Job's Syndrome: Establishing Mouse Models for a Complex Human Disease

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Hyper IgE Syndrome (Job's Syndrome)

First Patients were described in 1966
Classical Triad- **Eosinophilia**
Eczema
Recurrent Skin & Pulmonary Infections

1972: IgE was recognized as elevated in serum from patients

Classification expanded and in 1979
Recognition of skeletal and connective tissue abnormalities

Hyper IgE Syndrome (Job's Syndrome)

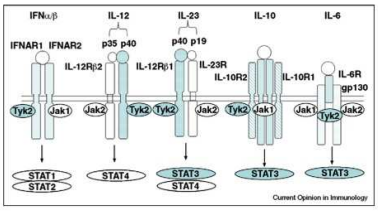
Two types of disease now recognized:

Type 1
Sporadic- more than 90% of cases
Skeletal and connective tissue abnormalities
Bone fractures, scoliosis
Retention of deciduous teeth
Hyperextensibility
Pneumatocele

Type 2
Familial with autosomal recessive inheritance
Lack developmental abnormalities
Viral infections (HSV, molluscum contagiosum)
absence of pulmonary cysts

Hyper IgE Syndrome Autosomal Recessive

- **Described in 2004 in a group of patients that lacked skeletal defects**
- **Intact TCR signaling, but with recurrent viral infections and the presence of infections with intracellular pathogens (mycobacterial) suggested cytokine signaling defects:**
  - **defective interferon alpha-mediated signaling**
  - **defective IL-12 signaling**
- **TYK 2 mutation found in a single patient in 2006**
- **Member of the JAK family kinases**
  - **Defective type 1 IFN, IL-12, IL-23, IL-10, IL-6**



Clinical inflammatory changes and susceptibility to viral infections led work to study IFN signaling pathways uncovering mutations in Tyk2 for Autosomal recessive patients

Minegishi 2008

Hyper IgE Syndrome Autosomal Dominant

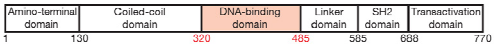
- **Multisystem disorder: immune system, skeletal/dental system, soft tissue/connective tissue changes (hyper extensibility)**
- **Abnormalities of head and face- distinctive facies with broad nose and forehead, deep set eyes**
- **Bone fractures, non-traumatic, hyper extensive joints, scoliosis, retained deciduous teet**
- **Recurrent skin abscesses and cyst forming pneumonia**
- **Infections and inflammation: usually Staphylococcal**
  - **Dysregulated local inflammatory changes**
- ***Cell signaling: Intact IL-12, IFN-alpha, defective for IL-6, IL-10 suggested Stat 3 signaling defects***

Clinical Features of Patients with AD-HIES

Immunologic (approximate percent frequency)
Peak serum IgE >2000 IU/mL (97)
Recurrent pneumonias (87)
Parenchymal lung abnormalities (bronchiectasis/pneumatocoele) (70)
Boils (87)
Moderate-severe eczema (95)
Newborn rash (80)
Mucocutaneous candidiasis (83)
Recurrent sinusitis or otitis (80)
Eosinophilia (90)
Lymphoma (5)
Somatic (approximate % frequency)
Characteristic face (85)
Hyperextensibility (70)
Retained primary teeth (70)
Minimal trauma fractures (65)
Scoliosis >10 degrees (60)
Coronary vasculature anomalies (60)
Arnold Chiari I malformations (40)
Focal hyperintensities on brain MRI (75)

Mineguishi and co-workers found STAT3 mutations affecting the DNA-binding domain,
associated w/ clinical manifestations of HIES
(Mineguchi et al., Nature 2007)

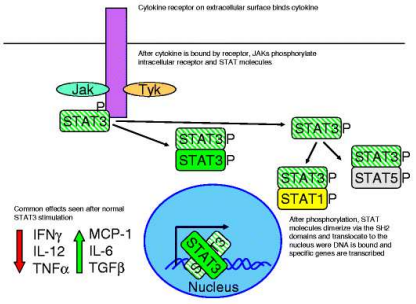
STAT3



Stat 3 has a central signaling roles for multiple pathways including:

