MOLECULAR BIOLOGY INTELLIGENCE UNIT 20

Alister C. Ward

The Jak-Stat Pathway in Hematopoiesis and Disease

EUREKAH.COM

LANDES BIOSCIENCE

MOLECULAR BIOLOGY INTELLIGENCE UNIT 20

The Jak-Stat Pathway in Hematopoiesis and Disease

Alister C. Ward, B.A., B.Sc.(Hons), Ph.D.
Centre for Cellular and Molecular Biology
Deakin University
Burwood, Victoria, Australia

LANDES BIOSCIENCE
GEORGETOWN, TEXAS
U.S.A.

EUREKAH.COM
AUSTIN, TEXAS
U.S.A.

THE JAK-STAT PATHWAY IN HEMATOPOIESIS AND DISEASE

Molecular Biology Intelligence Unit

Eurekah.com
Landes Bioscience
Designed by Lori Keyes and Celeste Carlton

Please address all inquiries to the Publishers:
Eurekah.com / Landes Bioscience, 810 South Church Street
Georgetown, Texas, U.S.A. 78626
Phone: 512/ 863 7762; FAX: 512/ 863 0081
www.Eurekah.com
www.landesbioscience.com

ISBN: 1-58706-074-4-CD (hardcover)
ISBN: 1-58706-114-7-CD (softcover)

Library of Congress Cataloging-in-Publication Data

The Jak-Stat pathway in hemtopoiesis and disease / [edited by] Alister C. Ward.
p. ; cm. -- (Molecular biology intelligence unit ; 20)
Includes bibliographical references and index.
ISBN 1-58706-074-4 (hardcover) -- ISBN 1-58706-114-7 (softcover)
1. Hematopoiesis--Regulation. 2. Cytokines--Receptors.
[DNLM: 1. Hematopoiesis--physiology. 2. Cytokines--physiology. 3. Leukemia--physiopathology. 4. Receptors, Cytokine--physiology. 5. Signal Transduction. WH 140 J 25 2001] I. Ward, Alister C. II. Series.
QP92 .J35 2001
612.4'1--dc21

2001005045

Dedication

To my wife, Tania, for her unwavering support, and our son, Samuel, for making it all worthwhile.

CONTENTS

1. Jaks and Normal Hematopoiesis 1
Tetsuya Nosaka
Introduction 1
Modes of Jak Activation in Cytokine Signaling 1
Jak Function Determined by Targeted Gene Disruption 3
Conclusions 6

2. Jak3-Dependent Pathways in Hematopoiesis and SCID 10
Nicholas A. Cacalano and James A. Johnston
Introduction 10
Jak3-Regulated Signal Transduction Pathways 11
Models of Jak3 Deficiency 19
Jak3 in Positive and Negative Selection of Thymocytes 25
Role of Jak3 in Innate Immunity 26
Role of Jak3 in the Periphery 28
Knockouts of Jak3-Dependent Signaling Components 30
Conclusions 41

3. Stat Activation by Src, Fes and Btk Tyrosine Kinases 51
Thomas E. Smithgall
Introduction 51
Src Kinases 52
The c-Fes Tyrosine Kinase 62
The Btk Kinase Family 68
Conclusions 71

4. Stats and Hematopoiesis 78
Alister C. Ward
Introduction 78
Stat Proteins 78
Role of Stats in Hematopoietic Processes 81
Mechanisms of Stat Action 84
Unanswered Questions 85
Conclusions 86
Acknowledgements 86

5. Stat Signaling in Leukemia: Implications for Pathogenesis and Treatment 92
David A. Frank
Introduction 92
Human Leukemia 93
Stat Activation in Leukemias 93
Targeting Stats in Anti-Leukemia Therapy 101
Conclusion 106

6. The Role of the Jak-Stat Pathway in Hematopoiesis and Immune Responses in Drosophila 112
Erika A. Bach and Norbert Perrimon
Introduction 112
The Jak-Stat Pathway 113
The Drosophila Jak-Stat Pathway 114
Hematopoiesis in Drosophila 116
Signaling Pathways Involved in Larval Hematopoiesis 120
Immune Responses in Drosophila 121
Conclusions 122

7. SOCS Proteins in Hematopoiesis 127
Sandra Nicholson and Warren Alexander
Introduction 127
SOCS Proteins 128
Mechanisms of Action of SOCS Proteins 129
Physiological Roles of the SOCS Proteins 132
Conclusions 135

Index 139

EDITOR

Dr. Alister C. Ward, B.A., B.Sc.(Hons), Ph.D.
Centre for Molecular and Cellular Biology
Deakin University
Burwood, Victoria, Australia
Chapter 4

CONTRIBUTORS

Warren Alexander
The Walter and Eliza Hall Institute for Medical Research
Parkville, Victoria, Australia
E-mail: alexander_w@wehi.edu.au
Chapter 7

Erika A. Bach
Genetics Department
Harvard Medical School
Boston, Massachusetts, U.S.A.
E-mail: ebach@genetics.med.harvard.edu
Chapter 6

Nichola A. Cacalano
DNAX Research Institute
Palo Alto, California, U.S.A.
E-mail: cacalano@dnax.org
Chapter 2

David A. Frank
Department of Adult Oncology
Dana-Farber Cancer Institute
Harvard Medical School
Boston, Massachusetts, U.S.A.
E-mail: david_frank@dfci.harvard.edu
Chapter 5

James A. Johnston
Chair of Immunology
Queen's University Belfast
Belfast, Northern Ireland
E-mail: jim.johnston@qub.ac.uk
Chapter 2

Sandra Nicholson
The Walter and Eliza Hall Institute for Medical Research
Parkville, Victoria, Australia
E-mail: snicholson@wehi.edu.au
Chapter 7

Tetsuya Nosaka
Department of Donation Laboratories
Institute of Medical Science
The University of Tokyo
Tokyo, Japan
E-mail: tenosaka@ims.u-tokyo.ac.jp
Chapter 1

Norbert Perrimon
Genetics Department
Howard Hughes Medical Institute
Harvard Medical School
Boston, Massachusetts, U.S.A.
E-mail: perrimon@rascal.med.harvard.edu
Chapter 6

Thomas E. Smithgall
Department of Molecular Genetics and Biochemistry
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania, U.S.A.
E-mail: tsmithga@pitt.edu
Chapter 3

PREFACE

Blood cell development—or hematopoiesis—is a complex, multi-step process known to be controlled by a range of extracellular signals, including cytokines and growth factors. The elucidation of the Janus kinase-signal transducer and activator of transcription (Jak-Stat) pathway represents one of the great advances in our understanding of how these various signals can facilitate rapid alterations in gene expression in hematopoietic and other target cells. This book aims to describe the role of the Jak-Stat pathway in the normal development and function of hematopoietic cells, and to describe how perturbations of this pathway contribute to several hematopoietic disorders, including malignancy.

