

Defective Cytokine Signaling in Severe Combined Immunodeficiency

Disclaimer: I am one of three co-inventors on an NIH patent on “Diagnosis and Therapy of XSCID” and another patent related to γ_c -knockout mice.

Key Messages from my Talk

X-linked SCID, *JAK3*-deficient SCID, and *IL7R*-deficient SCID are diseases of defective cytokine signaling.

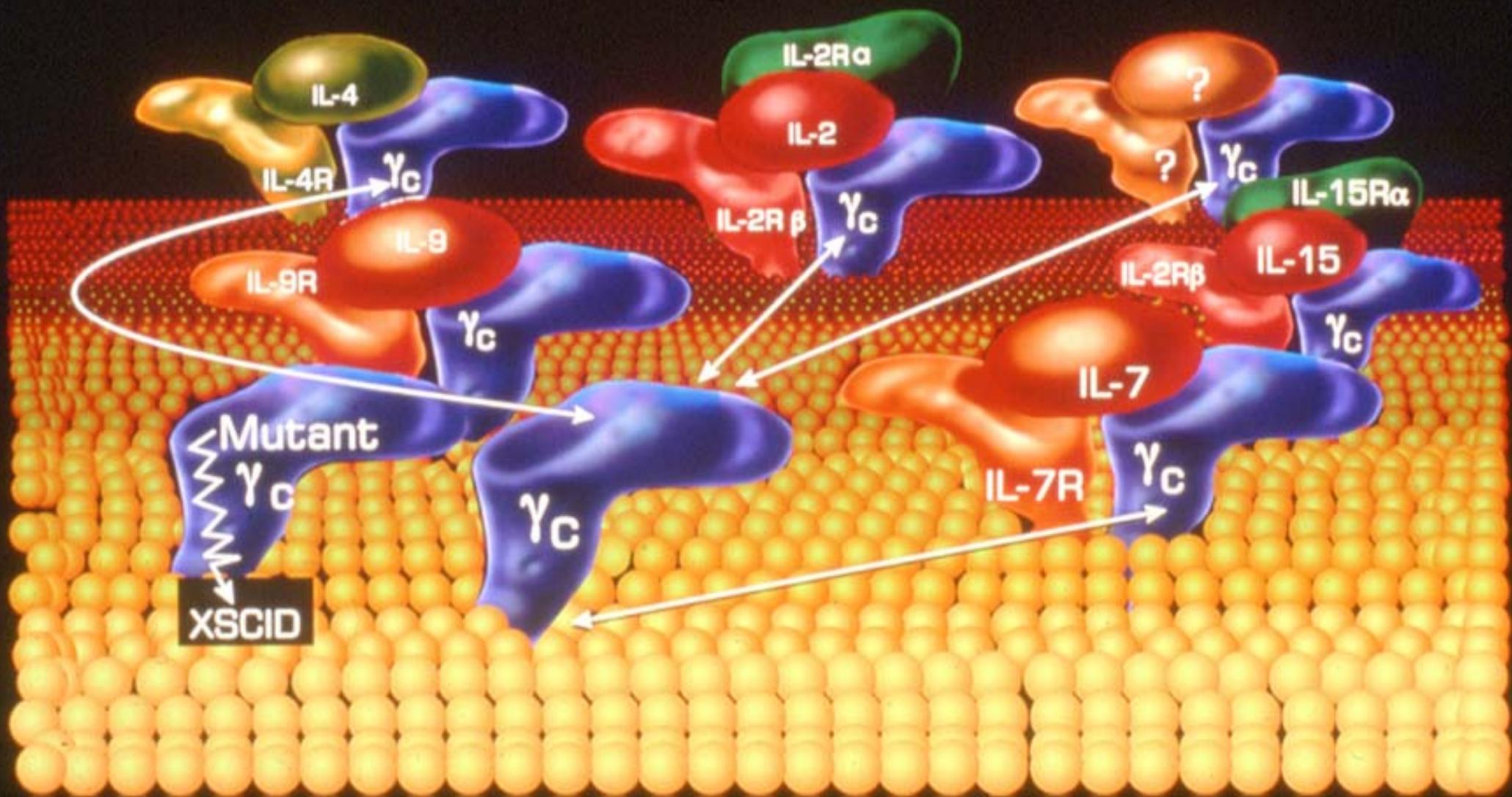
- 1. Defective IL-7 signaling appears to account for the T-cell developmental defect in these disorders.**
- 2. Defective IL-15 signaling appears to explain the NK-cell developmental defect in XSCID and Jak3 deficiency.**
- 3. IL-4 + IL-21 signaling defects may explain the intrinsic B-cell functional defect in XSCID and Jak3 deficiency.**
- 4. The contributions, if any, of defective IL-2 and IL-9 signaling remain unknown.**

Original Dilemma/Hypothesis

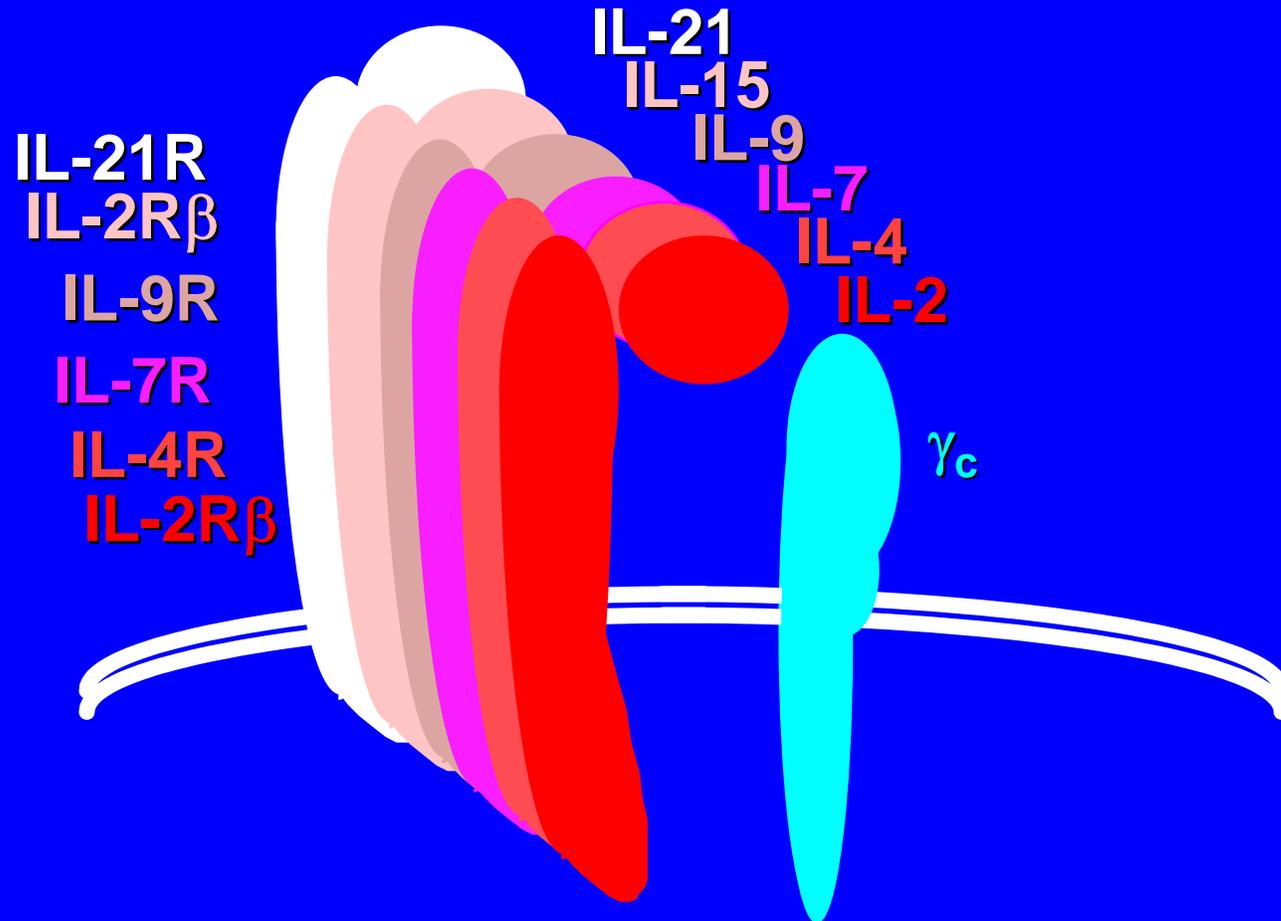
In 1993, we reported that the *IL2RG* gene mapped to the SCIDX1 locus at Xq13 and found that it was the defective gene in XSCID.

