Stat1 Targeted Mutation Mice

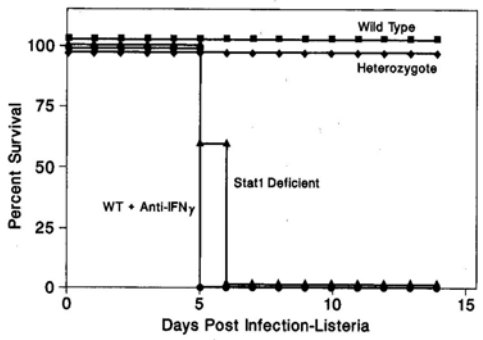
Homozygous disruption of the Stat1 gene eliminates the intracellular mechanism by which cells respond to interferons, resulting in a mouse model with extreme susceptibility to bacterial and viral infections, and with increased tumor formation

Applications for the Stat1 Targeted Mutation Mouse Model

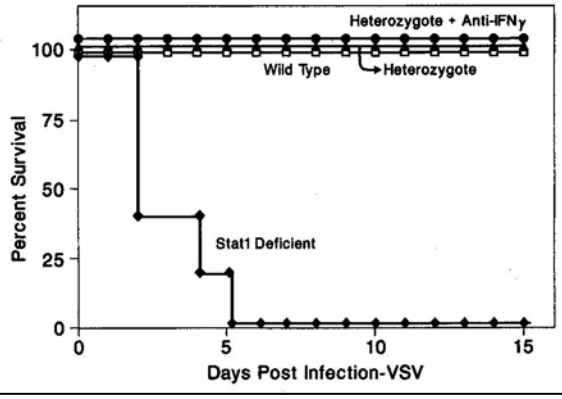
- Refining the role of STAT1 proteins in the general mechanism of interferon-induced gene activation
- Elucidating the specific actions of interferon on individual cell types through study of cell lines derived from Stat1 Targeted Mutation Mice
- Defining the role of interferons in immunity and inflammation
- Exploring the roles of interferons and STAT1 in tumorigenesis
- Defining the relative roles of tumor cells and immune cells in mediating tumor cell destruction
- Designing gene therapy strategies to "rescue" STAT1-deficient cells, such as tumor cells, to reinstate their susceptibility to immune destruction
- Refining the relative roles in cytokine signal transduction and gene expression of individual STAT proteins within the STAT family
- Clarifying the relative roles of malignant cells and immune cells in the destruction of tumors by immune surveillance
- Providing a genetic background into which additional genetic deletions or modifications can be bred, to investigate specific roles of genes in immunity and tumorigenesis
- Monitoring a murine colony's pathogen-free status, by using Stat1 Targeted Mutation Mice as sentinel animals for early detection of pathogens

Stat1 Targeted Mutation Mice Exhibit Defective Responses To Infection

Stat1-deficient mice and wild-type (WT) mice treated with anti-interferonγ (IFNγ) suffer fatal infections after exposure to non-lethal doses of the intracellular bacteria *Listeria monocytogenes* whereas WT and heterozygous mice survive.



Stat1-deficient mice died rapidly after exposure to non-lethal doses of the vesicular stomatitis virus (VSV) whereas WT and heterozygous mice treated with anti-IFNγ survive.



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Features of the Stat1 Targeted Mutation Mouse Model (model 2045) [1,2]

- Complete lack of functional STAT1 proteins
- Absence of gene activation that depends on IFN-α and IFN-γ, and no up-regulation of the synthesis of proteins that would be transcribed from those genes
- Extreme susceptibility to microbial and viral infections, associated with a cellular-level un-responsiveness to IFN-α and IFN-γ among leukocytes of the inflammatory and immune systems
- Accelerated and amplified development of chemically-induced and spontaneous tumors due to changes in tumor cell phenotype that results from disruption of the interferon signaling pathway
- Normal development and growth
- Normal *in vivo* responsiveness to other cytokines, including those that have been shown to activate *Stat1* in an *in vitro* setting

