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Signaling of c-kit in dendritic cells influences adaptive immunity

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Abstract

The binding of the receptor tyrosine kinase, c-kit, to its ligand, stem cell factor (SCF), mediates numerous biological functions. Important roles for c-kit in hematopoiesis, melanogenesis, erythropoiesis, spermatogenesis, and carcinogenesis are well documented. Similarly, activation of granulocytes, mast cells, and of eosinophils in particular, by c-kit ligation has long been known to result in degranulation with concomitant release of pro-inflammatory mediators, including cytokines. However, recent work from a number of laboratories, including our own, highlights previously unappreciated functions for c-kit in immunologic processes. These novel findings strongly suggest that signaling through the c-kit–SCF axis could have a significant impact on the pathogenesis of diseases associated with an immunologic component. In our own studies, c-kit upregulation on dendritic cells via T helper (Th)2- and Th17-inducing stimuli led to c-kit activation and immune skewing toward these T helper subsets and away from Th1 responses. Others have shown that dendritic cell treatment with inhibitors of c-kit activation, such as imatinib mesylate (Gleevec), favored breaking of T-cell tolerance, skewing of responses toward production of Th1 cytokines, and activation of natural killer cells. These data all indicate that deeper understanding of, and ability to control, the c-kit–SCF axis could lead to improved treatment modalities aimed at redirecting unwanted and/or deleterious immune responses in a wide variety of conditions.

Keywords

c-kit; SCF; interleukin-6; Jagged-2; Notch; dendritic cells; adaptive immunity

Identification of c-kit and its ligand stem cell factor

The cloning of c-kit, a type III tyrosine kinase receptor, came close on the heels of the identification of the v-kit oncogene as the transforming gene in the Hardy–Zuckerman 4 feline virus.^{1–3} c-kit bears close resemblance both with respect to sequence homology and function to platelet-derived growth factor receptor and macrophage colony stimulating factor receptor.⁴ All type III receptors are characterized by the five immunoglobulin-like domains in the extracellular region, followed by a 70–100 amino acids long, intracellular, kinase domain. Similar to most tyrosine kinase receptors, the extracellular domain facilitates the binding of the ligand and the cytoplasmic domain serves to transduce the signal.^{2,3,5,6}

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Alternate splicing of murine c-kit mRNA results in two isoforms characterized by the presence or absence of a glycine-asparagine-asparagine-lysine (GNNK; residues 510–513) tetrapeptide in the juxtamembrane region of the extracellular domain.^{7,8} In humans, the expression of similar splice variants has also been documented. These isoforms of c-kit are expressed in different ratios in various cell types and also differ in their signaling capabilities.^{9,10}

The ligand of c-kit, stem cell factor (SCF), is encoded by the Steel (Sl) locus on chromosome 12 in humans and chromosome 10 in mice.^{11,12} Like c-kit, SCF also exhibits two distinct isoforms that arise from alternative splicing of exon 6 of the mRNA.^{13,14} The primary translation product of 248 amino acids contains a proteolytic cleavage site encoded by exon 6, and posttranslational processing at this site results in the soluble form of SCF (sSCF) comprising 165 amino acid residues.^{13–15} The membrane-bound SCF (mSCF), on the other hand, is 220 amino acid residues long and results from an alternatively spliced mRNA. This alternately spliced SCF mRNA lacks the proteolytic cleavage site encoded by exon 6, which causes the anchoring of the protein to the membrane. mSCF may also yield sSCF by proteolytic cleavage.^{16,17}

Certain lineages of cells that express c-kit also express SCF, indicating a self-regulated¹⁸ feedback to enhance receptor expression. c-kit signaling has profound effects in various biological functions, such as spermatogenesis, melanin formation, and erythropoiesis.^{18,19} Mutations in c-kit result in the development of various tumors as a result of aberrant signaling of the receptor, which necessitates a complete understanding of c-kit structure, the initiation of signaling events,^{2,19} as well as characterization of downstream targets of the receptor.^{2,20} As discussed below, the expression of mSCF versus sSCF may have different biological ramifications.

