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Physiological significance of STAT proteins: investigations through gene disruption in vivo

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Abstract. Signal transducers and activators of transcription (STATs) were discovered as mediators of type I interferon-induced gene expression. This family of transcription factors has been found in widespread signaling pathways, especially those involving cytokines regulating the immune response. Because a plethora and often confusing set of activators for STAT proteins was observed in cell culture models, it became important to define the physiologically relevant actions of these molecules. One approach to this question has been through the targeted disruption of STAT genes in transgenic mice. Now that all seven STAT genes have been disrupted, both the high degree of STAT selectivity as well as many surprising and unexpected complexities are beginning to be characterized.

Key words. Cytokine signaling; interferon; protein tyrosine phosphorylation; SH2 domain; transcription factors; immune response; T lymphocyte development.

Introduction

Signal transducers and activators of transcription (STATs) were first defined in the context of the interferon (IFN) signaling system as an outgrowth of studies aimed at elucidating the molecular determinants of signal transduction specificity. The motivation for these studies derived from evidence that IFN-α and IFN-γ induced rapid but largely distinct changes in gene expression in treated target cells [1–5], reflecting their largely distinct biology of inducing direct antiviral action versus modulation of lymphocyte and monocyte physiology, respectively. The discovery of a family of transcription factors that could be activated by a cell surface receptor and deliver information directly to the nucleus to modulate gene expression with the same specificity inherent in the ligand-receptor interaction provided an appealing solution to the problem of specificity in signal transduction [6]. Therefore, it came as something of a surprise and perhaps a disappointment to discover that these two cytokines acted through signaling pathways that were not only analogous but that used overlapping components, in particular the STAT1 protein [7]. Furthermore, it was found that STAT proteins were activated by a broader array of cytokines and growth factors than just IFNs, with reports that STAT1, STAT2, and/or STAT3 were activated by epidermal growth factor (EGF), platelet-derived growth factor (PDGF), growth hormone (GH), prolactin (PRL), fibroblast growth factor (FGF), insulin, many interleukins, angiotensin 2, and tyrosine kinase oncoproteins, among others, as described elsewhere in this review collection. These observations led to questions concerning the degree to which STAT proteins explained the specificity of cytokine signaling.

Because the initial observations of STAT protein activation were made using tissue culture model systems, it became important to determine the action of these transcription factors in vivo. One approach to understanding STAT physiology has been to study the phenotype of null mutations in the mouse. To date, all seven mouse STAT genes have been disrupted in transgenic animals, and mice with homozygous null mutations in more than one STAT gene are now being generated. Most of these 'knockout' animals have been bred in more than one laboratory, and the consensus that is emerging reinforces the notion that STAT proteins lie at the heart of cytokine signaling specificity. In addition, interesting and unexpected insights into STAT and cytokine biology have been uncovered.

STAT1 and STAT2

The first STAT gene to be disrupted in mice, appropriately, was STAT1. STAT1 mutant mice were created which expressed either a truncated and crippled STAT1 protein that displayed no activity [8] or that expressed no detectable STAT1 protein [9]. Results obtained with these two mutant strains thus far have been entirely consistent, with the major defect associated with the null phenotype being unresponsiveness to both type I and type II IFN. This loss of IFN responsiveness would be predicted from the involvement of the STAT1 protein in signaling from both the IFN-α and the IFN-γ receptors [7, 10] and is consistent with findings from mutant tissue culture cell lines [11]. Remarkably, however, these initial studies of STAT1-null mice did not reveal phenotypes consistent with defects in any other cytokine or growth factor signaling pathway. Specifically, these animals showed no gross developmental defects as might be predicted from impaired EGF or PDGF signaling [12–14], and no impaired responses to signals that had been found to cause STAT1 activation in cell culture models, such as GH, interleukin (IL)-10, or colony-stimulating factor (CSF)-1. These results suggested that STAT1 utilization is remarkably specific to the IFN system in vivo. The expanded range of STAT1 activation observed in cell culture systems not reflected in the null phenotype could have resulted either from a loss of specificity inherent in these in vitro systems or from redundancy due to similar proteins still functional in vivo, perhaps other STATs. Many of the non-IFN activation events involving STAT1 observed in cell culture have not been observed in vivo or in primary cells ex vivo, arguing for the first possibility. However, some non-IFN activators are functional even on primary cells (for example, activated STAT1 in IL-12-treated lymphocytes that might be redundant with activated STAT4), suggesting that animals with compound lesions in multiple STAT genes will be required to definitively address this issue.

