic lung pathology, which leads to their rapid death after infection with virulent

MTB^{10,11}. We generated a congenic mouse strain C3H.B6-*sst1* (*sst1*^R) that carries 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 intravenously (i.v.) with a high dose of MTB (Fig. 1A), or with a low dose of MTB via 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, as compared to the resistant parental strain C57BL/6J (B6), clearly indicates that the *sst1* locus is responsible for a significant portion, but not the whole, of the tuberculosis resistance phenotype of the B6 mice.

[00224] The specific effect of the *sst1* locus on progression of tuberculosis was related to more efficient control of MTB multiplication primarily in the lungs after both respiratory challenge by aerosolized MTB (Fig. 1C), and systemic i.v. infection (Fig. 5A). The development of large necrotic lung lesions within 4 weeks after i.v. 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 approximately one third of the lung lobe (Fig. 1E), which resembled tuberculosis cavities in human lungs. Mycobacterium were present both extracellularly within necrotic central areas surrounded by the fibrotic capsule as well as within macrophages of the granuloma wall (Fig. 10). In the *sst1*^R mice, lung lesions were much smaller and contained fewer macrophages infected with MTB.

[00225] Although the most dramatic effect of the *sst1* polymorphism on progression of tuberculosis was observed in the lungs; bone marrow transplantation experiments demonstrated that bone marrow-derived, but not lung cells, were responsible for the effect of the *sst1* locus (Fig. 5B). It is known that T lymphocytes and macrophages play a major role in host resistance to tuberculosis. We have found that, while T lymphocytes are functionally unaffected by the *sst1* polymorphism (Jobe, in preparation), the *sst1* disparate macrophages display considerable differences in their ability to control MTB *in vitro*. The rate of MTB multiplication was significantly higher in the *sst1*^S macrophages (Fig. 1G, left panel). There was also a clear distinction in mechanism of macrophage cell death after the infection: the *sst1*^S macrophages showed characteristic necrosis, while the *sst1*^R macrophages underwent apoptosis (Fig. 1F, upper

left and upper right panels, respectively). The effect of the *sstI* locus was much more pronounced upon macrophage infection with virulent MTB, since avirulent vaccine strain of *M. bovis* BCG failed to multiply in (Fig. 1G, right panel) and to induce necrosis of the *sstI*^S macrophages (Fig. 1F, lower left panel).

[00226] *In vivo*, many cells within the tuberculosis lung granulomas of the *sstI*^R mice contained TUNEL-positive apoptotic nuclei and no necrosis was observed, while the apoptotic nuclei were largely absent from the necrotic lesions of the *sstI*^S mice (Figure 8). Thus our studies, both *in vivo* and *in vitro*, indicated that the extreme susceptibility to virulent MTB of *sstI*^S mice was associated with necrotic death of the susceptible macrophages. Virulent MTB was shown to cause necrosis of the infected epithelial cell lines¹². This necrosis-inducing propensity is specific for virulent MTB and is lost in RD1 mutants of MTB that have also dramatically decreased virulence^{12,13}. It appears from our studies 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 *sstI* locus.

[00227] To identify the critical gene(s), we employed a positional cloning strategy (see section under the heading Identification of a candidate gene, *Ipr1*, within the *sstI* locus and Fig. 6 for details). First, the *sstI* minimal region was reduced to an interval between D1Mit439 and D1Mit49 markers on mouse chromosome 1 (Fig. 6A). 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 and 6 Mb^{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 *sstI* candidate region encompasses part of the HSR repeat region and a region of mouse chromosome 1 immediately downstream of the repeat, i.e. between the repeat region and the *NppC* gene. A total of 22 known and predicted genes are encoded within the *sstI* critical region according to Ensembl and Celera databases of the mouse genome (Figure 7). It was impossible further to reduce the *sstI* critical region by genetic recombination. Therefore, in the next step we tested expression of each of the *sstI*-encoded candidate genes in the lungs during tuberculosis infection *in vivo* and in macrophages infected with MTB *in vitro* using RT-PCR and RACE. The *Ifi75* gene appeared from our studies as the most likely candidate (Fig. 6B and Fig. 6E).

[00228] As shown in Fig. 2B, the 5' RACE products of the *Ifi75* in the lungs of tuberculosis-infected mice was strikingly different between the *sst1* congenic strains: a major single band was amplified from the lungs of the *sst1* resistant mice, whereas this band was absent from the lungs of the *sst1* susceptible strain and, instead, multiple weak products were obtained. Although some aberrant transcripts were present in the lung tissue of the *sst1*^R animals as well, the majority of the *Ifi75*-related transcripts in their tuberculosis lung lesions were represented by a single isoform, which we named as *Ipr1* (for intracellular pathogen resistance) 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 the *Mus caroli* *Ifi75*. It contains an Sp100-like domain in its N-terminus, LXXLL-type nuclear receptor binding motif (NRB), bipartite nuclear localization signal (NLS), and a SAND domain in its C-terminus (Fig. 2C).

[00229] Using DNA probes specific for the Sp100 and SAND domains of the *Ipr1*, we have analyzed the kinetics of its expression by Northern hybridization in the lungs of the *sst1* congenic mouse strains during progression of tuberculosis (Fig. 2D). Expression of the *Ipr1* gene was detectable in the lungs of the naive *sst1*^R mice, and its expression increased significantly 2 weeks after intravenous infection with MTB and remained at elevated levels 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 region, *Sp100-rs*, was elevated in the lungs of the *sst1*^S mice (Fig. 2D).

[00230] To investigate expression of the *Ipr1* gene in macrophages, we used five overlapping combinations of the *Ipr1*-specific PCR primers that cover the full length *Ipr1* transcript (Fig. 2E). The *Ipr1* gene was expressed in non-activated *sst1*^R macrophages and the level of its expression increased after infection with both avirulent BCG and virulent MTB. No expression of the full length transcript of the *Ipr1* gene 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, while those from the *sst1*^S mice did not (data not shown). We were unable to detect additional *Ipr1*-related transcripts induced in macrophages upon infection *in vivo* and *in vitro* or stimulation with IFN- γ . Despite the fact that the *Ipr1* gene is encoded within the

HSR repeat region, our data suggest that a single major isoform of this gene is expressed in *sstI^R* macrophages either before or during tuberculosis infection and this isoform is not expressed in the *sstI^S* C3HeB/FeJ mice.

[00231] 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 after the infection displaying severe lung pathology. In our experiments, the survival time of other substrains of C3H was considerably longer and similar to the *sstI^R* congenic strain C3H.B6-*sstI* (Fig. 3A). The bacterial loads in the lungs of the C3HeB/FeJ mice at 3 weeks post infection were 50 to 100 fold higher as compared to other substrains of C3H and the *sstI^R* congenics (Fig. 3B), suggesting a unique allele at the *sstI* locus. We compared expression of the *sstI*-encoded candidate genes in the lungs of mice of four C3H substrains and the *sstI^R* congenics and found that the lack of expression of the *Ipr1* gene differentiated the C3HeB/FeJ from all other substrains of C3H (Fig. 3C). Since all the C3H substrains originate from a common ancestor¹⁷, it is likely that they are genetically identical within the *sstI* region and a unique *de novo* mutation has led to the defect of the *Ipr1* gene expression in a C3HeB/FeJ mice and is responsible for a severe defect in their tuberculosis resistance.

[00232] 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 control of the human scavenger receptor A promoter (SR-A). Mature bone marrow-derived macrophages, as well as resident peritoneal macrophages obtained from those mice, expressed the *Ipr1* transgene (Fig. 4A). Despite the fact that the regulation of *Ipr1* gene 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 MTB, a statistically significant difference in the bacterial loads between the *sstI^S* (Tg -/-) and the *Ipr1* transgenic (Tg +/-) animals was observed in the lungs (Fig. 4B). *In vitro*, the *Ipr1* transgenic macrophages also controlled multiplication of MTB more effectively (Fig. 4C) and turned on the apoptotic pathway of cell death upon interaction with virulent MTB (Fig. 4D, right panels). The growth of another intracellular pathogen, *L. monocytogenes*, was dramatically suppressed in the *Ipr1* transgenic macrophages by 50 to 100 fold (Fig. 4E). Similar to the MTB infection, necrotic death accompanied infection of the *sstI^S* macrophages with virulent *L.*

monocytogenes (Fig. 4F, left panel), while the *Ipr1* transgenic macrophages displayed markers of apoptotic death (Fig. 4F, right panel).

[00233] Thus expression of a single gene, *Ipr1*, in the *sst1^S* macrophages restored key functions related to pathogenesis of tuberculosis, which are encoded within the *sst1* locus: greater control of multiplication of virulent MTB *in vivo* and *in vitro* as well as an apoptotic mechanism of MTB-induced macrophage cell death. Moreover, the *Ipr1* gene mediated macrophage resistance to another intracellular pathogen, *L. monocytogenes*, suggesting that the *Ipr1* product controls a common mechanism of innate resistance against several intracellular pathogens.

[00234] The closest homologue of the predicted Ipr1 protein in humans (41% of identity) is SP110b¹⁸, which localizes to a region of human chromosome 2 syntenic with the *sst1* minimal region on mouse chromosome 1. Both the 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 nuclear receptor binding (NRB) motif LXXLL. Recent evidence suggests that human SP110 protein, may function as a nuclear hormone receptor transcriptional cofactor¹⁸ and directly bind the retinoic acid receptor²³. Signaling through nuclear receptors, such as the corticosteroid receptor, retinoic acid receptor, PPARs and vitamin D plays an important role in control of various aspects of the macrophage life cycle, including differentiation, activation, response to pathogens and apoptosis²⁴. Expression of both the *Ipr1* gene and its human homologue SP110, is regulated by interferons²⁵, additionally implicating a role in immunity in both species. Moreover, polymorphisms in *SP110* gene have been associated with susceptibility to the Hepatitis C virus²⁶, and the SP110b protein has been shown to physically interact with viral proteins, such as Epstein-Barr virus SM protein and Hepatitis C virus core protein^{23,27}. It is an intriguing possibility that the Ipr1 and SP110 proteins mediate cross talk between nuclear receptors, interferon signaling and pathogens. The viruses and, perhaps, intracellular pathogens might have evolved mechanisms to interfere with or exploit the *Ipr1*/SP110 function. Taken together, these data show that in mammals, since no *Ipr1* homologues were found in yeasts or insects, the Ipr1-related proteins play a novel role in integrating signals generated by intracellular pathogens or viruses with mechanisms regulating activation, gene expression and cell death of host cells²⁸.

[00235] *Identification of a candidate gene, Ipr1, within the sst1 locus.*

[00236] *Fine mapping of the sst1 locus.*

[00237] Previously we have determined that the B6-derived *sst1*^R allele was dominant. Therefore, for fine mapping of the *sst1* locus we analyzed progeny of males that carried recombinant chromosome 1 backcrossed to the *sst1*-susceptible parental strain C3HeB/FeJ. Approximately 30 to 40 backcross progeny of each male were generated and genotyped within the *sst1* locus. Progeny with no new recombination events within the *sst1* locus were infected with MTB for phenotyping. The recombinants were used for generating progeny only, but were not characterized phenotypically themselves. The *sst1* alleles of all recombinants were deduced based on testing of their progeny for tuberculosis susceptibility. Progeny of each recombinant was tested independently. To deduce the *sst1* alleles of a recombinant, we compared the median survival time (MST) of its progeny that carry the B6-derived segment within the *sst1* locus with the C3H homozygous littermates using t test. The MST of the progeny that carried the *sst1* resistant allele varied between 60 - 80 days in independent experiments, while the MST of the C3H homozygous littermates (*sst1* susceptible) was 27 -30 days. The threshold of significance was established as p=0.001, but typically the p value was lower. Progeny that carried chromosome 1 with new recombination events within the *sst1* locus, were not infected, but used for the next backcross. Thus, appearance of double crossovers in Figure 6A was a result of two recombination events which occurred sequentially in two subsequent backcrosses: a new recombination event between D1Mit415 and D1Mit49 occurred in a progeny of a male that already carried a recombinant chromosome with a breakpoint between D1Mit49 and D1Mit10. Initially, the candidate region was reduced to an interval between the D1Mit439 and D1Mit49 (Fig. 6A). In that figure, each column of boxes represents a genotypic class and each row of boxes represents genotypes for an individual microsatellite marker within the *sst1* candidate region (specified on the left of each row). Solid boxes represent heterozygous (B6 and C3H-derived) and opened boxes homozygous genotype (C3H only). The number of recombinant males, which were tested per each genotypic class, is denoted under each column. The two horizontal lines drawn between D1Mit 439 and D1Mit49 designate the *sst1* candidate region. To further reduce the *sst1* candidate region we have tested an additional 1102 meioses and identified 17 new recombination events between D1Mit439 and D1Mit80, and 5 new recombination events between D1Mit 415 and D1Mit49 (Fig. 2A). Each recombinant was used to

generate progeny, which were analyzed for tuberculosis susceptibility. We established that the proximal boundary of the *sstI* candidate region was delimited by the D1Mit438 marker and the distal boundary was delimited by recombinations between D1Mit415 and the *NppC* gene (Fig. 2A). Eight recombination events between D1Mit438 and WI_WGS_1_86,182,722 (SNP1, Fig. 2A) separated the *sstI* resistant allele from the D1Mit438. The region between D1Mit438 and WI_WGS_1_86,182,722 contains approximately 0.6 Mb of finished sequence and a gap. This gap is due to a presence of a so-called HSR (for homogeneously stained region) repeat. The HSR repeat region is, arguably, the largest repetitive region in the mouse genome and it remains unfinished by both mouse genome projects. Considering the size of the repeat region in inbred mouse strains, which was estimated to be between 3.5 and 6 Mb, it is likely that most of the eight recombination events between D1Mit438 and WI_WGS_1_86,182,722 (SNP1) occurred within the HSR repeat region. No recombination events were found between the *sstI* resistant allele and polymorphic markers within a 634 kb region between the WI_WGS_1_86,182,722 (SNP1) and D1Mit415 (Fig. 2A). Therefore, we concluded that the minimal candidate region encompasses the distal part of the HSR repeat region and a region of mouse chromosome 1 immediately downstream of the repeat, i.e. between the repeat region and the *NppC* gene.