From hematopoietic stem cells to dendritic cells, c-kit serves special functions

The discovery of the c-kit proto-oncogene^{2,3} marked an important milestone in understanding the biology of this receptor, which is widely expressed not only in hematopoietic stem cells but in many other cell types. In many cell types, such as in B and T cells, the expression of c-kit is lost upon cell differentiation. However, mast cells, natural killer (NK) cells, and dendritic cells (DCs) of the immune system retain their expression of c-kit, suggesting an important role for this molecule in these cell types.^{11,18} In addition to the well-described role of c-kit in mast cell development and function, other cell types that express c-kit include melanocytes, germ cells, and interstitial cells of Cajal.²¹

The expression of c-kit has been shown to be of critical importance as mice completely lacking c-kit die within the first 10 days of life.²² The ligand for c-kit, SCF, is expressed on a wide variety of cells, but c-kit expression is much more restricted. A major site of expression of c-kit is on hematopoietic stem cells (HSCs) in the bone marrow, suggesting an important role in cell differentiation and maturation.^{23,24} Indeed, c-kit is critical for the development of myeloid cells, although it is somewhat dispensable for lymphopoiesis.²⁵ Although co-expression of c-kit and SCF on the same cell has been described,¹⁸ HSCs express only c-kit but not SCF.^{18,26–28} There fore, in the bone marrow hematopoiesis mediated via c-kit appears to occur through expression of SCF on other types of cells, for example, stromal fibroblasts.

While c-kit is ubiquitous on HSCs and is important for their differentiation, expression is lost on most cells upon maturation, with several notable exceptions.²⁹ Importantly, c-kit expression on these fully differentiated cells has functional significance for each but also has

implications in a number of disease processes. Eosinophils, mast cells, and a subset of NK cells all retain c-kit expression in the periphery.¹⁸ Additionally, we have found that under certain conditions DCs can be induced to express c-kit, and this has direct bearing on the qualitative function of these cells.³⁰ The role of c-kit on each of these cell types and its known implications in a number of diseases is discussed below.

Mast cells

The cell type that is most extensively studied in regards to c-kit function is mast cells.¹⁸ c-kit plays a crucial role in mast cell development, survival, and function through interactions with its ligand SCF.^{29,31} The large number of reports in the literature is no doubt a result of the dramatic effects that c-kit ligation by SCF has on many aspects of mast cell biology. Briefly, mice deficient in either c-kit (white spotting locus; W) or SCF (Sl) completely lack mast cells, thus demonstrating the pivotal role each has in mast cell differentiation.^{32–36} In addition, expression of c-kit on fully mature mast cells in the periphery has functional importance. c-kit ligation results in mast cell degranulation with release of mediators, such as histamine, and also expression of inflammatory cytokines and chemokines.

The consequences of c-kit activation on mast cells are dramatically demonstrated in mastocytosis.²⁹ In this condition, a gain-of-function mutation in c-kit, resulting in uncontrolled activation even in the absence of SCF, leads to upregulation of the anti-apoptotic gene *bcl-2* and unchecked mast cell proliferation. Greatly increased numbers of activated mast cells are seen in many organs, with the most common and/or prominent being the skin. Mast cells in affected skin lesions can be induced to degranulate simply by touch. Another hallmark of mastocytosis is overexpression of IL-6, the level of which is correlated with the degree of organ involvement. This c-kit–IL-6 connection is maintained in DCs as well, as our recent study has established.³⁰

Eosinophils

SCF has a number of effects on eosinophils, in addition to promoting their generation from bone marrow progenitors, a process that is enhanced by granulocyte colony stimulating factor (G-CSF).³⁷ A microarray analysis of eosinophils reportedly resulted in upregulation of over 150 inflammatory genes, including CCL5, CCL22, CCL4, and CCL6, by SCF.³⁸ In addition, similar to its effects on mast cells, SCF causes degranulation of eosinophils and release of soluble mediators, such as eosinophil peroxidase and leukotriene C₄.³⁸

Dendritic cells

Various lineages of myeloid-type DCs have been described as arising from c-kit-expressing bone marrow precursors.³⁹ However, c-kit does not appear to be absolutely required as many, if not all, of these lineages can also arise from bone marrow cells lacking c-kit. However, there are reports that DCs, as well as macrophages, derived from precursor cells in the presence of c-kit blockade do not mature fully.^{40,41} Our recent work has shown that DC c-kit and SCF expression are upregulated by Th2/Th17- (but not by Th1-) inducing stimuli.³⁰ We found that c-kit expression was directly related to IL-6 production, which in turn promoted both Th2 and Th17 differentiation. Bone marrow-derived DCs upregulated c-kit upon treatment with cholera toxin (CT) or house dust mite (HDM) antigen, and this was associated with IL-6 production. When these treated cells were sorted into c-kit-expressing and c-kit-negative subpopulations, the former induced much greater production of Th2 and Th17 cytokines from naive CD4⁺ T cells than the latter. Furthermore, the c-kit-negative population enhanced production of the Th1 cytokine interferon (IFN)- γ even following CT or HDM treatment.