In Chapter One, Tetsuya Nosaka describes the function of the four members of the mammalian Jak tyrosine kinase family and details their role in normal hematopoiesis as revealed by gene targeting. In Chapter Two, Nicholas Cacalano and James Johnston focus more specifically on Jak3, revealing its close association with both interleukin-2 receptor and common gamma chain signaling in hematopoiesis, and the consequences of its ablation in immunodeficiency syndromes. In Chapter Three, Thomas Smithgall explores an exciting new area involving activation of Stats by non-Jak tyrosine kinases: specifically Src, Fes and Btk. In Chapter Four, I detail the function of Stats in normal hematopoietic processes, which is extended by David Frank in Chapter Five to include their role in leukemia and the implications of this for potential therapeutic intervention. In Chapter Six, Erika Bach and Norbert Perrimon describe the role of the Jak-Stat pathway in hematopoiesis and immune responses using Drosophila as a model organism. Finally, in Chapter Seven, Sandra Nicholson and Warren Alexander describe important negative regulators of the Jak-Stat pathway, the SOCS family of proteins, with emphasis on their function in hematopoiesis.

These contributions highlight the central role played by the Jak-Stat pathway in hematopoiesis. Importantly, it lies downstream of receptors for the clinically-relevant cytokines granulocyte colony-stimulating factor, erythropoietin, thrombopoietin, and the interferons. In addition, this pathway is perturbed in a variety of malignancies and hematopoietic disorders. Therefore, it can be safely anticipated that therapeutics currently under development which target this pathway will have wide and important hematological application.

Alister C. Ward

Chapter 1

Jaks and Normal Hematopoiesis

Tetsuya Nosaka

Introduction

Cytokines regulate cell fate including proliferation, differentiation, and apoptosis of hematopoietic progenitor cells, while lineage commitment of the hematopoietic stem cells is determined stochastically. Cytokines exert their specific function through binding to specific members of the cytokine receptor superfamily. The specific expression of these receptors is a vital determinant of hematopoietic cell differentiation. The Jak tyrosine kinases, consisting of four members in mammals: Jak1, Jak2, Jak3, and Tyk2, were each "Just Another Kinase" until their biological functions were unveiled.[1] In 1993, Ihle and colleagues reported that Jak2 associates with the membrane-proximal region of the erythropoietin receptor (EpoR) —which itself lacks a tyrosine kinase domain —and phosphorylates it on tyrosine residues upon Epo stimulation.[2] Meanwhile, Jak1 and Tyk2, and Jak1 and Jak2 were found by genetic complementation experiments using mutant cell lines to be involved in the signaling of the receptors for IFNα/β and IFNγ, respectively.[3-5] Since then Jaks have been recognized as one of the most important tyrosine kinases in cytokine signaling. A number of experiments in vitro have disclosed that Jak family tyrosine kinases are indispensable for signal transduction via the cytokine receptor superfamily. In addition, generation of Jak-deficient mice has revealed the biologically important and nonredundant roles of Jaks in vivo.

Modes of Jak Activation in Cytokine Signaling

The specificity of cytokine-mediated Jak-Stat signaling derives from the specific interaction of the phosphotyrosine of the cytokine receptor and the SH2 domain of the Stat protein.[6,7] Although preferential usage of a Jak for a subunit of the cytokine receptor exists (Table 1), the particular Jak engaged does not appear to determine the specificity of the downstream signaling. In contarst, Stats directly regulate the target gene expression in a cytokine receptor-specific manner.[8-10] On the other hand, since Jak activation is required for all signaling pathways including the Ras/Raf/Mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3-k)/Akt cascades, loss of Jak function leads to a wide range of aberrant signaling, in contRrst to loss of Stat function which results in a more restricted phenotype.[11]

The patterns of Jak activation in signaling via the cytokine receptor superfamily can be classified into three modes (Fig. 1 and Table 2). In the case of the receptors for Epo and thrombopoietin (TPO) which consist of a single receptor chain, and the receptors which use a common β chain as a single signaling chain (IL-3, IL-5, GM-CSF), Jak2 is activated

The Jak-Stat Pathway in Hematopoiesis and Disease, edited by Alister C. Ward.

Table 1. Jak requirements for function of cytokines essential for normal hematopoiesis and immune responses against pathogens

IL-6	IL-2	IL-15	IL-7	IFNγ	IFNα/β	IL-12	Epo
gp130	IL-Rβ		IL-7Rα	GR1(α)	AR2(β)	βII	EpoR
			Jak1			Jak2	
	γc			GR2(β)	AR1(α)	βI	
	Jak3			Jak2	Tyk2		