[00238] *Expression profiling of the sstI-encoded genes during the course of tuberculosis infection.*

[00239] Since it was impossible to further reduce the *sstI* critical region by the genetic recombination, the next step of our positional cloning strategy was based on analysis of expression the *sstI*-encoded genes during the course of tuberculosis infection. Based on the *sstI* functional studies, we anticipated that the *sstI* candidate gene would be expressed in the lungs during tuberculosis infection *in vivo* and in macrophages infected with MTB *in vitro*. RNA samples were isolated from the lungs of C3H and C3H.B6-*sstI* congenic mouse strains before and 3 weeks after infection with MTB and from the bone marrow-derived macrophages, which were obtained from the *sstI* congenic mice and infected with MTB *in vitro*. A total of 22 genes are encoded within the *sstI* critical region according to Ensembl and Celera databases of mouse genome (Figure 7). The sequences of the *Sp100-rs* and *Ifi75* genes, which are encoded within the repeat region, were described previously¹. Initially the expression analysis was done using RT-PCR (Fig. 6B, left panel) and Affymetrix GeneChip arrays. To prioritize the *sstI*-encoded

genes we used the following criteria: the gene is expressed in critical tissue (lung) and cell type (macrophage), expression of the gene is modulated by the MTB infection, and the gene might be differentially expressed between the *sst1* disparate animals or cells. Based on priority, all the genes within the *sst1* region were divided into 5 categories (Fig. 6E): not expressed in the lungs and macrophages (unlikely candidates, 9 genes); expressed in the lungs, not in macrophages (low priority, 3 genes); expressed in the lungs and macrophages, not induced by MTB infection (medium priority, 10 genes); upregulated by the MTB infection (high priority, 2 genes); differentially expressed between the *sst1* congenic macrophages (highest priority, 1 gene). To further study the transcripts of the genes that received priority scores of 2 and higher, we used the Rapid Amplification of cDNA Ends (RACE) technique using mRNA isolated from the total lung tissue of the *sst1* congenic mice at 2, 3 and 4 weeks after infection with MTB. The gene-specific primers for RACE and RT-PCR were designed to anneal to conserved regions of each cDNA, which were identified by sequence alignment of all homologous sequences in the NCBI Unigene database. Both the 5' and the 3' ends of mRNA transcripts were amplified using several gene specific and anchor primers and the amplification products were analyzed by gel electrophoresis (Fig. 6B, right panel). The *Ifi75* gene-specific transcripts demonstrated differential expression by both RT-PCR and RACE and this gene was considered to be the most likely *sst1* candidate gene.

[00240] Below is a table of genes within the *sst1* critical region.

Table 1. Genes within the *sst1* critical region.

Number	Start Position	End Position	Strand	Ensembl Gene ID	Gene name	AFFY probe
1	87269325	87317661	1	ENSMUSG00000053971.1	lpr1	131389_at
2	87492420	87583040	1	ENSMUSG00000026222.2	Sp100	
3	87675962	87733755	1	ENSMUSG00000036707.2	Cab39	93976_at
4	87776843	87790871	1	ENSMUSG00000026223.2	Itm2c	96283_at
5	87811183	87813704	1	ENSMUSG00000026224.1	4933407L21Rik	129895_at
6	87823133	87824035	-1	ENSMUSG00000049608.2	Gpr55	
7	87904081	87912093	1	ENSMUSG00000026226.2	Tsag1	
8	87928455	87937590	1	ENSMUSG00000026227.1	2810459M11Rik	131707_at
9	87946897	88021344	1	ENSMUSG00000026229.2	Psmd1	95483_at
10	87981411	87994110	-1	ENSMUSG00000026228.1	Htr2b	95332_at
11	88036892	88161362	1	ENSMUSG00000026230.2	4930438O05Rik	
12	88158616	88159071	-1	ENSMUSG00000053417.1	No description	
13	88186190	88190027	1	ENSMUSG00000047290.1	B3gnt7	134061_r_at
14	88205989	88206315	1	ENSMUSG00000026233.1	No description	
15	88232353	88242086	-1	ENSMUSG00000026234.1	Ncl	100144_at
16	88269044	88270891	-1	ENSMUSG00000026237.1	Gpr66	99417_at

17	88309051	88310762	1	ENSMUSG00000036574.1	1700019O17Rik	
18	88356381	88357217	1	ENSMUSG00000056535.1	Similar to ribosomal L30	
19	88408488	88411591	1	ENSMUSG00000026238.2	Ptma	100718_at
20	88425056	88474833	-1	ENSMUSG00000026239.1	Pde6d	160602_at
21	88479473	88499438	1	ENSMUSG00000026240.1	Cops7b	102765_at
22	88558001	88562282	-1	ENSMUSG00000026241.1	Nppc	94156_at

[00241] *Isolation and characterization of the Ifi75-related transcripts from the lung tuberculosis lesions of the sst1^R mice.*

[00242] The *Ifi75* gene is encoded within the HSR repeat region, also known as long-range repeat cluster D1Lub1. Weichenhan et al isolated two principal component genes encoded within the repeat: *Sp100-rs* and *Ifi75*. The *Sp100-rs* is a chimeric gene that arose by fusion of the *Sp100* and *Csprs* genes. The mouse *Ifi75* gene encodes a putative nuclear hormone receptor co-activator, which is homologous to human "nuclear dot" protein IFI75, also designated SP110. Several *Mus musculus* cDNA and EST sequences in the GenBank database have significant homology to the *Mus caroli Ifi75* cDNA (accession number AJ401361). The genome of *M. caroli* contains single copies of *Sp100*, *Csprs* and *Ifi75* genes, while both the *Sp100-rs* and *Ifi75* genes are amplified in *M. musculus*. It was estimated that a number of the repeat copies ranged from about 50 in the inbred mouse strain C57BL/6, to about 2,000 copies in some isolates of wild mice, which accounts for 0.2 – 6.7% of the haploid mouse genome. Rearranged copies of *Ifi75* are homogeneously spread along the repeat region. We considered a possibility that different isoforms of *Ifi75*, which we called the *Ifi75*-related sequences (*Ifi75-rs*), might exist within individual repeat elements and might be expressed in different cell types or under different physiological conditions. To isolate the *Ifi75-rs*, relevant to tuberculosis resistance phenotype, we performed RACE using RNA isolated from tuberculosis lung lesions of the *sst1* resistant mice. Several oligonucleotide primers were designed based on a large and most conserved region, which is present in known *Ifi75-rs* sequences, and 5' and 3' cDNA sequences were amplified, cloned and sequenced. As shown in Fig. 2B, the 5' RACE products of the *Ifi75-rs* in the lungs of tuberculosis-infected mice was strikingly different between the *sst1* congenic strains: a major single band was amplified from the lungs of the *sst1* resistant mice, whereas this band was absent from the lungs of the *sst1* susceptible strain and, instead, multiple weak products were obtained. The sequences of the *sst1^R* RACE clones were used to assemble a full length sequence and to design a set of primers based on conserved regions to amplify the full length transcripts of

Ifi75. Location of the primers is presented in Figure 2c and the primer sequences are presented in the Methods. The full length products were possible to obtain only from the lesions of the *sst1^R* mice using end-to-end PCR. Although some of the aberrant transcripts were present in the lung tissue of the *sst1^R* animals as well, the majority of the *Ifi75-rs* transcripts in the tuberculosis lung lesions were represented by a single isoform encoding 12 exons, which was 92% identical to the *Mus caroli Ifi75*. To differentiate between the previously identified *Ifi75* transcripts, we named the *Ifi75* isoform isolated in our studies from the tuberculosis lung lesions of the C3H.B6-*sst1* mice as *Ipr1* (for intracellular pathogen resistance1, GenBank accession No. AY845948). The predicted *Ipr1* protein contained a Sp100-like domain in its N-terminus, a LXXLL-type nuclear receptor binding motif (NRB), a bipartite nuclear localization signal (NLS), and a SAND domain in its C-terminus (Fig. 2C). In addition, we have identified another isoform of the *Ifi75* in the lungs of the resistant mice that was most likely the product of alternative splicing and encoded a transcript containing a stop codon after exon 10. In Fig. 2D and e, we demonstrate that a single major isoform of the *Ifi75-rs*, the *Ipr1* gene, is expressed in *sst1^R* macrophages, and is absent from the *sst1^S* macrophages obtained from the C3HeB/FeJ mice.

[00243] Using DNA probes specific for the Sp100 and SAND domains of the *Ipr1*, we have analyzed the kinetics of its expression by Northern hybridization in the lungs of the *sst1* congenic mouse strains during progression of tuberculosis (Fig. 2D). Expression of the *Ipr1* gene was detectable in the lungs of the naive *sst1^R* mice, and its expression increased significantly 2 weeks after intravenous infection with MTB and remained at elevated levels 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 region, *Sp100-rs*, was elevated in the lungs of the *sst1^S* mice (Fig. 2D). This was detected using a probe that is specific for the *Csprs* portion of the *Sp100-rs*, which does not hybridize with a normal transcript of the *Sp100* gene. We have also performed RT-PCR of the RNA isolated from the *sst1* congenic macrophages using primers that specifically amplify the *Sp100-rs* transcripts, but not the *Sp100* gene, because the reverse primer was specific for the *Csprs* sequence that is not present in the *Sp100* cDNA. As shown in Fig. 4A, *Sp100-rs*, as well

as the aberrant *Ifi75-rs*, are expressed in the *sst1^S* homozygous (S), heterozygous (SxR and RxS), and the *Ipr1* transgenic macrophages (Tg). However, they are not prominently expressed in the macrophages that are homozygous for the B6-derived resistant allele of the *sst1* locus (R). The *sst1* resistant allele in our model is dominant and the tuberculosis resistance of the F1 hybrid mice that are *sst1* heterozygous is similar to that of the *sst1^R* homozygous mice (data not shown). It is, therefore, most likely that the expression of the full length *Ipr1* gene is necessary for tuberculosis resistance and expression of the *Sp100-rs* does not confer susceptibility.

[00244] *Genetic basis of the Ipr1 silencing in the sst1^S mice.*

[00245] To investigate the lack of the *Ipr1* gene expression in the *sst1^S* C3HeB/FeJ mice, first we used quantitative Real Time PCR of the genomic DNA to compare the number of individual exons of the *Ipr1* gene encoded in the genomes of the *sst1^R* and the *sst1^S* mice. The genomic DNA of *Mus caroli*, which contains a single copy of the *Ifi75* gene¹, was used as a reference. We found that both C3H-derived and B6-derived chromosomes contain the HSR repeat. As shown in Fig. 6C, the number copies of individual exons in the *sst1* resistant mice (C3H.B6-*sst1*) varied between 40 copies for exon 8 (maximal copy number) and 5 copies for exon 12 (minimal copy number) suggesting that most of the copies of the *Ifi75-rs* contain less than a complete set of 12 exons. Thus, the HSR repeat, even in the *sst1^R* mice, contains mostly aberrant forms of the *Ifi75-rs*, and very few, perhaps one copy of the full length *Ipr1* gene. The number of exons 1, 2, and 4 was lower in the genome of the *sst1^S* mouse strain. Single Strand Conformation Polymorphism (SSCP) analysis of individual exons of the *Ipr1* gene in *sst1^R* and *sst1^S* congenics demonstrated a clear difference between the congeneric mouse strains in genomic DNA encoding exon 1 (Fig. 6D). We hypothesize that a mutation, perhaps a deletion, within the 5' region of a rare full length copy of the *Ipr1* gene resulted in the lack of this gene expression in the *sst1*-susceptible C3HeB/FeJ mice.

[00246] To summarize, aberrant transcripts of the *Ifi75-rs* are weakly expressed in macrophages of the *sst1* susceptible C3HeB/FeJ mice, and the full length *Ipr1* transcript is missing. In contrast, a major *Ifi75-rs* isoform, which is expressed in macrophages of the *sst1* resistant mice, is represented by a full length *Ipr1* transcript containing all 12 exons.

EXAMPLE II

[00247] We have generated an additional strain of *sst1* congenic mice, C57BL/6J.C3H-*sst1* (susceptible). C57BL/6J.C3H-*sst1* (susceptible) was generated by transfer of an approximately 20 cM segment of mouse chromosome 1, which contains the susceptible allele of the *sst1* locus, on the resistant genetic background (C57BL/6J, or B6). Therefore, the B6.C3H-*sst1* mice, as opposed to the parental B6 mice, do not express the *Ipr1* gene. This is a unique strain, which was generated in our laboratory by 10 backcrosses on B6 genetic background, which is a standard genetic background for testing genetically engineered mice. Since the B6.C3H-*sst1* mice do not express the *Ipr1* gene, this strain is equivalent of the *Ipr1* knockout mouse. However, generation of the *Ipr1* knockout mouse by standard homologous recombination technique would be currently impossible, because of highly repetitive nature of the chromosomal region encoding the *Ipr1* gene. Thus, the B6.C3H-*sst1* mouse strain represents a unique resource for studies of the *Ipr1* gene function. For example, it can be used for generation of transgenic mice that express human homologue of the *Ipr1* gene to study its function, as well as for screening and testing of potential activators and inhibitors of the human *Ipr1* homologue in a whole animal.

[00248] In addition these mice are more resistant to tuberculosis infection than the C3HeB/FeJ parental mice, which also do not express the *Ipr1* gene. Therefore, it can be used for identification of compensatory immune mechanisms that function in the absence of the *Ipr1* gene product. Although the B6.C3H-*sst1* mice survive longer than the C3HeB/FeJ parental mice, a significant proportion of these mice does develop tuberculosis cavities in their lungs. Therefore, this strain represents a unique model of chronic lung tuberculosis, resembling a key element of human pulmonary tuberculosis in adults, such as formation of tuberculosis cavities in the lungs. Although adult pulmonary tuberculosis is the most common and infectious form of tuberculosis infection in humans, until now, no adequate experimental mouse model of this clinical form of tuberculosis existed and progress in developing effective therapeutic and preventive strategies targeting adult pulmonary tuberculosis has been slow. Meanwhile, methods for prevention of formation of the tuberculosis cavities in the lungs and/ or for effective drug delivery to the bacteria within these cavities, could greatly reduce transmission of tuberculosis and would have a significant epidemiological impact.

[00249] Availability of this mouse model of chronic pulmonary tuberculosis with formation of cavities in the infected lungs provides a model for testing of new anti-tuberculosis drugs, treatment regimens, vaccines and methods of immunomodulation. This strain can also be used in studies of pathogenesis of tuberculosis, because the B6.C3H-sst1 mice can be bred with genetically engineered mice that carry knockout of other relevant genes on the same C57BL/6J genetic background, thus permitting generation of double and triple knockouts.