We additionally found that c-kit expression was important for the expression of Jagged-2 on dendritic cells. This finding is particularly important in relation to Th2 generation because while IL-6 promotes the Th2 phenotype, it is not considered a Th2-skewing cytokine. Jagged-2, on the other hand, by virtue of its binding to Notch 1 or Notch 2 on T cells has been associated with Th2 differentiation, although the precise role of the Jagged ligands 1 and 2 in this process needs additional work.^{42,43} This newly identified relationship between c-kit and Jagged-2 is discussed below in light of the recent developments in the Notch field in relation to adaptive immunity. To confirm the link between c-kit upregulation/signaling, IL-6 production, Jagged-2/Notch, and Th2/Th17 differentiation, we found that mice with functional defects in c-kit had impairment in both Jagged-2 and IL-6 expression and the ability to drive Th2/Th17 differentiation.³⁰ Finally, and most significantly, c-kit deficient DCs were unable to induce experimental airway inflammation upon adoptive transfer into naive animals.

Considering that Pulendran and colleagues⁴⁴ documented the expression of c-kit on spleen DCs in 1997, it is surprising to note the paucity of research addressing the role of c-kit in DC biology. Investigations of c-kit function in DCs have lagged because the *in vitro* development of bone marrow-derived DCs from progenitor cells stamped c-kit as a stem cell-specific marker. In fact, several studies reported a decrease in c-kit expression with DC development and maturation in line with the general expression profile of c-kit, with expression maintained in HSCs but lost upon differentiation into most lineages.^{29,45,46} This limited documentation of the expression of c-kit on tissue DCs can also be explained by the fact that naive DCs have minimal expression of this receptor and that this is indeed an area of underexplored research. For example, a prior study showed that microglia express c-kit and that sSCF secreted by neurons can modulate microglial functions.⁴⁷ Interactions between neurons and microglia via c-kit and SCF raise several pertinent research questions, which can have profound implications in disease pathogenesis given that IL-17 is implicated in the development of experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis, and a role for microglia in mediating the pathology in EAE has already been suggested.⁴⁸ Specifically targeting c-kit on microglia could significantly ameliorate the disease condition and offer c-kit as a therapeutic target beyond mast cells.

Recently, several groups have begun investigations addressing the origin of DCs in various tissues from distinct precursor populations and the ability of specific populations to repopulate in the tissue and give rise to DCs.⁴⁹ Using sophisticated *in vivo* techniques with chimeric mice expressing green fluorescent protein (GFP)-tagged CX3CR1, the origin of DCs has been investigated and, interestingly, in the lung the expression of c-kit is distributed on both CD103⁺ and CD103⁻ cells.³⁹ Furthermore, the adoptive transfer of CX3CR1⁺c-kit⁺ bone marrow cells into irradiated mice subjected to a model of allergic inflammation resulted in active recruitment of these cells into the lungs and the conversion of these cells into both CD103⁺ and CD103⁻ cells. Collectively, these studies establish that c-kit-expressing precursors are efficient in homing into lymphoid and nonlymphoid tissues and have the capacity to differentiate into distinct DC populations. It is intriguing to note that even though DC subtypes in different tissues, such as the lung and spleen, may serve different tissue-specific functions, expression of c-kit drives IL-6 production irrespective of the source of the DCs.³⁰ An additional role for c-kit expression on DCs appears to be to drive NK cell activation, as discussed below.