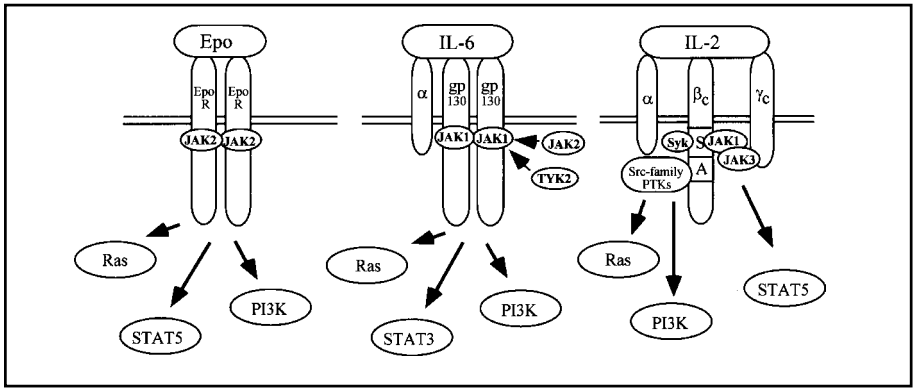

Figure. 1. Three modes of Jak activation through cytokine receptors. **EpoR**: single signaling chain-single Jak. Epo stimulation induces dimerization of EpoR, resulting in autophosphorylation of Jak2. Activated Jak2 phosphorylates EpoR on tyrosine residues, followed by recruitment of Stat5. Then Jak2 phosphorylates Stat5, resulting in dimerization of Stat5. Dimerized Stat5 enters the nucleus to regulate target gene expression. Tyrosine phosphorylation of EpoR also activates both Ras/Raf/MAP kinase and PI3K/Akt pathways. **IL-6R**: single signaling chain-multiple Jaks. IL-6 stimulation induces dimerization of gp130. Although Jak1, Jak2, and Tyk2 are phosphorylated after receptor chain aggregation, only Jak1 plays a major role in phosphorylation of gp130, while Jak2 and Tyk2 are dispensable.[12] The α chain of the IL-6R contributes to the ligand-specific binding of the receptor, since gp130 is shared among the other cytokines. **IL-2R**: multiple signaling chains-multiple Jaks. IL-2 stimulation induces transphosphorylation of Jak1 and Jak3 which associate with a serine-rich region (S) of the β chain and C-terminus of the common γ chain, respectively.[13,14] Activation of both Jak1 and Jak3 is essential for subsequent activation of Stat5 and signal transducing adaptor molecule (STAM) which activates c-*Myc* transcription (not shown).[15] The other tyrosine kinase Syk which associates with a serine-rich region of the β chain, and Src-family tyrosine kinases such as Lck, Fyn, and Lyn which associate with an acidic region (A) of the β chain, are also activated upon IL-2 stimulation.[16] Src-family tyrosine kinases activate pathways of both Ras/Raf/MAP kinase and PI3K/Akt, whereas Syk induces c-*Myc* expression.

via dimerization of the single receptor chain or the common β chain. In the latter case, a ligand-specific α chain does not associate with Jaks and is not directly involved in intracellular signaling. The second mode of activation is typified by receptors of the IL-6 family —IL-6, IL-11, oncostatin M (OSM), leukemia inhibitory factor (LIF), ciliary neurotrophic factor, cardiotrophin-1, and G-CSF—where multiple Jaks are activated with a single signaling chain. However, in this case, only Jak1 appears to be essential for signaling, with activation of Jak2 or Tyk2 not mandatory in spite of the activation of these kinases upon ligand stimulation.[12] The third mode is typified by signaling of the cytokines which use a

Table 2. Three modes of cytokine signaling using Jaks in hematopoietic cells

1. Single signaling chain-single Jak: (Jak2 is used) Epo, TPO, βc family (IL-3, IL-5, GM-CSF)
2. Single signaling chain-multiople Jaks: (Jak1 is essential) IL-6 family (IL-6, IL-11, OSM, LIF), G-CSF
3. Multiple signaling chains-multiple Jaks: γc family (IL-2, IL-4, IL-7, IL-9, IL-15), IFNα/β, IFNγ, IL-10, IL-13, TSLP, IL-12

common γ chain (γ_c) family (IL-2, IL-4, IL-7, IL-9, and IL-15), as well as IFNα/β, IFNγ, IL-10, IL-13, thymic stromal lymphopoietin (TSLP), and IL-12. The receptors for these cytokines consist of multiple signaling chains and multiple Jaks are used. In the case of IL-2 signaling, for example, Jak1 and Jak3 associate with the β chain of the IL-2R and the γ_c chain, respectively,[13,14] and transphosphorylate each other upon the ligand binding. Therefore, deletion of either Jak1 or Jak3 abolishes the response to IL-2. Because Jak activation by auto- or trans-phosphorylation is indispensable for tyrosine phosphorylation of the hemopoietin receptor superfamily in each of the modes described above, Jaks inevitably play critical roles in hematopoiesis.[17,18]

Jak Function Determined by Targeted Gene Disruption

Since cytokines and hematopoietic growth factors are somewhat redundant in their function,[19] mice deficient for single factors generally display no severe developmental abnormalities, except for rare cases such as Epo,[20] stem cell factor,[21,22] IL-7,[23] and platelet derived growth factor.[24-26] Of these, Epo and IL-7 exert their function via receptors with no intrinsic tyrosine kinase activity, and so rely entirely on Jak function. The phenotype of Jak2-deficient mice[27,28] (to be described later) is recapitulated in Epo- or EpoR-deficient mice.[20] Likewise, the phenotype of Jak3-deficient mice[29-31] is very similar to that of IL-7-[23], IL-7R α chain-,[32] or γ_c chain-[33,34]deficient mice.

Jak1 Performs Nonredundant Functions in Three Classes of Cytokine Receptor-Mediated Biological Responses

Activation of Jak1 is essential for signal transduction of class II cytokine receptors as well as those of the γ_c and gp130 famililies. Jak1-deficient mice die within 24 hr of birth, probably by neuronal defects due to impaired gp130-mediated signaling.[35] Hematologically, $Jak1^{-/-}$ mice exhibit severely impaired lymphoid development. B cell differentiation is blocked at the transition step of pro-B to pre-B cells. Thymocyte numbers are greatly reduced, but CD4/CD8 profile is not perturbed. These profiles of lymphoid development are very similar to those of $Jak3^{-/-}$ mice, indicating that IL-7 signaling, which is essential for lymphoid development, requires activation of both Jak1 and Jak3 as obligate partners to one another. In addition, $Jak1^{-/-}$ embryonic fibroblasts fail to respond to IFNα/β and IFNγ, resulting in impaired antiviral activity, and $Jak1^{-/-}$ macrophages fail to respond to IL-10 in inhibiting production of tumor necrosis factor α induced by LPS. Interestingly, colony forming assays using fetal liver cells from $Jak1^{+/+}$ and $Jak1^{-/-}$ embryos revealed that Jak1 is dispensable for G-CSF responses, in contrast to the results obtained from in

vitro experiments.[36] Thus Jak1 appears indispensable for lymphoid development principally mediated by IL-7, and immune responses mediated by IFNs.