EXAMPLE III

[00250] *The sst1 locus modulates production of inflammatory mediators by the MTB-infected macrophages in vitro.*

[00251] To determine whether the *sst1* locus also modulates production of inflammatory mediators by macrophages, we measured production of a panel of cytokines and chemokines by *sst1^S* and *sst1^R* bone marrow-derived macrophages (BMDM) 6 and 30 hours after infection with virulent MTB *in vitro*. The *sst1^R* macrophages produced more TNF α within the first 6 hours post infection. At that time concentrations of IL-10, MCP-1, IP-10, KC and MIP-1 α increased to a similar level on both genetic backgrounds. During the next 24 hours, however, the *sst1* congenic macrophages demonstrated dichotomous trends: the *sst1^R* macrophages (either non-treated or treated with IFN γ) continued to produce higher levels of TNF α , while the susceptible macrophages produced higher levels of IL-10, MCP-1 and IP-10. Concentrations of TGF β in these samples were low and similar for both genotypes. Higher levels of TNF α and lower level of IL-10 production by the *sst1^R* macrophages were also observed at low MOI of 1 bacterium per 10 macrophages.

[00252] Six hours after MTB infection both *sst1^R* and *sst1^S* macrophages showed approximately 80-fold increases in TNF α mRNA levels, while the increase in IL-10 mRNA was slightly higher in the *sst1^S* cells (12-fold as compared to 8-fold in the *sst1^R* cells). To test whether the *sst1^S* macrophages responded to MTB via an alternative activation pathway, we measured expression of arginase, Ym1 and FIZZ1 gene markers of this pathway³⁵. MTB infection caused increased expression of arginase, but had no effect on the expression of Ym1 and FIZZ1 genes in macrophages of either genetic background. In control experiments macrophages of both strains expressed Ym1 and FIZZ1 genes after treatment with IL-4. When the *sst1^R* and *sst1^S* macrophages were

stimulated with IFN γ (30 U/ml) and LPS (50 ng/ml) they produced equal amounts of TNF α , but no detectable IL-10.

[00253] We sought to determine which cytokines are produced by the MTB-infected macrophages themselves, and which are produced by non-infected bystander macrophages upon interaction with the infected cells. We employed a genetically modified strain of virulent MTB that constitutively expresses green fluorescent protein (GFP) or MTB Erdman that was labeled with fluorochrome CFSE (for short term experiments). This allows identification of MTB-infected macrophages by green fluorescence using flow cytometric analysis. The cytokine production by MTB-infected (GFP-positive) and non-infected (GFP-negative) cells was analyzed using intracellular cytokine staining (ICC) at 24, 48 and 72 hours after infection with MTB *in vitro*. A major effect of the *sstI* locus on TNF α and IL-10 production was observed specifically in the cells which harbored the bacteria. At 24 h post infection, all of the MTB-infected *sstI*^R macrophages produced TNF α , but very few of them produced IL-10. In contrast, the *sstI*^S macrophages infected by MTB mostly produced IL-10, and very few of them produced TNF α . Importantly, production of both TNF α and IL-10 by the bystander macrophages at that time was similar on both genetic backgrounds. The MTB-infected *sstI*^R and *sstI*^S macrophages also differed in MCP-1 production. However, this difference was observed later, at 48 hours post infection. At 24 hours post infection, the bacterial load per infected macrophage was similar in the *sstI*^R and *sstI*^S cells, in which the majority of infected macrophages contained a single bacterium. Therefore, the difference in cytokine production by the MTB-infected macrophages cannot be simply accounted for by a difference in bacterial load. It more likely reflects intrinsic differences in regulation of cytokine gene expression in the infected cells.

[00254] In parallel, we used GFP-labelled MTB to examine kinetics of cell death in infected macrophages. MTB-containing (GFP-positive) macrophages were analyzed for membrane damage (PI staining) and apoptosis (Annexin V staining) at 24 h intervals. At 72 hours after a low multiplicity infection, evidence of cell death was primarily limited to MTB-infected cells. The MTB-infected *sstI*^R cells were undergoing apoptosis (PI-negative, Annexin V-positive), while the *sstI*^S macrophages typically died by necrosis, i.e. they displayed membrane damage (PI-positive) in the absence of apoptotic membrane transformation (Annexin V-negative). Of note, in both the *sstI*^R and *sstI*^S macrophage cultures signs of cell death were observed only 72 hours after the infection and later.

Thus, the earliest macrophage-specific phenotypic expression of the *sst1* locus *in vitro* was its effect on cytokine production specifically by MTB-infected cells.

[00255] *Production of TNF- α and IL-10 by macrophages infected with virulent MTB is mediated by the Ipr1 gene.*

[00256] Production of TNF α , IL-10 and IL-12 by macrophages isolated from the *Ipr1* transgenic C3H.Tg(*Ipr1*) mice and from the *sst1*^S C3H mice, which lack the *Ipr1* expression, was compared at 12 and 24 hours after the MTB infection *in vitro*. The MTB-infected *Ipr1* transgenic macrophages produced higher levels of TNF- α at both time points, similar to the *sst1*^R congenic macrophages, which were also tested in parallel. Production of IL-10 was delayed, as compared to TNF- α , but at 24 hours fewer transgenic macrophages produced IL-10 as compared to wild-type C3H, while IL-12 production increased similarly in MTB-infected macrophages of both genotypes. Thus, the MTB-infected *Ipr1* transgenic macrophages that genetically differ from C3H macrophages only by expression of a single gene (*Ipr1*), demonstrated a pattern of cytokine production similar to that of the *sst1*^R congenic macrophages that differed from C3H by a 20 cM segment of chromosome 1.

[00257] We compared cytokine production by the *Ipr1* disparate macrophages that were infected either with virulent MTB or avirulent vaccine strain of *Mycobacterium bovis* BCG (BCG). Infection with BCG induced production of TNF α in both *Ipr1*-positive and *Ipr1*-negative macrophages at 12 hours, which declined at 24 hours after the BCG infection. The *Ipr1* transgenic and the *sst1*^R macrophages produced higher levels of TNF α when they were infected with MTB then after the BCG infection. On the contrary, production of TNF α by the MTB-infected *sst1*^S macrophages was lower as compared to the same cells infected with BCG. Interestingly, at 24 hours post infection with either mycobacteria, the bystander macrophages in these cultures also started to produce TNF α . We have demonstrated that activation of the bystander cells was mediated by TNF α , since neutralization of TNF α using specific antibodies substantially decreased TNF α production by these macrophages.

[00258] The proportion of IL-10 producing cells among the BCG-infected macrophages was low in both genetic backgrounds. Elevated IL-10 production was observed only in macrophages infected with MTB, and proportion of the IL-10 producing cells was significantly higher in the *Ipr1*-negative C3H macrophages. Conversely, IL-12 was rapidly induced by the BCG infection on both genetic backgrounds, less so by MTB,

and we did not observe any impact of the *Ipr1* gene on regulation of this cytokine neither in BCG nor in MTB-infected cells.

[00259] Neutralization of TNF α by specific antibodies significantly enhanced growth rates of MTB in the *sstI*^R and the *Ipr1* transgenic macrophages to the levels similar to the MTB growth in the *sstI*^S cells, while having little effect on the bacterial growth in the *sstI*^S macrophages. This treatment also blocked apoptosis of the MTB-infected *Ipr1* transgenic macrophages leading to appearance of Annexin V-negative, PI-positive necrotic cells, similar to the cell death phenotype of the *sstI*^S macrophages. In both cases, signs of cell death were predominantly associated with the MTB-infected GFP-positive cells.

[00260] TNF α is known to induce apoptosis via caspase 8 activation. Therefore, we compared caspase 8 activity in the *sstI*^R congenic, the *Ipr1* transgenic and the *sstI*^S C3H macrophages 72 hours after infection with MTB. Activation of caspase 8 was observed in macrophages that expressed the *Ipr1* gene. The caspase 8-positive cells were also PI-negative, i.e. at this point they had not undergone membrane damage. Conversely, most of the *sstI*^S macrophages were PI-positive and caspase 8-negative consistent with a necrotic mechanism for their cell death. Neutralization of TNF α using specific antibodies blocked caspase 8 activation, as did the specific peptide inhibitor of caspase 8 zIETD-fmk. Both of these treatments also inhibited apoptotic cell death of the MTB-infected *Ipr1* transgenic macrophages, which resulted in appearance of Annexin V-negative, PI-positive necrotic cells.

[00261] Thus, blockade of TNF α switched the phenotype of the *Ipr1*-transgenic and the *sstI*^R macrophages to the phenotype characteristic for *Ipr1*-negative *sstI*^S C3HeB/FeJ macrophages, when control of bacterial multiplication and mechanism of cell death were assessed. However, neutralization of TNF α failed to upregulate production of IL-10 by the *sstI*^R macrophages to the levels observed in the MTB-infected *sstI*^S cells suggesting that production of IL-10 is regulated by the *Ipr1* gene via a TNF α -independent mechanism.

[00262] We combined the *sstI*^S macrophages with the *sstI*^R macrophages, which are capable of TNF α production after MTB infection, infected the mixed population of the *sstI*^R and *sstI*^S macrophages with MTB and studied mechanism of their cell death. To differentiate between the *sstI*^R and the *sstI*^S macrophages in a mixed culture, we stained

macrophages of one genotype with CFSE. Reciprocal staining was performed to control for potential adverse effect of CFSE on macrophages, but this switch did not affect the outcome of the experiment. The susceptible phenotype was dominant in mixed cultures: apoptosis was blocked and necrotic death was observed in macrophages of both *sstI^S* and *sstI^R* genotypes. Necrosis was abolished by IL-10-specific neutralizing antibodies. Importantly, we observed appearance of equal numbers of Annexin V-positive cells apoptotic cells among macrophages of both *sstI^R* and *sstI^S* genotypes. These experiments demonstrated that, in principle, the *sstI^S* macrophages were capable of entering apoptotic pathway, but this was blocked by IL-10 secreted by the susceptible cells.

[00263] Blockade of IL-10 also initiated the apoptotic cell death pathway in isolated cultures of MTB-infected *sstI^S* macrophages. This effect was TNF α -mediated since apoptosis was blocked again, when the *sstI^S* macrophages, were also treated with anti-TNF α antibodies in addition to neutralizing anti-IL-10. This suggested that either the low amount of TNF α , which was initially produced by the MTB-infected *sstI^S* macrophages, was sufficient, upon IL10 neutralization, to induce apoptosis, or that the inhibition of IL-10 restored production of TNF α in the MTB-infected *sstI^S* cells.

[00264] The later possibility was tested using intracellular cytokine staining. Initially, neutralization of IL-10 resulted in increased TNF α production by both the MTB-infected cells and the non-infected *sstI^S* macrophages at 12 hours after the infection. However, by 24 hours, the majority of the infected cells ceased TNF α production, while the by-stander macrophages still produced some. Thus, TNF α production by the MTB-infected *sstI^S* cells was not sustainable over longer period even in the presence of IL-10-neutralizing antibodies. This is clearly different from the kinetics of TNF α production by the MTB-infected *sstI^R* macrophages, in which high levels of this cytokine were observed within 24 - 48 hours after the MTB infection.

[00265] Anti-IL-10 treatment improved control of MTB replication by the *sstI^S* macrophages, although this effect was only partial. No effect of IL-10 blockade on MTB control by the *sstI^R* and the *Ipr1* transgenic macrophages was registered in this experiment, which is consistent with low levels of IL-10 production by the resistant cells.

[00266] *Cell autonomous control of TNF α production by the sst1 locus.*

[00267] To avoid possible artifacts related to incomplete inhibition of specific cytokines with antibodies in the above experiments, we studied production of cytokines by the MTB-infected macrophages in co-cultures of the *sst1*^R and *sst1*^S cells. In these experiments macrophages of one genotype were labeled with red fluorescent dye (SNARF1), mixed with non-labelled macrophages of the opposite *sst1* genotype and the mixed population was infected with CFSE-labeled MTB. Production of TNF α , IL-10 and IL-12 by cells of each genotype in mixed population was determined by intracellular cytokine staining and plotted separately for the MTB-infected and non-infected macrophages of each genotype. This approach eliminates environmental variables and highlights cell specific phenotypes. Two major observations were made regarding TNF α production in these settings. First, 24 hours after infection the MTB-infected *sst1*^R macrophages as well as the non-infected macrophages of both *sst1* genotypes produced similar amounts of TNF α . Meanwhile TNF α production by the *sst1*^S MTB-infected cells was profoundly depressed. This effect of the *sst1* locus on TNF α production was restricted to MTB -infected *sst1*^S macrophages. Interestingly, IL-10 production by the *sst1*^R and the *sst1*^S cells in co-cultures was similar and intermediate between the two genotypes, indicating that production of this by the *sst1*^R macrophages could be upregulated by soluble mediators or cell-cell contact with the *sst1*^S cells. Production of IL-12 by the *sst1*^S and the *sst1*^R macrophages in these settings was also similar. Thus, production of TNF α by the MTB-infected macrophages is strictly controlled by the *Ipr1* gene in a cell autonomous manner, and this effect perhaps constitutes mechanistic basis of an important role that this genes plays in innate immunity to infection with virulent MTB.

[00268] We have shown that the *Ipr1* gene plays a role in cytokine production by macrophages infected with virulent cytosolic pathogens, such as, *Mycobacterium tuberculosis*. We found that the *Ipr1* gene controlled cytokine gene expression specifically in macrophages that contained intracellular MTB. *Ipr1* gene expression was necessary for sustainable production of TNF α , while the MTB-infected macrophages that lack *Ipr1* produced IL-10. Our findings that production of TNF α and IL-10 production is reciprocally controlled in the MTB-infected macrophages by the *Ipr1* gene demonstrates that the balance of these cytokines is also influenced by the genetic makeup of the host.

[00269] The references cited herein are incorporated by reference in their entirety.

