# Mutagenetix Phenotypic Mutation 'riogrande'

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<td>Anne Murray</td>
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<tr>
<td>Authors</td>
<td>Kuan-Wen Wang, Ming Zeng, Bruce Beutler</td>
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<tr>
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Riogrande mice exhibit decreased frequencies of peripheral T cells. Flow cytometric analysis of peripheral blood was utilized to determine T cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (µ) and standard deviation (σ) are indicated.

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Figure 2. Homozygous *riogrande* mice exhibit diminished T-dependent IgG responses to recombinant Semliki Forest virus (rSFV)-encoded β-galactosidase (rSFV-β-gal). IgG levels were determined by ELISA. Raw data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (?) and standard deviation (?) are indicated.

The *riogrande* phenotype was initially identified among G3 mice of the pedigree R0012, some of which exhibited decreased frequencies of T cells (Figure 1) and a diminished T-dependent antibody response to recombinant Semliki Forest virus (rSFV)-encoded β-galactosidase (rSFV-β-gal) (Figure 2).
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Whole exome HiSeq sequencing of the G1 grandsire identified 85 mutations. Both of the above anomalies were linked by continuous variable mapping to a mutation in Fasl: an A to G transition at base pair 161,788,164 (v38) on chromosome 1, or base pair 5,651 in the GenBank genomic region NC_000067. The strongest association was found with an additive model of inheritance to the normalized T cell frequency, wherein 8 variant homozygotes departed phenotypically from 43 unaffected mice that were either heterozygous (n = 26) or homozygous (n = 17) for the reference allele with a P value of 4.65 x 10^{-4} (Figure 3). The mutation corresponds to residue 332 in the mRNA sequence NM_010177 within exon 1 of 4 total exons.

316 GGGCCTAGGAGCCGGAACAAAGGAGACCGCCA
36   -G--P--R--G--P--D--Q--R--R--P--P--

The mutated nucleotide is indicated in red. The mutation results in an aspartic acid (D) to glycine (G) substitution at position 41 (D41G) in the FasL protein. The mutation is predicted by Polyphen-2 to be benign (score = 0.00).

Protein Prediction

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**Figure 4. Domain structure of FasL.** The *riogrande* mutation (D41G) is shown. The sites of SSPL2a and ADAM10/MMP3/MMP7 cleavage are shown. See the text for more details. Abbreviations: PRD, proline-rich domain; Poly-Pro, polyproline domain; TM, transmembrane domain; SA, self-assembly region; THD, tumor necrosis factor homology domain; ICD, intracellular domain. Click on the image to view other mutations found in FasL. Click on each mutation for more specific information.

*Fasl* encodes Fas ligand (FasL; alternatively, CD95L, TNFSF6, or CD178), a type II membrane protein and member of the tumor necrosis factor (TNF) family [reviewed in (1,2)]. For information about other members of the TNF family see the records for *PanR1* (*Tnf*) and *walla* (*Cd40lg*). The 279 amino acid mouse FasL consists of an intracellular domain (ICD; amino acids 1-78), a transmembrane domain (amino acids 79-100), and an extracellular TNF homology domain (THD; amino acids 101-279) that contains the Fas receptor-binding site [Figure 4; (3); reviewed in (1)].