Jak2 is Essential for Definitive Erythropoiesis and Signaling of TPO, IL-3, GM-CSF, and IFNγ, but not for the Generation of Lymphoid Progenitors

Jak2 also plays nonredundant roles in signaling via a variety of cytokine receptors. Jak2-deficient mice[27,28] die around day 12.5 postcoitum due to lack of definitive erythropoiesis. The phenotype of Jak2-deficiency is similar to that of Epo or EpoR-deficiency,[20] but the reduction in the numbers of erythrocytes is more severe in $Jak2^{-/-}$ mice than in $Epo^{-/-}$ or $EpoR^{-/-}$ mice. This is because Jak2 is involved not only in signaling of Epo, but also that of TPO which also contibutes to the expansion of early erythroid progenitor cells. Furthermore, $Jak2^{-/-}$ fetal liver cells are unable to trasduce sigrals upon stimulation with GM-CSF, IL-3, and IL-5, whose functions in hematopoiesis are redundant as reported in mice doubly-deficient for common β chain and IL-3 ligand.[37] Jak2 is also required for IFNγ signaling, but not for IFNα/β signaling in vitro and in vivo. Thus fibroblasts from Jak2-deficient embryos are no longer protected by IFNγ from the cytopathic effect of viral infection. On the other hand, fetal liver cells from Jak2-deficient embryos contain lymphoid progenitor cells which can reconstitute lymphoid development in sub-lethally irradiated Jak3-deficient mice, as well as hematopoietic stem cells which co-express CD34 and c-kit. $Jak2^{-/-}$ fetal liver cells and $Jak2^{-/-}$ fibroblasts respond normally to G-CSF and IL-6, respectively. Taken together, Jak2 seems to play a crucial role in definitive erythropoiesis and is essential for signaling through a distinct group of cytokines which are involved in erythroid/myeloid differentiation, but is dispensable for lymphoid development. Other studies have shown that aberrant signaling through Jak2 causes dysregulated cell growth. For example, constitutive activation of Jak2 by oligomerization as a result of a *TEL-Jak2* fusion generated by chromosomal translocation leads to acute lymphoid and chronic myeloid leukemias in humans.[38,39]

Jak3 is Essential for Lymphoid Development

Among the Jak family members, Jak3 is unique in that it is predominantly expressed in hematopoietic tissues, particularly in lymphoid cells, and is only activated by the cytokines of γ_c family.[40-42] In contrast, Jak1,rJak2, and Tyk2 are expressed ubiquitously and involved in signaling of more than two types of cytokine receptors. Mutations in the human Jak3 gene were first reported in 1995 in patients with autosomal recessive SCID[43,44] whose phenotype is indistinguishable from that of X-linked SCID (XSCID) which is caused by mutations in γ_c chain.[45] At the same time, Jak3-deficiency was proven to be sufficient to develop SCID in mice by gene targeting experiments.[29-31] Furthermore, a mutation of γ_c found in a family of patients with a moderate phenotype (XCID) was shown to result in partial loss of Jak3 association,[13] suggesting that the γ_c-Jak3 pathway is pivotal for driving lymphoid development. Jak3-deficient mice show severe lymphopenia (reduction in B and T cell numbers and no NK cells) with tiny thymuses. However, in spite of the extremely low thymic cellularity, $Jak3^{-/-}$ thymocytes do not display any block of differentiation, although the CD4/CD8 ratio is increased. Bone marrow and spleen cells from $Jak3^{-/-}$ mice show markedly diminished numbers of mature B cells and a developmental blockade of B cell differentiation at the pro-B to pre-B stage. However, a few cells with a mature B cell phenotype do exist in $Jak3^{-/-}$ mice. Hence, the B cell differentiation blockade in

Jak3$^{-/-}$ mice is incomplete, in comparison with the complete arrest of lymphoid differentiation observed in recombination activating gene (RAG)-1-[46] or RAG-2-[47] deficient mice. One major functional defect of Jak3$^{-/-}$ lymphocytes is an inability to proliferate upon stimulation with the γ_c family of cytokines. Among these, IL-7 plays a critical role for lymphoid development. Lack of responses to IL-7 results in a failure of lymphoid progenitor expansion in early hematopoietic development. The phenotype of deficiency in γ_c or Jak3 is nearly identical to that in IL-7 except that IL-7$^{-/-}$ mice retain NK cells which respond to IL-15. Jak3$^{-/-}$ splenocytes show impaired response to antigenic stimulation by polyclonal activators such as concanavalin A, anti-CD3 antibody, and lipopolysaccharide (LPS), due to a lack of response to IL-2 and IL-4 that are required for maximal proliferation of T and B cells. However, the frequency of generation of immunologically competent mature lymphocytes can be theoretically increased by expanding early lymphoid progenitor cells as a reservoir, although Jak3$^{-/-}$ mature lymphocytes lack the ability to expand with γ_c family cytokines. For this purpose, IL-3 which is similar to IL-7 in the effect on expansion of early lymphoid progenitors can be used, based on the usage of Jak2 for IL-3 signaling. In fact, IL-3 treatment of new-born Jak3$^{-/-}$ mice partially restores lymphoid development.[48] On the other hand, complete rescue of lymphoid development in Jak3$^{-/-}$ mice is achieved by bone marrow transplantation after retroviral gene transfer of a wild type Jak3 gene into Jak3$^{-/-}$ bone marrow cells.[49] This finding has opened a possibility of gene therapy for Jak3-deficient SCID and γ_c-deficient XSCID patients. In 2000, XSCID patients were reported to be successfully treated for at least 10 months of follow-up period by retroviral gene transfer of the γ_c gene.[50] This is one of the most exciting medical advances which has eventuated from the study of signal transduction via cytokine receptors. Of course, further improvements of the delivery system for more efficient introduction of the deficient gene into hematopoietic stem cells would be required for a complete cure.