Unexpectedly, the phenotypes in XSCID (IL-2R γ deficiency) and IL-2 deficiency were very different-- in XSCID, T-cell and NK-cell development are profoundly diminished, but IL-2-deficient patients and mice had normal lineage development.

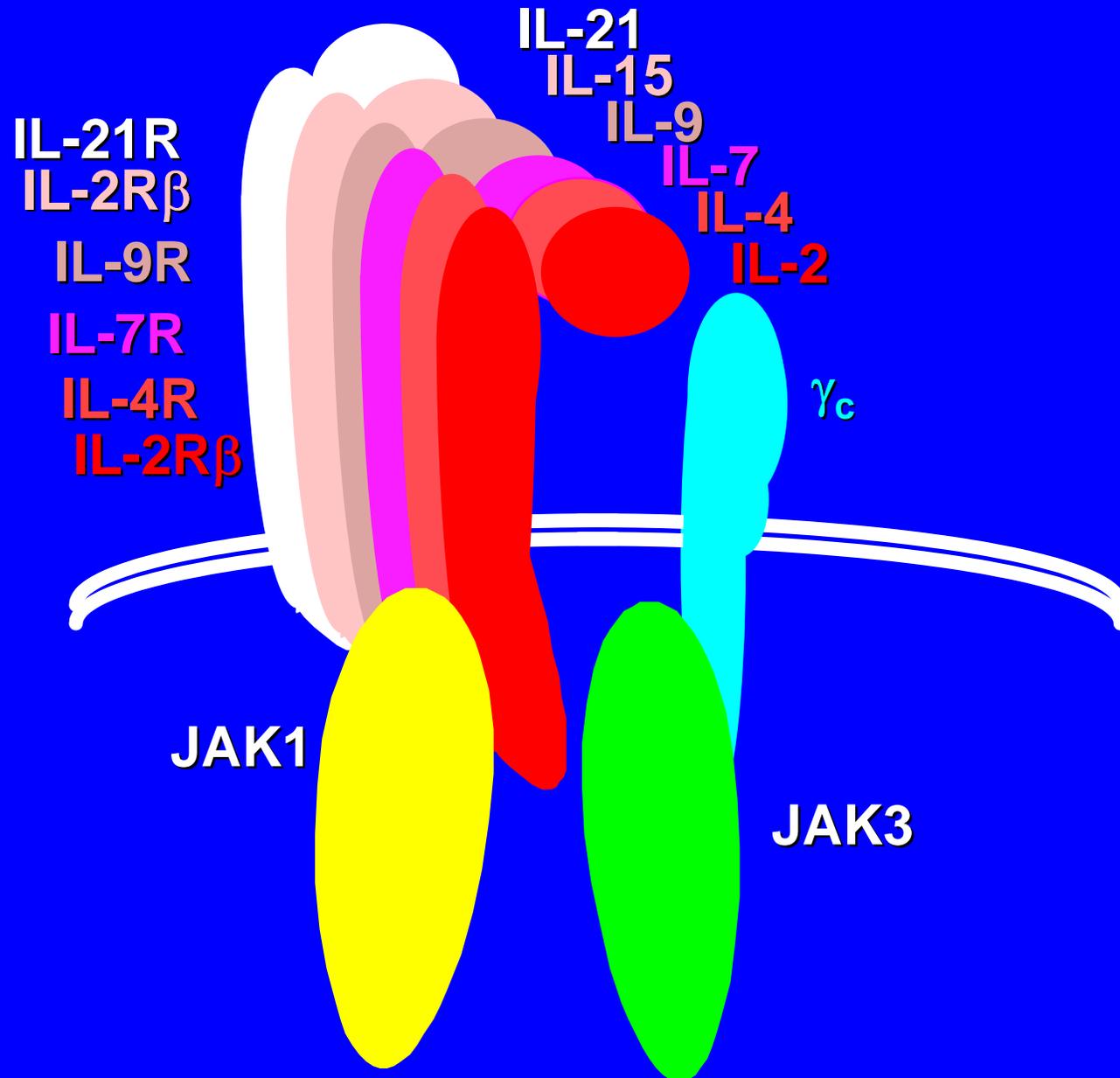
Hypothesis: IL-2R γ is a component of additional cytokine receptors.



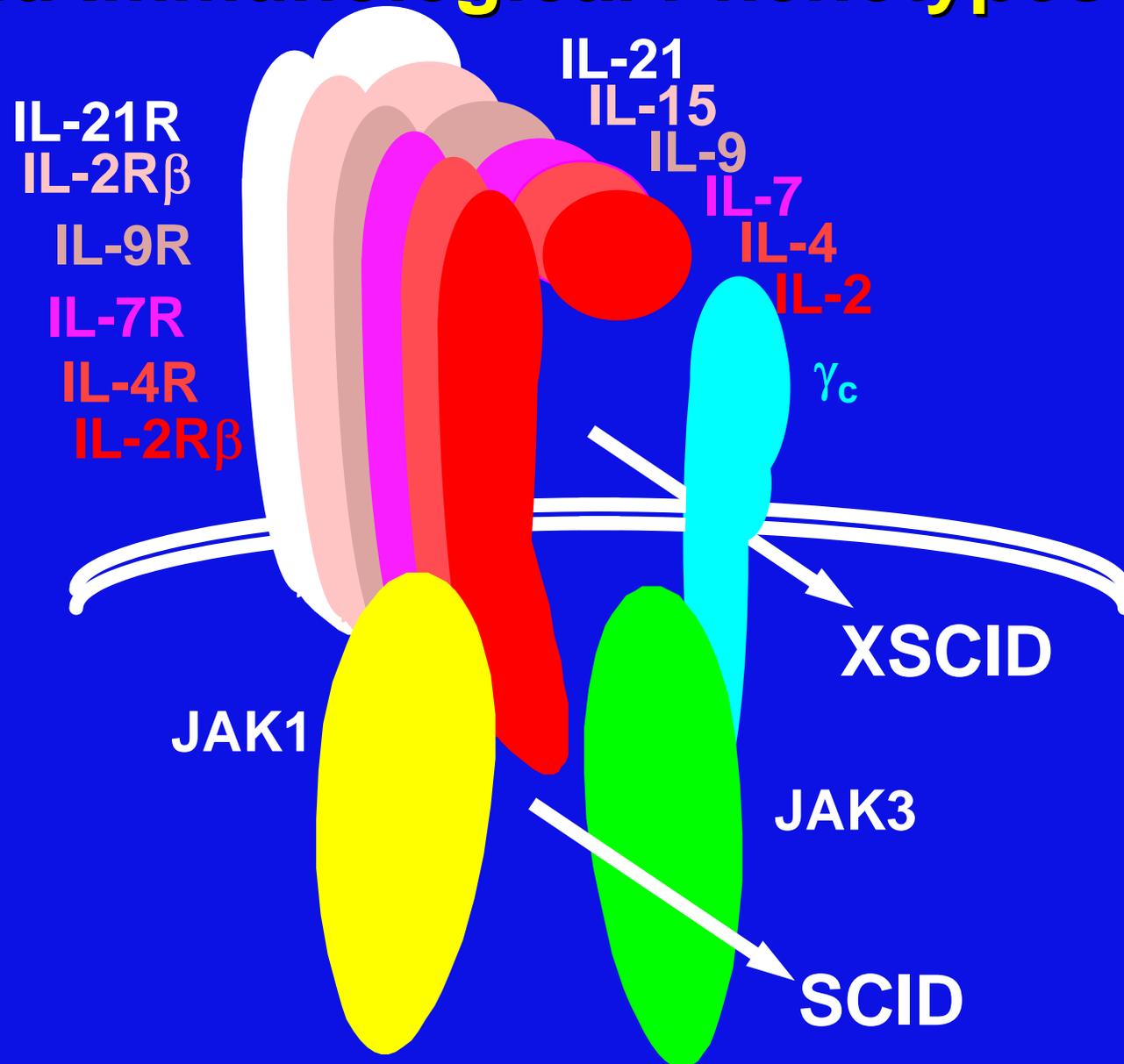
γ_c is a Component of the Receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21