A proline-rich domain (PRD) and polyproline domain within the ICD (amino acids 4-69 and 45-51, respectively) facilitates the binding of Src homology 3 (SH3) or WW domain-containing proteins (e.g., Fyn, Nck, SNX9, SNX18, SNX33, and CD2BP1) to FasL [35606 Ghadimi,M.P. 2002; reviewed in (1,2)]. FasL association with SH3 domain-containing proteins facilitates FasL-mediated reverse signal transduction, FasL sorting to the lysosomal compartment in T and natural killer (NK) cells, trafficking to the membrane, and the recruitment of FasL and FasL-associated vesicles to the T cell/target cell interface (see “Background” and “Putative Mechanism” for more details on the function of FasL) (4-8). Members of the sorting nexin 9 protein family (e.g., SNX9, SNX18, SNX33) are endocytic and endosomal sorting proteins that regulate FasL trafficking (9;10). CD2BP1, a member of the pombe cdc15 homology (PCH) protein family, interacts with FasL and regulates actin reorganization to mediate immune synapse formation and T cell activation upon interaction with an antigen presenting cell (APC) (11-14). A comprehensive list of FasL interacting proteins is available in [reviewed in (1)]. In nontransformed murine CD8+ T cells, a construct containing only the FasL ICD was sufficient to recruit FasL to lipid rafts as well as to activate nuclear factor of activated T cells (NFAT) and activator protein 1 (AP1), mediate an increase in AKT levels and interferon-? production as well as increase phosphorylation of ERK1/2, JNK, and FasL (15). Signaling via the FasL ICD dampened the lymphocyte activation-induced proliferative response by impairing the phosphorylation of PLC? (see the record for *queen*), protein kinase C (PKC), and ERK1/2 (16).
motifs (amino acids 17-21) [reviewed in (1)]. TCR-induced CKI phosphorylation of FasL is correlated with FasL-associated reverse signaling in CD8+ T cells (15). Tyr7, 9, and 13 in human FasL and Tyr7 in mouse FasL are putative tyrosine phosphorylation sites [(17); reviewed in (1)]. Tyrosine phosphorylation as well as ubiquitination at lysines flanking the PRD, support the sorting of FasL to secretory lysosomes, resulting in FasL localization to the cell surface (18). The extracellular THD domain contains four putative Asn-linked glycosylation consensus sequences (Asn-X-Ser/Thr; Asn117, Asn182, Asn248, and Asn258) [(3;19); reviewed in (1;2)]. Asn-linked glycosylation is predicted to regulate FasL storage, stability, maturation, surface appearance, and secretion (20;21). Mutations of any of these residues did not significantly affect self-association or receptor binding, but did result in reduced FasL expression [reviewed in (1)]. A self-assembly (SA) region (amino acids 137-183) within the FasL extracellular domain mediates the formation of homotrimeric or hexameric complexes [(22); reviewed in (1)].

FasL occurs in both a membrane-bound (mFasL) and soluble form (sFasL) as a result of proteolytic processing of FasL. A disintegrin and metalloproteinase domain 10 (ADAM10; for information about ADAM family member, Adam17, please see the record wavedX) cleaves FasL between Lys127 and Gln128; ADAM10-induced cleavage of FasL in T cells regulated both FasL-induced cytotoxicity and activation-induced cell death (AICD; see the “Background” section for more information about AICD) (23). Following cleavage by ADAM10, FasL is further processed by signal peptide peptidase-like 2a (SPPL2a; amino acids 79-80), an intramembrane cleaving protease specific for type II transmembrane proteins [(23;24); reviewed in (1)]. Metalloproteinase-3 (MMP3) and MMP7 (see the record cartoon for information about MMP family member Mmp14) have also been linked to the cleavage of FasL between Lys127 and Gln128 in some cells (e.g., glandular epithelial cells) (25). The MMP7-induced cleavage is proposed to reduce the death-promoting activity of FasL and to subsequently promote tumor survival and the resistance to cytotoxic drugs [reviewed in (1)]. The function of the released N-terminal fragment is unknown, but it may enter the nucleus to regulate the transcription of target genes (24).

The riogrande mutation (D41G) is within the intracellular PRD domain.
FasL is expressed in T cells (26), NK cells (27), and mature B cells (28) as well as in sites of immune privilege such as the eye, thyroid, lung, brain, placenta, and testis (29-34). Several tumor types including colorectal carcinoma, melanoma, head and neck carcinomas, hepatocellular carcinoma, lung carcinoma, and myeloma express FasL (35-39). In the rat, Fasl is moderately expressed in the small intestines, kidney and lung (3). Fasl expression in activated T cells and NK cells is transient (e.g., Fasl expression in NK cells is increased upon stimulation with CD16 ligation or cytokines such as IL-2 and IL-12), but Fasl expression in non-immune cells is typically constitutive [(17); reviewed in (40)].

In NK cells, FasL is stored in secretory lysosomes that mediate the secretion of cargo proteins upon stimulation (8). CD8+ cytotoxic T cells and some activated CD4+ T cells express mFasL on the cell surface (41). In activated CD4+ T helper 1 (TH1) cells, the surface expression of FasL is induced by several factors including antigens, anti-T cell receptor/CD3 antibodies, and mitogenic lectins (17); stimulation of CD4+ TH2 cells does not change FasL expression (3;42). In monocytes, FasL is localized intracellularly, but is subsequently released after exposure to either phytohemagglutinin (PHA), a lectin that induces T cell proliferation, or to immune complexes (7). FasL is released from cells by both proteolytic cleavage (in the form of sFasL) and in microvesicles in activated T cells (sFasL and mFasL) (43). In fibroblasts and epithelial cell lines, FasL is predominantly trafficked to the plasma membrane (44;45).