Scientific Profile of the Stat1 Targeted Mutation Mouse Model

Many of the cytokines that influence target cell activities utilize the JAK-STAT signaling pathway to elicit changes in gene transcription. The JAK family is a group of tyrosine kinases that become activated following binding of any of several cytokines to their specific surface receptors. One model of how this leads to a cellular response is that JAK activation causes phosphorylation of an intracellular domain on the cytokine receptor that becomes a docking site for a STAT protein. The STAT proteins (signal transducers and activators of transcription) are a family of several related proteins which, once activated, form homodimers or heterodimers that translocate to the nucleus and promote transcription of genes. Activation appears to require their attachment to the cytokine receptor's docking site, and involves phosphorylation of the molecule. Thus, JAK and STAT proteins are essential
as a second-messenger mechanism for the transduction of the surface-binding signal to the nucleus. Elimination of either the JAK or the STAT proteins disrupts the cytokine signal, with profound effects at cellular and physiologic levels.

Among the cytokines that utilize the JAK-STAT mechanism are interferon alpha (IFN-α) and interferon gamma (IFN-γ). Both cytokines stimulate an intracellular increase in activated STAT1 protein. The several actions of interferons continue to be elucidated, and include an essential immune-stimulating function that provides defense against pathogens (a variety of bacteria and some viruses) and the development of tumors. Animals that lack the JAK-STAT mechanism are dramatically compromised in both these immune activities. In addition, several human tumors have been found to lack essential components of the receptor-JAK-STAT system.[2]

Stat1 Targeted Mutation Mice produce an abnormal STAT1 protein in all tissues that have been assayed because they are homozygous for a targeted mutation of the *Stat1* gene.[1] As a result, the mice lack IFN-mediated biological responses, as evidenced both by cellular assays and by a dampened immune activity *in vivo*. For example, while IFN-induced up-regulation of MHC class I proteins on T lymphocytes and of MHC class II proteins on macrophages is a normal response of cells from wild-type mice, these responses are lacking in cells from Stat1 Targeted Mutation Mice (although the latter had a baseline level of expression similar to that of cells from normal mice before IFN stimulation).[1] In addition, the activation of transcription of several genes that is stimulated in wild-type mice by IFN-α or IFN-γ is absent in Stat1 Targeted Mutation Mice, as measured *in vitro* or *in vivo*. Such genes include interferon regulatory factor 1, guanylate-binding protein 1, MHC class II transactivating protein, complement protein C3, and complement protein factor B.[1] Thus, Stat1 Targeted Mutation Mice show, by several assays, a disrupted IFN signaling pathway that impacts transcription of several genes and the production of immune-regulatory proteins.

Stat1 Targeted Mutation Mice show evidence at a cellular and physiologic level of non-responsiveness to IFN-induced immune activities.[1] Macrophages of Stat1-deficient mice cannot produce nitric oxide by the IFN-inducible enzyme, nitric oxide synthetase, which normally provides an important pathogen-destroying mechanism in murine cells, and fibroblasts are lacking an IFN-mediated resistance to viral infection. In addition, the STAT1 deficiency produces striking physiologic alterations of the immune system. Mutant mice succumbed to bacterial (*Listeria monocytogenes*) and viral (vesicular stomatitis virus) infections and died within 5 days of inoculation, whereas the same doses stimulated a curative response in wild-type mice and in those heterozygous for the Stat1 mutation.[1] These same kinds of pathogen susceptibilities have been described in another mouse model carrying a disrupted *Stat1* gene, with mice easily succumbing to illness and death from otherwise innocuous pathogens.[3]

Stat1 Targeted Mutation mice show normal responses to other cytokines that have been shown *in vitro* to activate the STAT1 pathway. Specifically, growth hormone, epidermal growth factor, and interleukin-10 all generate normal responses in cells from Stat1-deficient mice, both *in vitro* and *in vivo*, compared to wild type mice.[1] This suggests that the activation in wild-type cells of STAT1 by these cytokines, as previously has been documented *in vitro*, does not mean that STAT1 is a required participant in the signaling pathway *in vivo*. In another Stat1-deficient mouse model,[3] as in the Stat1 Targeted Mutation Mouse, transcriptional responses that would be expected in cells treated with IFN were absent, but responses to other cytokines remained intact. Studies with both models thus support the conclusion that the STAT1 signaling pathway exhibits a high degree of IFN-specificity *in vivo*. The normal cellular response to growth hormone in Stat1 Targeted Mutation Mice accounts, at least in part, for a normal developmental weight gain compared to wild-type mice.