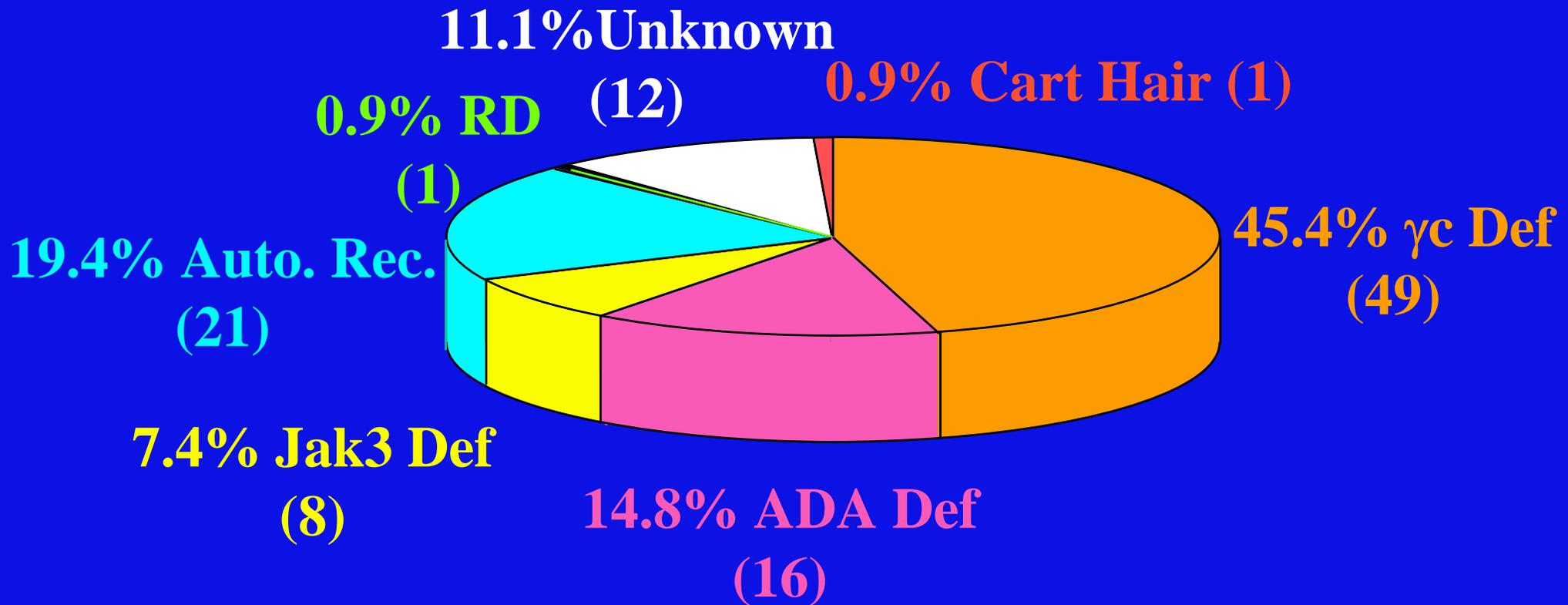
The Janus kinase, Jak3, Associates with γ_c



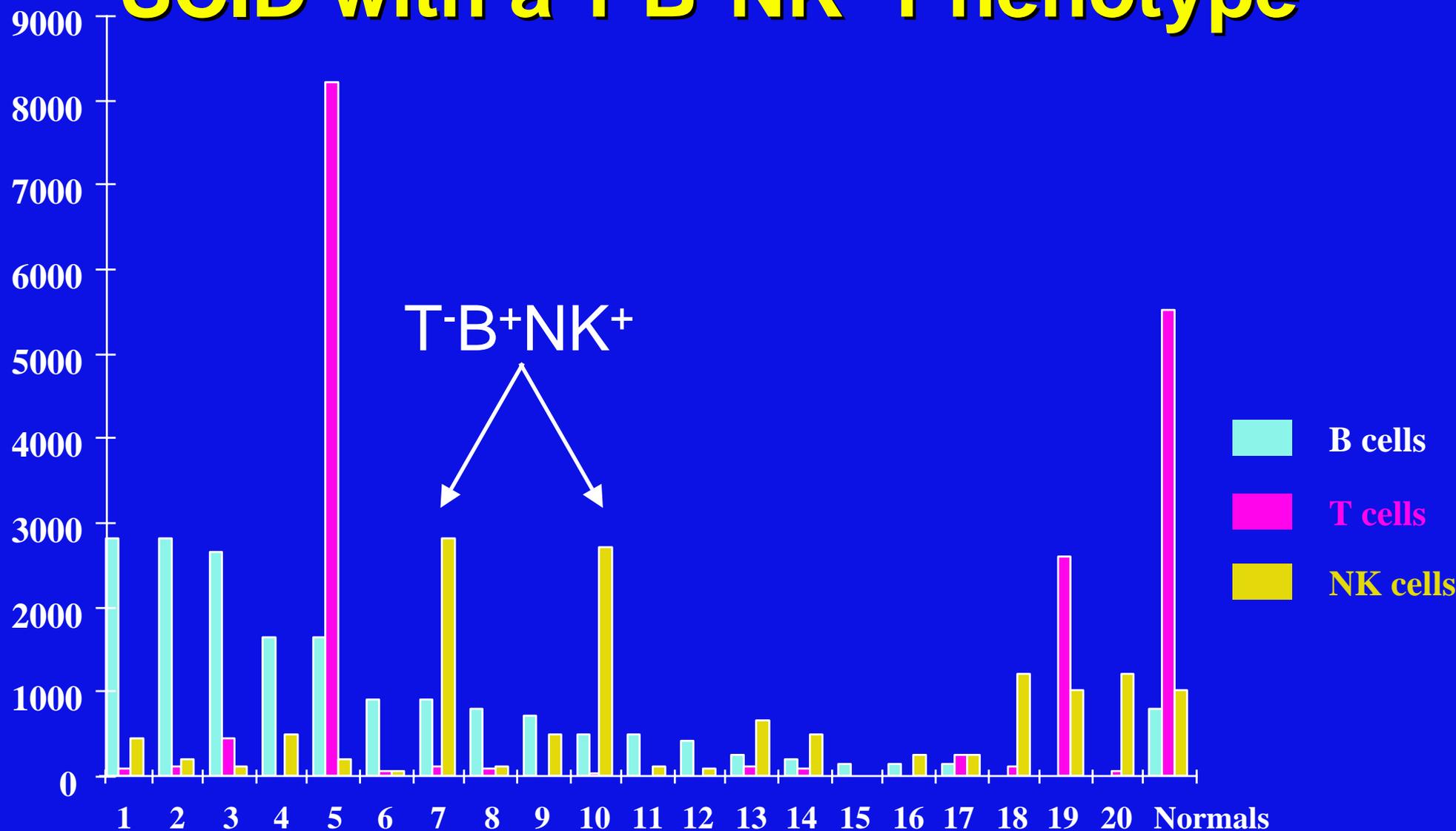
Mutation of γ_c or Jak3 Result in Similar Clinical and Immunological Phenotypes