FasL expression is regulated at both the transcriptional and posttranslational levels. Factors including NFAT, NF-κB (see the record for finlay), c-Myc, IRF-1 (see the record for Endeka), stimulating protein 1 (SP-1), cyclin B1/Cdk1, and early growth response 3 (Egr-3) mediate the induction of Fasl upon T cell activation (17;46). The Fasl promoter has two NFAT sites; the more distal NFAT site is more essential for TCR-mediated Fasl expression in CD4+ T cells (47). Egr-3 is induced by NFAT and is predicted to act synergistically with NFAT to induce Fasl expression (48;50). SP-1 regulates basal Fasl expression in Jurkat T cells and the constitutive expression of Fasl in Sertoli cells (51) as well as the expression of Fasl in smooth muscle cells (52;53). After withdrawal of growth factors, forhead family transcription factors induce the expression of Fasl (17). AP-1 mediates the stress- and TCR-induced expression of Fasl (17;54). The transcription of Fasl is negatively regulated by c-Fos (17). Class II transactivator (CIITA), a major histocompatibility complex (MHC) class II transactivator, and retinoic acid block NFAT function and subsequently inhibit Fasl transcription (55;56). TGF-β downregulates c-Myc expression and subsequently inhibits TCR-induced Fasl expression (57).

Background

The FasL/Fas receptor system has several functions: (i) acting as a pro-apoptotic factor, (ii) facilitating the removal of target cells by NK and cytotoxic T lymphocytes (CTLs), (iii) maintaining immune-privileged sites, (iv) preventing autoimmunity, (v) regulating resting T cell activation by acting as a non-apoptotic costimulatory ligand/receptor, (vi) acting as a proinflammatory signal, (vii) acting as a proliferative/promigratory signal, and (viii) assisting in tumor cell survival [reviewed in (1;58)]. Several of these functions are described in more detail, below.
Figure 5. FasL/Fas receptor-associated signaling induces apoptosis. In the extrinsic apoptosis pathway (left), activated caspase-8 directly activates downstream effector caspases such as caspase-3, -6, and -7. FasL/Fas-mediated apoptosis can also occur through the intrinsic apoptosis pathway. In the intrinsic pathway (right), caspase-8 mediates the cleavage of the pro-apoptotic Bcl-2 family member Bid to tBid and tBid translocates to the outer mitochondrial membrane (OMM) to activate Bcl-2 family members Bak and Bax. Bak oligomerizes, forming pores in the OMM that allow for the release of pro-apoptotic proteins such as cytochrome c and SMAC/DIABLO from the intermembrane space to the cytoplasm. Binding of cytochrome c and SMAC/DIABLO activate the apoptosome.
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c and cytosolic ATP to apoptosis promoting factor-1 (Apaf-1) results in the formation of the apoptosome, a complex of cytochrome c, Apaf-1, and procaspase-9. The apoptosome activates caspase-9 which will then cleave caspase-3 and caspase-7.