IL-6
gp 130 family (leptin, LIF)
MAPK
SHP pathways in T cells
malignant transformation of cells (Ras)
mutations associated with Crohn's disease
mutations associated with lymphoma

Mutant Stat 3: Mechanism



Holland 2009

TH17 T cell differentiation is defective in patients With AD HIES

Absent TH17 T cells in patients with AD-HIES
Reported by multiple groups- accounts for defective immunity

Infections however are primarily lung and skin
Keratinocytes and bronchial epithelial cells are more dependent on TH17-mediated cytokines for function

Other T cell subsets involved?
Defective antigen specific antibody production:
encapsulated pathogens

TH17 T cell defect does not explain the multiple immune defects

Characteristics of patients with Stat 3 mutations in AD HIES

TABLE I. Number and percentage of patients positive for the 17 evaluated features

	all HIES		HIES STAT3 wild-type		HIES STAT3 mutated	
	No.	%	No.	%	No.	%
Recurrent pneumonia	85/100	85	24/36	66.7	61/64	95.3
Eczema	90/100	90	32/36	88.9	58/64	90.6
Recurrent skin abscesses	86/100	86	28/36	77.8	58/64	90.6
Characteristic face	82/99	82.8	24/35	68.6	58/64	90.6
Failure to shed deciduous teeth	60/86	68.9	16/31	51.6	44/55	80.0
Lung cyst formation	61/97	62.9	14/34	41.2	47/63	74.6
Eosinophilia	68/94	72.3	27/36	75.0	41/58	70.7
Newborn rash	52/86	60.5	15/29	51.7	37/57	64.9
Other unusual infections	47/94	50	13/34	38.2	34/60	56.7
Increased interalar distance	37/83	44.6	10/31	32.3	27/52	51.9
Cathedral palate	41/84	48.8	12/31	38.7	29/53	54.7
Hyperextensibility	37/87	42.5	8/32	25.0	29/55	52.7
Pathologic bone fractures	32/94	34.0	5/35	14.3	27/59	45.8
Recurrent upper respiratory infections	41/92	44.6	14/33	42.4	27/59	45.8
Candidiasis	37/91	40.6	12/33	36.4	25/58	43.1
Scoliosis	20/83	24.1	7/33	21.2	13/50	26.0
Midline anomaly	12/86	14.0	5/34	14.7	7/52	13.5

Features were considered as present if more than 1 point of the NIH score[9] was achieved. The 5 cardinal clinical features (recurrent pneumonia, newborn rash, pathologic bone fractures, characteristic face, and cathedral palate) are shaded.

1/3 of AD HIES patients have no Stat 3 mutation

Woellner et al 2010

Spectrum of mutations in Stat3

No. of patients	Protein domain	Site of mutation	DNA sequence change	Predicted amino acid change
1	Coiled-coil	Exon 3	c.172C>T	H58Y
1	DNA-binding	Exon 10	c.982_990dupTGCATGCCC	C328_P330dup
1	DNA-binding	Exon 10	c.1025G>A	G342D
2	DNA-binding	Intron 11	c.1110-2A>G	D371_G380del
1	DNA-binding	Intron 11	c.1110-1G>T	D371_G380del
1	DNA-binding	Intron 12	c.1391 1G>T	D371_G380del
1	DNA-binding	Intron 12	c.1139C>T	D371_G380del
14	DNA-binding	Exon 13	c.1144C>T	R382W
2	DNA-binding	Exon 13	c.1145G>T	R382L
9	DNA-binding	Exon 13	c.1145G>A	R382Q
2	DNA-binding	Exon 13	c.1150T>C	F384L
1	DNA-binding	Exon 13	c.1166C>T	T389I
1	DNA-binding	Exon 14	c.1268G>A	R423Q
1	DNA-binding	Exon 16	c.1387_1389delGTG	V463del
1	DNA-binding	Exon 16	c.1396 A>G	N466D
1	DNA-binding	Exon 16	c.1397A>G	N466S
1	DNA-binding	Exon 16	c.1397A>C	N466T
1	DNA-binding	Exon 16	c.1398C>G	N466K
1	DNA-binding	Exon 16	c.1407G>T	Q469H
1	SH2	Exon 20	c.1721A>G	K574E
1	SH2	Exon 20	c.1865C>T	T622I
1	SH2	Exon 21	c.1907C>A	S636Y
10	SH2	Exon 21	c.1909G>A	V637M
1	SH2	Exon 21	c.1910T>C	V637A
1	SH2	Exon 21	c.1915T>C	F639S
1	SH2	Exon 21	c.1970A>G	Y657C
1	SH2	Exon 21	c.2003C>T	S668F
1	Transactivation	Exon 22	c.2124C>G	T708S
1	Transactivation	Exon 22	c.2129T>C	I710C
1	Transactivation	Exon 22	c.2141C>G	T714A
1	Transactivation	Intron 22	c.2144 1G>A	p.?