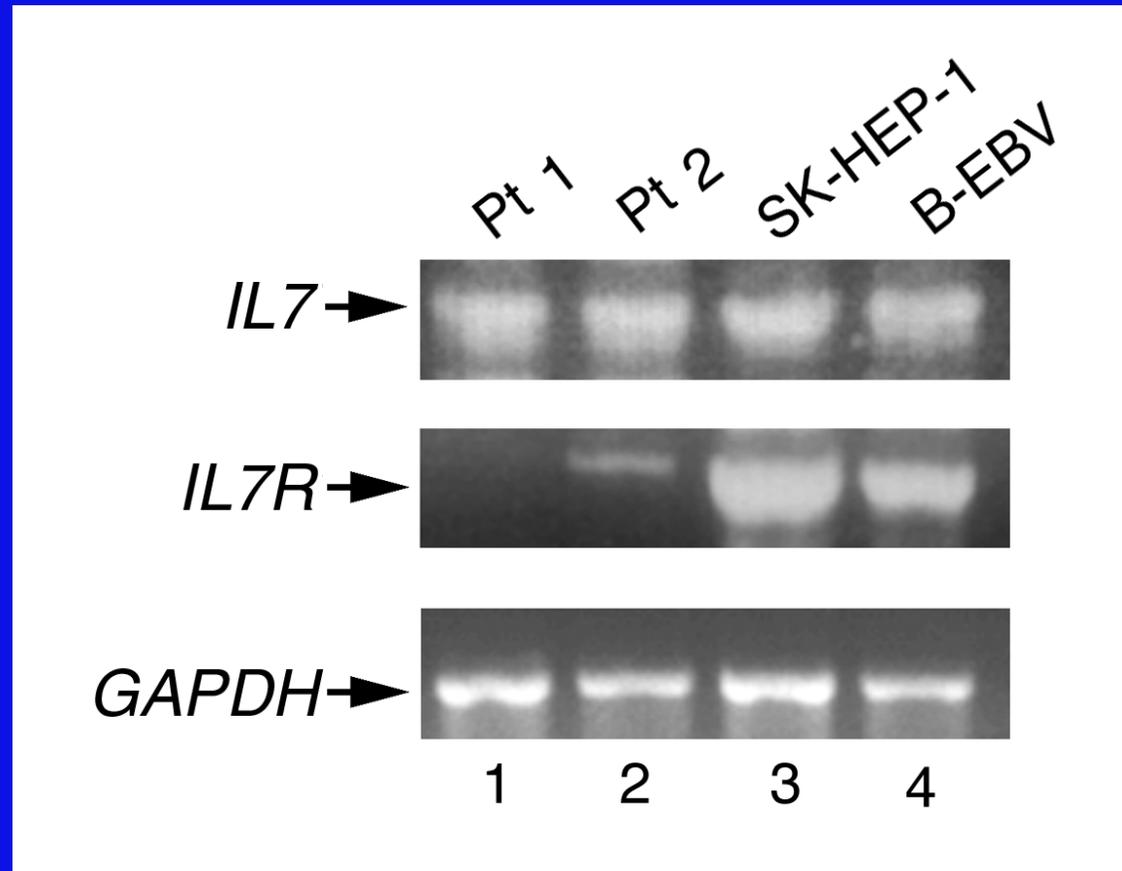
Frequencies of Different Forms of SCID in 108 Infants with SCID



Two Patients with Autosomal Recessive SCID with a T-B⁺NK⁺ Phenotype



Defective *IL7R* mRNA Levels in Two Patients with T-B⁺NK⁺ SCID



Conclusions

1. Defective IL-7R α expression causes T-B⁺NK⁺ SCID. Thus, IL-7R α is essential for T-cell but not B or NK-cell development in humans.
2. In contrast, both B cells and T cells are greatly diminished in *Il7ra*^{-/-} mice, indicating partially different roles for IL-7 in humans and mice.
3. Defective IL-7R α signaling likely explains the T-cell defect in XSCID.

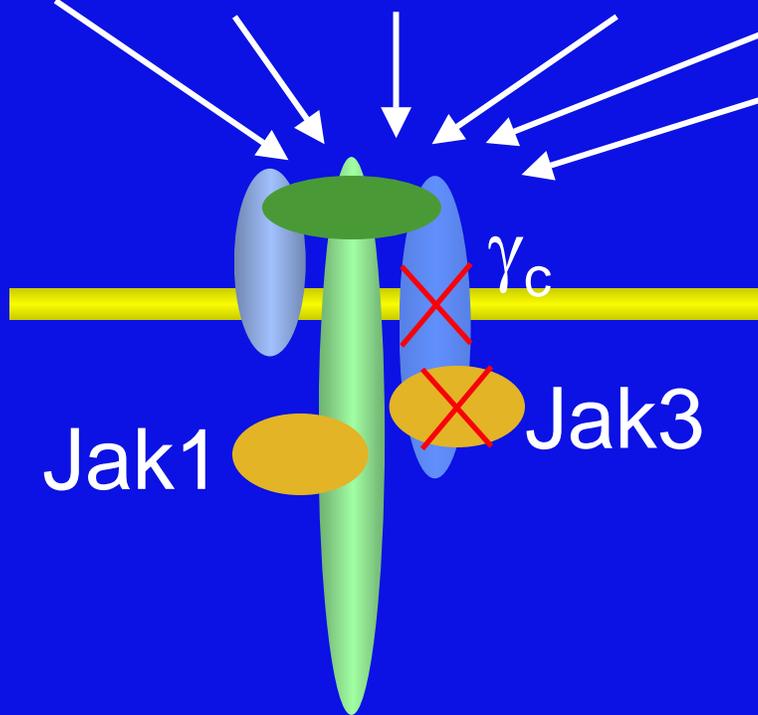
Can *IL7* deficiency also cause T-B⁺NK⁺ SCID?

1. Not yet reported.

2. Hypothesis: *IL7*-deficient patients will have a similar phenotype but will not achieve engraftment of transplanted bone marrow due to lack of stromal IL-7.