Apoptosis
FasL/Fas receptor-associated signaling induces apoptosis and mediates AICD of reactive T cells, a process that results in the termination of an immune response. AICD is essential in the prevention of lymphoid neoplasia (e.g., lymphoma and leukemia) and autoimmunity ([59]; reviewed in [20]). In Fas/FasL-induced apoptosis, binding of FasL to the Fas extracellular domain results in the formation of receptor microaggregates and a conformational change in the cytosolic domain of Fas (60; 61). The conformational change in Fas results in the recruitment and binding of the adaptor protein Fas-associated protein with death domain (FADD) to the cytosolic death domain of Fas. Subsequently, FADD recruits the initiator caspase, caspase-8 (or -10), which interacts with the FADD N-terminal death effector domain; the Fas, FADD and caspase-8/-10 complex is referred to as the death-inducing signaling complex (DISC) [reviewed in (62)]. Formation of the DISC and subsequent auto-activation of caspase-8 through self-cleavage (63) requires the internalization of Fas to an endosomal compartment (64) or to lipid rafts to form signaling protein oligomeric transduction structures (SPOTS) (65). In the extrinsic apoptosis pathway in “type 1” cells, activated caspase-8 directly activates downstream effector caspases such as caspase-3, -6, and -7. The effector caspases subsequently cleave structural proteins and enzymes and the cell undergoes cell death [Figure 5; (29)]. In “type 2” cells (e.g., during negative selection of T cells and in B-cell maturation), low caspase-8 expression and/or caspase inhibitory molecules cause insufficient caspase activation [reviewed in (2)]. In these cells, FasL/Fas-mediated apoptosis occurs through the intrinsic apoptosis pathway (66). In the intrinsic pathway, caspase-8 mediates the cleavage of the pro-apoptotic Bcl-2 family member Bid to tBid and tBid translocates to the outer mitochondrial membrane (OMM) to activate Bcl-2 family members Bak and Bax (67;68). Bak oligomerizes, forming pores in the OMM that allow for the release of pro-apoptotic proteins such as cytochrome c and SMAC/DIABLO from the intermembrane space to the cytoplasm (69;70). Binding of cytochrome c and cytosolic ATP to apoptosis promoting factor-1 (Apaf-1) results in the formation of the apoptosome, a complex of cytochrome c, Apaf-1, and procaspase-9 [Figure 5; reviewed in (2)]. The apoptosome activates caspase-9, which subsequently cleaves caspase-3 and caspase-7 (71).

Constitutively expressed FasL protects immune privileged sites by triggering apoptosis of Fas-sensitive activated inflammatory cells entering into the sites (30). For example, constitutive expression of FasL in the eye is proposed to limit ocular inflammation (30). In FasL-deficient mice (Fasl<sup>−/−</sup>; generalized lymphoproliferative disease (gld); MGI:1856384), exposure to herpes simplex virus or Toxoplasma gondii resulted in a more severe ocular inflammation than in wild-type mice (30;72).

For certain tumor cells, the constitutive expression of FasL, or its secretion from tumor-derived exosomes, can produce tumor-associated immune privilege, subsequently allowing malignant cells to evade immune surveillance (73). Tumor cells are rendered resistant to Fas-mediated apoptosis at several stages of Fas-associated signaling by secreting soluble decoy receptors (74), mediating apoptosis of tumor-infiltrating lymphocytes (TILs) (75;76), regulating Fas surface expression (77), altering the apoptosis signal at the DISC level, and/or by altering the expression of c-FLIP, FADD, XIAP, and/or caspase-8 ([78;79]; reviewed in (2)].

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sFasL often downregulates part of the apoptotic activity of mFasL (81). In a mouse model of glaucoma, administration of mFasL led to retinal neurotoxicity, while administration of sFasL protected against apoptosis of retinal ganglion cells (82). In transfected lymphoma cells, sFasL blocked the apoptotic activity as well as opposed the pro-inflammatory activity of mFasL (83). Receptor-interacting protein 1 (RIP1) is required for activation of downstream caspases after stimulation with mFasL. Cross-linking sFasL resulted in the formation of a larger, less efficient DISC complex than mFasL and bypassed the requirement for RIP1 (29). The association of sFasL with matrix proteins during physiological conditions would prevent cell death due to a reduced recruitment of RIP1 to the DISC, subsequently promoting the activation of non-apoptotic signaling pathways (84).

\[\text{Figure 6. The FasL ICD interacts with PSTPIP to recruit PTP-PEST, a negative regulator of ERK1/2/MAPK. After posttranslational processing by ADAM10 and SPPL2a, the FasL ICD binds Lef-1 to negatively regulate Lef-1-dependent pro-proliferative Wnt signaling, subsequently impeding proliferation.}\]