Woellner et al 2010

Generate a mouse model of Job's Syndrome

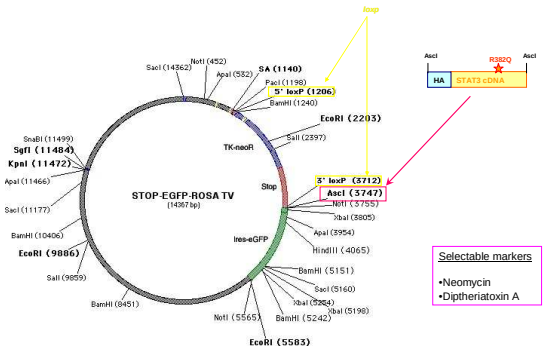
Challenges:

-Stat 3 is important in maintaining ES cell pluripotency, thus mutant Stat3 cannot be expressed in targeted ES cells for germline expression

-Job's Syndrome affects many tissues, it would be useful to express the mutation in a tissue specific fashion

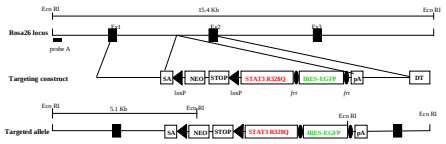
-Stat3 null mutant mice are embryonic lethal, heterozygous mice have no phenotype c/w Job's Syndrome

R382Q-STAT3 in ROSA26

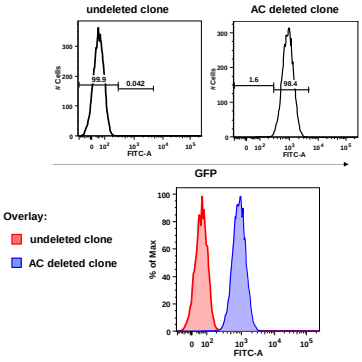


TARGETING ES CELLS

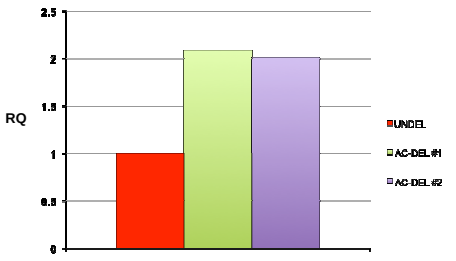
R382 Stat 3 is targeted into the Rosa 26 locus, and driven off the endogenous promoter



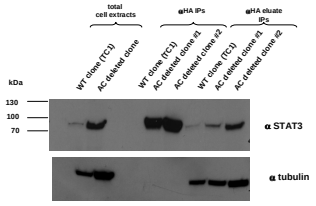
GFP expression by FACS- gate on ES population defined by FSC vs SSC



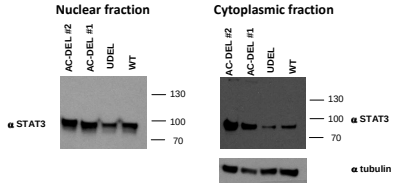
R382Q Stat 3 is overexpressed in a 1:1 ratio with wildtype Stat 3