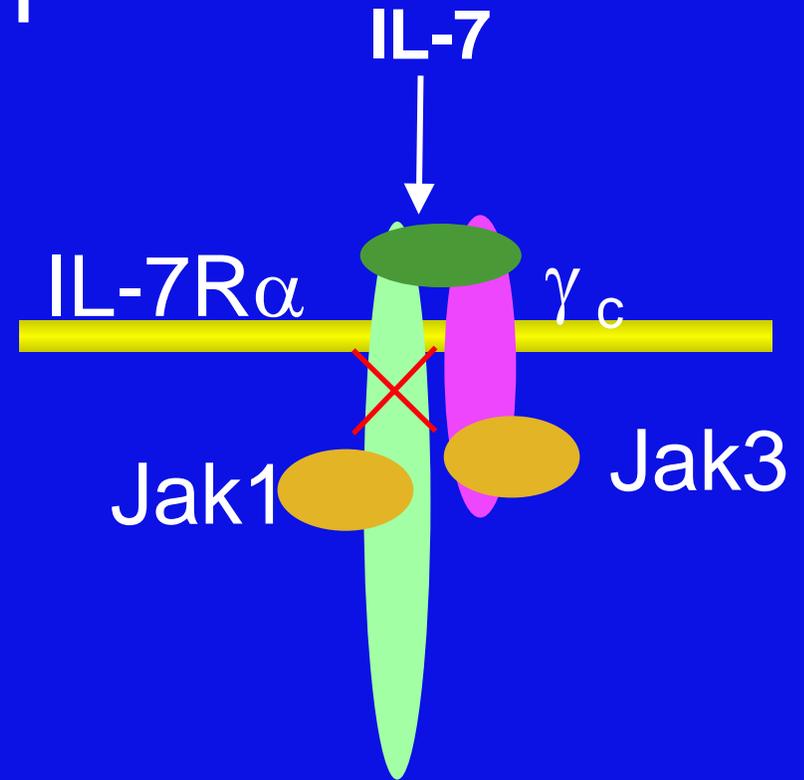
Causes of T-B⁺NK⁻ and T-B⁺NK⁺ SCID

IL-2 IL-4 IL-7 IL-9 IL-15 IL-21



Jak1 Jak3

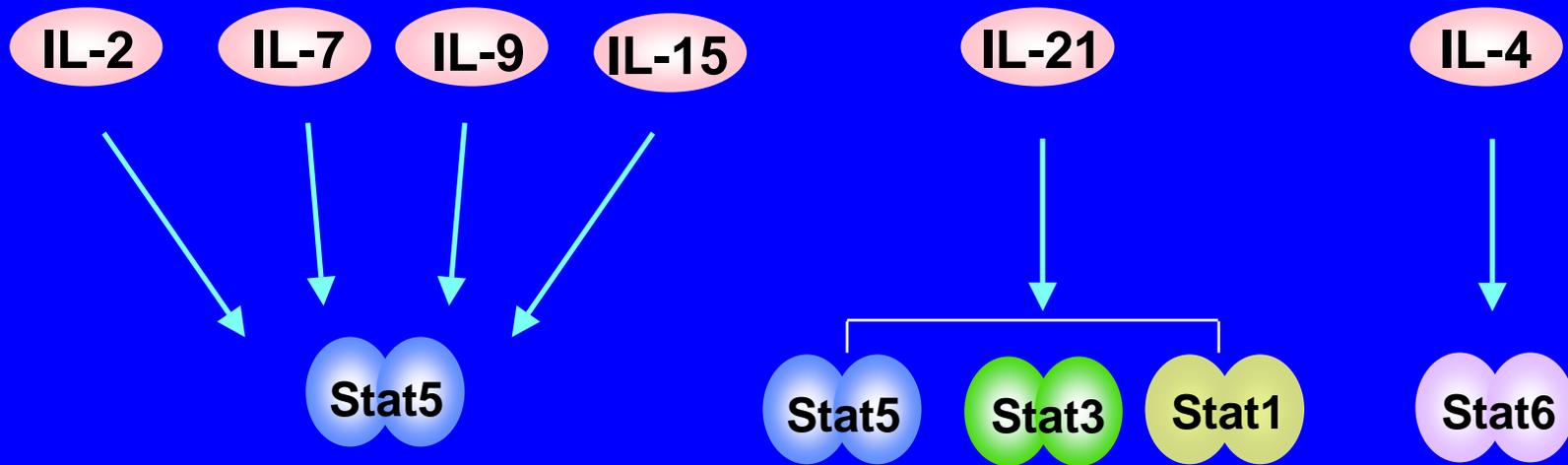
T-B⁺NK⁻ SCID



Jak1 Jak3

T-B⁺NK⁺ SCID

STAT Proteins that are Potently Activated by Cytokines whose Receptors Share γ_c



Above, Stat5 refers to both Stat5a and Stat5b

Stat5 Deficiency Causes T-cell and NK-cell Defects

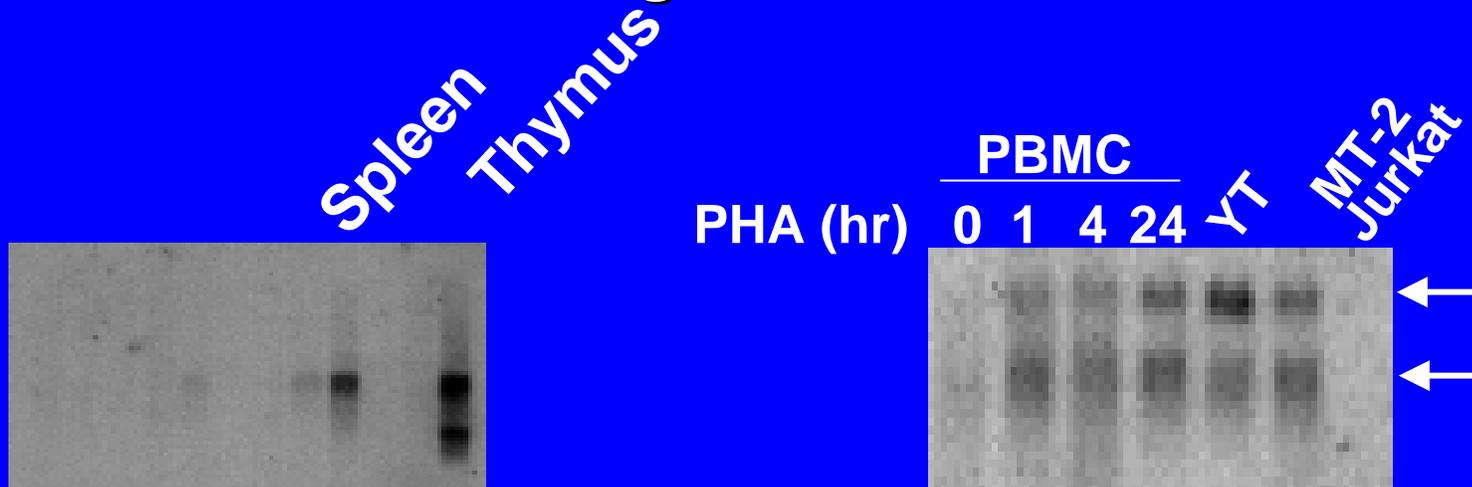
- 1. Stat5a and Stat5b >90% identical at the amino acid level.**
- 2. Defects in either or both proteins cause partial defects in T-cell development.**
- 3. Simultaneous deletion of both Stat5a and Stat5b prevents NK-cell development.**

What Causes the T, NK, and B-cell Defects in XSCID

1. Because T-B⁺NK⁺ SCID can result from *IL7R*-deficiency, defective IL-7R α signaling can explain the T-cell defect in XSCID.
2. Based on the phenotype of mice lacking IL-15 or IL-15 receptor components, defective IL-15 signaling appears to explain the NK-cell defect in XSCID. Based on the Stat5a/5b double KO phenotype, the IL-15 defect appears to be IL-15-mediated activation of Stat5 proteins.
3. However, the basis for the B-cell defect in XSCID has been unclear-- role for IL-4 + IL-21.

Properties of IL-21/IL-21R

1. IL-21 is the most recently discovered γ_c -dependent cytokine. IL-21 is produced by CD4⁺ T cells after antigen activation.
2. IL-21R is expressed on T, B, and NK cells. It is most similar to IL-2R β in sequence. Like IL-2R β , expression of IL-21R is lympho-hematopoietic restricted and is induced in T cells by antigen/mitogen. IL-21R is also expressed in HTLV-I-transformed T cells. The *IL21R* gene is immediately downstream of the *IL4RA* gene.