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CLAIMS

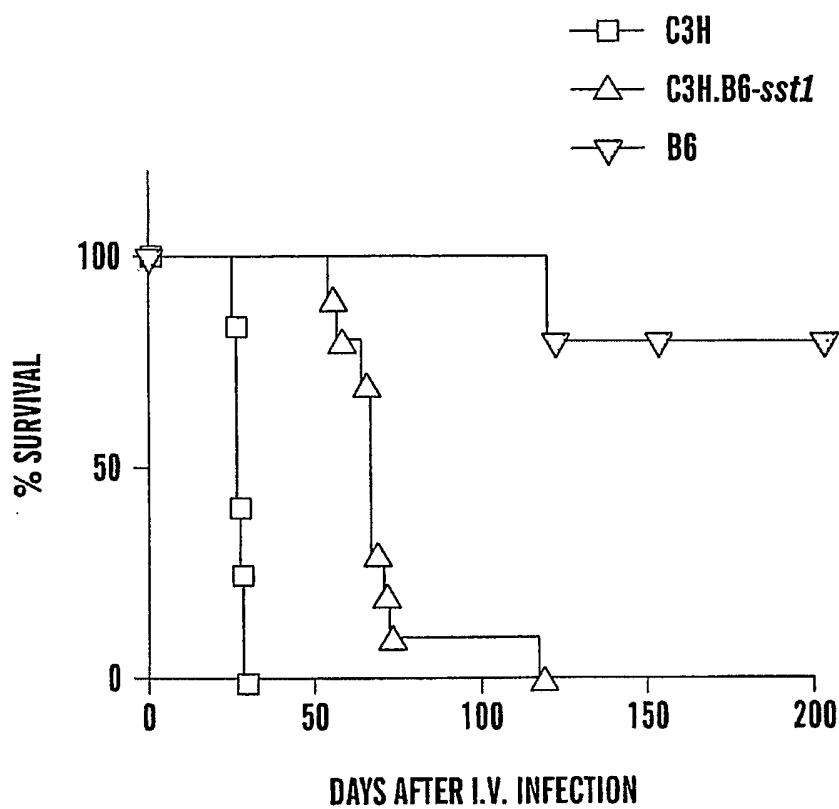
We claim:

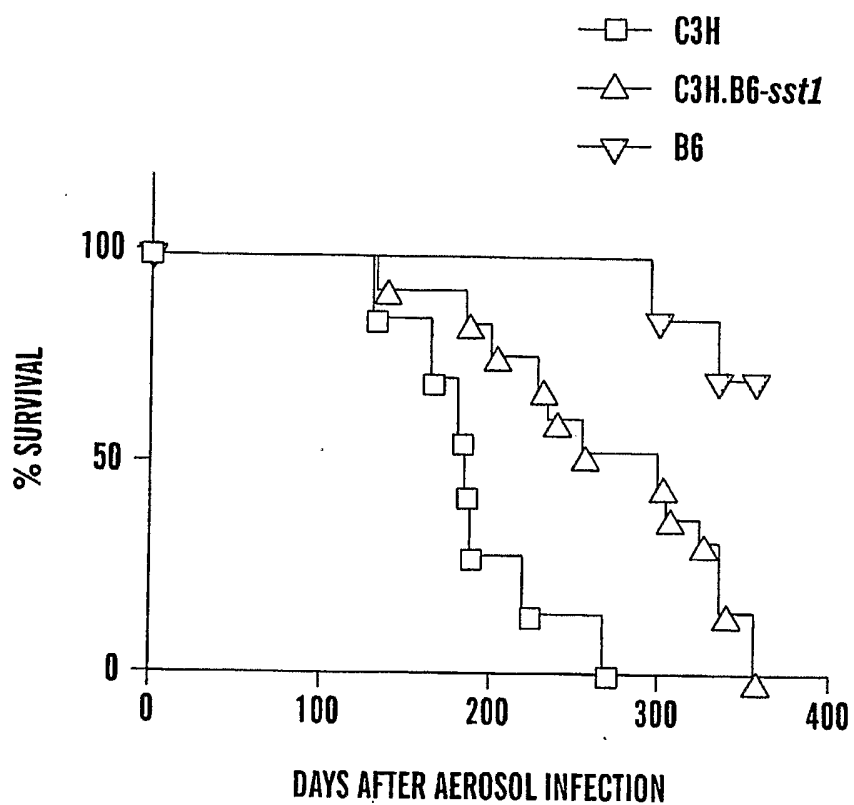
1. A method for determining a patients susceptibility to infection by a cytosolic pathogen comprising:
 - i. obtaining a biological sample from a patient; and
 - ii. analyzing gene expression and/or detecting the presence of a mutation or polymorphism of a human homologue to *Ipr1* (SEQ ID NO:11) in the biological sample as compared to a control group, wherein a reduction in gene expression, presence of a mutation or polymorphism, or expression of a protein that has impaired antimicrobial or antiviral activity indicates that the individual has an increased risk of infection by cytosolic pathogens.
2. The method of claim 1, further comprising analyzing the biological sample for tumor necrosis factor-alpha (TNF- α) and interleukin-10 (IL-10) expression, as compared to a control group, wherein a decrease in TNF- α expression or an increase in IL-10 expression indicates that the individual has an increased risk of infection by cytosolic pathogens.
3. The method of claims 1 or 2, wherein the human homologue to *Ipr1* is *SP110b* (SEQ ID NO:1).
4. The method of claims 1, 2, or 3, wherein the biological sample is a peripheral blood sample or a tissue sample.
5. The method of claims 1, 2, or 3, wherein gene expression is analyzed by analyzing levels of mRNA.
6. The method of claim 5, wherein the levels of mRNA are determined by quantitative RT-PCR.
7. The method of claim 5, wherein the levels of mRNA are determined by using a gene expression array.
8. The method of claims 1, 2, or 3, wherein the gene expression levels is analyzed by analyzing levels of protein.

9. The method of claims 1, 2, or 3, wherein the cytosolic pathogen is a bacterial pathogen or viral pathogen.
10. The method of claim 9, wherein the bacterial pathogen is selected from the group consisting of *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Listeria monocytogenes*, and *Brucella abortus*.
11. The method of claim 9, wherein the viral pathogen is selected from the group consisting of a Hepatitis C Virus and a retrovirus.
12. A method for determining a patients susceptibility to infection by a cytosolic pathogen comprising:
 - i. obtaining a biological sample from a patient; and
 - ii. analyzing the rate of protein degradation of a human homologue to *Ipr1* (SEQ ID NO:11) in the biological sample as compared to a control group, wherein an increase in the rate of degradation indicates that the individual has an increased risk of infection by cytosolic pathogens.
13. The method of claim 12, further comprising analyzing said biological sample at a first and second time point for protein degradation.
14. A method for treating a patient infected with a cytosolic pathogen comprising administering to said patient an effective amount of a compound or agent that upregulates gene expression of a human homologue to *Ipr1* (SEQ ID NO:11) or a portion thereof resulting in enhancing immune reactions to cytosolic pathogens.
15. A method for treating a patient infected with a cytosolic pathogen or preventing infection of an individual by a cytosolic pathogen comprising administering to said patient an effective amount of a human protein homologue to *Ipr1*.
16. The method of claims 14 or 15, wherein the human homologue to *Ipr1* is *SP110b* (SEQ ID NO:1).
17. The method of claims 14 or 16, wherein the compound is interferon- γ .
18. The method of claims 14, 15, or 16 further comprising administering to the patient an agent that increases tumor necrosis factor-alpha (TNF- α) and/or decreases interleukin-10 (IL-10).

19. The method of claim 18, wherein the agent that increases TNF- α is a TNF- α agonist and the agent that decreases IL-10 is an IL-10 antagonist.
20. The method of claim 19, wherein the TNF- α agonist is selected from the group consisting of an antibody, antibody fragment, monoclonal antibody, polyclonal antibody, humanized antibody, chimeric antibody, small molecule, aptamer, and nucleic acid.
21. The method of claims 19, wherein the IL-10 antagonist is selected from the group consisting of an antibody, antibody fragment, monoclonal antibody, polyclonal antibody, humanized antibody, chimeric antibody, small molecule, aptamer, IL-10 receptor antagonist, and nucleic acid.
22. The method of claims 15 or 16, wherein the human protein homologue to Ipr1 is a fusion protein.
23. The method of claim 22, wherein the human protein homologue to Ipr1 is a fragment that comprises a portion of Ipr1 responsible for antimicrobial or antiviral activity.
24. The method of claims 15 or 16, wherein said protein is administered by administration of a nucleic acid sequence that encodes human protein homologue to *Ipr1* or a functional fragment thereof.
25. The method of claim 24, wherein the nucleic acid is contained within a viral vector.
26. The method of claim 25, wherein the viral vector is selected from the group consisting of: adenoviral vector, pox virus vector, retroviral vector, lentiviral vector and an adeno-associated-viral vector (AAV).
27. The method of claim 14, 15, or 16, wherein the cytosolic pathogen is a bacterial pathogen.
28. The method of claim 14, 15, or 16, wherein the cytosolic pathogen is a viral pathogen.
29. The method of claim 27, wherein the bacterial pathogen is selected from the group consisting of: *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Listeria monocytogenes*, and *Brucella abortus*.
30. The method of claim 28, wherein the viral pathogen is selected from the group consisting of: Hepatitis C Virus, and a retrovirus.

31. The method of claim 28, wherein the viral pathogen is not an Epstein Barr virus.
32. The method of claim 14, 15, or 16, wherein the patient is immunocompromised.
33. The method of claim 14, 15, or 16, wherein the patient is chronically infected with the cytosolic pathogen.

***FIG. 1a***

**FIG. 1b**

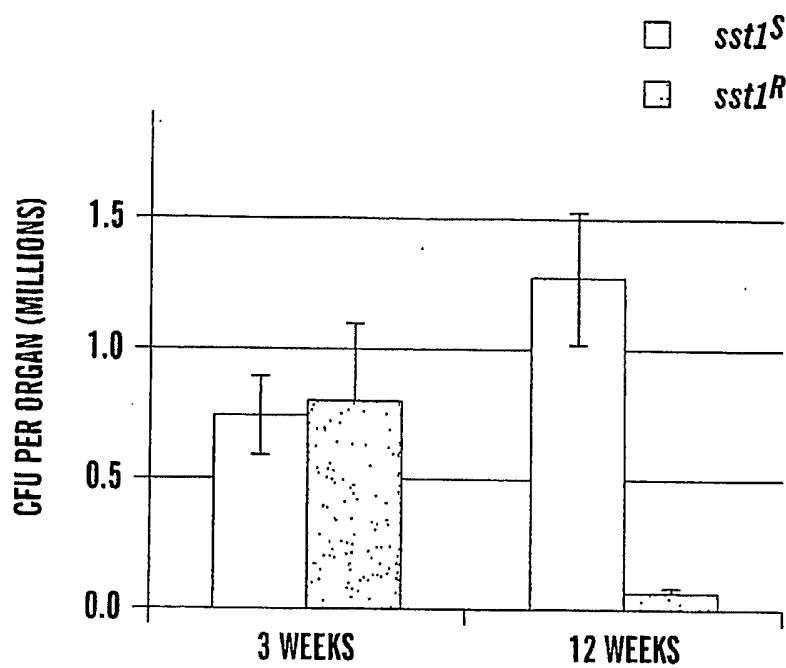
***FIG. 1c***



FIG. 1d

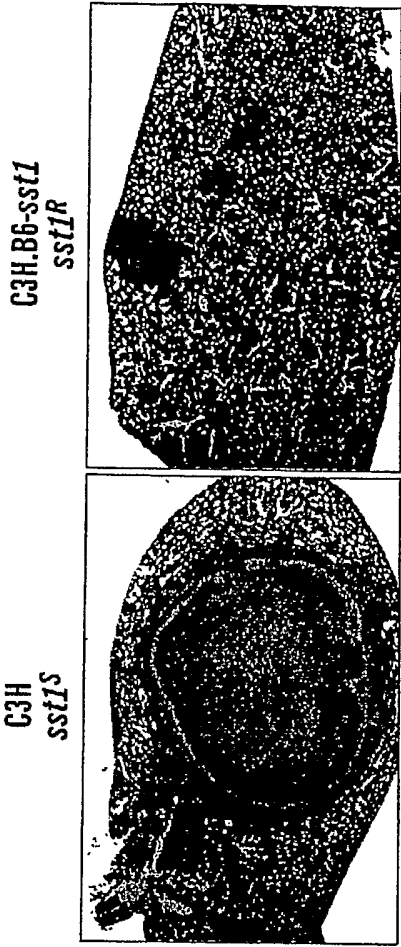
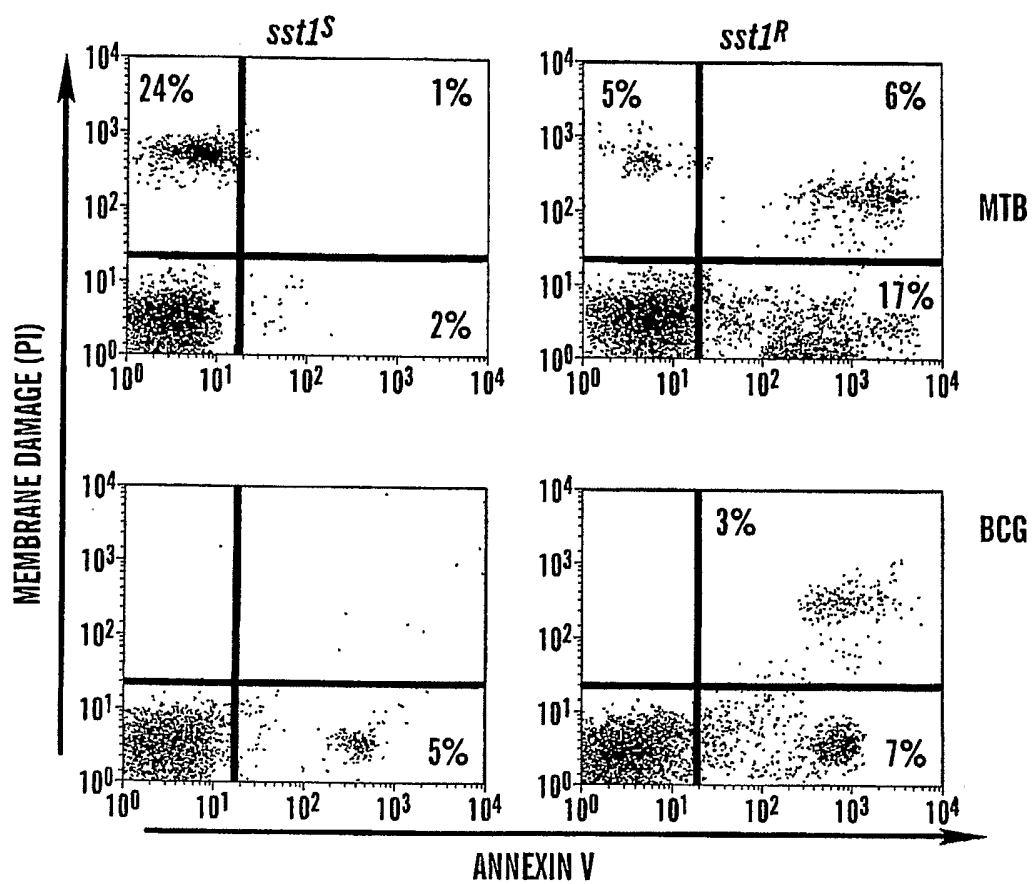