A lack of response to IL-15 in Jak3-deficient mice appears to be responsible for the absence of NK cells and intestinal intraepithelial γδ T cells—which is also seen in IL-15-deficient mice.[51] The phenotype of the IL-7 receptor α chain-deficient mice is more severe than that of the Jak3- or IL-7-deficient mice in the aspect of lymphoid development, probably due to abrogation of TSLP signaling which also uses the α chain of the IL-7 receptor together with the TSLP-specific second chain of the receptor (also called δ1/CRLM-2) in conjunction with Jak1 and Jak2, respectively.[52-55] Reconstitution experiments of Jak3$^{-/-}$ mice by interbreeding the transgenic mice expressing Jak3 in the thymus but not in peripheral T cells revealed that Jak3 expression in the thymus restores normal T cell development, but not the function of peripheral T cells.[56] This finding indicates that constitutive expression of Jak3 in peripheral T cells is required to maintain T cell function. Interestingly, transgenic expression of *Bcl*-2 in γ_c-deficient mice rescues T lymphopoiesis, but not B or NK cell development,[57] and retroviral gene transfer of *Bcl*-2 into Jak3$^{-/-}$ bone marrow cells followed by transplantation into Jak3-deficient mice also improves peripheral T cell numbers.[58] These findings suggest that γ_c/Jak3-mediated signaling contributes to survival of T lineage cells. Curiously, Jak3$^{-/-}$ splenic T cells show a spontaneously "activated" phenotype without antigenic stimulation. These cells actively synthesize DNA and proliferate in vivo. Transgenic expression of Jak3 on mature T cells in Jak3-deficient mice rescues the quiescent state of peripheral T cells, suggesting that Jak3 not only contributes to expansion and survival of thymocytes, but also plays an important role for T cell homeostasis.[59] An unexpected expansion of cells of the myeloid lineages caused by Jak3$^{-/-}$ T cells was also reported in Jak3-deficient mice, suggesting that Jak3 is also involved in downregulating a myeloproliferative signal.[60] Finally, immunoglobulin class switching is

impaired in human SCID patients with Jak3 mutations, due to lack of IL-4 response, resulting in readily detectable levels of serum IgM with greatly diminished levels of IgG, IgA, and IgE.[44]

Tyk2 is Required for the Full Response to IL-12, but Plays a Restricted Role in IFNα/β Signaling

The function of Tyk2 was originally demonstrated to be an essential tyrosine kinase for IFNα/β signaling by genetic complementation of a mutant cell line.[3] However, Tyk2-deficient mice[61,62] are developmentally normal and, unexpectedly, $Tyk2^{-/-}$ cells show only partially impaired responses to IFNα/β stimulation. Reduced signaling of IFNα/β in $Tyk2^{-/-}$ cells is overcome by increased amounts of IFNα/β, suggesting that Tyk2 plays only a restricted role in IFNα/β signaling. Another unexpected finding was that the response to IFNγ was also reduced in $Tyk2^{-/-}$ cells, demonstrating a cross-talk between IFNα/β and IFNγ signaling components.[63] It should be noted that this cross-talk is unidirectional; IFNγ signaling depends on IFNα/β signaling, while IFNα/β signaling is not affected by IFNγ signaling as observed in $Jak2^{-/-}$ mice.

In contrast to the semi-redundant role of Tyk2 in IFNα/β signaling, $Tyk2^{-/-}$splenocytes show a markedly decreased IL-12-stimulated production of IFNγ, although it is not completely abolished. On the other hand, IL-12-induced proliferation of $CD3^{+}$ splenocytes is not significantly affected by the absence of Tyk2. Consistent with this finding is that the cytoplasmic region of IL-12RβII alone was shown to be sufficient to deliver a proliferative signal by using Jak2[64] (see Table 1). Thus Tyk2 plays an important role in IL-12-mediated IFNγ production in association with IL-12RβI. However, there is no obligatory requirement for it in IFNα/β signaling in vivo.

Conclusions

Gene targeting experiments in mice has clarified that Jak1, Jak2, and Jak3 play nonredundant and critical roles in cytokine signaling and hematopoietic development, whereas Tyk2 is not absolutely required for any cytokine signaling and is dispensable for the normal development of the hematopoietic system (summarized in Table 3). Now it is quite obvious that the three Jaks are indispensable for normal hematopoiesis, and aberrant signaling of Jak2 or Jak3 due to structural alterations leads to leukemia or immunodeficiency, respectively. This makes Jaks one of the most attractive molecular targets for drug design[65] and gene therapy.[49]

Table 3. Phenotypes of Jak-deficient mice

Gene	Phenotype	Cytokines affected
Jak1	• **defective lymphoid development** • **perinatal lethality**	• **γc family (IL-2, IL-4, IL-7, IL-9, IL-15)** • **gp130 family (IL-6, IL-11, OSM,LIF)** • **class II cytokine receptor family (IFN α/β, IFNγ, IL-10)**
Jak2	• **no definitive erythropoiesis** • **embryonical lethality (day 12.5)**	• **Epo, TPO, IFNγ** • **β_c family (IL-3, IL-5, GM-CSF)**
Jak3	• **SCID** • **dysregulated myelopoiesis**	• **γ_c family (IL-2, IL-4, IL-7, IL-9, IL-15)**
Tyk2	• **reduced antiviral response** • **reduction in IL-12 induced IFNγ production**	• **IFNα/β, IFNγ (due to cross-talk), IL-12**

Cytokines with nonredundant roles in hematopoiesis and immune responses against pathogens are shown in bold.