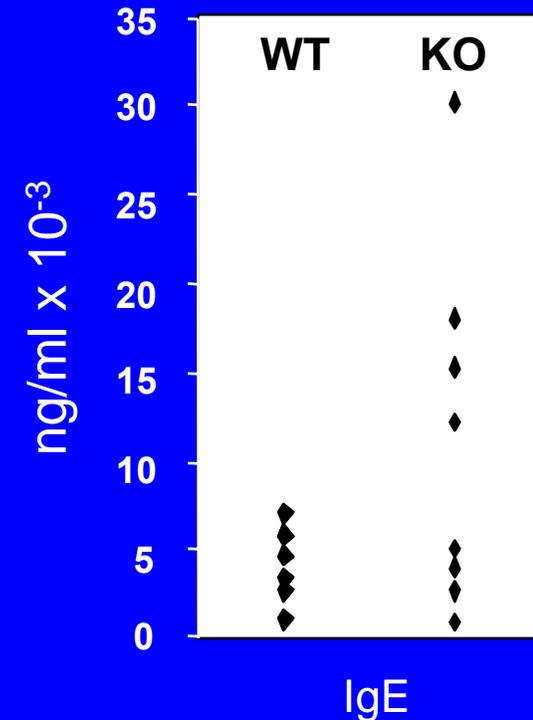
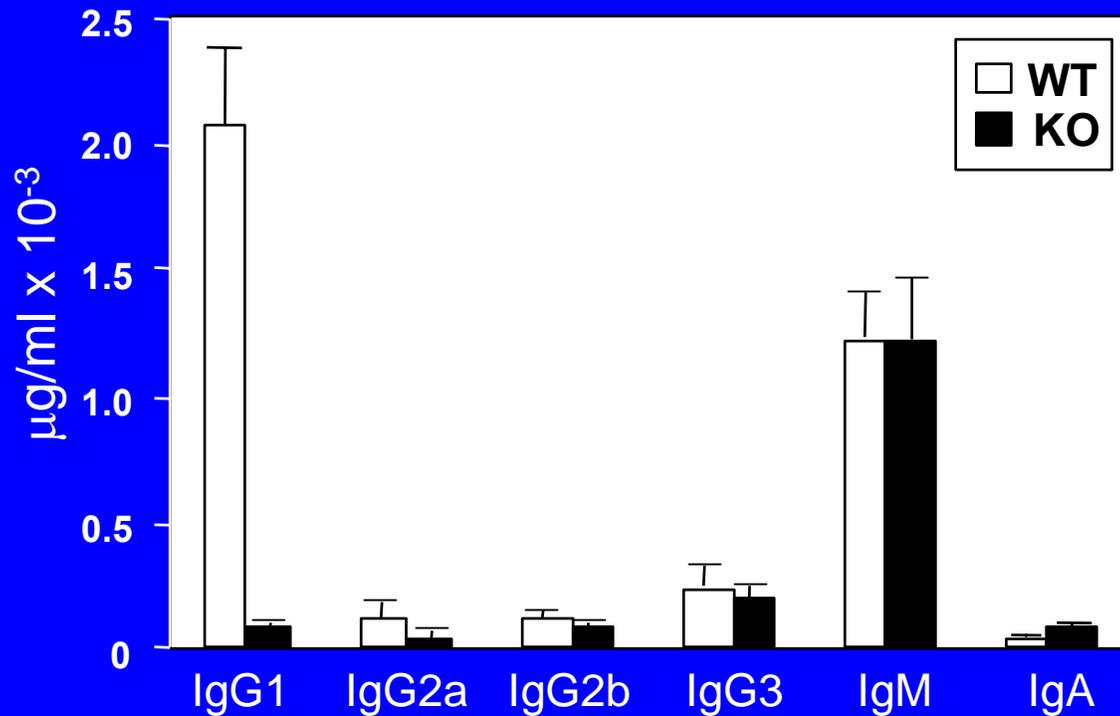


Properties of IL-21R^{-/-} Mice

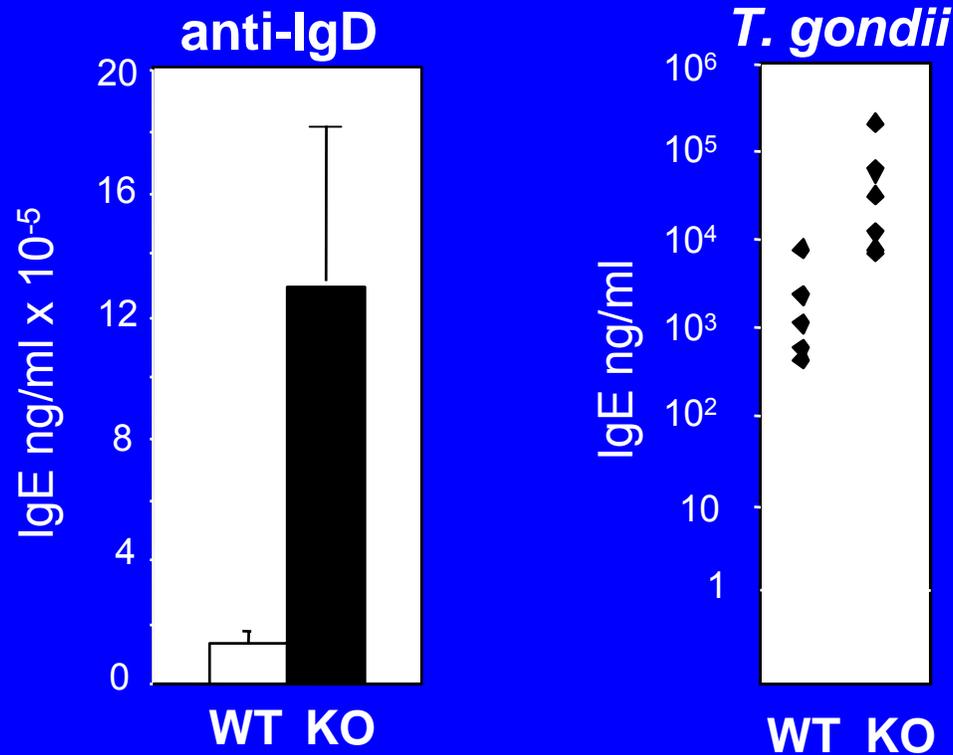
1. **Viable, fertile, normal Mendelian genetics, normal life span.**
2. **Normal T-cell, B-cell, and NK-cell development. No obvious abnormalities.**

Decreased IgG1 and Increased IgE in IL-21R^{-/-} Mice Immunized with Ovalbumin

Total Serum Ig /OVA Immunization



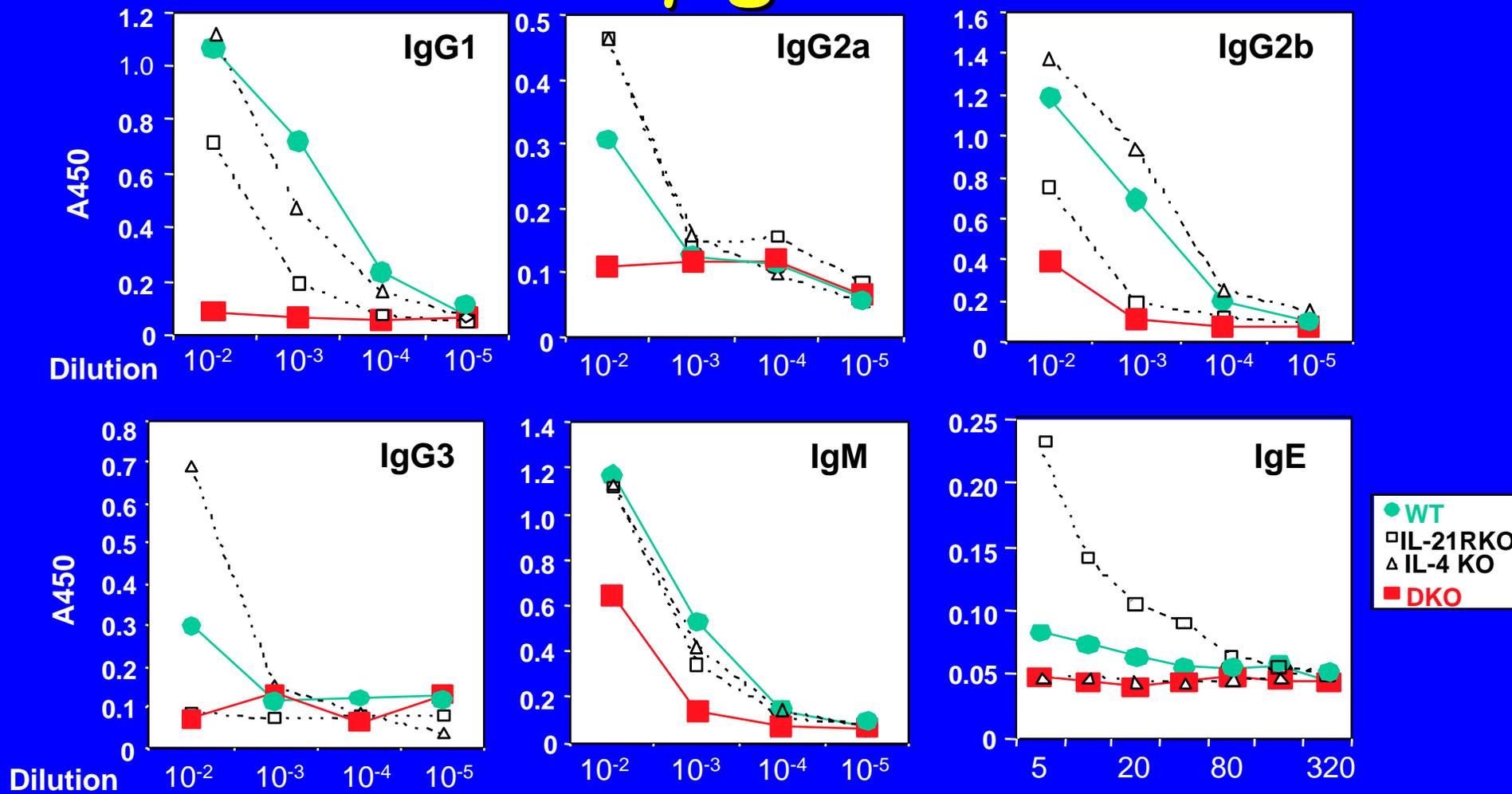
Potent Induction of IgE in IL-21R^{-/-} Mice in Response to anti-IgD or *T. gondii*



Is the increased IgE in IL-21R^{-/-} mice dependent on IL-4?