Natural killer cells

A small subset of NK cells expresses c-kit in the periphery.⁵⁰ These cells arise from HSCs where c-kit is important for the generation, proliferation, and maturation of NK cells, most of which lose c-kit expression. Interestingly, the small, residual, c-kit-expressing cells appear to exhibit less cytotoxic activity than c-kit⁺ NK cells, suggesting that they are

somewhat immature.^{50,51} Direct activation of c-kit-expressing NK cells with SCF leads to their increased survival, proliferation, and enhanced lytic activity comparable to c-kit⁻ NK cells.⁵¹ These data indicate that the c-kit-expressing NK cells in the periphery are indeed immature and that SCF functions to drive their final maturation. To date, there are no known reports showing that NK cell-specific c-kit ligation renders any functional advantage to the cells. Therefore, it seems that for some types of cells, c-kit/SCF is important for the generation of the particular lineage but not necessarily for the function of fully matured cells. While direct ligation of c-kit on NK cells may not have major functional consequences in the periphery, as discussed in a later section, a potentially very important indirect role involving DCs may have emerged from the studies of Zitvogel and colleagues who reported that inhibition of c-kit signaling by Gleevec (imatinib mesylate/STI571) in DCs imparted the ability for the cells to activate NK cells in the setting of gastrointestinal stromal tumors (GISTs).⁵²

Progenitor-T and B cells

Pro-T cells express high levels of c-kit, which is critical for the development of T cells. Recent studies have reported that Notch-mediated signaling during early T-cell development is pivotal for the maintenance of c-kit expression on precursor T cells and also necessary for the normal progression and commitment along the T-cell lineage.^{53,54} Pharmacological intervention of c-kit signaling by Gleevec abrogates this pathway, resulting in abortive T-cell development.⁵³ A recent study has also shown that specific deletions for the docking site for the Src family of kinases in c-kit abrogates the development of both T and B cells, reiterating the importance of signaling events transduced by this receptor for the development of these cell types.⁵⁵

The biological ramifications of soluble versus membrane-bound form of the ligand

mSCF has signaling properties distinct from that of the soluble form, and this results in varied biological functions mediated by the two isoforms.^{56,57} The regulation of c-kit signaling is also fine-tuned by whether SCF is expressed in the membrane-bound or soluble form. Association of c-kit with sSCF results in transient activation of the receptor, whereas mSCF prevents the internalization of the receptor and promotes sustained downstream activation.^{58,59} The majority of SCF expression is believed to be in the membrane-bound form. The expression of the membrane-bound form of SCF brings into play cell-cell interactions that underlie many of the biological functions of c-kit. For example, the expression of c-kit but not SCF is found on a significant population of hematopoietic stem cells, and the renewal of these cells is promoted by fibroblasts, which express SCF but not the receptor.²⁶⁻²⁸ Additionally, the fact that the expression of both ligand and receptor is altered during injury, infection, and inflammation reinforces the concept that selective expression of the ligand or the receptor is key in maintaining homeostasis. mSCF has been shown to provide better support for mast cells than sSCF.⁶⁰ The same is true for CD34⁺ progenitor cells. The brain produces high levels of sSCF;¹⁵ however, during brain injury, the level of mSCF is elevated, which has been shown to be important not only for recruiting neural stem cells to the site of injury but also for activating c-kit expressed on the stem cells, which contributes to the repair process.⁶¹ Our recent study has shown that the expression of c-kit on lung DCs from naive mice is low but the expression of both the membrane-bound ligand and receptor is significantly elevated in response to certain allergens causing persistent signaling downstream of c-kit as a result of cell-cell interactions.³⁰ Given that both receptor and ligand can be expressed by the same cell type under specific conditions, it is critical that such expression patterns are prevented under homeostatic conditions to minimize inadvertent activation of the receptor. By the same token, such interactions are an

integral part of development and repair after tissue injury and must be stringently regulated to prevent adverse effects, such as onco-genesis. In the setting of cancers, alteration of c-kit function and signaling has been studied extensively in association with GIST.⁶² In GIST, the gain-of-function mutation in exon 11 leads to constitutive activation of the receptor even in the absence of SCF.⁶² However, recent studies have demonstrated increased tyrosine phosphorylation of the receptor even in the absence of gain-of-function mutations in c-kit in GIST patients, which has also been associated with the presence of mSCF expressed by tumor cells.⁶³ Several other cancers have also shown altered or increased expression of the receptor and ligand, suggesting a paracrine or autocrine mechanism that induces an oncogenic signaling cascade in cells.⁶⁴ Given that most studies suggest a role for mSCF in chronic signaling via c-kit, it would be interesting to determine how production of mSCF versus sSCF is regulated in different cell types. This knowledge may also provide alternate drug targets to regulate c-kit signaling, either inhibiting it in cancers or allergic inflammation or promoting it in brain injury.