FIG. 1e

**FIG. 1f**

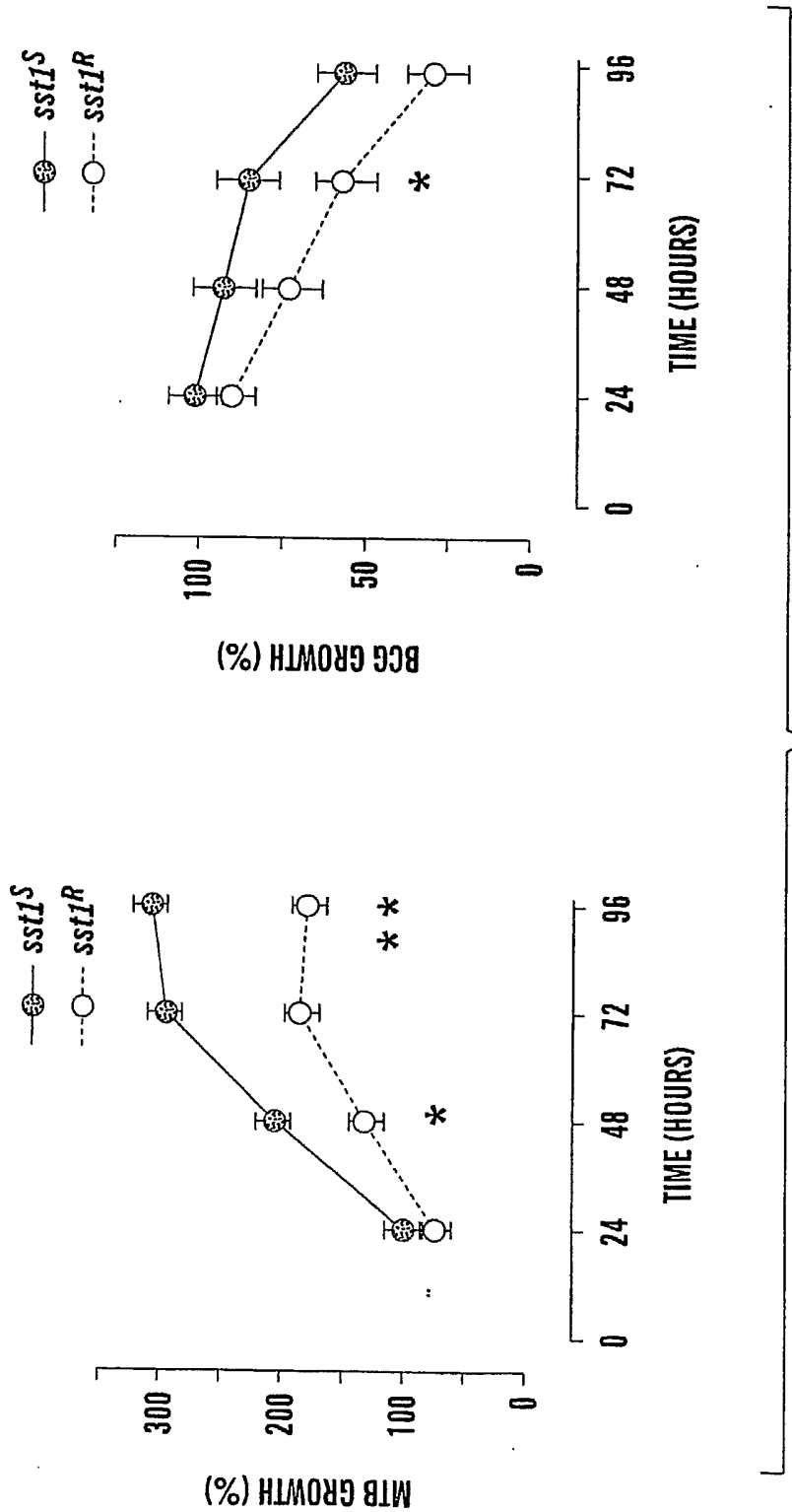


FIG. 1g

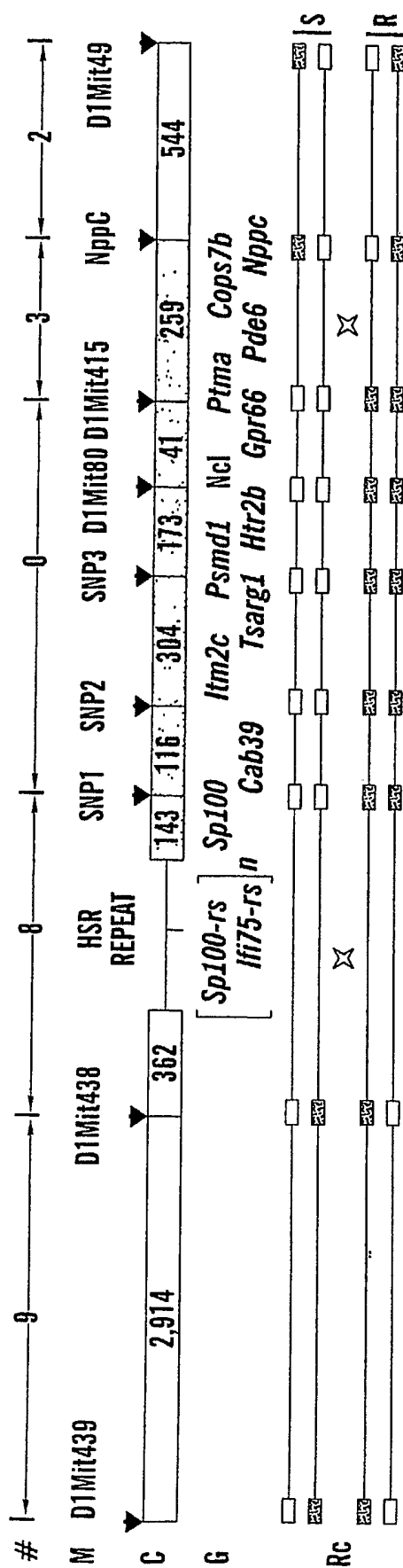


FIG. 2a

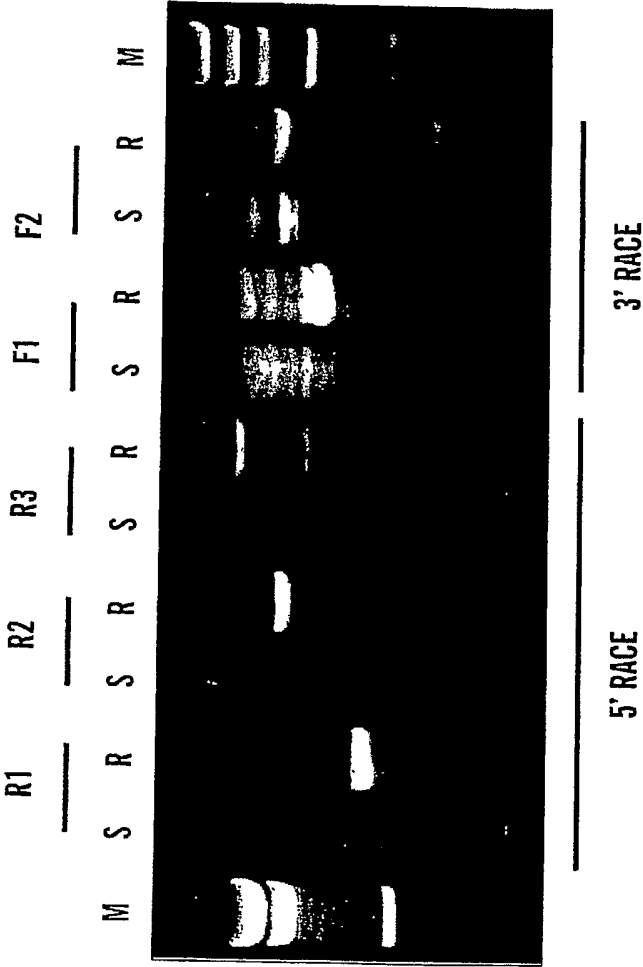


FIG. 2b

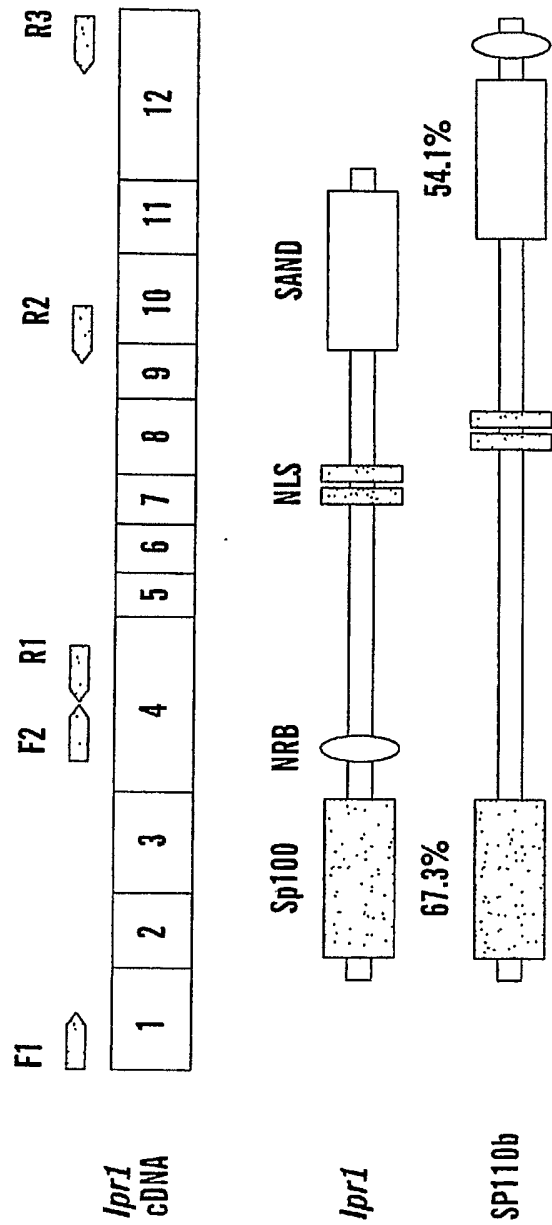