References

1. Ihle JN. Cytokine receptor signalling. Nature 1995; 377:591-594.
2. Witthuhn BA, Quelle FW, Silvennoinen O et al. Jak2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. Cell 1993; 74:227-236.
3. Velazquez L, Fellous M, Stark GR et al. A protein tyrosine kinase in the interferon α/β signaling pathway. Cell 1992; 70:313-322.
4. Muller M, Briscoe J, Laxton C et al. The protein tyrosine kinase Jak1 complements defects in interferon-α/β and -γ signal transduction. Nature 1993; 366:129-135.
5. Watling D, Guschin D, Muller M et al. Complementation by the protein tyrosine kinase Jak2 of a mutant cell line defective in the interferon-γ signal transduction pathway. Nature 1993; 366:166-170.
6. Heim MH, Kerr IM, Stark GR et al. Contribution of Stat SH2 groups to specific interferon signaling by the Jak-Stat pathway. Science 1995; 267:1347-1349.
7. Stahl N, Farruggella TJ, Boulton TG et al. Choice of Stats and other substrates specified by modular tyrosine-based motifs in cytokine receptors. Science 1995; 67:1349-1353.
8. Darnell JE Jr, Kerr IM, Stark GR. Jak-Stat pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994; 264:1415-1421.
9. Ihle JN. Stats: signal transducers and activators of transcription. Cell 1996; 84:331-334.
10. O'Shea JJ. Jaks, Stats, cytokine signal transduction, and immunoregulation: are we there yet? Immunity 1997; 7:1-11.
11. Ihle JN, Nosaka T, Thierfelder W et al. Jaks and Stats in cytokine signaling. Stem Cells 1997; 15(suppl 1):105-112.
12. Guschin D, Rogers N, Briscoe J et al. A major role for the protein tyrosine kinase Jak1 in the Jak/Stat signal transduction pathway in response to interleukin-6. EMBO J 1995; 14:1421-1429.
13. Russell, SM, Johnston JA, Noguchi M et al. Interaction of IL-2Rβ and γ_c chains with Jak1 and Jak3: implications for XSCID and XCID. Science 1994; 266:1042-1045.
14. Miyazaki T, KawaharaA, Fujii H et al. Functional activation of Jak1 and Jak3 by selective association with IL-2 receptor subunits. Science 1994; 266:1045-1047.

15. Takeshita T, Arita T, Higuchi M et al. STAM, signal transducing adaptor molecule, is associated with Janus kinases and involved in signaling for cell growth and c-Myc induction. Immunity 1997; 6:449-57.
16. Taniguchi T. Cytokine signaling through nonreceptor protein tyrosine kinases. Science 1995; 268:251-255.
17. Ward AC, Touw I, Yoshimura A. The Jak-Stat pathway in normal and perturbed hematopoiesis. Blood 2000; 95:19-29.
18. Nosaka T, Kitamura T. Janus kinases (Jaks) and signal transducers and activators of transcription (Stats) in hematopoietic cells. Int J Hematol 2000; 71:309-319.
19. Miyajima A, Kitamura T, Harada N et al. Cytokine receptors and signal transduction. Annu Rev Immunol 1992; 10:295-331.
20. Wu H, Liu X, Jaenisch R et al. Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor. Cell 1995; 83:59-67.
21. Flanagan JG, Chan DC, Leder P. Transmembrane form of the kit ligand growth factor is determined by alternative splicing and is missing in the Sld mutant. Cell 1991; 64:1025-1035.
22. Brannan CI, Lyman SD, Williams DE et al. Steel-Dickie mutation encodes a c-kit ligand lacking transmembrane and cytoplasmic domains. Proc Natl Acad Sci USA 1991; 88:4671-4674.
23. von Freeden-Jeffry U, Vieira P, Lucian LA et al. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. J Exp Med 1995; 181:1519-26.
24. Leveen P, Pekny M, Gebre-Medhin S et al. Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. Genes Dev 1994; 8:1875-1887.
25. Bostrom H, Willetts K, Pekny M et al. PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. Cell 1996; 85:863-873.
26. Lindahl P, Johansson BR, Leveen P et al. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science 1997; 277:242-245.
27. Parganas E, Wang D, Stravopodis D et al. Jak2 is essential for signaling through a variety of cytokine receptors. Cell 1998; 93:385-395.
28. Neubauer H, Cumano A, Muller M et al. Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. Cell 1998; 93:397-409.
29. Thomis DC, Gurniak CB, Tivol E et al. Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. Science 1995; 270:794-797.
30. Nosaka T, van Deursen JMA, Tripp RA et al. Defective lymphoid development in mice lacking Jak3. Science 1995; 270:800-802.
31. Park SY, Saijo K, Takahashi T et al. Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. Immunity 1995; 3:771-782.
32. Peschon JJ, Morrissey PJ, Grabstein KH et al. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J Exp Med 1994; 180:1955-1960.
33. Cao X, Shores EW, Hu-Li J et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor γ chain. Immunity 1995; 2:223-238.
34. DiSanto JP, Muller W, Guy-Grand D et al. Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor γ chain. Proc Natl Acad Sci USA 1995; 92:377-381.
35. Rodig SJ, Meraz MA, White JM et al. Disruption of the *Jak1* gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. Cell 1998; 93:373-383.
36. Shimoda K, Feng J, Murakami H et al. Jak1 plays an essential role for receptor phosphorylation and Stat activation in response to granulocyte colony-stimulating factor. Blood 1997; 90:597-604.
37. Nishinakamura R, Miyajima A, Mee PJ et al. Hematopoiesis in mice lacking the entire granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 functions. Blood 1996; 88:2458-2464.
38. Lacronique V, Boureux A, Valle VD et al. A TEL-Jak2 fusion protein with constitutive kinase activity in human leukemia. Science 1997; 278:1309-1312.
39. Peeters P, Raynaud SD, Cools J et al. Fusion of TEL, the ETS-variant gene 6 (ETV6), to the receptor-associated kinase Jak2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. Blood 1997; 90:2535-2540.
40. Johnston JA, Kawamura M, Kirken RA et al. Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. Nature 1994; 370:151-153.
41. Witthuhn BA, Silvennoinen O, Miura O et al. Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells. Nature 1994; 370:153-157.