Initial glimpses of c-kit–Notch partnership

Kit-mediated signal transduction is critical for the normal development and survival of hematopoietic progenitor cells.^{65,66} However, expression of c-kit is generally lost during hematopoietic cell differentiation.²⁹ Similar to c-kit, the protein Notch is also expressed by HSCs.^{67,68} Notch is a transmembrane protein and has four members, Notch 1–4, that interact with the ligands Jagged-1, Jagged-2, Delta-like 1, 3, and 4 that are also expressed on the cell surface.^{67,68} Notch activation also involves cell–cell interaction, which leads to a stepwise proteolytic processing of the receptor to generate Notch intracellular domain (NICD).^{67,68} NICD then translocates to the nucleus where it interacts with the DNA-binding protein RBP-Jk to induce target gene transcription via recruitment of histone acetylases and transcriptional co-activators, such as mastermind.^{67,68} During fetal and adult development, expression of Notch continues in the proliferative layers of several mature tissues. There is considerable interest in the events that trigger Notch–Notch ligand interactions, but relative concentrations appear to play an important role in deciding whether interactions occur intercellularly or intracellularly.^{67,68} More importantly, Notch ligands have been associated with both activation and inhibition of Notch signaling. Notch is involved in cell-fate decisions. During somite formation in vertebrates, repetitive cycles of Notch activation and inactivation cause specific pattern formation and segmental boundary in the presomitic mesoderm.^{69,70} High levels of ligand appear to inhibit Notch signaling, while lower levels promote Notch activation. This mechanism was initially appreciated in *Drosophila* during wing development.^{71,72} In higher eukaryotes, Delta-like 3 in *Xenopus* appears to only exert inhibitory activity.⁷³ In humans, it has been suggested that high levels of Delta expression during keratinocyte differentiation could act as an inhibitory mechanism of Notch signaling to maintain stem cell population.⁷⁴ The detailed mechanisms responsible for the inhibitory effects are unclear, although intercellular ligand–ligand interactions as well as intracellular receptor titration by ligand have been proposed.⁷⁵

Recent studies suggest functional interaction between the c-kit and Notch pathways. Using a Pax-5-deficient pro-B-cell line blocked in its B-cell potential as well as a bone marrow-derived lymphoid and myeloid progenitor, Notch signaling rapidly upregulates c-kit expression, which is required for T-cell development; however, the development of non-T cells (NK or myeloid) was found to be c-kit independent.⁷⁶ Conditional deletion of Delta-like 1 was found to block the development of marginal zone B cells but did not impair T-cell development.⁷⁷ In the developing nervous system, c-kit signaling is involved in survival, migration, proliferation, and differentiation of neural crest precursors.^{78,79} In the ciliary epithelium, which is derived from the central nervous system, c-kit signaling upregulates

Notch expression and cooperation between c-kit and Notch is required for the maintenance of neural stem cells.⁸⁰

Partnership of c-kit and Notch in the immune system

It is clear that the role of c-kit in the regulation of the immune system extends beyond its well-established function in mast cells.³¹ A role for c-kit in regulating DC function was largely unappreciated prior to our study in which we investigated what signals promote IL-6 production in these cells.³⁰ In our study linking c-kit to increased IL-6 production, basal Jagged-2 expression was blunted in DCs isolated from c-kit-mutant mice which, unlike in DCs isolated from wild-type mice, could not be up-regulated by CT.³⁰ Unlike CT, the Th1-promoting adjuvant CpG oligodeoxynucleotide (ODN) inhibited Jagged-2 expression but promoted expression of Delta-like 4, which inhibits Th2 and promotes Th1 development.^{42,81} The expression of Delta-like 4 or Jagged-1 was not affected by lack of functional c-kit in the DCs. Our study showed that both basal and induced Jagged-2 expression is dependent on c-kit expression in DCs. In additional studies, Jagged-2 expression has been shown to be upregulated under Th2-inducing conditions.^{42,82} However, in other studies of infection with *Schistosoma mansoni*, which induces a potent Th2 response, Jagged-2 knockdown *in vivo* did not diminish the Th2 response.^{83,84} Similarly, another study suggested an indirect role of Notch signaling in Th2 differentiation in which IL-4 production in NK and CD4 memory T cells triggered by Notch activation was shown to promote naive CD4⁺ T-cell differentiation.⁸⁵ The role of Notch versus IL-12 in Th1 differentiation is also not unequivocal. The relative importance of IL-12 versus Notch/Delta-4 signaling in regulating Th1 differentiation was addressed in a study that showed that in the absence of IL-12 production, Delta-4 is required to mount Th1 differentiation, the specific cell type in this study being LPS-stimulated CD8⁻ DCs.⁸⁶ Taken together, these observations suggest that the role of Notch in regulating T helper cell differentiation is prominent/critical under conditions that do not make available high levels of IL-12 or IL-4 to naive CD4⁺ T cells to induce differentiation along Th1 or Th2 lineage. Notch is also regulated by posttranslational modification in which the Fringe family of glycosyl transferases, which cause glycosylation of the extra-cellular domain of Notch, inhibits binding to Jagged proteins but not to the Delta members.^{87,88}