While some of these multiply mutant mouse strains have been produced through interbreeding (see below), others will be more difficult to produce due to the close genetic linkage of STAT gene clusters [15]. The primary defect in STAT1 mutant mice is susceptibility to microbial infections, similar to the combined phenotypes of IFNAR1 $-/-$ or IFNAR2 $-/-$ and IFNGR1 $-/-$ or IFNGR2 $-/-$ mice [16–18]. For example, STAT1 $-/-$ mice were exquisitely susceptible to vesicular stomatitis virus infection which produced lethal disease with extensive virus replication in mutant animals at challenge doses 10^8-fold lower than in their wild-type counterparts. This enhanced susceptibility reflects the primary role of type I IFN-induced cellular antiviral systems in preventing VSV replication in vivo. Likewise, Stat1 $-/-$ mice were unable to cope with infections by *Listeria monocytogenes* or *Leishmania major*, reflecting the known requirements for IFN-γ for defense against these pathogens [19–21]. Molecular markers of IFN action were also affected by mutation of the STAT1 gene. Macrophages were unable to upregulate nitric oxide production in response to IFN-γ; splenocytes and fibroblasts failed to show increased IFN-stimulated gene expression in response to either IFN-α or IFN-γ, and fibroblast growth was not arrested by IFN treatment [22]. However, other responses, such as those mediated by NF-κB, were largely intact. Analysis of chemical carcinogenesis in STAT1 mutant and IFN-γ receptor mutant animals revealed the existence of an IFN-γ-mediated tumor surveillance system that operates in immunocompetent animals [23]. This study demonstrated that endogenously produced IFN-γ was required for a tumor surveillance system capable of controlling development of both chemically induced and spontaneously arising tumors. Compared with wild-type mice, animals lacking sensitivity to either IFN-γ alone or all IFN family members (i.e., STAT1-deficient mice) developed tumors more rapidly and with greater frequency when challenged with different doses of the chemical carcinogen methylcholanthrene. In addition, IFN-γ-insensitive mice developed tumors more rapidly than wild-type mice when bred onto a background deficient in the p53 tumor-suppressor gene. IFN-γ-insensitive p53($-/-$) mice also developed a broader spectrum of tumors than mice lacking p53 alone. Studies of the growth of tumor cells derived from methylcholanthrene-treated IFN-γ-insensitive mice demonstrated that this enhanced tumor phenotype was at least partly mediated through indirect effects of IFN-γ on the tumor cell leading to enhanced tumor cell immunogenicity rather than direct antiproliferative effects on tumor cell growth. Interestingly, certain types of spontaneously arising human tumors were found to be selectively unresponsive to IFN-γ due to molecular defects in the STAT1 activation pathway, suggesting that a similar tumor surveillance system operates in humans as well. In addition to enhanced growth of IFN-γ-insensitive tumors, growth of transplanted tumors in STAT1-deficient or IFN-γ-insensitive mice demonstrated a defect in host response to tumors that was independent of the tumor genotype. Therefore, not only enhanced tumor immunogenicity but also the ability of the host to recognize and/or eliminate tumor cells was diminished in the absence of a functioning IFN-γ system.

Given the multitude of agents other than IFN capable of activating STAT1 in cell culture models, efforts have been made to find STAT1-dependent phenotypes not readily explained by loss of IFN responsiveness. One such phenotype involves the basal expression of MHC

class I antigens on peripheral T lymphocytes. MHC class I expression and many aspects of the class I antigen processing and presentation pathway are induced in response to IFN. However, most non-neuronal nucleated cells also constitutively express basal levels of MHC class I. This basal expression was found to be reduced on cells from STAT1 $-/-$ animals, particularly on mature T cells.