42. Kawamura M, McVicar DW, Johnston JA et al. Molecular cloning of L-Jak, a Janus family protein-tyrosine kinase expressed in natural killer cells and activated leukocytes. Proc Natl Acad Sci USA 1994; 91:6374-6378.
43. Macchi P, Villa A, Giliani S et al. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). Nature 1995; 377:65-68.
44. Russell SM, Tayebi N, Nakajima H et al. Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development. Science 1995; 270:797-800.
45. Noguchi M, Yi H, Rosenblatt HM. Interleukin-2 receptor γ chain mutation results in X-linked severe combined immunodeficiency in humans. Cell 1993; 73:147-157.
46. Mombaerts P, Iacomini J, Johnson RS et al. RAG-1-deficient mice have no mature B and T lymphocytes. Cell 1992; 68:869-877.
47. Shinkai Y, Rathbun G, Lam KP et al. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. Cell 1992; 68:855-867.
48. Brown MP, Nosaka T, Tripp RA et al. Reconstitution of early lymphoid proliferation and immune function in Jak3-deficient mice by IL-3. Blood 1999; 94:1906-1914.
49. Bunting KD, Sangster MY, Ihle JN et al. Restoration of lymphocyte function in Janus kinase 3-deficient mice by retroviral-mediated gene transfer. Nature Med 1998; 4:58-64.
50. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 2000; 288:669-672.
51. Kennedy MK, Glaccum M, Brown SN et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. J Exp Med 2000; 191:771-780.
52. Pandey A, Ozaki K, Baumann H et al. Cloning of a receptor subunit required for signaling by thymic stromal lymphopoietin. Nat Immunol 2000; 1:59-64.
53. Park LS, Martin U, Garka K et al. Cloning of the murine thymic stromal lymphopoietin (TSLP) receptor: Formation of a functional heteromeric complex requires interleukin 7 receptor. J Exp Med 2000; 192:659-670.
54. Fujio K, Nosaka T, Kojima T et al. Molecular cloning of a novel type 1 cytokine receptor similar to the common gamma chain. Blood 2000; 95:2204-2211.
55. Hiroyama T, Iwama A, Morita Y et al. Molecular cloning and characterization of CRLM-2, a novel type I cytokine receptor preferentially expressed in hematopoietic cells. Biochem Biophys Res Commun 2000; 272:224-229.
56. Thomis DC, Berg LJ. Peripheral expression of Jak3 is required to maintain T lymphocyte function. J Exp Med 1997; 185:197-206.
57. Kondo M, Akashi K, Domen J et al. Bcl-2 rescues T lymphopoiesis, but not B or NK cell development, in common γ chain-deficient mice. Immunity 1997; 7:155-162.
58. Wen R, Wang D, McKay C et al. Jak3 selectively regulates Bax and Bcl-2 expression to promote T-cell development. Mol Cell Biol 2001; 21:678-689.
59. Sohn SJ, Forbush KA, Nguyen N et al. Requirement for Jak3 in mature T cells: its role in regulation of T cell homeostasis. J Immunol 1998; 160:2130-2138.
60. Grossman WJ, Verbsky JW, Yang L et al. Dysregulated myelopoiesis in mice lacking Jak3. Blood 1999; 94:932-939.
61. Karaghiosoff M, Neubauer H, Lassnig C et al. Partial impairment of cytokine responses in Tyk2-deficient mice. Immunity 2000; 13:549-560.
62. Shimoda K, Kato K, Aoki K et al. Tyk2 plays a restricted role in IFNα signaling, although it is required for IL-12-mediated T cell function. Immunity 2000; 13:561-571.
63. Takaoka A, Mitani Y, Suemori H et al. Cross talk between interferon-γ and -α/β signaling components in caveolar membrane domains. Science 2000; 288:2357-2360.
64. Zou J, Presky DH, Wu CY et al. Differential associations between the cytoplasmic regions of the interleukin-12 receptor subunits β1 and β2 and Jak kinases. J Biol Chem 1997; 272:6073-6077.
65. Meydan N, Grunberger T, Dadi H et al. Inhibition of acute lymphoblastic leukaemia by a Jak-2 inhibitor. Nature 1996; 379:645-648.

CHAPTER 2

Jak3-Dependent Pathways in Hematopoiesis and SCID

Nicholas A. Cacalano and James A. Johnston

Introduction

Hematopoiesis is a critical developmental process that depends on highly regulated growth and differentiation of pluripotent bone marrow stem cells. The process of hematopoiesis regulates the production of myeloid, erythroid, and lymphoid cells from bone marrow progenitors and maintains the correct balance of different blood cell lineages. The control of hematopoiesis depends on signals delivered through surface receptors of the hematopoietic superfamily present on progenitor cells in response to a complex cocktail of cytokines in the bone marrow microenvironment and peripheral blood.[1-4] Members of the cytokine receptor superfamily lack endogenous tyrosine kinase activity, but bind members of the Jak family of tyrosine kinases including Jak1, Jak2, Jak3, and Tyk2. Jak3 expression is restricted to cells of hematopoietic lineage, and binds specifically to the common gamma chain (γ_c) component of several multisubunit hematopoetic receptor complexes. γ_c-dependent cytokines include IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Signaling through all of these receptors requires functional Jak1 and Jak3, and mutation of either kinase or their associated receptor chains ablates functional signal transduction. The importance of this signaling pathway has been underlined by naturally occurring mutations in γ_c or Jak3 which disrupt kinase-receptor interaction and block signal transduction. Naturally occuring mutations in Jak3 or γ_c cause Severe Combined Immmunodeficiency (SCID). This type of SCID is the most common inherited immunodeficiency, appearing in 1:50,000 live births. SCID mice and humans have profound defects in lymphocyte development and development of secondary lymphoid organs. They have a significant block in productive TCR and BCR gene rearrangements and markedly reduced survival of lymphoid progenitor cells.[5-8]

It has been found that Jak3-dependent cytokine signaling is critical for multiple, opposing effects on hematopoietic cells. Jak3 is required for delivering survival and proliferative signals to lymphocyte progenitor cells derived from the bone marrow, by activating cell cycle progression through transcriptional regulation of cell cycle activators, cyclins D1, D2 and D3,[9-12] while simultaneously delivering potent survival signals by increasing expression of the anti-apoptotic Bcl-2 family of proteins and activation of the cell survival kinase Akt.[13] Interestingly, at later stages of lymphocyte development, Jak3 signal transduction ensures proper control of the immune system by delivering critical differentiation

The Jak-Stat Pathway in Hematopoiesis and Disease, edited by Alister C. Ward.

and cell death signals to mature, activated peripheral T cells. Jak3 is required in the periphery for deletion of self-reactive T cells, generation of memory antigen-specific CD8+ T cells, and for triggering the Fas ligand-dependent activation induced cell death (AICD) pathway, crucial for the proper negative control of immune responses and prevention of autoimmune reactions. Indeed, gene-targeting experiments in mice have demonstrated the dual requirement for Jak3-dependent activation of cell cycle progression and cell survival, as well as for the negative control of lymphocyte activation.[14,15] Jak3, γ_c, and IL-7 receptor deficiency results in profound defects in T, B, and NK cell development and cytokine-dependent proliferative responses of peripheral lymphocytes.[16-20] The T cells that develop in Jak3, γ_c, Stat5a/b, IL-2, and IL-2 receptor knockout mice are activated peripheral T cells, and in some cases, can cause fatal autoimmune disease.[17,20] This chapter will discuss our current knowledge of the multiple roles of Jak3 in hematopoiesis and peripheral lymphocyte function by detailing the function of specific cytokine-activated biochemical pathways in T, B and natural killer (NK) cell development. In addition, we will analyse the results of gene targeting experiments as well as the phenotypes of humans with mutations in Jak3. Finally, the function of individual Jak3-dependent receptors will be discussed through the analysis of mice lacking single receptor chains, but retaining all other Jak3 signaling complexes.