The relationship between c-kit and Jagged-2 and increased IL-6 production revealed in our study was similar to observations of Jagged-2-promoted IL-6 secretion from malignant plasma cells from multiple myeloma (MM) patients that may involve paracrine or autocrine mechanisms.^{89,90} MM is a plasma cell malignancy associated with increased accumulation of monoclonal plasma cells in the bone marrow. Interestingly, while MM is characterized by increased IL-6 secretion, which functions as an autocrine growth factor for these cells, approximately 30% of MM patients also express c-kit; c-kit is not expressed by plasma cells present in healthy individuals. The role of c-kit in disease prognosis in MM is also fraught with controversy. In one study, the expression of c-kit was associated with better disease prognosis.⁹¹ In a different study, however, MM cells with expression of the c-kit isoform lacking the GNNK tetrapeptide in its juxtamembrane domain were more resistant to the antimyeloma drugs bortezomib and melphalan.⁹² In both GNNK⁺ and GNNK⁻ MM cells, SCF promoted protein kinase B (AKT) phosphorylation, although the kinetics and duration of phosphorylation were different between cells expressing the two isoforms.⁹² Activation of ERK^{1/2} was low in response to SCF in both types of cells and explained the weak mitogenic effect of SCF on MM cells in general.⁹² Another phase II clinical trial with Gleevec found limited therapeutic effect of this drug in MM.⁹³ These studies suggest that the function of c-kit in different cell types may depend on the specific isoform of c-kit expressed by the cell. It will be interesting to determine whether c-kit⁺ plasma cells in MM patients exhibit increased Jagged-2 expression and also whether Jagged-2 expression is

related to a specific isoform of c-kit. Thus, the success of therapies targeting c-kit in specific malignancies may depend on which isoform of c-kit is expressed in an individual. For the same reason, what regulates expression of the isoforms of c-kit (and SCF) will be important to elucidate in order to develop better therapeutics.

While a role for c-kit in Jagged-2 expression was not recognized until recently, co-expression of c-kit and Jagged-2 was noted in other contexts in previous studies. For example, hematopoietic progenitors were shown to express c-kit and Jagged-2,⁹⁴ and the latter promoted survival and proliferation of the progenitors and their development into NK cells.⁹⁵ As noted above, c-kit can promote both development and activation of NK cells, the latter involving DC-NK cell interaction. Jagged-2 is expressed in multiple tissues in adult mice, and homozygous Jagged-2 mutant mice display limb and craniofacial deformities along with altered Tab:Tyd ratios.⁹⁶ Jagged-2-expressing, mobilized, Lin-Sca-1⁺c-kit⁺ hematopoietic progenitor cells (HPCs), unlike HSCs, can directly promote expansion of CD4⁺CD25⁺Foxp3⁺ T regulatory cells (Treg cells), and this was found to be associated with Notch signaling.⁹⁷ In an earlier study, Jagged-2 overexpressing antigen-presenting cells when adoptively transferred into mice induced tolerance in the recipients.⁹⁸ Collectively, these reports beg the question as to why c-kit and Jagged-2 in one context promote Th2/Th17 but induce Treg cells/immune suppression in another. Although the reason is unclear at the present time, on the basis of prior literature it may be speculated that the level of Jagged-2 expression in a DC or hematopoietic progenitor cell dictates immune activation versus suppression. One reason for this may lie in the expression of relative levels of soluble and membrane-bound forms of Jagged-2 in DCs similar to the expression characteristics of SCF. Jagged-2, similar to other Notch ligands, is expressed in both membrane-bound and soluble forms. Interestingly, the soluble form binds efficiently to Notch 1 but not to Notch 2,⁹⁹ both Notch proteins being expressed on T cells, albeit Notch 1 may be expressed at higher levels. Although it is unclear whether the soluble form binds to Notch 1 better than the membrane-bound form, it might be speculated that overexpression of Jagged-2 causes increased production of the soluble form, which preferentially binds (over the membrane-bound form) to Notch 1 to promote Treg rather than T helper cell differentiation. It will also be important to determine why c-kit specifically influences Jagged-2 expression. Based on recent findings,^{92,95} one possible reason is to use Jagged-2, like mSCF,⁶⁰ for cell survival and maintenance of a specific phenotype.