FIG. 2c

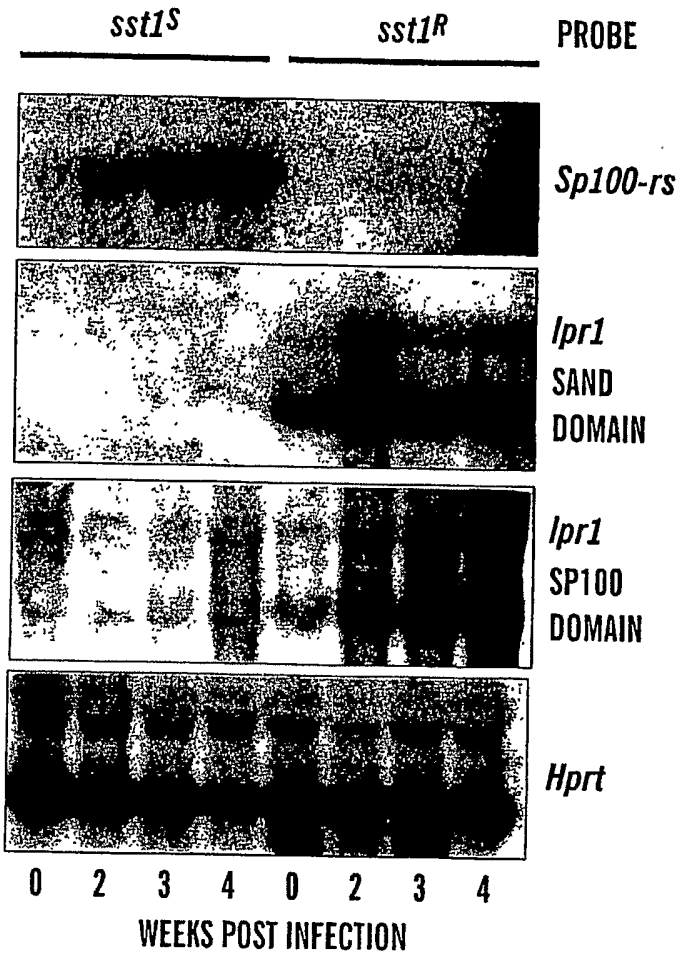
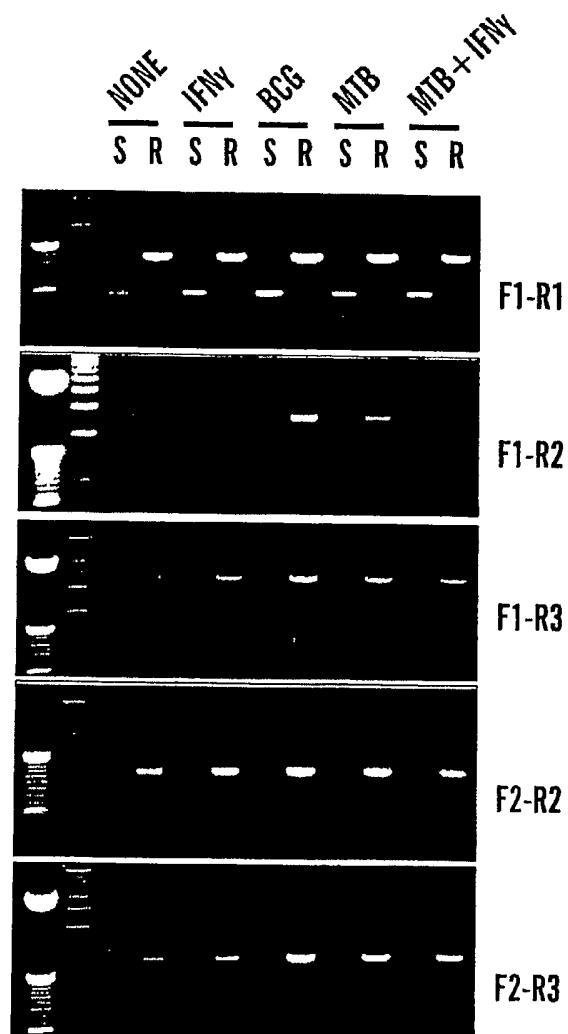
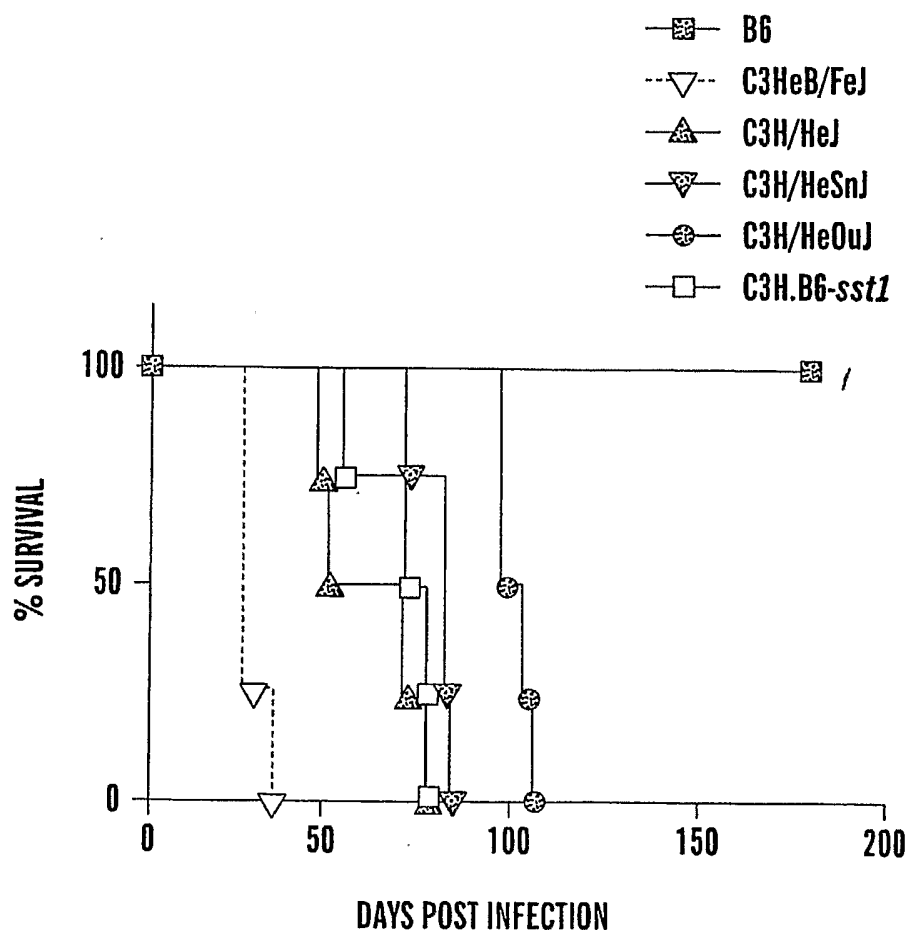
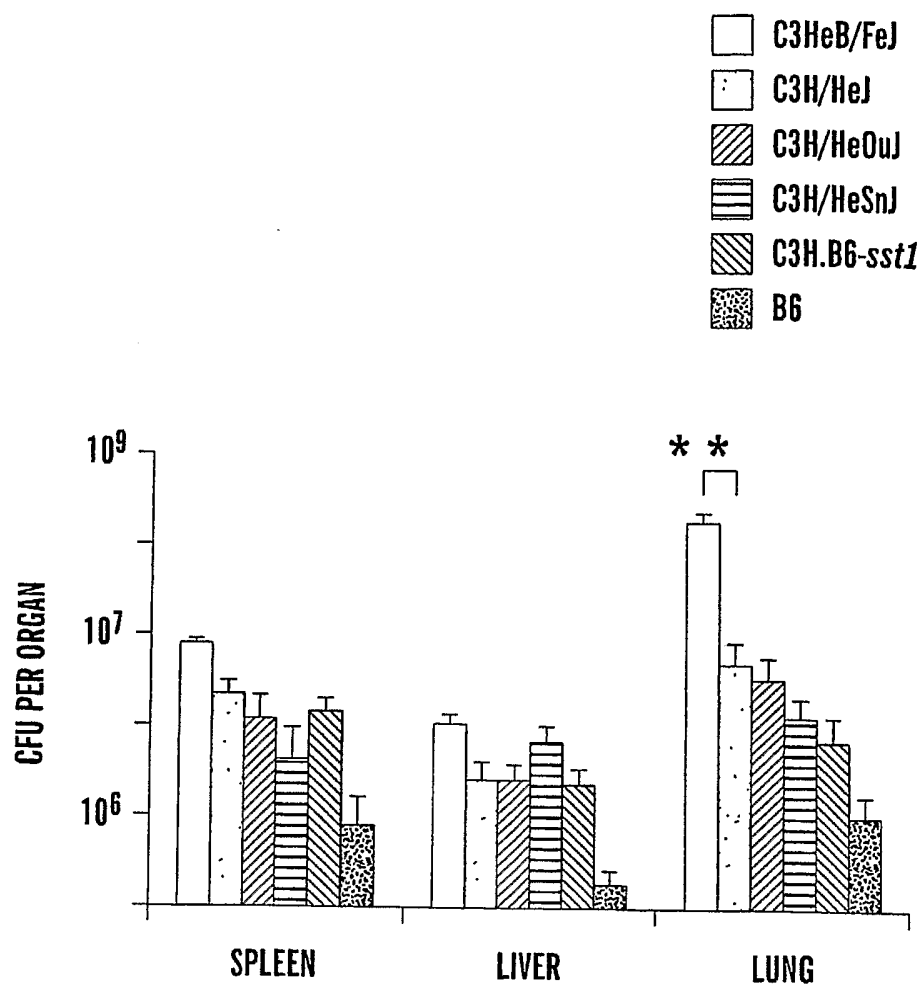
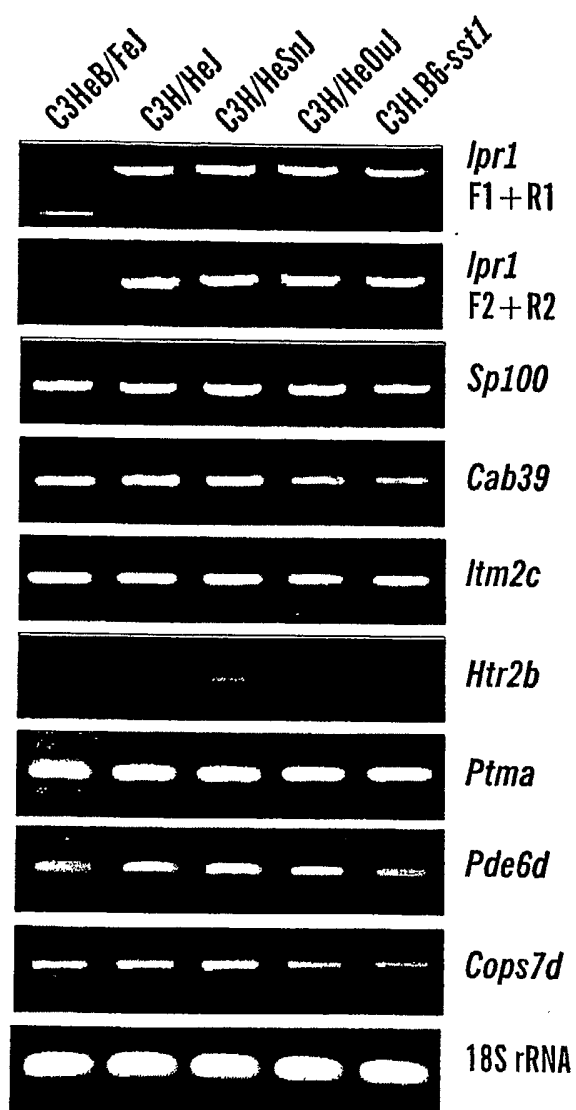