Confirmation of R382Q protein expression of Stat3 in Gene-targeted ES cells



Nuclear fraction of Stat 3 is maintained at wildtype levels in the R382Q over expressing ES cells

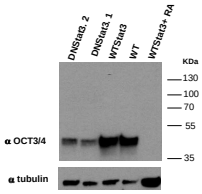


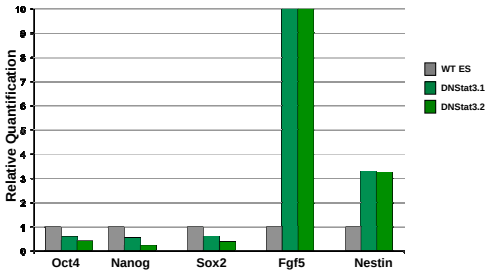
Role of Stat3 R382Q in ES cell differentiation

Does DNStat3 R382Q alter differentiating patterns of ES cells, account for the variable phenotypes seen in patients?

What lineages are affected most during differentiation of R382Q ES cells

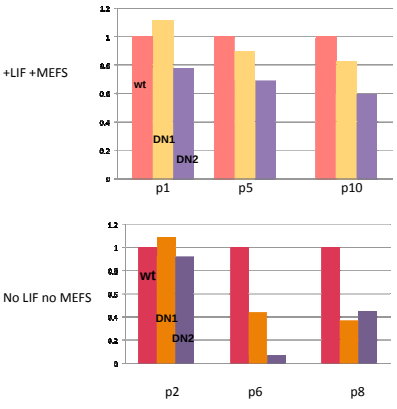
Oct4 is downregulated at early passages in R382Q ES cells



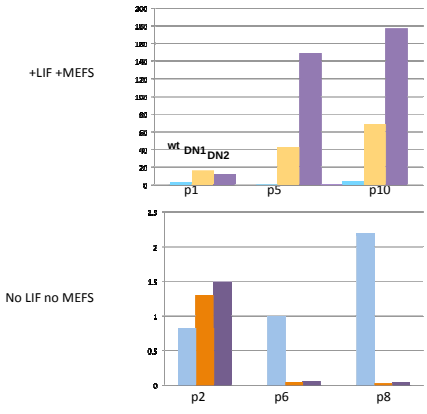


Mesenchymal fate differentiation program is preferentially affected

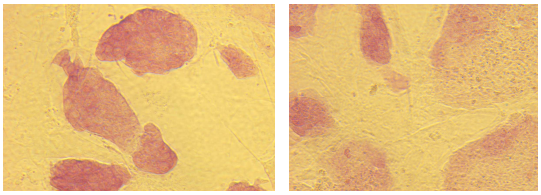
OCT4



Fgf5



WT **R382Q Stat3**



Alkaline phosphatase staining of ES cell colonies (passage 10)

ES Cell Conclusions:

R382Q ES cells differentiate faster than WT ES cells, via impaired LIF signaling

LIF independent pathways exist to maintain pluripotency

Lineage that is most affected is mesenchymal (microarray data)

Neural progenitor differentiation is maintained

Implications for clinical syndrome:
- **Chimerism seen inpatients**
- **Implications for ability of HSC cells to maintain pluripotency**

Generation of B cell Mouse Model

Deletion of Stat3 in mice is embryonic lethal:
conditional deletion- completely lack Stat 3 (unlike AD-HIES)

"Knock-in" of mutation in mouse ES cells- marked chimerism and selection against mutation

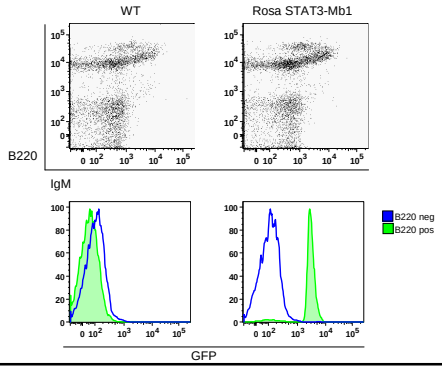
Expression of R382Q is induced in a Cre specific fashion