Reverse signaling/costimulatory molecule for T cell activation

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Without co-stimulation, the TCR/CD3-stimulated naïve CD4\(^+\) T cells undergo apoptosis\(^{29}\). FasL can function as an accessory/costimulatory molecule during T cell activation, replacing the conventional CD28 co-stimulatory signal required for naïve T cell proliferation, and participates in reverse signaling (alternatively, retrograde signal transduction) in FasL-expressing cells\(^{[29]}\); reviewed in \(^{(1)}\). In reverse signaling, the Fas receptor acts as the ligand for FasL, transmitting a signal into the FasL-expressing cell. Activation of FasL results in the recruitment of SH3-containing proteins including Fyn, Grb2, and the p85 subunit of PI3K to the cytoplasmic domain of FasL\(^{(5;15)}\). CD8\(^+\) T cells require FasL co-stimulation to progress through the cell cycle, to proliferate, and to acquire cytolytic effector function, while FasL engagement in CD4\(^+\) T cells results in inhibition of the TCR-associated cell cycle progression and increased rates of apoptosis\(^{(85;86)}\). During positive selection in the thymus, FasL-induced reverse signaling occurs upon the interaction of FasL-expressing thymocytes with Fas-positive stroma cells or thymic antigen-presenting cells (APCs)\(^{(29;87)}\). In isolated human peripheral T cells, TCR/CD3/CD28-induced proliferation and activation were blocked by anti-FasL antibodies\(^{(88)}\). In contrast to reports showing a costimulatory function for FasL\(^{(86;89)}\), Luckerath \textit{et al.} demonstrated that FasL plays an inhibitory role in reverse signaling in T cell proliferation\(^{(85;88)}\). Luckerath \textit{et al.} showed that the FasL ICD interacted with PSTPIP to recruit PTP-PEST, a negative regulator of ERK1/2/MAPK [Figure 6; \(^{(16)}\)]. In addition, after posttranslational processing by ADAM10 and SPPL2a, the FasL ICD bound Lef-1 to negatively regulate Lef-1-dependent pro-proliferative Wnt signaling, subsequently impeding proliferation\(^{(16)}\). Luckerath \textit{et al.} propose that the conflicting costimulation results were due to the use of different FasL ICD constructs and expression systems between the studies and/or cross-linking induced by anti-FasL that led to a possibly non-physiologically strong (or weak) signal\(^{(16;85;86;89)}\).

FasL-associated reverse signaling has also been documented in nonhematopoietic cells. In cancer cells, FasL-associated signaling enhanced cell migration and metastasis through activation of the hepatocyte growth factor (HGF) receptor Met\(^{(90)}\). In the nervous system, FasL-associated signaling in Schwann cells resulted in activation of the Src and MAPK ERK1/2 pathways as well as the induction of nerve growth factor (NGF) secretion and subsequent NGF-stimulated neurite outgrowth\(^{(91)}\).

**Suppression of calcium influx upon T cell activation**

TCR-mediated activation of T cells results in the triggering of calcium release from the endoplasmic reticulum (ER)\(^{(29)}\). Emptying of the calcium stores of the ER subsequently results in the influx of extracellular calcium through calcium-release-activated calcium (CRAC) channels in the plasma membrane and a sustained calcium influx\(^{(92)}\). Following T cell activation by an antigen, the elevated levels of intracellular free calcium results in activation of the transcription factor NFAT and the production of IL-2. Cross-linking of Fas on T cells before stimulation with anti-CD3 inhibited TCR-associated calcium influx indicating that Fas/FasL-mediated inhibition of calcium influx through the CRAC channels prevented T cell activation through an impairment of NFAT activation and IL-2 synthesis\(^{(93)}\).

**Mediation of CTL activity**

Following target cell recognition, storage granules are transported to the site of cell-to-cell contact, where they fuse with the plasma membrane and release cytotoxic effector molecules including granzymes and perforin (see the record for Prime) into the immunological synapse\(^{(94)}\). FasL expressed on activated CTLs or NK cells functions as a part of the perforin-independent effector machinery of cytotoxic immune cells and supplements the granzyme/perforin-mediated Fas\(^+\) target cell lysis pathway\(^{(17;95)}\).

**Triggers inflammation**
B pathways to regulate cell proliferation, migration, and inflammation (29). FasL/Fas-mediated induction of NF-κB signaling in non-lymphoid cells such as hepatocytes, synoviocytes, macrophages, fibroblasts, and colonic and lung epithelial cells leads to the expression and release of inflammatory cytokines and chemokines such as IL-6, IL-1, IL-8, TNF-α, MCP-1 (monocyte chemoattractant protein-1), IP-10 (interferon-gamma-induced protein 10) and PGE2 (prostaglandin E2) (96-99). The release of inflammatory factors may subsequently recruit inflammatory cells. FasL/Fas-associated inflammation is linked to several diseases including cystic fibrosis (100), arthritis (101;102), cancer (103), and acute respiratory distress syndrome (104).