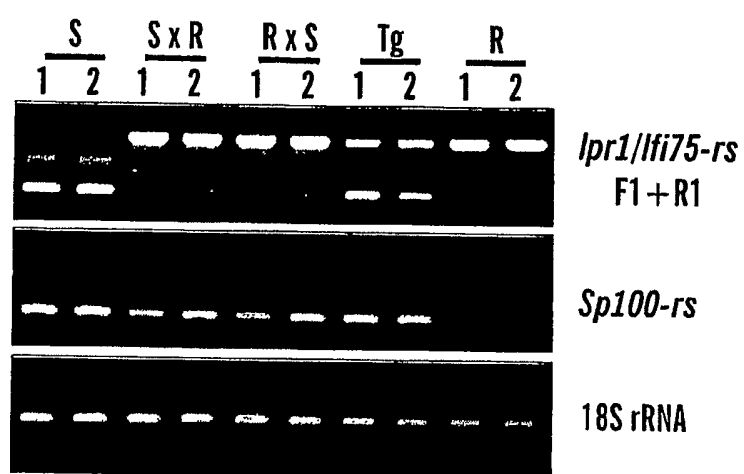
FIG. 2d

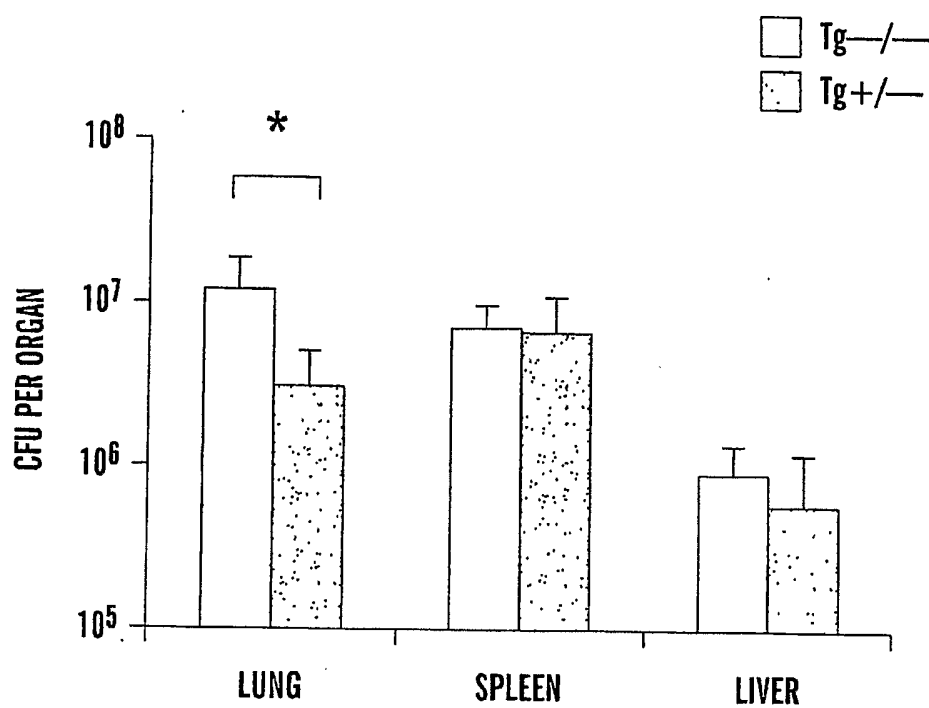
***FIG. 2e***

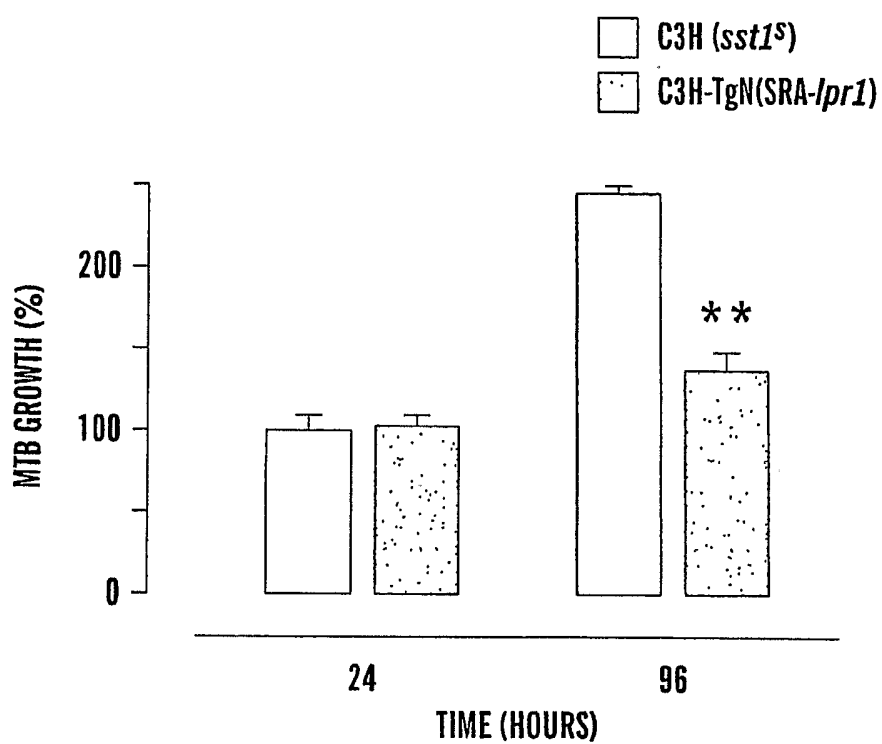
**FIG. 3a**

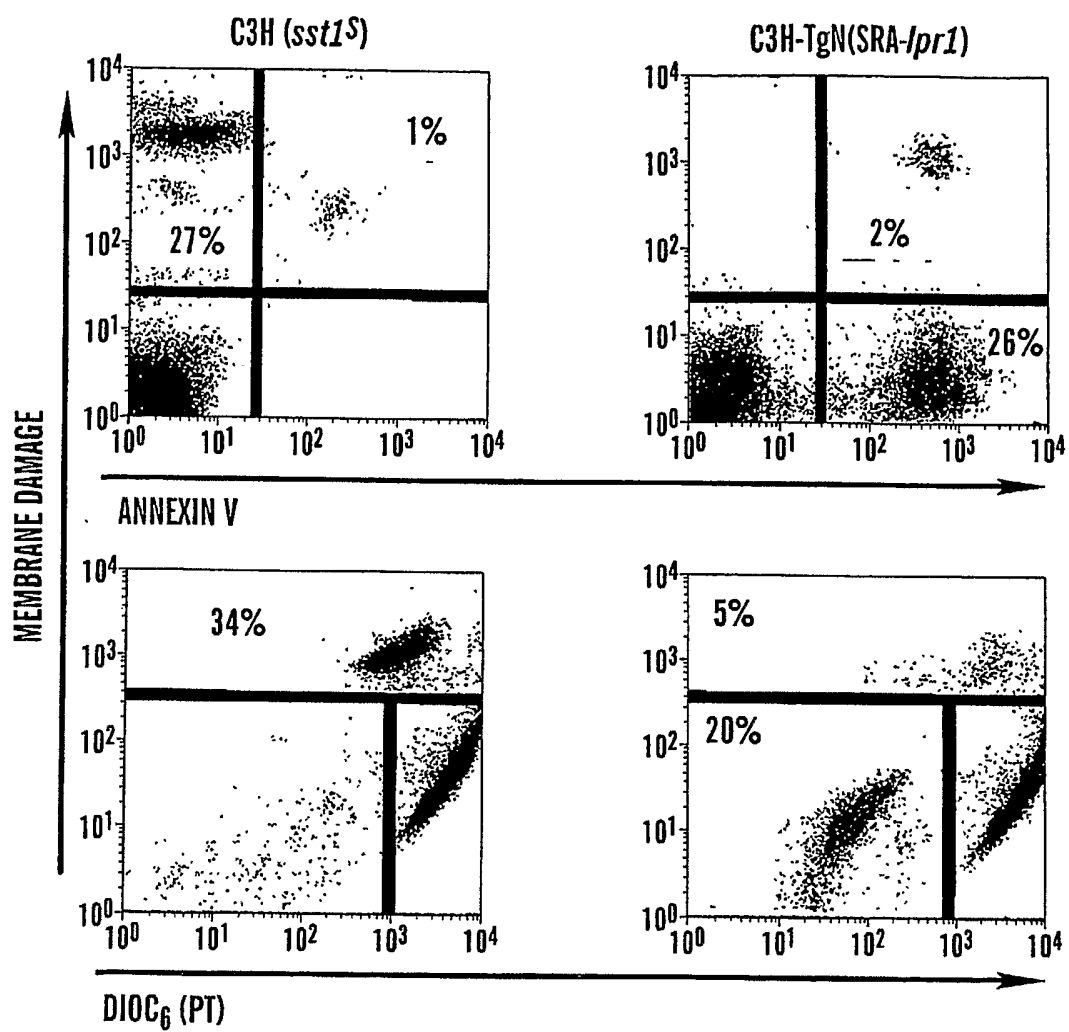
**FIG. 3b**

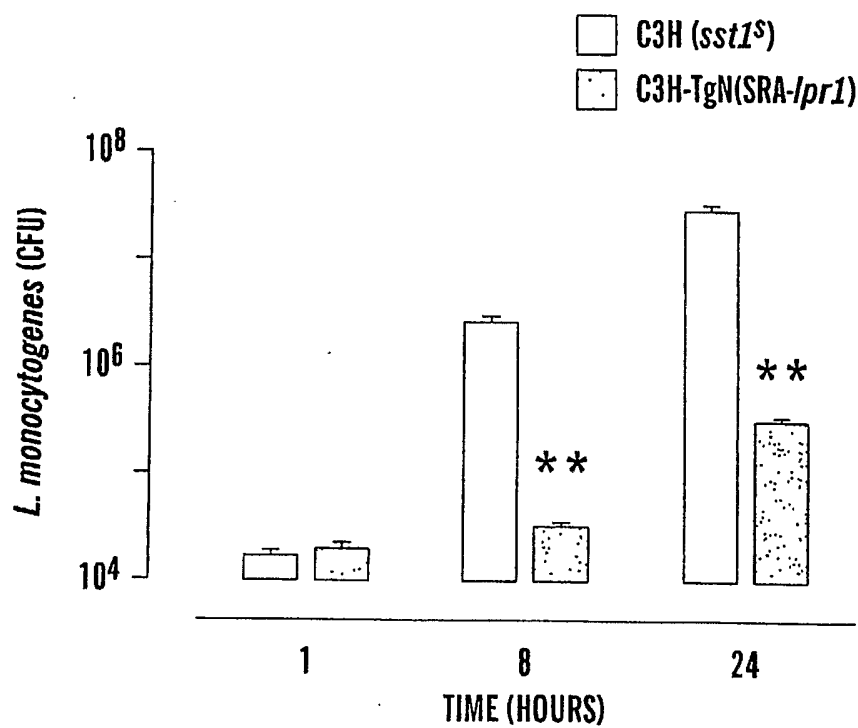
**FIG. 3c**

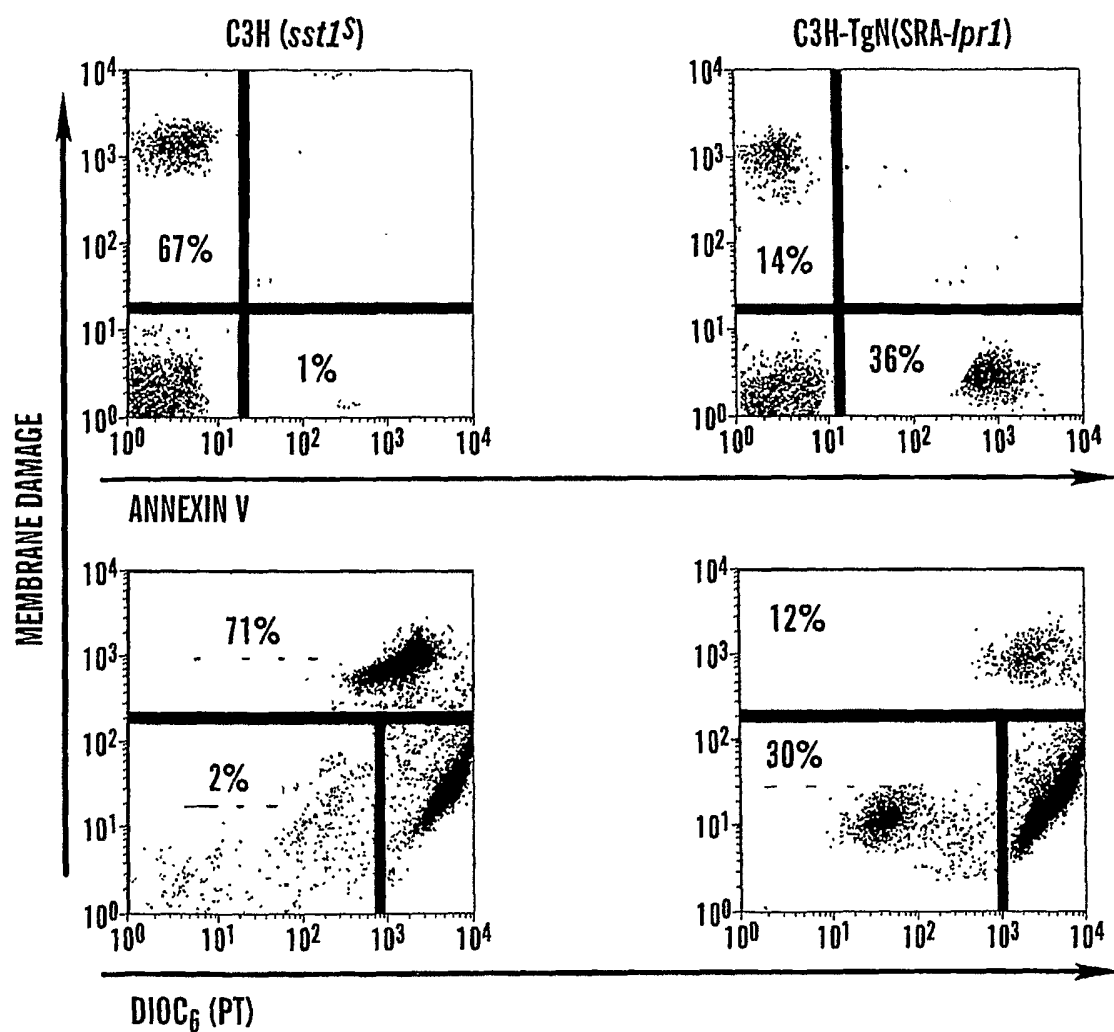
**FIG. 4a**

**FIG. 4b**

**FIG. 4c**

**FIG. 4d**

**FIG. 4e**

**FIG. 4f**

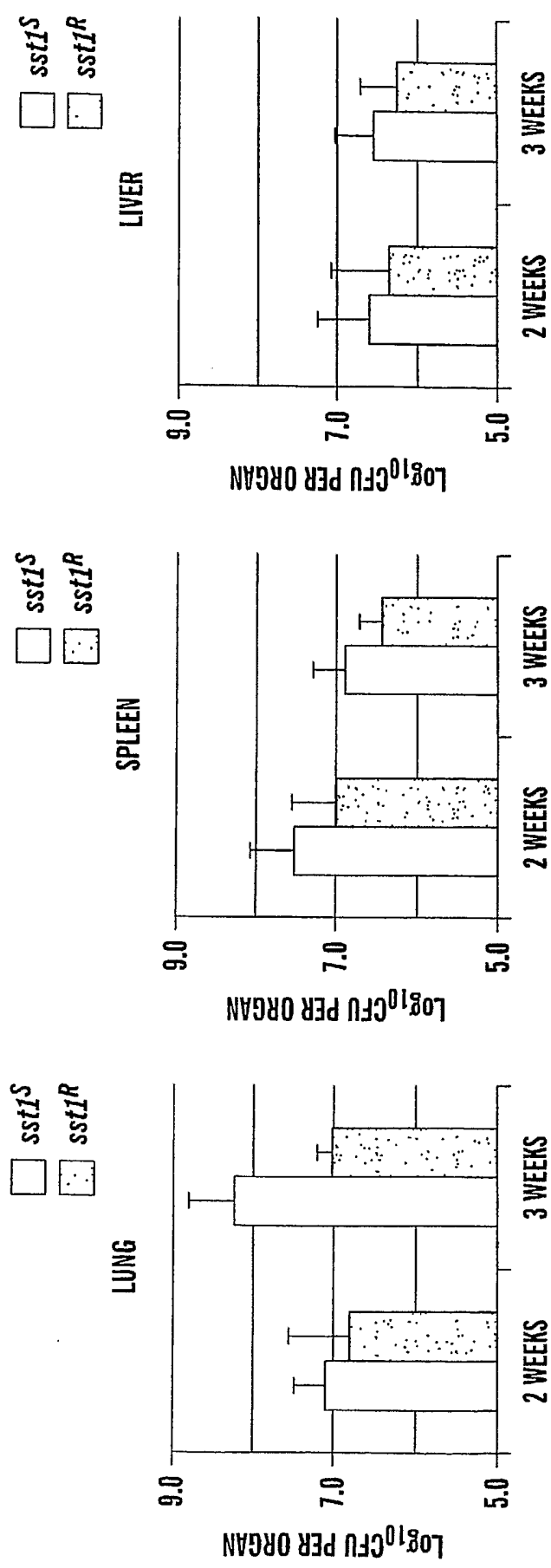


FIG. 5a

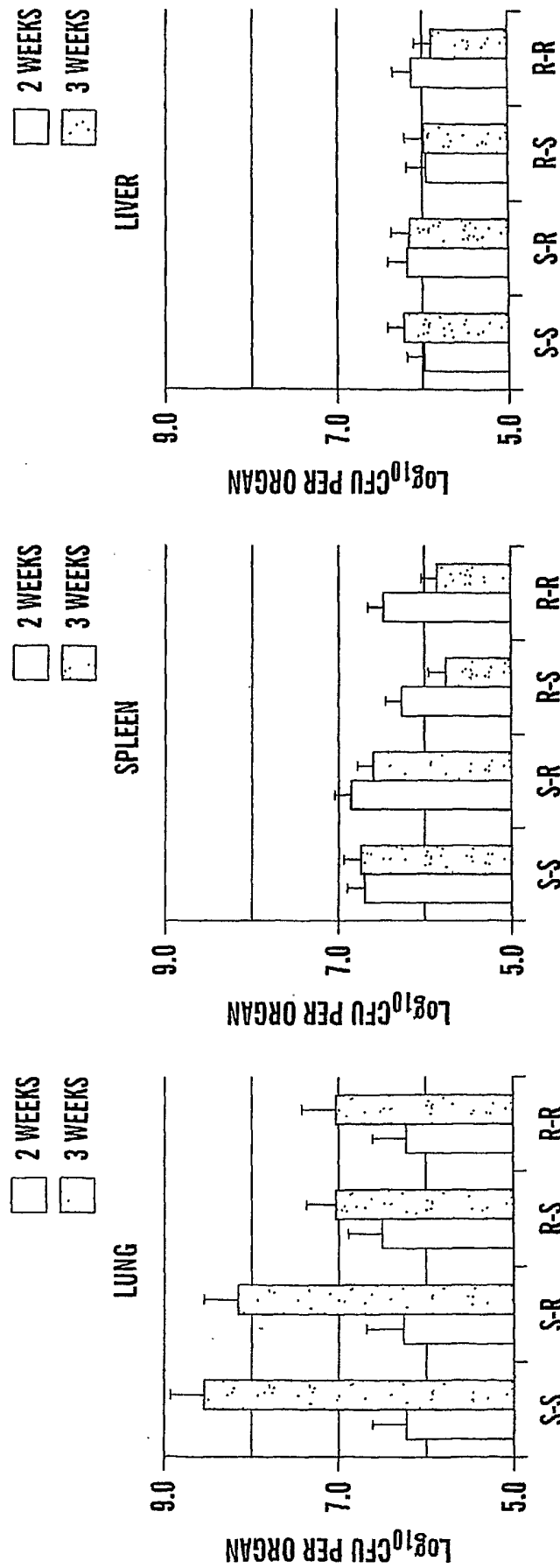


FIG. 5b

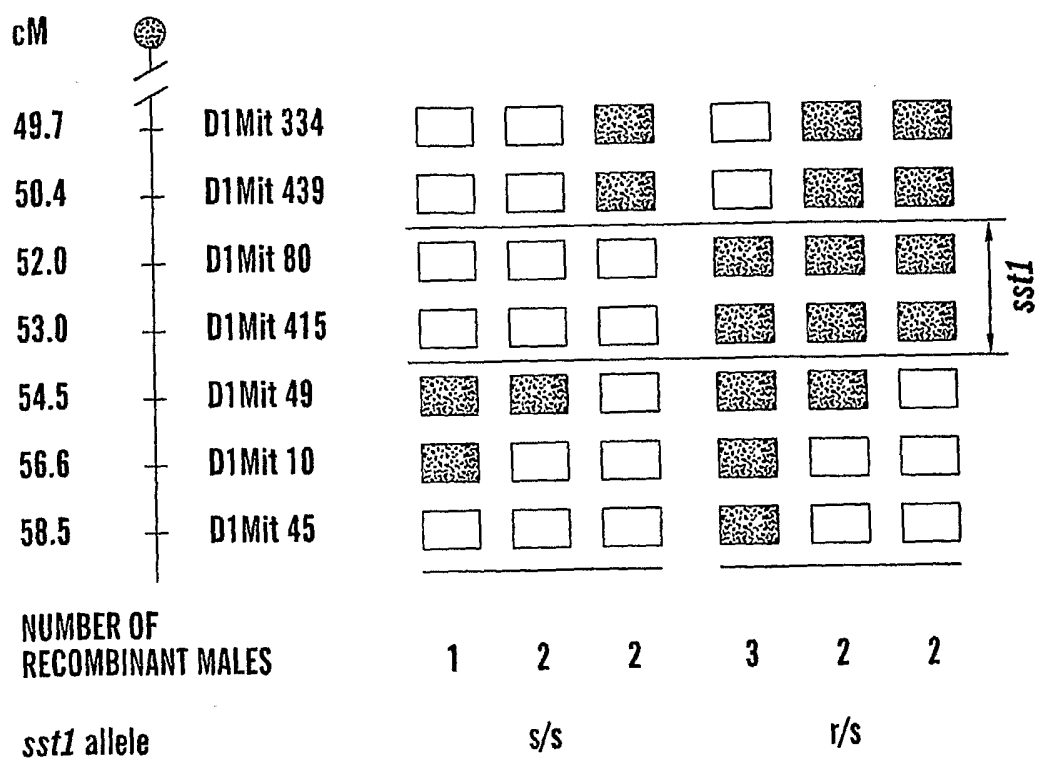
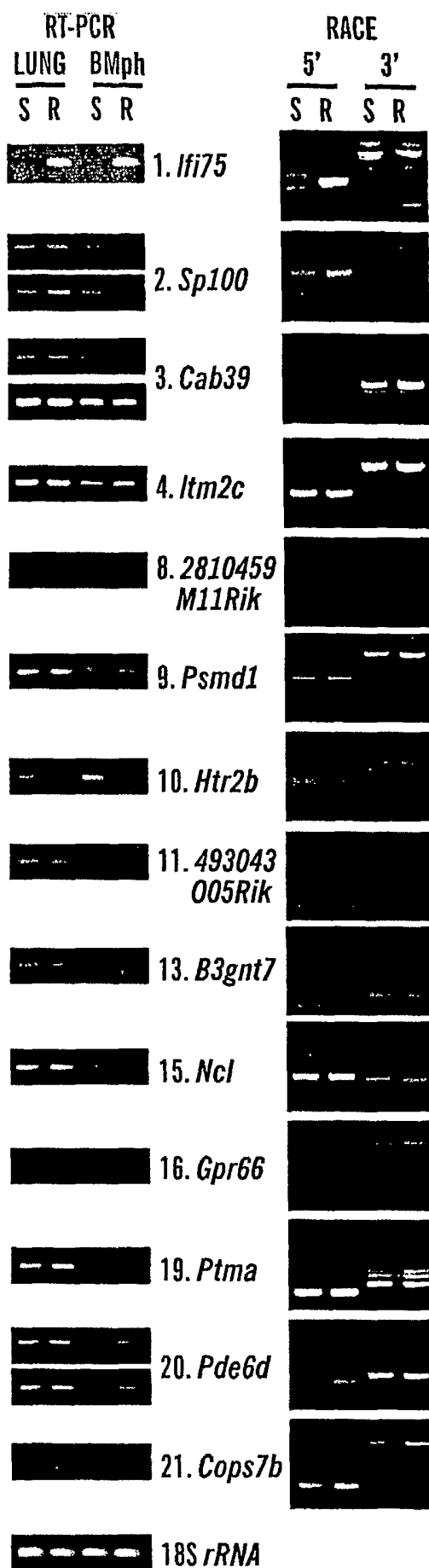
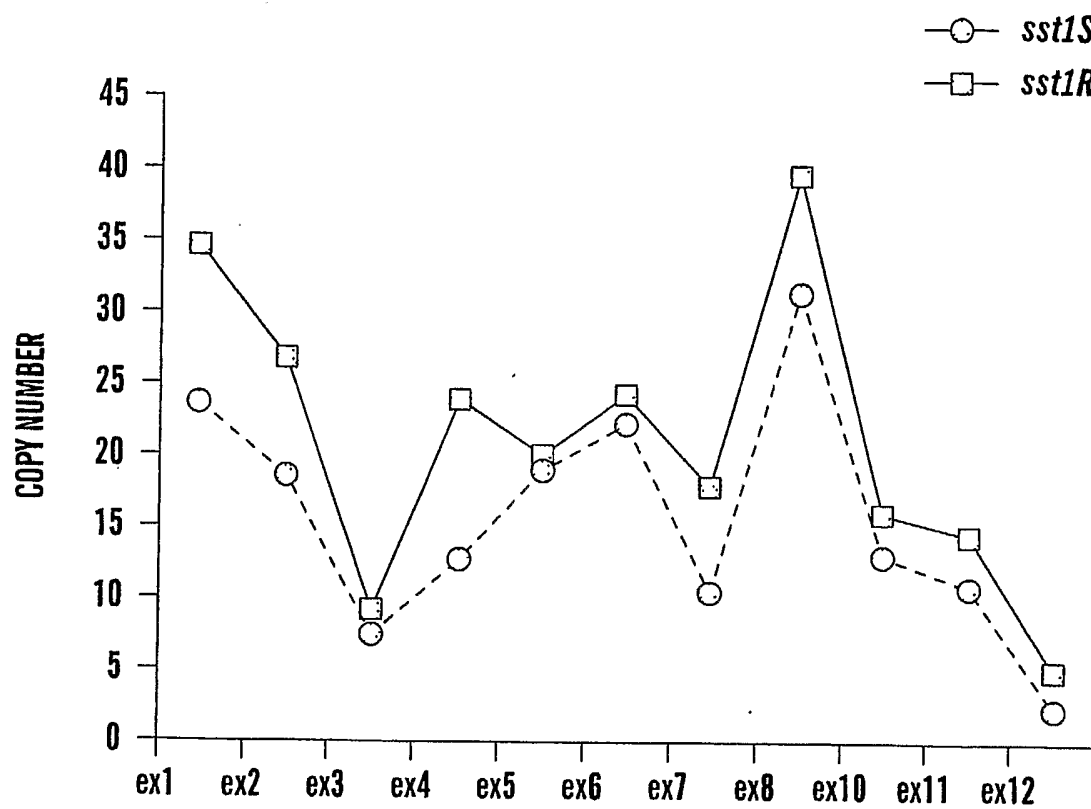
**FIG. 6a**

FIG. 6b

***FIG. 6c***

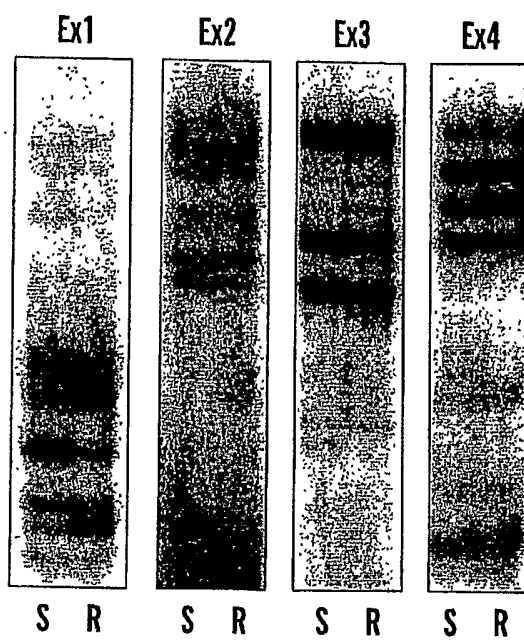


FIG. 6d

CRITERIA		CELLS OR TISSUE		GENE EXPRESSION																						PRIORITY SCORE	
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22		
DIFFERENTIAL EXPRESSION IN <i>sst1</i> CONGENICS	BM MACROPHAGES																										4