Mb-1 (Ig-alpha)-Cre mice, express only in B cells, at the pro-B cell stage of development

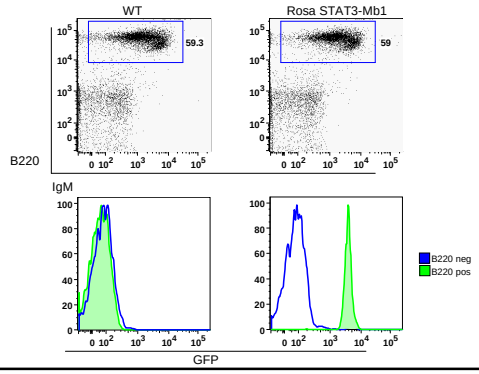
All B cells in this mouse will express Dominant Negative Stat3

Exploit the ability to exploit the DN Stat3 in a tissue specific fashion

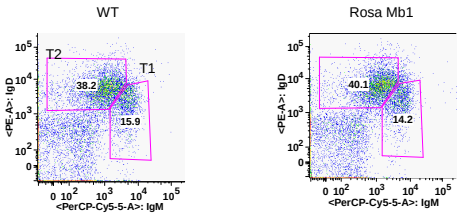
BM IgM B220- GFP expression



SPLEEN IgM B220- GFP expression

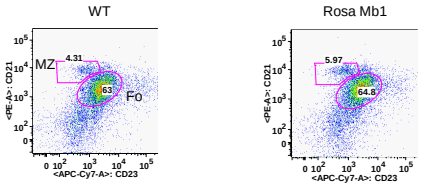


IgM IgD spleen



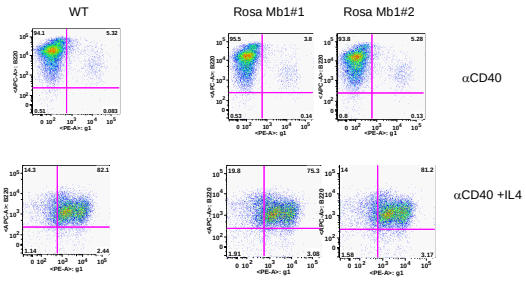
Mature B cell compartment appears intact in 8 week old mice

CD21CD23 on B220 pos -spleen

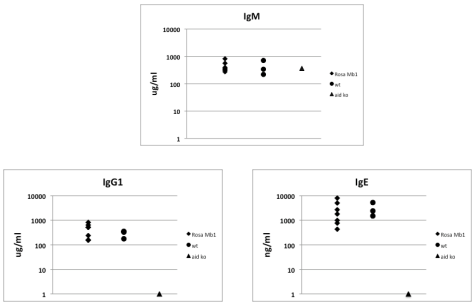


Marginal zone and Follicular cell B cell compartments appear intact

Class switch assay day4



Serum levels of Immunoglobulin in R382Q MB-1 Mice are normal



Conclusions from B cell Mouse Model

B cell numbers are unaffected- in resting unimmunized mice

DN Stat 3B cells do not intrinsically undergo increased class switching to IgE or other Isotypes (need immunization studies)

Fewer plasma cells are found in older mb-1 mice (4 months), based on immunohistochemical staining of the bone marrow

Future work:
- **CD4 T cell specific expression**
- **Bone specific expression**
- **Skin specific expression**

Summary:

AD-HIES is a heterogeneous disease- genetically and clinically
not all patients have Stat 3 mutations

Most patients with Stat 3 mutations are sporadic in nature
indicating importance in embryonic development

Developmental selection is severely influenced by DN-Stat 3
murine ES cells severely affected

Preliminary studies suggest no intrinsic B cell defect for class switching
in context of normal T cells

Mouse models may elucidate therapeutic targets for non-immune defects
Bone and skin manifestations

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