Jak3-Regulated Signal Transduction Pathways

Treatment of cells with cytokines induces a marked increase in total cellular tyrosine phosphorylation. However, members of the cytokine receptor superfamily lack endogenous tyrosine kinase activity, which suggested that the intracellular domains of the signaling chains were non-covalently associated with cytoplasmic tyrosine kinases. In genetic complementation experiments using interferon-non-responsive mutant cell lines, interferon signaling could be reconstituted with cDNAs encoding tyrosine kinases of the Janus (Jak) family.[21,22] Four Jak family members were identified: Jak1, Jak2, Jak3, and Tyk2, all of which are important in signal transduction through a variety of cytokine receptors. In contrast to Jak1, Jak2, and Tyk2, which are ubiquitously expressed in all cell types, Jak3 expression is restricted to cells of hematopoietic lineage, suggesting an important, specific role for this kinase in hematopoiesis.[23-26] Indeed, stimulation of lymphoid cells with several different γ_c-dependent cytokines was found to induce rapid tyrosine phosphorylation of Jak3 as well as other downstream signaling molecules.[27-29] The structures of the receptor complexes that use Jak3 as a signaling component are shown in Figure 1. All of these receptors are heteromeric complexes containing either two or three polypeptide chains.[30] Each receptor contains a ligand-specific high affinity binding chain as well as a chain shared by all members of this subfamily of hematopoietic receptors, p64, which was subsequently named the common gamma chain (γ_c)(Fig. 1)[31-39] An important finding which implicated Jak3 in signal transduction through this family of receptors was the finding that Jak3 was physically associated with γ_c, and immunoprecipitating anti-γ_c antibodies can co-precipitate Jak3 with γ_c.[40,41] The receptors for IL-4, IL-7 and IL-9 are all heterodimers containing γ_c as well as their respective ligand-specific chains that are non-covalently associated with another family member, Jak1. By comparision, the receptors for IL-2 and IL-15 are trimeric complexes which contain ligand-specific components (IL-2Rα and IL-15Rα), as well as two shared chains, γ_c and IL-2Rβ. In these complexes, the shared IL-2Rβ chain is associated with Jak1 (Fig. 1). Signaling through all of these receptors requires functional Jak1 and Jak3, as mutation of either kinase or their associated receptor chains ablates functional signal transduction.[42-46] Although there is only weak sequence

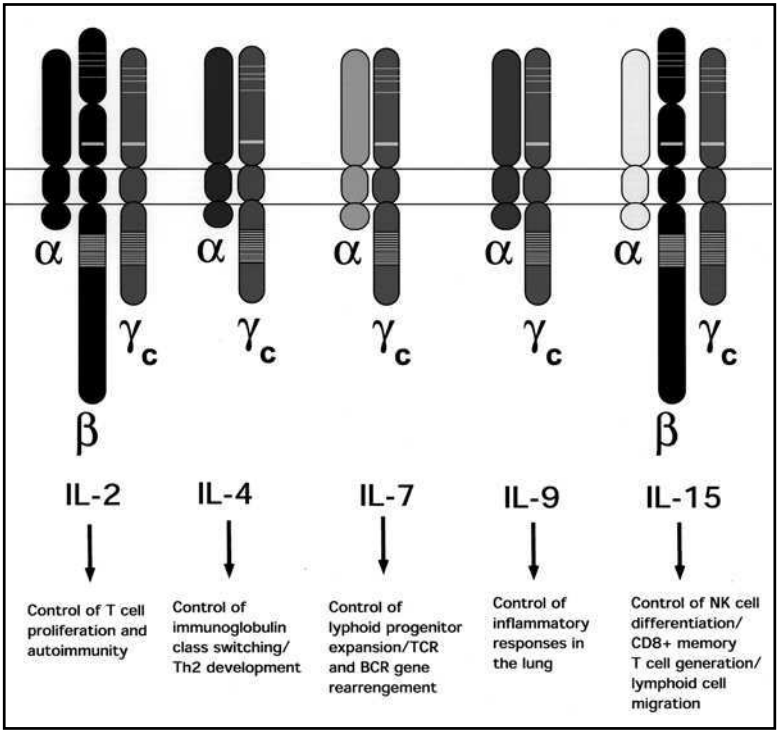

Figure 1. IL-2-related cytokine receptors.

homology among members of the cytokine receptor superfamily, there is a loosely homologous region in the membrane-proximal portion of the cytoplasmic tail, known as the Box1/Box2 motif, which encodes conserved proline-containing motifs that have been shown to be critical for the association of the receptors to the amino-terminal regions of Jak kinases.[47-49] The importance of these sequences have been demonstrated by the identification of naturally occurring mutations in γ_c or Jak3 which disrupt the association and cause human genetic disease.[50]

A critical event in cytokine signal transduction is the Jak kinase-mediated phosphorylation of tyrosine residues on the signaling components of the receptor complex. Phosphorylated tyrosines serve as docking sites for signaling molecules that contain Src homology (SH) 2 and phosphotyrosine binding (PTB) domains and initiate a cascade of protein-protein interactions that are required for the activation of survival and proliferative pathways. Extensive analysis of receptor mutants that fail to activate one or more of these pathways has demonstrated the role of these biochemical signals in cell survival, proliferation, and differentiation at many stages of hematopoietic development.[51-53] Shown in Figure 2 are some of the most well studied signaling pathways activated by cytokine receptors.

Stat Proteins

One of the best studied pathways involves the latent cytoplasmic SH2-containing transcription factors known as Stats (signal transducers and activators of transcription). Early experiments in IFN α/β-non-responsive mutant cell lines that identified Jak kinase