c-kit signaling pathway

The binding of SCF induces the homodimerization of c-kit, resulting in selective phosphorylation of tyrosine residues in c-kit, thereby unmasking docking sites for the Src-homology2 (SH2)-containing signal transducers.¹⁰⁰ Site-specific mutagenesis studies have revealed a hierarchical importance in the phosphorylation of tyrosine residues. Some mutations completely abrogate c-kit signaling, while others only dampen the overall signaling.^{101,102}

The involvement of PI3 phosphoinositide kinase in c-kit signaling has been extensively characterized by using molecular mutagenesis studies and biochemical analyses. The p85 regulatory subunit of PI3 kinase specifically associates with phosphorylated tyrosine residue 719 of c-kit, resulting in the recruitment and phosphorylation of AKT.¹⁰³ Transgenic mice harboring a point mutation in the tyrosine residue have revealed the physiological importance of this residue.¹⁰⁴ Substitution of tyrosine with phenylalanine resulted in both reduced spermatogenesis and impaired follicular development. Phosphorylated AKT enhances the survival and proliferation of the primordial cells and specifically mediates several downstream functions through nuclear factor (NF)- κ B signaling as well as phosphorylation of the pro-apoptotic molecule BAD.¹⁰⁰ Phosphorylation of BAD inhibits

the pro-apoptotic function of the molecule, which is one of the reasons why impaired c-kit signaling results in reduced proliferation and survival of several cell types. The c-kit gene maps to the W locus in mice.¹⁰⁵ Mutations in the W locus cause deficiency in melanocytes^{20,36,104,106,107} as well as reduced PI3-kinase activity.¹⁰⁸ It is interesting to note that extracellular c-kit mutations result in hyperactivation of c-kit, marked by prolonged PI3-kinase activation and enhanced cell survival and proliferation.¹⁰⁷ In addition to PI3-kinase activation, binding of SCF has been shown to induce activation of multiple additional pathways, including phospholipase C- γ , Src kinase, Janus kinase (JAK)/signal transducers and activators of transcription (STAT), and mitogen-activated protein (MAP) kinase pathways.¹⁸ In a recent study, distinct roles of the Src-kinase and the PI3-kinase pathways were noted with regard to c-kit function. A mutation in the Src kinase-docking site blocked pro-B and pro-T-cell development, while that in the PI3-kinase binding site affected spermatogenesis.⁵⁵

There are mechanisms in place in cells that downregulate c-kit signaling to prevent chronic activation of the receptor, which can promote cancer. Protein kinase C, a known negative regulator of PI3-kinase pathways, phosphorylates residues S741 and S746 and downregulates c-kit signaling.^{100,109} This is evident from these mutational studies where substitution of these serine residues to alanine resulted in prolonged c-kit signaling. Suppressor of cytokine signaling (SOCS) SOCS1, SOCS6, and SOCS8 bind to c-kit and dampen downstream signaling of the receptor.^{106,110,111} Additional negative regulators of c-kit signaling include SH2-domain-containing inositol 5-phosphatase (SHIP).^{112,113} and the guanosine-5' triphosphate (GTP) ase-activating protein neurofibromin-1.¹¹⁴ Furthermore, ubiquitination of c-kit via Cbl, a ubiquitin ligase, and subsequent proteasomal degradation may also be involved in c-kit downregulation.¹¹⁵ While chronic activation of c-kit may promote tumorigenesis, loss of function of c-kit can also be deleterious as studies suggest that loss of c-kit may allow melanoma cells to escape SCF-mediated apoptosis, thus allowing tumor growth and metastasis.¹¹⁶ Thus, deregulation of either activation or inhibitory pathways of c-kit has adverse effects, often resulting in tumor formation.