Stat1 Targeted Mutation Mice also show a compromised immune surveillance capacity against tumor cells.[2] The lack of an intact STAT1 signaling pathway in these mice is associated with a heightened sensitivity to tumor induction by chemicals as well as an increased frequency of spontaneous tumor development. Further examination of this response has revealed that the tumor cells themselves are responsible for their ability to escape immune destruction, possibly by disruption of an IFN-γ-inducible synthesis of MHC proteins.[2]

Origin of the Model

Stat1 Targeted Mutation Mice were originated by Dr. Robert Schreiber and colleagues at Washington University School of Medicine.[1] The mutation was generated by electroporation into a GS-1 embryonic stem cell line of a targeting vector that carried a neomycin resistance cassette designed to replace the first three translated exons of the *Stat1* gene.

Successfully transfected cells were used to generate male chimeras that then were bred to 129/Sv females. Offspring that transmitted the disrupted allele were interbred to generate mice that were homozygous for the mutation. The model came to Taconic in 1997 and was embryo-transfer derived. The original Stat1 model (000679-M) differed from the Taconic 129S6 at two biochemical loci, *Pgm1* and *Gpi1*. In January 2002, the original (000679-M) Stat1 line was backcrossed to the Taconic 129S6/SvEvTac and animals were selected to match the $Pgm1^{c}$ *and* $Gpi1^{b}$ alleles of the 129S6. The selected heterozygotes were intercrossed to achieve homozygosity and the line is currently maintained by incrossing of homozygous mice. This improvement makes the 129S6/SvEvTac a more perfectly matched control for the Stat1 model (002045-M).

Ready for Your Experiments

Taconic's Stat1 Targeted Mutation Mouse Model is maintained in Isolator Barrier Unit (IBU™) facilities. Mice are shipped in Taconic Transport Cages (TTC™) and come with an up-to-date health report documenting their Murine Pathogen Free (MPF™) health status. Barrier

housing conditions are recommended for maintenance of Stat1 Targeted Mutation Mice.

Related Mouse Models from Taconic

Taconic provides a number of mouse models relevant to immune system function and tumorigenesis, including mutational, knockout, and microinjected models on a variety of backgrounds. Contact Taconic to inquire about the following additional models:

- **nude (models B6NU, B6NBO, BALBNU, BLBANU, NCRNU, NSWNU and NMRINU) –** heterozygous or homozygous for the nude gene, conferring in homozygous mice a T-lymphocyte deficiency due to absence of a functional thymus, but no altered T-cell immunity in carrier (heterozygous) mice
- **scid (models CB17SCRF, CB17SC, ICRSC and NODSC) –** homozygous for the *scid* (severe combined immunodeficiency) gene, lacking both T- and B-lymphocytes, serves as a model for immunity research and a host for xenotransplanation studies
- **scid-beige (model CBSCBG)** – homozygous for the *scid* (severe combined immunodeficiency) gene and *beige* mutation, lacking both T- and B-lymphocytes and exhibiting impaired macrophage and NK cell function; serves as a model for immunity research and a host in xenotransplantation studies
- **beige-nude-xid (model NIHBNX)** – homozygous for *nude* and *beige* mutations, and carrying an X-linked *xid* mutation; lacking normal thymic development and T-lymphocytes, exhibiting impaired function of macrophages, NK cells, and B-lymphocytes; serves as a model for immunity research and a host in xenotransplantation studies
- **Abb Targeted Mutation (models 004026 and ABBN12)** – exhibiting depletion of $CD4^+$ T-lymphocytes and deficiency in MHC Class II proteins due to disruption of the *Abb* gene, useful in transplantation, gene therapy, and immunological disease research
- **B2m Targeted Mutation (models 004020 and B2MN12)** – depleted of $CD8^+$ T-lymphocytes due to disruption of the *B2m* gene; useful in transplantation, gene therapy, and immunological disease research
- **Abb/B2m Double Targeted Mutation (model 004080)** – lacking MHC Class II and reduced in MHC Class I expression, deficient in both $CD4^+$ and $CD8^+$ T-lymphocytes
- **Fcer1g (FcRγ) Targeted Mutation (models 000584 and 000583)** – exhibiting impaired function of macrophages, neutrophils, mast cells, basophils, and NK cells due to lack of the gene encoding the γ subunit of the cell surface receptor proteins, FcγRIII and FcεRIγ
- **Fcgr2b (FcγRII) Targeted Mutation (models 000579 and 000580)** – exhibiting dysfunctional immune inhibitory pathways due to lack of the gene encoding FcγRIIβ, a low affinity IgG receptor
- **Pfp Targeted Mutation (model PFPN12)** – exhibiting a deficiency in perforin, a protein essential for cytotoxic activities of NK cells; useful for studies of immune suppression and transplantation
- **Rag2 Targeted Mutation (models 000461, 000601, RAG2 and RAGN12)** – lacking mature B- and T-lymphocytes, due to inactivation of the *Rag2* (recombination activating 2) gene required for V(D)J rearrangement; useful for vaccine development, transplantation studies, and hematopoiesis research
- **Pfp/Rag2 Double Targeted Mutation (model 001177)** – lacking both *Pfp* and *Rag2* genes, exhibiting a severe depletion of functional NK cells and of B- and T-lymphocytes; useful for vaccine development, transplantation studies, and studies of the immune system
- **Rag2 Targeted Mutation-HY Microinjected Mouse (model 004079)** – lacking endogenously derived mature B- and T-lymphocytes but with expression of receptors for the HY antigen, which rescues $CD8^+$ cell development in females
- **K6-ODC Microinjected Mouse (models 000993 and 003000)** – over-expressing ornithine decarboxylase (ODC) in epidermal root sheath keratinocytes, with resulting skin abnormalities and epidermal papilloma formation after exposure to initiators only (promoters not required); allows for dramatic

reduction in time, expenditure, and animals required for effective carcinogenicity testing

- **TSG-p53® Targeted Mutation Mouse (models P53N4, P53N5 and P53N12)** – carrying one or two nonfunctional copies of the well-established tumor suppresser and pro-apoptotic *p53* gene, leaving mice highly susceptible to tumorigenesis; allows for dramatic reduction in time, expenditure, and animals required for effective carcinogenicity testing
- **v-Ha-ras (TG.AC) OncoMouse™ Micro-injected Model** – carrying an activated *v-Ha-ras* oncogene fused to a murine zeta-globulin promoter, conferring high sensitivity to tumor promotion through a *v-Ha-ras* mechanism; allows for dramatic reduction in time, expenditure, and animals required for effective carcinogenicity testing

References Cited:

1. Meraz, M.A., et al. (1996) Targeted Disruption of the *Stat1* Gene in Mice Reveals Unexpected Physiologic Specificity in the JAK-STAT Signaling Pathway. Cell, Vol. 84, pp. 431-442.
2. Kaplan, D.H., et al. (1998) Demonstration of an Interferon γ-Dependent Tumor Surveillance System in Immunocompetent Mice. *Proc. Natl. Acad. Sci. USA*, Vol. 95, pp. 7556-7561.

Durbin, J.E., et al. (1996) Targeted Disruption of the Mouse *Stat1* Gene Results in Compromised Innate Immunity to Viral Disease. *Cell*, Vol. 84, pp. 443-450.