Comparison of class I levels on lymphocytes from IFN-unresponsive strains due to loss of IFN-α receptors, IFN-γ receptors, or both demonstrated an IFN-dependent component to this response. However, there was also a significant loss of MHC expression in STAT1-deficient animals that was not explained by loss of IFN responsiveness. These results revealed both a role for IFN in the absence of overt pathogen infection as well as an IFN-independent role for STAT1 in the normal physiological regulation of gene expression [C. K. Lee, R. Gimeno, D. E. Levy, unpublished results].

Studies of STAT1-mutant mice have also help elucidate the role of type I IFN during viral infections. For example, influenza virus infections are usually confined to the respiratory tract due to the tropism of the virus for lung epithelial cells. However, virus replicated not only in the lungs of STAT1 $-/-$ mice but developed into a fulminant systemic influenza virus infection following either intranasal or intraperitoneal inoculation [24]. Replicating virus was found in many organs of STAT1 $-/-$ mice, including spleen, liver, and brain. Influenza virus could replicate in fibroblasts derived from STAT1 $-/-$ mice or from IFN-α-receptor-deficient mice, showing the IFN type dependence of this effect. These results indicated that in all organs except the lung, influenza virus infection and replication are apparently prevented by an intact type I IFN response which plays an important role in determining the pathogenicity and tissue restriction of the virus. Moreover, the cellular antiviral response induced by IFN secreted by a virus-infected cell can inhibit virus replication in that same infected cells, thus effectively eliminating viral disease.

FGF was one of the cytokines found to activate STAT1 under certain cell culture conditions, but no phenotypes were obvious in STAT1 $-/-$ mice that could be ascribed to this activity. However, it was suggested that hyperactivation of STAT1 might be involved in the growth impairment observed in genetic forms of dwarfism involving activated mutations in FGF receptor [25]. Primary chondrocytes derived from STAT1-deficient embryos were found to be defective in FGF-mediated growth inhibition, even though normal levels of FGF receptors were expressed [26]. Furthermore, organ cultures of bone rudiments from mouse embryos showed that FGF treatment produces a drastic impairment of chrondrocyte proliferation and bone development in wild-type but not in STAT1 $-/-$ rudiments. Thus, FGF signaling can inhibit specifically the proliferation of chondrocytes, despite its growth-promoting activity on other cell types. Moreover, this inhibition of proliferation requires STAT1, though the biochemical basis for this cell type selectivity remains unclear.

Studies in STAT1-deficient mice have provided compelling evidence for its primary role in mediating responses to both IFN-α and IFN-γ. Similar studies have been initiated recently using mice deficient in the STAT2 gene through gene targeting [C. Schindler, personal communication]. Although these studies are still in progress, initial results have confirmed its importance in type I IFN signaling. Again, like the STAT1-deficient mice, these animals are viable and fertile and exhibit no overt developmental abnormalities. It will be of interest to determine if additional roles for STAT2 outside the IFN system are revealed by further studies of this mutant mouse strain.

STAT3

STAT3 has been primarily characterized as a target for cytokines from the IL-6 family that use gp130-type receptors [27–29], and these findings have been largely confirmed by studies in cell culture using dominant-inhibitory constructs [30–33]. Initial attempts to study STAT3 function by gene disruption were complicated by the discovery that lack of STAT3 produces embryonic lethality in mice [34]. Although STAT3 hemizygous mice were viable, no viable STAT3-deficient mice could be obtained from interbreeding these animals. Analysis of embryos at several gestation times revealed that STAT3-deficient embryos showed a rapid degeneration between embryonic days 6.5 and 7.5, although they developed normally into the egg cylinder stage until embryonic day 6. These results were rather surprising in light of the phenotypes of IL6-deficient, gp130-deficient, and LIFRβ-deficient animals [35–37]. Mice missing IL-6 genes are viable, while disruption of LIFRβ causes perinatal lethality. While gp130 is also required during embryogenesis, gp130 $-/-$ mice survive to a significantly later stage of embryogenesis, dying from severe defects in myocardial development and hematopoiesis that occur between embryonic day 12.5 and term. Even mice lacking Janus kinase (JAK)1, which is thought to be the primary mediator of STAT3 activation in response to gp130 ligands [38], do not die as early embryos but survive until term [39]. Therefore, it would appear that STAT3 mediates an essential process that is distinct from those requiring its known activators. Interestingly, constitutively activated STAT3 has been found during early postimplantation develop-