In a model of heart disease, mFasL triggered pro-inflammatory cytokine production by monocytes and macrophages (105), while cardiac-specific expression of sFasL inhibited macrophage activation and pro-inflammatory cytokine production (106). To study the different functions of mFasL and sFasL in inflammation, mouse models were generated that solely expressed either mFasL (Fast1s/s; Fast1m2.1Ast; MGI:4366051) or sFasL (Fas1tm7/m, Fas1tm1.1Ast, MGI:4366050) (80). The Fas1tm7/m mice exhibited lymphadenopathy, splenomegaly, hyper-gammaglobulinaemia, and auto-nuclear antibodies, similar to gld mice (80). The titers of anti-DNA auto-antibodies were higher in the Fas1tm7/m mice compared to gld mice and homozygotes had increased rates of developing hepatic tumors (80). The Fas1s/s mice were phenotypically normal and had a normal life span (80). Taken together, sFasL appeared to be responsible for the pro-inflammatory function of FasL (80).

Autoimmunity and allograft rejection
The role of Fasl in autoimmunity has been conflicting. Ectopic expression of Fasl prevented autoimmune-mediated destruction of the thyroid (107). In contrast, an accelerated development of autoimmune diabetes was observed in nonobese diabetic mice upon Fasl overexpression (108).

During kidney, heart, blood vessel, liver, lung, skin, thyroid, pancreatic islet cells, and bone marrow allograft rejection, recipient activated CD4+ and CD8+ T cells recognize the MHC molecules expressed on the allograft [reviewed in (109)]. Fasl protects allogeneic grafts from immune rejection either through Fasl-transduced allogeneic donor dendritic cells or by expressing Fasl on the allogeneic tissue [reviewed in (109)]. The display of a chimeric streptavidin-Fasl protein on cardiac vasculature prolonged graft survival through the selective deletion of alloreactive immune cells (110). In contrast, other studies found enhanced neutrophil recruitment and accelerated graft rejection upon ectopic overexpression of Fasl in tissue and tumor allograft studies (111-113). In the mouse, corneal transplants from gld mice were more prone to graft failure than those from wild-type mice (114). In addition, acute graft-versus-host disease in mice can be prevented with treatment with antibodies to both Fasl and TNF-α (115;116).

Recovery after injury
Fas/Fasl-associated signaling regulates neuronal development, growth, and differentiation (117). After sciatic nerve injury, Fasl/Fas signaling triggered neurite outgrowth and branching as well as accelerated nerve regeneration (118). Following traumatic brain injury, the activation of Fasl initially induced apoptosis, but later promoted regeneration by mediating neuronal branching (119). Fas/Fasl-induced neurite growth required the recruitment of ezrin, a protein that links transmembrane proteins to the cytoskeleton, to Fasl. Ezrin recruitment subsequently activated the small GTPase Rac1, resulting in process growth (119). Fasl-associated activation of ERK further promoted process elongation and branching (118;119). Uprogulation of Fasl has been noted in axotomy-induced motor neuron death (120) and brain damage caused by ischemia (121;122).

Fas activation promoted liver regeneration after partial hepatectomy in mice (123). In contrast, massive hepatocyte apoptosis occurred in mice after intraperitoneal injection of Fas-activating antibodies (124). Fasl/Fasl activation also induced proliferation of quiescent hepatic stellate cells through Fasl-induced phosphorylation of the EGFR and subsequent ERK activation (29).

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In the heart, FasL/Fas signaling is proposed to mediate tissue damage after acute myocardial infarction (125;126). FasL overexpression in endothelial cells attenuated ischemia-reperfusion injury in the heart and led to a reduction in myocardial infarct size and neutrophil infiltration (127). In addition, FasL regulated Trypanosoma cruzi-induced acute myocarditis. Chronic infection of the heart resulted in a persistent inflammatory reaction of the myocardium, loss of cardiomyocytes, and development of fibrosis. In gld mice, cardiac infiltration and cardiomyocyte destruction were reduced in T. cruzi-induced acute myocarditis compared to BALB/c controls (128).