	TOTAL LUNG																										3
INDUCED BY MTB	BM MACROPHAGES																										2
	TOTAL LUNG																										1
EXPRESSED IN	BM MACROPHAGES																										1
	TOTAL LUNG																										1
AFFYMETRIX ARRAY	BM MACROPHAGES																										1
	TOTAL LUNG																										1

FIG. 6e

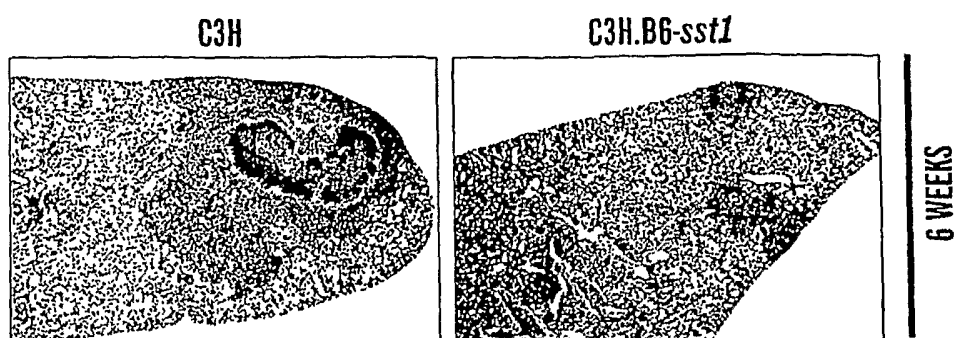


FIG. 7a

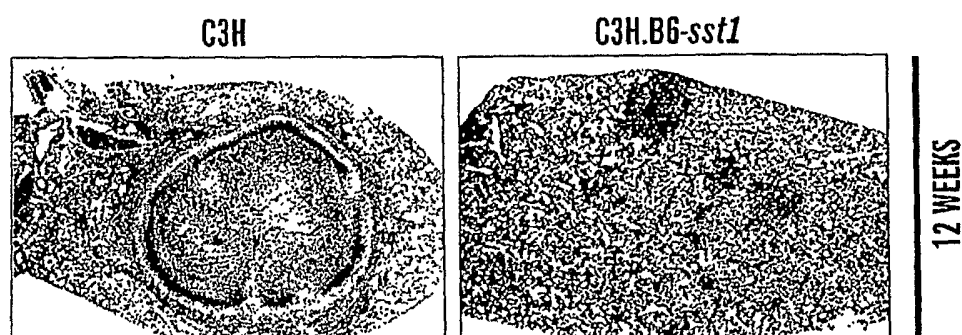


FIG. 7b

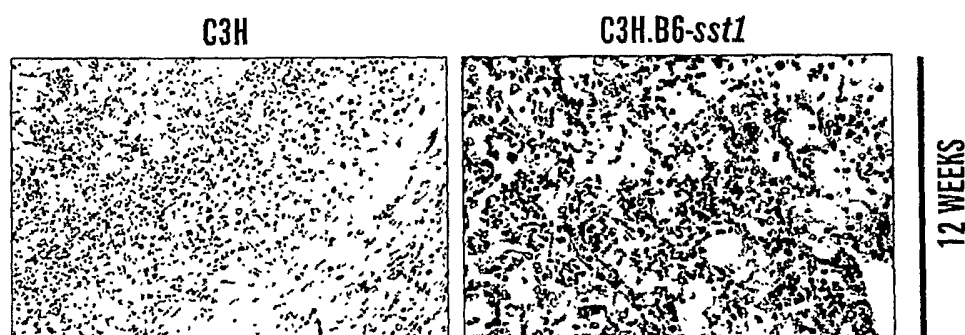


FIG. 7c

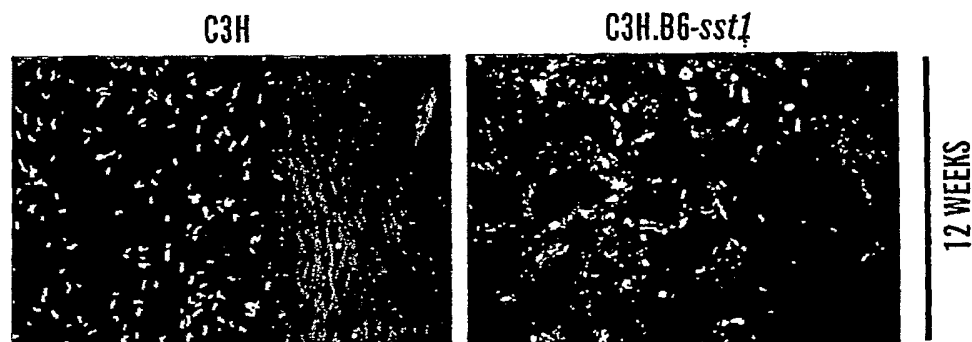


FIG. 7d

TUNEL STAINING OF THE TUBERCULOSIS LUNG LESIONS OF THE *sst1^R* AND *sst1^S* CONGENIC MICE.

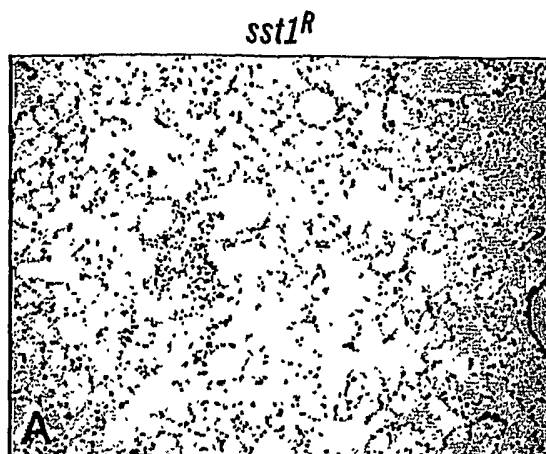


FIG. 8a

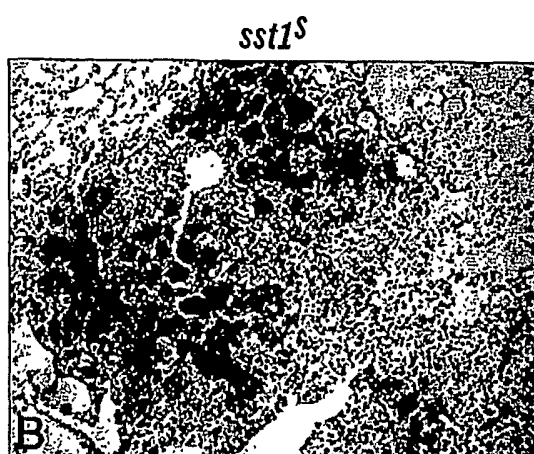


FIG. 8b

TUNEL STAINING OF THE TUBERCULOSIS LUNG LESIONS OF THE *sst1^R* AND *sst1^S* CONGENIC MICE.



FIG. 8c



FIG. 8d