ment [40], although none of the known STAT3 activators would account for its phosphorylation at this stage. To circumvent the early postimplantation lethality of the STAT3 nullizygous phenotype, several groups have undertaken conditional gene targeting approaches using the loxP-Cre recombinase system. Mice have been produced carrying an allele of STAT3 in which exon 21 was flanked by loxP sites such that Cre recombinase-mediated rearrangement result in removal of the exon-21-encoded tyrosine phosphate acceptor site [41]. The mutant form of STAT3 expressed from the rearranged allele appears to function as a dominant inhibitor of the wild-type protein, resulting in two simultaneous mechanisms to produce loss of function. Using tissue-specific expression of Cre recombinase, STAT3 loss-of-function/dominant-negative effects have been examined.

STAT3-deficient T cells were obtained by interbreeding with an lck-Cre transgenic line. IL-6 induced proliferation of thymic and splenic T cells, largely due to loss of IL-6-induced protection from apoptosis. Since the anti-apoptotic protein, BCL-2, was normally induced in response to IL-6 in STAT3-deficient T cells, it was concluded that STAT3 activation is required for an unknown survival mechanism mediated by IL-6 not involving BCL2 [41]. Given the recent finding that dominant-negative STAT3 constructs inhibit myeloma cell survival through inhibition of BCL-XL [42], it is tempting to speculate that a similar mechanism may operate in normal T cells.

A partial defect in IL-2-induced proliferation was also detected in STAT3-deficient T cells [43]. This effect correlated with impaired induction of the high-affinity IL-2 receptor complex through reduced expression of the IL2Rα protein. Similar findings have been made for STAT5-deficient T cells (see below), suggesting that multiple STAT proteins may be involved in regulating the increased IL2Rα expression characteristic of proliferating cells. Much of the analysis of STAT3 function has occurred using monocytic cell lines due to their sensitivity to modulation by IL-6-type cytokines. The corresponding in vivo phenotype has now been elucidated through interbreeding STAT3-loxP mice with a monocyte/granulocyte-specific Cre transgenic line that exploits the lysozyme M gene to drive the recombinase [44]. The mutant mice produced following Cre-mediated recombination were highly susceptible to endotoxin shock, showing increased production of inflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1, IFN-γ, and IL-6. Augmentation of endotoxin-induced production of inflammatory cytokines appeared to be caused by failure of the normally suppressive effects of IL-10. The ability of IL-10 to suppress inflammatory cytokine production from macrophages and neutrophils was completely abolished in the absence of STAT3. The mice also showed a polarized immune response toward the Th1 type, and developed chronic enterocolitis with age. Interestingly, STAT3-deficient monocytes also showed constitutive activation of STAT1, which could either be due to the constitutive cytokine production or could reflect a degree of compensation by STAT1 for the loss of STAT3. These results suggest that the major function of STAT3 in the monocyte lineage is to mediate the suppressive effects of IL-10, a surprising finding after the many cell-culture-based experiments that suggested roles for STAT3 in monocyte proliferation, differentiation, and survival. They also suggest that IL-10 plays an important role as a Th2-inducing cytokine through its ability to inhibit production of Th1-type cytokines by monocytic cells.

STAT4 and STAT6

STAT4 and STAT6 are best considered together because they are both highly specific in terms of their cytokine activation profile and to some degree they display opposing functions. Both STAT4 and STAT6 have been best characterized in T cells where STAT4 is primarily activated by IL-12 and STAT6 is primarily activated by IL-4 and its relative, IL-13. In accordance with this activation profile, disruption of the genes for STAT4 and STAT6 resulted in animals unresponsive to IL-12 and IL-4, respectively, and largely mimicking the phenotypes of IL-12-deficient or IL-4-deficient mice.