To address this, we generated IL-4/IL-21R double KO mice.

Defective Ig Production in IL-4/IL-21R double KO mice Immunized with TNP-Chicken γ -globulin



The B-cell Phenotype in the IL-4/IL-21R DKO Mice Appears to Mimic that Found in Human XSCID

γ_c KO mice do not have B cells, because in contrast to humans, IL-7 is essential for B-cell development in mice. By leaving IL-7 signaling intact, but by simultaneously inactivating IL-4 and IL-21 signaling, we appear to have mimicked in mice the human XSCID B-cell defect.

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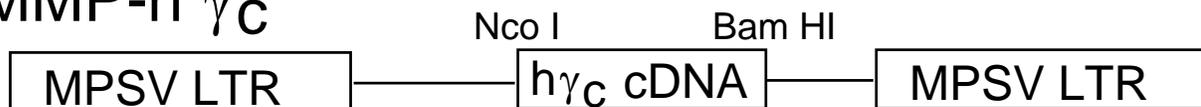
These data suggest that defective signaling by IL-4 and IL-21 may explain the B-cell defect in human XSCID.

Gene Therapy of γ_c -Deficient Mice

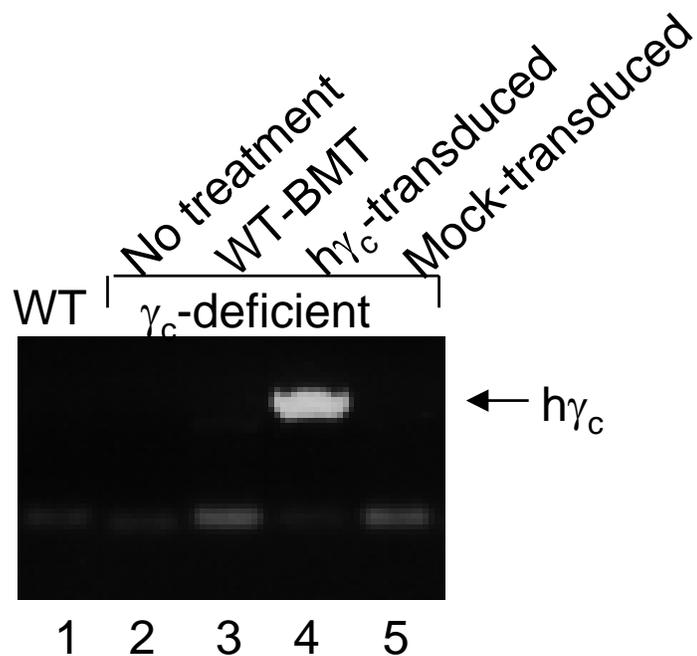
1. Our study showed reconstitution of T-cells, B-cells, and NK-cells, as well as IL-2-induced proliferation and immunoglobulin production.
2. Study of the Fischer/DiSanto group showed similar reconstitution of γ_c /RAG-double KO mice.
3. No adverse events observed in either study.

A

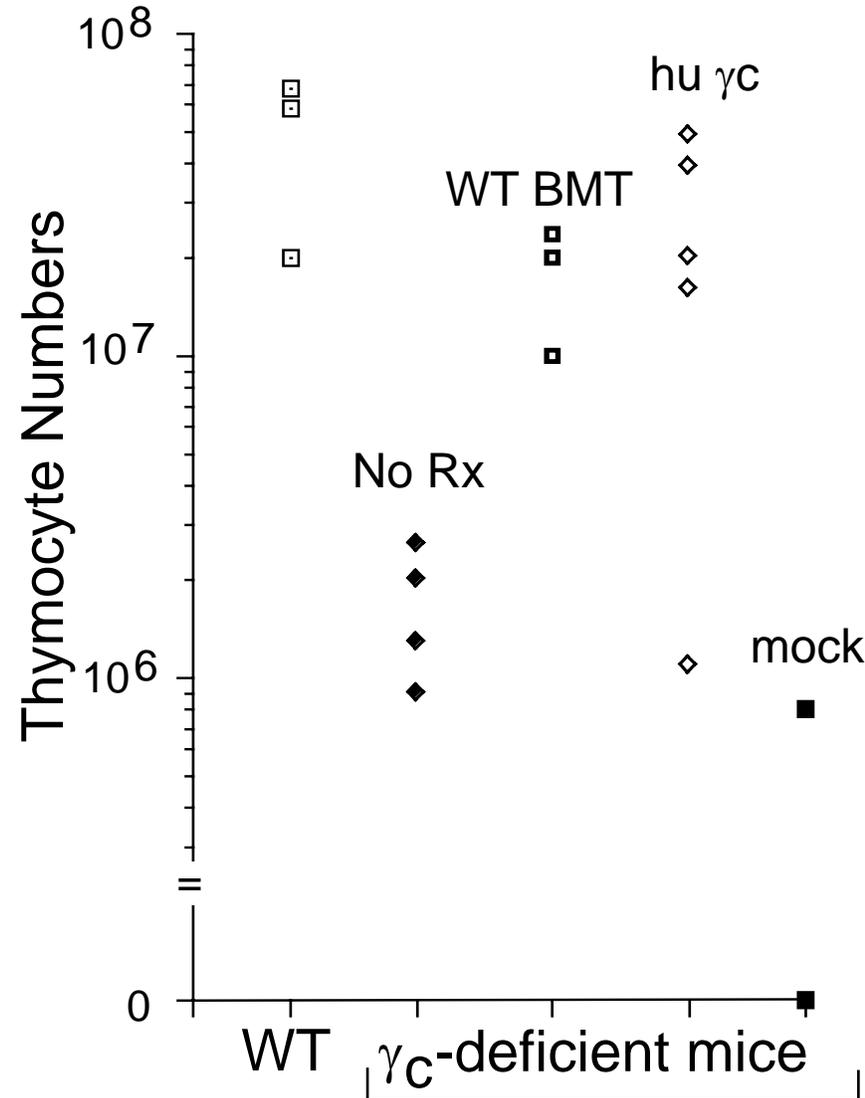
MMP-h γ_c



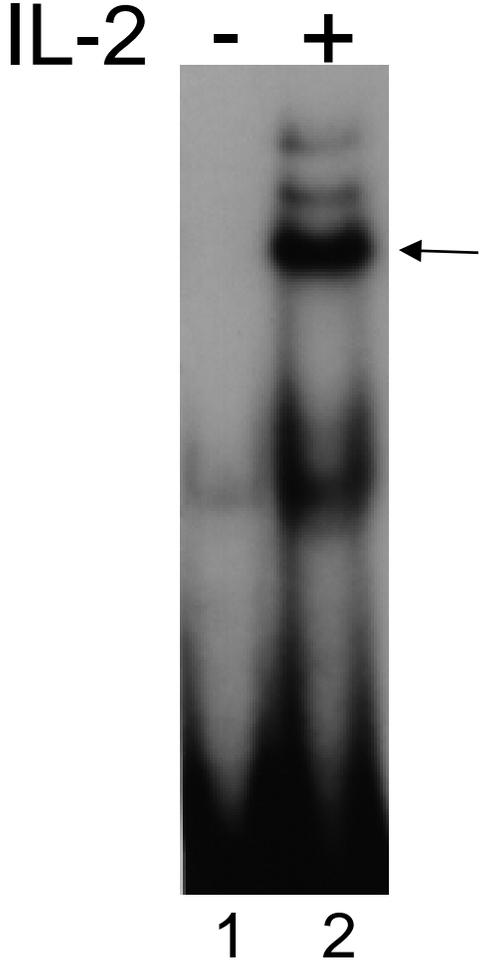
B



Reconstitution of Thymocyte Numbers after Retroviral Transduction of γ_C into γ_C -Knockout Mice