Mouse models
Several mutant mouse models of Fasl have been generated including the spontaneous gld model (83;129;130), an induced Fasl deletion (Fast+/−; Fastl−/−; gld, MGI:3032864) (131), a second spontaneous Fasl mouse model that lacks the transmembrane and THD domains (Fastl−/−; Fastl−/−gld, MGI:3803664) (132), and a knockin model that expresses a FasL protein that lacks the ICD but expresses a functional, truncated mFasL (Fast?Intra; Fastl−/−−/−; MGI:4888490) (16). Loss of Fasl expression results in leukocytosis, splenomegaly, enlarged peripheral lymph nodes, and premature death by 4-5 months (131-133); examination of the gld mice determined that they had an inflammatory disease of the lung similar to interstitial pneumonitis (133). Approximately 40% of gld mice were anemic or exhibited significant leukocytosis (133). The livers of the Fast−/− mice were larger than wild-type mice and had frequent moderate-to-severe lymphocytic infiltration (131). The glomerular size and cellularity of the kidneys of the Fast−/− mice were increased compared to wild-type mice. At 9-10 weeks of age, tumors were evident at the cervical lymph nodes of the Fast−/− mice (132). Fast−/− mice exhibited larger lymph nodes than age matched homozygous gld mice at 3- to 5-months of age (131). Gld mice exhibited an increased occurrence of plasmacytoid tumors (134). The majority of thymocytes in younger (6-week) Fast−/− thymocytes were mostly CD4+CD8− cells in wild-type and Fast−/− mice, while in older Fast−/− mice the cell population was mainly abnormal CD3+B220+CD4+CD8− T cells that were proposed to be derived from conventional T cells that were repeatedly activated via the TCR complex (131;132). The gld and Fast−/− mice produced autoantibodies, anti-DNA, and rheumatoid factor as well as developed a lupus-like autoimmune disease with symptoms that include glomerulonephritis, vasculitis, arthritis, and hypergammaglobulinemia (129;131-133;135;136).

Humans
Mutations in FASL are linked to autoimmune lymphoproliferative syndrome, type IB (ALPS; OMIM: #601859) (137). In ALPS, patients exhibit nonmalignant lymphadenopathy with splenomegaly (66). ALPS is an autosomal dominant disorder of FasL-induced apoptosis, resulting in the accumulation of autoreactive lymphocytes and the production of autoantibodies (138). Patients with ALPS may also exhibit includes Coombs-positive hemolytic anemia, chronic immune thrombocytopenic purpura, and neutropenia (66). ALPS patients have increased incidences of Hodgkin and non-Hodgkin lymphoma (139;140). A heterozygous mutation in FASL has been identified in a patient with systemic lupus erythematosus (SLE) ([141-143]; reviewed in (144)). The mutant FasL, an 84-bp deletion within exon 4 that resulted in a 28 amino acid in-frame deletion, exhibited decreased activity, decreased activation-induced cell death, and increased T cell proliferation upon activation (141). Mutations in FASL have also been linked to cervical carcinogenesis (145) as well as increased susceptibility to lung cancer (OMIM: #211980), breast cancer (146), and esophageal cancer (147-150). Changes in the levels of sFasL in the serum are considered to be prognostic or diagnostic marker for immune disorders (e.g., HIV infection and rheumatoid arthritis) and cancer (151). Patients with Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) exhibit increased sFasL concentrations compared to healthy controls (152). The interaction of FasL with Fas is essential in keratinocyte apoptosis in SJS and TEN patients (153). In patients with SJS and TEN, the interaction of sFasL with keratinocyte Fas leads to widespread epidermal apoptosis with blister formation and skin detachment (152;154). A role for the FasL-Fas system has been proposed in autoimmune thyroiditis, multiple sclerosis, experimental allergic encephalomyelitis, and the pathogenesis of type-1 and type-2 diabetes [reviewed in (2)].
In both B and T cell development, apoptosis is essential to maintain immune cell homeostasis. Immature cells that fail to undergo proper V(D)J rearrangement and subsequently fail to express surface antigen receptors (BCR or TCR) undergo apoptosis. Fas/FasL-induced apoptosis is essential for the deletion of autoreactive thymocytes and immature B cells in the bone marrow ([155;156]; reviewed in [41]). BCR crosslinking or CD40 stimulation protect B lymphocytes from Fas-induced apoptosis through activation of Rel-NF-κB transcription factors (157) and the expression of c-FLIP, a regulator of caspase 8 activation (158). Activated B lymphocytes that bear low-affinity BCRs undergo apoptosis when Fas on their surface is ligated to FasL on intra-follicular CD4+ T cells and when they fail to receive a pro-survival signal through the BCR and CD40 (159). In combination with BCR-crosslinking and CD40 activation, Fas ligation is proposed to contribute to the activation, proliferation, and differentiation of B cells [reviewed in (41)].