STAT4 is a major determinant of Th1 polarized responses by mediating the effects of IL-12. STAT4 is activated in response to treatment of T cells with IL-12 [45], and is not activated in response to a large number of other growth factors and cytokines that have been tested [46, 47]. STAT4 was disrupted in mice by two independent groups [48, 49], and resulted in a loss of IL-12 responsiveness, biased T cell development along a Th2 pathway, and susceptibility to infections that normally elicit a Th1 response. In particular, IL12-induced increases in the production of IFN-γ, cellular proliferation, and natural killer (NK) cell cytotoxicity were abrogated in lymphocytes from STAT4-deficient mice. The development of Th1 cells in response to either IL-12 or to *L. monocytogenes* infection was also impaired in the absence of STAT4. Interestingly, STAT4 was only required for IFN-γ production by CD4+ helper T cells and not by CD8+ cytotoxic T cells [50], reflecting the CD4 cell requirement for IL-12/IL-18-dependent induction of IFN-γ. In contrast, cytotoxic T cells stimulated through their antigen receptors produced IFN-γ through a pathway not dependent on IL-12 and therefore not dependent on STAT4.

STAT6 mediates responses opposite those of STAT4, at least to the extent that T lymphocyte development can be considered a dichotomy of Th1 and Th2 pathways

[51]. STAT6 had been shown in cell culture models to be activated exclusively by IL-4 and IL-13; therefore, it came as no surprise that when three groups produced STAT6-deficient mice they exhibited defects similar to those observed in IL4-deficient or IL4-receptor-deficient animals [52–54]. IL4-induced increases in the cell surface expression of MHC class II antigens, CD23, and IL4 receptor were completely abrogated, and B lymphocytes from STAT6-deficient animals failed to proliferate in response to IL-4 or to costimulation with anti-IgM. STAT6-deficient B cells did not produce IgE following in vivo immunization with anti-IgD. Ig switch recombination, normally stimulated through transcriptional responses in response to IL-4, was deficient in STAT6 $-/-$ B cells, though stimulation by CD40 was unaffected [55]. In addition, STAT6-deficient T lymphocytes failed to differentiate into Th2 cells in response to either IL-4 or IL-13. The T cell proliferative responses were also notably reduced, and IgE and IgG1 responses after nematode infection were profoundly reduced. Despite the existence of multiple signaling pathways activated by IL-4, STAT6 appears to be essential for mediating responses to IL-4, at least in lymphocytes.

The role of STAT6 in cell proliferation has also been investigated because lymphocyte proliferation in response to cytokine stimulation is essential for a variety of immune responses. Lymphocyte proliferation in response to IL-4 was of particular interest because of the variety of signaling pathways activated by this cytokine, including STAT6, insulin receptor substrates, PI3 kinase and MAP kinases, and the finding that IL-4-induced proliferation was impaired in STAT6-deficient lymphocytes. The impaired IL4-induced proliferative response of STAT6-deficient lymphocytes was not found to be due to an inability to activate these alternate signaling pathways [56]. Cell cycle analysis showed that the percentage of STAT6-deficient lymphocytes that transit from the G1 to the S phase of the cell cycle following IL-4 stimulation was lower than that of control lymphocytes, and that protein levels of the CDK inhibitor $p27^{Kip1}$ were dysregulated. Levels of $p27^{Kip1}$ were significantly higher in STAT6-deficient lymphocytes than in control cells following IL-4 stimulation, correlating with decreased CDK2-associated kinase activity. A similar increase in $p27^{Kip1}$ was observed in IL-12-stimulated STAT4-deficient lymphocytes with an associated impaired proliferation. Increases in $p27^{Kip1}$ levels appeared to result from increased protein accumulation rather than altered mRNA expression. These results indicate that IL-4 and IL-12, through STAT-dependent pathways, stimulate proliferation by counteracting an opposing increase in CDK inhibitors stimulated through a non-STAT pathway.