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Taconic Transgenic Models
Publication Reference List
Stat1 Targeted Mutation Mice

Agrawal, S., Agarwal, M.L., Chatterjee-Kishore, M., Stark, G.R., Chisolm, G.M. (2002) **Stat1-Dependent, p53-Independent Expression of p21^{waf1} Modulates Oxysterol-Induced Apoptosis.** *Molecular Cell Biology*, Vol. 22, No. 7, pp. 1981-1992.

Chin YE, Kitagawa M, Su WC, You ZH, Iwamoto Y, Fu XY. (1996) **Cell growth arrest and induction of cyclin-dependent kinase inhibitor p21 WAF1/CIP1 mediated by STAT1.** *Science*, 272(5262):719-722.

Collazo, C.M., Yap, G.S., Hieny, S., Caspar, P., Feng, C.G., Taylor, G.A., Sher, A. (2002) **The Function of Gamma Interferon-Inducible GTP-Binding Protein IGTP in Host Resistance to *Toxoplasma gondii* Is Stat1 Dependent and Requires Expression in Both Hematopoietic and Nonhematopoietic Cellular Compartments.** *Infection and Immunity*, Vol. 70, No. 12, pp. 6933-6939.

Durbin, J.E., Hackenmiller, R., Simon, M.C., Levy, D.E. (1996) **Targeted Disruption of the Mouse *Stat1* Gene Results in Compromised Innate Immunity to Viral Disease**, *Cell*, Vol. 84, pp. 443-450.

Gavrilescu, L.C., Butcher, B.A., Del Rio, L., Taylor, G.A., Denkers, E.Y. (2004) **STAT1 Is Essential for Antimicrobial Effector Function but Dispensable for Gamma Interferon Production during *Toxoplasma gondii* Infection.** *Infection and Immunity*, Vol. 72, No. 3, pp. 1257-1264.

Gongora R, Stephan RP, Schreiber RD, Cooper MD. (2000) **Stat-1 is not essential for inhibition of B lymphopoiesis by type I IFNs.** *J Immunol*, 165(5):2362-6.

Halford, W.P., Maender, J.L., Gebhardt, B.M. (2005) **Re-evaluating the role of natural killer cells in innate resistance to herpes simplex virus type 1.** *Virology Journal*, Vol. 2, pp. 56.

Halford, W.P., Weisend, C., Grace, J., Soboleski, M., Carr, D.J., Balliet, J.W., Imai, Y., Margolis, T.P., Gebhardt, B.M. (2006) **ICP0 antagonizes Stat 1-dependent repression of herpes simplex virus: implications for the regulation of viral latency.** *Virology Journal*, Vol. 3, pp. 44.

Hancock, W.W., Szaba, F.M., Berggren, K.N., Parent, M.A., Mullarky, I.K., Pearl, J., Cooper, A.M., Ely, K.H., Woodland, D.L., Kim, I.J., Blackman, M.A., Johnson, L.L., Smiley, S.T. (2004) **Intact type 1 immunity and immune-associated coagulative responses in mice lacking IFNγ-inducible fibrinogen-like protein 2.** *Proceedings of the National Academy of Science*, Vol. 101, No. 9, pp. 3005-3010.

Kaplan, D.H., Shankaran, V., Dighe, A.S., Stockert, E., Aguet, M., Old, L.J., Schreiber, R.D. (1998) **Demonstration of an Interferon γ-Dependent Tumor Surveillance System in Immunocompetent Mice,** *Proc. Natl. Acad. Sci. USA*, Vol. 95, pp. 7556-7561.

Kotelkin, A., Belyakov, I.M., Yang, L., Berzofsky, J.A., Collins, P.L., Bukreyev, A. (2006) **The NS2 Protein of Human Respiratory Syncytial Virus Suppresses the Cytotoxic T-Cell Response as a Consequence of Suppressing the Type I Interferon Response.** *Journal of Virology*, Vol. 80, No. 12, pp. 5958-5967.