Homozygous Fasl-/-Intra mice exhibited elevated plasma cells and increased generation of germinal center B cells, leading to increased titers of NP-specific IgM antibodies in the serum after immunization with 3-hydroxy 4-nitrophenylacetyl chicken gamma globulin (NP-CGG) (16). T-independent immunization of homozygous Fasl-/-Intra mice with NP-Ficoll resulted in increased plasma cell number as well as NP-specific IgM titers (16). In addition, the Fast-/- and FastDel mice exhibited increased IgG and IgM levels compared to wild-type levels (131;132). The riogrande mice exhibit reduced T-dependent IgG responses to rSFV. These findings indicate that the activation of B and/or T cells in the riogrande mice may be negatively affected by the Fasl mutation.

The riogrande mice also exhibited a semi-dominant increase in the frequencies of peripheral T cells. The gld mice exhibit an increase in the numbers of peripheral blood lymphocytes (133;135). In addition, activation-induced proliferation of B cells, CD8+ cytotoxic T lymphocytes, and CD4+ T cells were increased in homozygous Fasl-/-Intra mice (16). The levels of several proliferation-associated genes including Irf4, a regulator of plasma cell differentiation, as well as Lef-1 target genes Nfat, Nfkb, Cycd1, and Fgf4, were upregulated in Fasl-/-Intra B cells (16). Luckerath et al. proposed that the differences in the immune responses of the Fasl-/-Intra mice compared to those in wild-type mice was due to a loss of inhibitory FasL reverse signaling in the Fasl-/-Intra.

The riogrande mutation within the PRD of FasL may be altering the association of FasLriogrande with a SH3 or WW-containing protein needed for B and/or T cell activation. SH3-containing proteins that interact with the PRD of FasL include Src-related kinases (e.g., Fyn, Lyn, Lck, Hck, Fgr, Src and Abl) and TCR-associated signaling adapter proteins (e.g., Grb2, Gads, the p85 subunit of PI3 kinase, and Nck) (4;160).
**Riogrande** genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transition.

**PCR Primers**

Riogrande(F): 5’- ACATGGGCCATAGCAAGTCCCTAC-3’
Riogrande(R): 5’- TGACACCTGAGTCTCCTCCACAAG-3’

**Sequencing Primer**

Riogrande_seq(F): 5’- TGCTAATCCATGTGCTAGGC-3’
Riogrande_seq(R): 5’- CCTCCACAAGGCTGTGAGAAG-3’

**PCR program**

1) 94°C 2:00  
2) 94°C 0:30  
3) 55°C 0:30  
4) 72°C 1:00  
5) repeat steps (2-4) 40X  
6) 72°C 10:00  
7) 4°C ?

The following sequence of 493 nucleotides is amplified (Chr.1: 161787854-161788346, GRCm38):

```
1   acatggccca taqcaagtcc ctactccccac gcagcagagt caggttgcag ccaggaacgc 
61  gaggctgctaatcccatgtgctagg  
121 gtggaagagc tgatacattc ctaatccat tccaaccaga gccaccagaa ccatgaaaaa 
181 taccaccggt agccacagat ttgtgttgtg gtccttcttc tttagaggg tcagtgccc 
241 cagtggagtgtttagtgat gcgggtgtag tgggtgacaca ggtgtgggtt gagggtgccc 
301 ttctcttttgctcc gcagcccaag tagtgcgcag gcagacagac gcagcttgacat tgaattcat 
361 agcctagggctagcccgctag cggagcgcag tggggttccc ttttaggaga gacgctgctg 
421 gggtcctgctg atggcaccaca gccccagga aaggcttttc ttcagcttgg tgaaggg 
481 gacctcggtgc tcc
```

Primer binding sites are underlined and the sequencing primer is highlighted; the mutated nucleotide is shown in red text (T>C, Chr. (+) strand; A>G, sense strand).

**References**


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Mutagenetix Phenotypic Mutation 'riogrande'


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