STAT4- and STAT6-deficient mice provide strong support for the Th1/Th2 polarization paradigm. For example, mice normally susceptible to infection by Leishmania due to a strong Th2 response became resistant after disruption of the STAT6 gene, due to development of a Th1 response [57]. Similarly, allergen-induced airway inflammation and the resulting eosinophilia, hallmarks of a Th2-mediated response, were abrogated in the absence of STAT6 [58, 59], and development of *Schistosoma mansoni* ova-induced granulomas and the resulting tissue-destructive fibrotic pathology required STAT6-mediated responses but not STAT4 [60]. These results suggested that Th1 development represents a default pathway for CD4 + T lymphocytes. This notion has been investigated using STAT4/STAT6 doubly deficient mice [61]. STAT4-STAT6 $-/-$ lymphocytes failed to differentiate into IL4-secreting Th2 cells. However, in contrast to STAT4 $-/-$ lymphocytes, T cells from STAT4-STAT6 $-/-$ mice produced significant amounts of IFN-γ when activated in vitro. Although STAT4-STAT6 $-/-$ lymphocytes produced less IFN-γ than IL-12-stimulated control lymphocytes, the number of IFN-γ-secreting cells generated in cultures of STAT4-STAT6 $-/-$ lymphocytes activated under neutral conditions was largely equivalent to the number of IFN-γ-secreting cells produced by wild-type lymphocytes activated under Th1-cell-promoting conditions. Moreover, STAT4-STAT6 $-/-$ mice mounted an in vivo Th1-cell-mediated delayed-type hypersensitivity response. These results support a model of T helper lymphocyte differentiation in which the generation of Th2 cells requires STAT6, the development of Th1 cells is augmented through the action of STAT4, but the existence of an additional STAT4-independent pathway allows the development of Th1 cells in the absence of either STAT4 or STAT6.

The concept that Th1 development is a default pathway that can be diverted toward a Th2 pathway through STAT6-dependent signaling has been challenged by recent results involving the STAT6 antagonist BCL6. BCL6 is a zinc finger protein that binds DNA sequences also recognized by STAT6, is expressed in germinal-center B lymphocytes, and can inhibit IL-4-responsive, STAT6-dependent gene transcription, at least in cell culture models [62, 63]. However, STAT6-BCL6 $-/-$ double-mutant mice developed the same Th2-type inflammations characteristic of BCL6-/- mice, suggesting that in the absence of BCL6, T lymphocyte development defaulted to a Th2 pathway. Furthermore, a Th2 cytokine response developed in STAT6-BCL6 $-/-$ and IL-4-BCL-6 $-/-$ mice after immunization with a conventional antigen in adjuvant. In contrast to these in vivo findings, STAT6 was required for the in vitro differentiation of BCL-6 $-/-$ T cells into Th2 cells. These studies revealed an important role for the transcriptional repressive effects of BCL-6 in the regulation

of Th2 responses in vivo by a pathway independent of both IL-4 and STAT6 [64].

STAT5

Analysis of the role for STAT5 in vivo has been complicated by the existence of the two nearly identical genes STAT5a and STAT5b closely linked on chromosome 11 [15]. Due to the difficulty in producing a double knockout, deficiencies for STAT5a and STAT5b were first examined individually. As expected from the original characterization of STAT5 as mammary gland factor [65], it was not surprising that the phenotype of STAT5a-deficient mice showed impaired mammary gland development and lactogenesis [66]. STAT5a-deficient mice developed normally and were indistinguishable from hemizygous and wild-type littermates in size, weight, and fertility. However, mammary lobuloalveolar outgrowth during pregnancy was curtailed, and females failed to lactate after parturition due to a failure of terminal differentiation, suggesting that the presence of STAT5b was insufficient to compensate for the loss of STAT5a. One potential explanation for the lack of redundancy could be the significant reduction in STAT5b protein levels observed in the absence of STAT5a. While the effects of STAT5a deficiency on mammary gland development were striking, the expression of only some milk protein genes was affected. Whereas the promoters of many genes for milk proteins are dependent on STAT binding sites for PRL-induced expression, only production of whey acidic protein was severely impaired in the absence of STAT5a. Presumably, the reduced levels of STAT5b were able to sustain expression of other milk protein genes, providing evidence for discrete functions for these highly related transcription factors. Overall, the mammary development phenotype of STAT5a-deficient mice resembled that of PRL-receptor-deficient mice [67], suggesting that STAT5a is indispensable for this aspect of PRL function.