Lighvani, A.A., Frucht, D.M., Jankovic, D., Yamane, H., Aliberti, J., Hissong, B.D., Nguyen, B.V., Gadina, M., Sher, A., Paul, W.E., O'Shea, J.J. (2001) **T-bet is rapidly induced by interferon-γ in lymphoid and myeloid cells.** *Proceedings of the National Academy of Science*, Vol. 98, No. 26, pp. 15137-15142.

Loke, P., Allison, J.P. (2003) **PD-L1 and PD-L2 are differentially regulated by Th1 and Th2 cells.** *Proceedings of the National Academy of Science*, Vol. 100, No. 9, pp. 5336-5341.

Lucas, S., Ghilardi, N., Li, J., de Sauvage, F.J. (2003) **IL-27 regulates IL-12 responsiveness of naïve CD4^{+} T cells through Stat1-dependent and -independent mechanisms.** *Proceedings of the*

National Academy of Science, Vol. 100, No. 25, pp. 15047-15052.

Lugo-Villarino G, Ito SI, Klinman DM, Glimcher LH. (2005) **The adjuvant activity of CpG DNA requires T-bet expression in dendritic cells.** *Proceedings of the National Academy of Science*, 102(37):13248-53.

Lugo-Villarino, G., Maldonado-López, R., Possemato, R., Peñaranda, C., Glimcher, L.H. (2003) **T-bet is required for optimal production of IFN-γ and antigen-specific T cell activation by dendritic cells.** *Proceedings of the National Academy of Science*, Vol. 100, No. 13, pp. 7749-7754.

Meraz, M.A., White, J.M., Sheehan, K.C.F., Bach, E.A., Rodig, S.J., Dighe, A.S., Kaplan, D.H., Riley, J.K., Greenlund, A.C., Campbell, D., Carver-Moore, K., DuBois, R.N., Clark, R., Aguet, M., Schreiber, R.D. (1996) **Targeted Disruption of the *Stat1* Gene in Mice Reveals Unexpected Physiologic Specificity in the JAK-STAT Signaling Pathway,** *Cell*, Vol. 84, pp. 431-442.

Mullarky, I.K., Szaba, F.M., Berggren, K.N., Kummer, L.W., Wilhelm, L.B., Parent, M.A., Johnson, L.L., Smiley, S.T. (2006) **Tumor Necrosis Factor Alpha and Gamma Interferon, but Not Hemorrhage or Pathogen Burden, Dictate Levels of Protective Fibrin Deposition during Infection.** *Infection and Immunity*, Vol. 74, No. 2, pp. 1181-1188.

Nishibori, T., Tanabe, Y., Su, L., David, M. (2004) **Impaired Development of $CD4^+$ $CD25^+$ Regulatory T Cells in the Absence of STAT1: Increased Susceptibility to Autoimmune Disease.** *Journal of Experimental Medicine*, Vol. 199, No. 1, pp. 25-34.

Pasieka TJ, Lu B, Leib1 DA. (2008) **Enhanced Pathogenesis of an Attenuated Herpes Simplex Virus for Mice Lacking Stat1.** *J Virology*, p. 6052–6055.

Wang J, Schreiber RD, Campbell IL. (2002) **STAT1 deficiency unexpectedly and markedly exacerbates the pathophysiological actions of IFN-alpha in the central nervous system.** *Proc Natl Acad Sci USA*, 99(25):16209-14.

Walters, D.M., Antao-Menezes, A., Ingram, J.L., Rice, A.B., Nyska, A., Tani, Y., Kleeberger, S.R., Bonner, J.C. (2005) **Susceptibility of Signal Transducer and Activator of Transcription-1-Deficient Mice to Pulmonary Fibrogenesis.** *American Journal of Pathology*, Vol. 167, No. 5, pp. 1221-1229.

Wobus, C.E., Karst, S.M., Thackray, L.B., Chang, K.O., Sosnovtsev, S.V., Belliot, G., Krug, A., Mackenzie, J.M., Green, K.Y., Virgin, H.W. (2004) **Replication of *Norovirus* in Cell Culture Reveals a Tropism for Dendritic Cells and Macrophages.** *PloS Biology*, Vol. 2, No.12, pp. e432.