Reconstitution of IL-2-induced STAT Protein Activation in γ_c -transduced Mice



Reconstitution of T, B, and NK Cells after Retroviral Transduction of γ_C into γ_C -Knockout Mice

Table 1: Number of T cells, B cells, and NK cells in wild type and reconstituted γ_C KO Mice

Mice	BMT	CD3 ⁺ B220 ⁻ (T cells)	CD3 ⁻ B220 ⁺ (B cells)	TCR β ⁻ NK1.1 ⁺ (NK cells)
Wild type (n=4)	–	25.9 \pm 1.1	54.2 \pm 2.7	2.1 \pm 0.22
γ_C KO (n=6) *	–	7.8 \pm 2.5	0.6 \pm 0.1	0.04 \pm 0.01
γ_C KO (n=4)	Wild type BM	13.5 \pm 3.8	40.7 \pm 9.6	1.17 \pm 0.35
γ_C KO (n=8)	γ_C -transduced γ_C KO BM	14.6 \pm 2.8	45.5 \pm 12.7	0.62 \pm 0.1
γ_C KO (n=4)	mock-transduced γ_C KO BM	1.1 \pm 0.2	0.1 \pm 0.1	0.03 \pm 0.02

Values are presented as mean number of cells $\times 10^{-6} \pm$ SEM.

*n=5 for NK cell numbers

Abbreviations: BM, bone marrow; BMT, bone marrow transplantation

Reconstitution of Ig levels after Retroviral Transduction of γ_C into γ_C -Knockout Mice

Table 2: Serum Immunoglobulin Levels in wild type and reconstituted γ_C KO Mice

Mice	BMT	Serum Immunoglobulin Levels ($\mu\text{g/ml}$)		
		IgG	IgA	IgM
Wild type (n=4)	–	5300 \pm 1041	2574 \pm 873	540 \pm 192
γ_C KO (n=6)	–	198 \pm 151	153 \pm 8	309 \pm 101
γ_C KO (n=4)	Wild type BM	6953 \pm 132	5504 \pm 1615	1273 \pm 513
γ_C KO (n=8)	$h\gamma_C$ -transduced γ_C KO BM	4554 \pm 696	2890 \pm 1097	1610 \pm 183
γ_C KO (n=4)	mock-transduced γ_C KO BM	151 \pm 63	102 \pm 43	372 \pm 190

Values are presented as mean \pm SEM.

Abbreviations: BM, bone marrow; BMT, bone marrow transplantation.

Does Constitutive Expression of γ_c Predispose to Development of Leukemia?

1. Cells expressing γ_c should have a growth advantage over cells not expressing γ_c . However, γ_c normally is constitutively expressed in T cells and NK cells. It is unknown whether γ_c levels are limiting.

Queries:

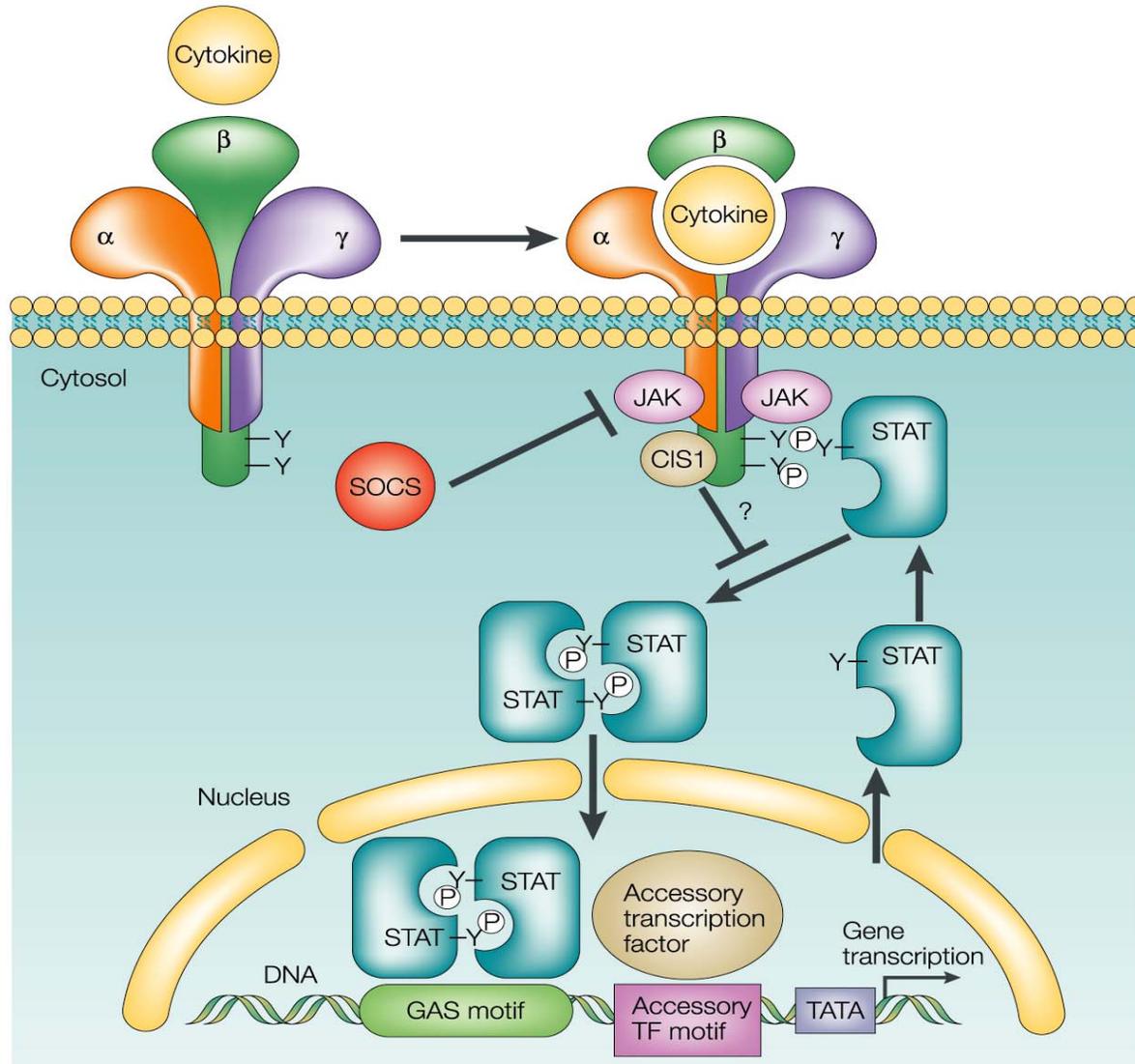
1. Are the levels of transduced γ_c higher than the normal level at any stage of development?
2. Is there evidence of constitutive signaling (e.g., constitutively activated Jak kinases or STAT proteins, as these have been associated with various malignant states)? IL-7, IL-9, and IL-15 transgenic mice have been reported to develop leukemias or lymphomas.

Credit Slide

Masayuki Noguchi
Sarah Russell
Anne Puel
Hiroshi Nakajima
Kazunori Imada
John Kelly
Panu Kovanen
Jian-Xin Lin
Mindy Lo
Katsutoshi Ozaki
Rosanne Spolski
Hai-Hui Xue
Rong Zeng

Collaborators

O. Wesley McBride
Lisa Filipovich
Howard Rosenblatt
Rebecca Buckley
Helen Davey, AgResearch
Lothar Hennighausen, NIDDKD
Michael Bloom, formerly of NHLBI
Sandy Morse, NIAID
Andreas Rosenwald, NCI
Pam Schwartzberg, NHGRI
Kristy Kikly, formerly of SmithKlineBeecham
Peter Young, formerly of SmithKlineBeecham



γ_c is a Component of the Receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21