Disruption of STAT5b provided a very different picture, suggesting an indispensable role in GH action [68]. STAT5b gene disruption led to a major loss of multiple, sexually differentiated responses associated with the sexually dimorphic pattern of pituitary GH secretion. Male-characteristic body growth rates and male-specific liver gene expression were decreased to wild-type female levels in STAT5b $-/-$ males, while female-predominant liver gene products were increased to a level intermediate between those of wild-type males and females. Pulsatile but not continuous GH exposure has been proposed to play a key role in regulating the sexual dimorphism of liver gene expression [69]. Although the responses observed in STAT5b $-/-$ mice were similar to those observed in GH-deficient Little mice, STAT5b $-/-$ mice were not GH deficient, suggesting that they may only be impaired in sensing GH pulses. Indeed, dwarfism, elevated plasma GH, low plasma insulin-like growth factor I, and development of obesity were observed in STAT5b $-/-$ mice, and these are all characteristics of Laron-type dwarfism, a human GH resistance disease generally associated with a defective GH receptor. The requirement of STAT5b to maintain sexual dimorphism of body growth rates and liver gene expression suggests that STAT5b may be the major, if not the sole, STAT protein that mediates the sexually dimorphic effects of GH pulses in liver and perhaps other target tissues.

One of the surprises from disruptions of either STAT5a or STAT5b was the apparently normal development of the hematopoietic system, despite the many studies showing STAT5 activation in response to such hematopoietic cytokines as erythropoietin (EPO), IL-2, IL-3, IL-5, granulocyte-macrophage-colony stimulating factor (GM-CSF), IL-7, and CSF-1. However, an impairment in peripheral T lymphocyte proliferation was observed in the absence of STAT5a [70]. This defect appeared to be due to the loss of induced expression of the IL-2 receptor α-subunit which is necessary for maintenance of high-affinity cell surface receptors. T cell proliferation in the absence of induced α-subunit expression could be overcome by increased IL-2, demonstrating that the defect in proliferation was due to an indirect effect of STAT5a on receptor expression. Interestingly, this defect was reflected by reduced T cell proliferation in vivo in response to antigenic stimulation, underscoring the importance of α-subunit induction during an immune response. A similar defect in NK cell proliferation was also observed in the absence of STAT5a [71]. However, in this case, the defect could not be rectified by high-dose IL-2 and likely reflects an additional role for STAT5a in NK cells.

The results described for the individual inactivation of STAT5a and STAT5b were surprising, given the high degree of homology of these two proteins, their very similar patterns of expression, and the almost indistinguishable set of activating cytokines. In fact, it was suspected that simultaneous inactivation of both genes would be required to produce a profound phenotype of STAT5 deficiency. However, production of a STAT5a-STAT5b doubly deficient mouse strain was also a surprise. The genes for both STAT5a and STAT5b were individually disrupted in a single ES cell clone and used to produce mutant mice [72]. Again, it was possible to derive viable, homozygous-null mice and hematopoiesis in these animals was largely intact. Moreover, mice with individual STAT5a or STAT5b mutations displayed phenotypes that were somewhat discordant from the results reported previously from other laboratories, pos-

sibly due to differences in the strain backgrounds used or the exact mutation constructed. Nonetheless, the phenotypes of these mice demonstrated an essential though often redundant role for the two STAT5 proteins in a spectrum of physiological responses associated with GH and PRL signaling; and yet, the responses to most STAT5-activating cytokines, including EPO, were largely unaffected.

