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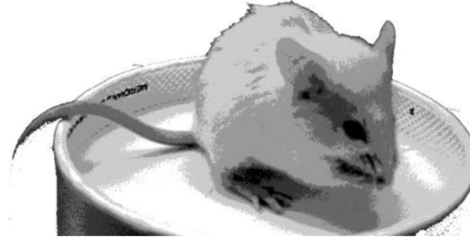
Maintained mouse lines



RAG-1^0/N
Recombination Activating Gene 1 Knock Out

Development

A neomycin resistance cassette was inserted in the RAG-1 gene, resulting in a 1356 bp deletion in the 5' end of the coding sequence into 129-derived AB1 ES cell line. The C57BL/6J strain was generated by backcrossing mice carrying the Rag-10 mutation 10 times to C57BL/6J inbred mice. (Mombaerts P *et al*, Papaioannou VE., 1992).
Rag-1 deficient mice were transferred to the NOD/LtSz strain background. (Carnaud C. *et al*, Bendelac A., 1999; Shultz *et al* Greiner, 2000). The line is currently at the 18th backcross to NOD.

Description

RAG-1deficient mice are viable and fertile.They are unable to initiate V(D)J recombination in Ig and TCR genes and lack functional T and B lymphocytes. Although RAG-1 expression has been reported in the central nervous system of the mouse, no obvious neuroanatomical or behavioral abnormalities have been found in the RAG-1-deficient mice. (Mombaerts P *et al*, Papaioannou VE., 1992).
On the NOD background, NOD.Rag10 mice are devoid of mature T or B cells. NOD/LtSz-Rag10 recipients of adoptively transferred spleen cells from diabetic NOD/Lt+/+ mice rapidly develop diabetes. (Carnaud C. *et al*, Bendelac A., 1999; Shultz *et al* Greiner, 2000).

Foxp3^{DTR}/N
Foxp3-Human DTR-eGFP transgene

Development

Transgene-directed expression of the human Diphteria Toxin Receptor (DTR) can be used for lineage ablation studies, to determine the role of particular cell types. Mouse cells are resistant to DT for lack of a receptor, but targeted expression of DTR as a transgene renders DTR-expressing cells sensitive to DT treatment in vivo (Saito et al., 2001). Mice expressing the human DTR under the control of foxp3 transcriptional control elements were generated by BAC transgenesis. The BAC construct spanned from 150 kb upstream to 70kb downstream of the Foxp3 transcription start site. A DTR-eGFP cDNA with a stop codon was inserted between the first and second codon of the Foxp3 open reading frame.
The recombinant Foxp3-DTR-eGFP BAC was injected into fertilized NOD oocytes, and offspring were genotyped by PCR. (Feuerer *et al*, 2009).

Description

Heterozygous NOD.Foxp3.DTR + transgenic mice show no obvious phenotype. The eGFP component can be used for identification of Treg cells, although the fluorescence intensity is lower than that of common reporter lines.
DT administration into young adults results in 80%-90% depletion of Foxp3+ T cells in secondary lymphoid organs after a few days. Treg cell pools recover quickly after the end of DT treatment, and Treg cell numbers recovered to 60% of normal by three days after the last injection. (Feuerer *et al*, 2009)
The pancreatic islet of the DT-injected mice showed a strong immune infiltrate compared to littermate control when DT is injected every other day in young adult for 9 days. Very mild infiltrate can be detected in lung and liver of Foxp3 DTR trangenic mice after such a regimen (Feuerer *et al*, 2009).

FoxP3-I-GFP/N
Forkhead Box P3 - IRES-GFP Knock-in

Development

FoxP3-I-GFP mice were generated in the Kuchroo laboratory (Betteli et al, 2006). An enhanced fluorescent protein reporter (EGFP) was introduced by recombination into the Foxp3 locus, creating a bi-cistronic transcript that expresses both Foxp3 and GFP proteins. An IRES-EGFP derived from pMSCV-IRES-EGFP was subcloned into a TKPbs-LoxP-Neo cassette with an SV40 polyadenylation sequence. A 5.3 kb fragment containing the 3' untranslated region and a 1.7 kb fragment containing exons 11, 12 and part of the exon 13 of C57BL/6 Foxp3 genomic DNA were generated by PCR amplification using a BAC clone (RP23-54C14) as a template, then inserted into the targeting construct. Targeted Bruce4 ES cells were injected into BALB/c blastocysts and male chimeras were initially bred with female C57BL/6. The line was subsequently backcrossed onto the NOD/MrkTac in the Kuchroo lab over 12 times, intercrossed for 6 generations, then 3 generations in NOD/Ltj in the T1 Resource (ongoing).

Description

Description

Knock-in mice express a functional chimeric GFP protein only in cells where the Foxp3 gene is transcriptionally active (Betteli et al, 2006, Xu et al, 2010). FoxP3 is expressed in Treg cells, which constitutes about 13% of CD4 alpha/beta T cells in NOD mice. The GFP fluorescent protein is readily detectable by flow cytometry, providing an easy identification of Treg cells in lymphoid organs and pancreas and spleens of NOD mice, useful for tracking or purification (Betteli et al, 2006, Xu et al, 2010).

Ealpha16/N Tg
E Alpha 16 Transgene

Development

A cloned Ek alpha MHC Class II gene was introduced by microinjection into (C57BL/6 x SJL) F1 mouse oocytes. These strains, as NOD mice, are generally deficient in the MHC II E complex because of a large deletion in the promoter of he Ealpha gene. The transgene thus restore proper expression of the E alpha E beta complex, and immune reactivities linked to it (Le Meur *et al*, Mathis D., 1985).