Nonetheless, additional phenotypes were discovered in the absence of both isoforms of STAT5 that were not observed in either STAT5a $-/-$ or STAT5b $-/-$ mice. For example, the STAT5a/b mutant female mice, but not the STAT5a or STAT5b mutants, were infertile. Female infertility correlated with altered histology of the ovaries, with either few or no large corpora lutea evident in ovaries from mutant mice, and altered gene expression patterns characteristic of impaired PRL signalling [72]. In addition, the growth defects observed in STAT5b $-/-$ mice [68] were even more apparent in the doubly deficient animals. At 12 weeks of age, the male and female mice weighed 30–40% and 20–30% less than their wild-type littermates, while the STAT5b $-/-$ mice were consistently 20–30% smaller than their wild-type littermates. The STAT5a/b mutant phenotypes are quite similar to those observed in the absence of GH or of GH receptor, suggesting that most if not all GH signaling is mediated by STAT5a and/or b. For example, similar to GH-receptor-deficient mice, STAT5a and STAT5b mutant mice had somewhat reduced epidermal fat pads, and those of STAT5a/b mutant mice were approximately one-fifth that of wild type [72].

Impaired peripheral T lymphocyte proliferation was also observed in STAT5a/b $-/-$ mice, but this phenotype was ascribed to fundamental defects in cell cycle entry rather than to decreased IL-2 receptor expression [73]. While lymphopoiesis was normal, T cells from STAT5 doubly deficient mice were profoundly deficient in proliferation and failed to undergo cell cycle progression or to express some of the genes controlling cell cycle progression. In addition, the mice lacked NK cells, developed splenomegaly, and their T cells displayed an activated phenotype. These phenotypes are strikingly similar to those seen in IL-2-receptor-beta-chain-deficient mice. These phenotypes are not seen in mice lacking STAT5a or STAT5b alone, even though similar T cell and NK cell deficiencies were observed in STAT5a $-/-$ mice developed independently [70]. The reasons underlying the discrepancies between STAT5 mutant mice developed in different laboratories are currently unclear.

Concluding remarks

The initial promise following the discovery of Jak-STAT signaling in the IFN system for a straightforward explanation of signal transduction specificity appeared to be undermined by subsequent findings of the seemingly broad and indiscriminate activation of these signaling molecules. However, much of this early hope has been restored by physiological studies in vivo, particularly those derived from phenotypes of gene disruptions, which have indicated a high degree of selectivity in the requirements for individual STAT proteins. Thus, it is tempting to suggest that each STAT protein evolved for a particular response pathway, and even chromosomal clusters of STAT genes could be seen as serving discrete functions. STAT1 and STAT4 could be characterized as largely responsible for host defenses involving IFN-γ, STAT2 and STAT6 as highly specific responders to the cytokines IFN-α/β and IL-4/IL-13, respectively, and STAT3 and STAT5a/b being involved in terminal differentiation and tissue-specific gene expression, possibly through regulation of cell survival.

However, as the data reviewed here indicate, there are underlying complexities that belie any simple explanations of the functions or requirements for these transcription factors. The constitutive activity of STAT1, the embryonic lethality but very mild adult phenotype of STAT3 deficiency, the T cell phenotype of STAT5, regulation of CDK inhibitor protein abundance by STAT4 and STAT6, and the intricate interactions among STAT4, STAT6, and BCL6 in the regulation of Th1/Th2 polarization provide but a few glimpses of the complexities yet to be understood. Another question stimulated by the apparent high degree of selectivity in STAT protein utilization is how cytokine signaling pathways not regulated through STATs might function. For example, the function of myeloid and erythroid cytokines such as IL-3, IL-5, GM-CSF, EPO, and thrombopoietin do not appear to be impaired by STAT deficiencies. Will additional STAT proteins or new signaling pathways be uncovered that explain the action of these cytokines? Or will it be found that the phenotypes so far characterized represent only the unique aspects of individual STAT proteins while additional functions are provided redundantly and will only be revealed by compound mutation of several STAT genes simultaneously? The successful targeting of all known STAT genes, coupled with similar disruptions of all JAK genes, should provide the necessary tools for further characterization of cytokine signal transduction pathways.

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