The line is currently at the >99th backcross generation in NOD/LtJ (Bohme J *et al*, Mathis D, 1990).

Description

Transgenic mice are viable and fertile. Ealpha 16 abundance in the spleen of all transgenic mice is equivalent to that in BALB/c and B10-BR controls. E alpha 16 expresses the I-E complex on all cells that normally display class II molecules.

On the NOD genetic background, the transgene E alpha 16 confers almost complete protection from insulitis by preventing lymphocyte infiltration into the pancreatic islets of 10 week old mice (Bohme J *et al*, Mathis D, 1990).

MIP-GFP/N
Mouse insulin promoter(MIP), human Growth Hormone/EGFP transgene

NOD/ShiLtJ-Tg(Ins1-EGFP/GH1)14Hara/HaraJ

Development

This transgenic allele expresses Enhanced Green Fluorescent Protein fused to a 2.1kb fragment of human Growth Hormone under the control of Mouse Insulin Promoter 1. The transgenic construct was injected directly into NOD/LtJ oocytes. (Hara *et al*, (2003). The line was backcrossed onto the NOD/Ltj background.

Description

Transgenic mice develop normally and behave similarly to controls with respect to glucose tolerance and pancreatic insulin content. Histology confirms transgenic mice have normal islet architecture with co-expression of insulin and GFP. (Hara *et al*, 2003).The enhanced GFP reporter allows the beta cells to be easily identified and purified for further studies.Studies completed at The Jackson Laboratory (Leiter EH et al 2007) indicate there is strong non-mosaic expression of green fluorescent protein in NOD/LtJ-Tg(Ins1-EGFP/GH1)14Hara/HaraJ islets. 100% of homozygous Ins1-EGFP transgenic males and females, identified by qPCR, become diabetic by 9 weeks of age. Pancreatic histopathology of homozygous mice shows beta cell loss without insulitis.

Hemizygous Ins1-EGFP mice are viable and are used for breeding. A 30-week incidence study comparing NOD/LtJ controls with mice hemizygous for the Ins1-EGFP transgene shows severely depressed diabetes incidence in these hemizygotes, with males atypically at greater risk than females. Glucose tolerance in prediabetic, Ins1-EGFP transgenic hemizygous 8-week-old males is selectively impaired compared to wild type controls. Although these 8-week-old Ins1-EGFP transgenic and wild type males did not differ in plasma insulin content; a significant decline was noted in hemizygous Ins1-EGFP transgenic males compared to normoglycemic wildtype males when sampled at 30 wk. Adoptive transfer of highly diabetogenic CTL (AI4 TCRTg Rag) CD8+ T cells produced diabetes within 8 days post-injection into NOD/Lt females, but failed to produce any diabetes or even home to islets weeks after injection into hemizygous females. Pancreatic histopathology of 31 week old, non-diabetic, hemizygous Ins1-EGFP transgenic males and females indicate peri and intra islet fibrosis, peri-insulitis and depleted beta cell granulation in 70% of the animals, while only 30% of the mice have intra islet insulitis.

BDC2.5/N
TCR alpha /beta transgene

Development

BDC2.5/N mice carry both rearranged TCR alpha and beta genes from the diabetogenic H2-Ag7 restricted BDC2.5 CD4+ T cell clone BDC-2.5. The BDC2.5 TCRa and TCRb sequences were co-injected into (B6xSJL)F2 eggs. To achieve the natural expression of Tcra, the rearranged V alpha J alpha sequence from BDC2.5 was cloned into the cassette vector generated by Kouskoff *et al* (1996), which contains both the 5' upstream promoter and the 3' downstream enhancer regions of the Tcra gene. The integration site for these BDC2.5Tcr transgenes was localized to chromosome 13 near the D13mit125 marker (Katz *et al* , Mathis D. 1993). The line is currently at the >99 backcross generation to NOD/LtJ.

Description

On the NOD background, mice carrying the transgenes have a reduced incidence of diabetes relative to NOD/LtJ controls (12% incidence at age 30 weeks). BDC2.5/N animals show a generalized and very extensive islet infiltration after a few weeks of age, but most remain free of overt diabetes for long periods or develop forms of insulitis that does not cause beta cells destruction. When coupled with the homozygous Rag1 knock Out, mice develop diabetes extremely early (mean age of 25 days). (Katz *et al* Mathis D. 1993, Gonzalez *et al*, Benoist C., 2001, Mombaerts *et al*, Papaioannou VE., 1992).

BDC2.5/N Thy1.1
TCR alpha /beta transgene, Thymus Cell Antigen 1a variant

Development

The BDC2.5 TCR alpha and beta sequences were co-injected into (B6xSJL)F2 eggs. The integration site for these BDC2.5 TCR transgenes was localized to chromosome 13 near the D13mit125 marker (Katz *et al* , Mathis D. 1993). BDC2.5/N Thy1.1 mice are created by crossing BDC2.5/N with NOD.Thy1a (NOD.NON-Thy1a/1LtJ). Thy1.1 is an allogenic marker from PL/J back-crossed onto NOD. (Fabien N *et al*, Thivolet C. 1995). The BDC2.5/N Thy1.1 line is currently at the N22 F6 backcross generation.

Description

On the NOD background, mice carrying the transgenes have a reduced incidence of diabetes relative to NOD/LtJ controls (12% incidence at age 30 weeks). BDC2.5/N animals show a generalized and very extensive islet infiltration after a few weeks of age, but most remain free of overt diabetes for long periods or develop forms of insulitis that does not cause beta cells destruction. The BDC2.5/N Thy1.1 congenic strain carries the T lymphocyte specific Thy1a (Thy1.1) allele. Donor T cells can be easily distinguished from recipient T cells by both flow cytometric and histological analysis.