cAMP, the intracellular messenger in c-kit/SCF-induced biological functions

Recent studies have demonstrated a role for the intracellular second messenger cyclic adenosine monophosphate (cAMP) in the expression of both c-kit and SCF in different cell types. In several cancer cell lines, an elevated level of cAMP induced by agents, such as forskolin, has been associated with increased expression of c-kit.^{117–120} However, the elevation in c-kit expression mediated by cAMP is not solely restricted to tumor cells, as was revealed in our study of allergen-induced c-kit expression in DCs.³⁰ In DCs the increase in c-kit expression in response to both CT and HDM was inhibited by a cAMP antagonist.³⁰ Similarly, treatment with forskolin or CT promoted c-kit expression in human, ovarian, carcinoma cell lines, which constitutively express SCF.¹²⁰ In keeping with the antiproliferative effects of cAMP, the increase in intracellular cAMP level in these cells inhibited cell proliferation, which was not dependent on c-kit expression. Interestingly, cAMP has also been shown to directly activate the SCF promoter in Sertoli cells. An unidentified cAMP-induced factor has been shown to bind the SCF promoter, resulting in increased SCF expression.¹²¹ Because c-kit plays an important role in the development of various cell types, cAMP may play a dual role in upregulating c-kit/SCF expression and promoting cell differentiation.

c-kit signaling in DCs and cytokine balance

An immune response is a highly orchestrated event involving several key players and cross-regulated pathways, which are essential for mounting a balanced innate and adaptive response toward a foreign antigen or a danger signal generated within the host. Although

Toll-like receptors (TLRs) have long been considered as being involved in the identification of pattern recognition molecules on pathogens, recent studies highlight an equally important role for other molecules on DCs, and the inclusion of c-kit significantly furthers our understanding of DC–T-cell interactions.^{30,122,123} The induction of a Th2 response mediated by lung DCs is inherently tied to the nature of the allergen. Low levels of IL-12 production by DCs strongly favor the establishment of a Th2 response. Several Th2-skewing agents have selective mechanisms to inhibit IL-12 and unique pathways to promote Th2 response. For example, prostaglandin E2 (PGE2) and CT trigger elevated levels of cAMP in DCs through the action of G protein-coupled receptors. CT also inhibits the expression of IL-12 β 2 on CD4 T cells.¹²⁴ The downregulation of CD40 on DCs by PGE2 is another mechanism by which IL-12 is suppressed,¹²⁵ a low IL-12 being a necessary but not sufficient requisite for Th2 development.¹²³ Although the mechanisms to subvert a Th1 response involve direct inhibition of IL-12 or its signaling molecule, the pathways that promote a Th2 response are very diverse. This diversity is an important reason that explains the challenge in developing therapeutic intervention in allergies or asthma.

Unlike IL-12, a DC-derived cytokine that strongly influences Th1 response, a counterpart cytokine released from DCs that uniquely promotes Th2 response has not been identified. IL-6 is produced by DCs in response to an allergen or cholera toxin, which promotes the development of a Th2 response,^{126,127} and IL-6 has also been shown to play a key role in the development of Th17 responses in different immunologic contexts.^{128,129}

Allergens may also use the TLR pathway to establish a Th2 response, and low-level LPS contamination seen in most allergens facilitates the promotion of Th2 by activating the TLR4 pathway in DCs.¹³⁰ It is important to note that in our study, CT and HDM promote the expression of c-kit on lung DCs and neither of these Th2/Th17-promoting stimuli has been shown to elicit responses via TLRs. This is of significance because our findings assign specificity to c-kit for the induction of Th2 and Th17 responses given that a potent Th1-promoting agent, like CpG ODN, was unable to upregulate the expression of c-kit on DCs.³⁰ The low level of c-kit expression on naive lung DCs coupled with reduced c-kit expression following stimulation with CpG ODN suggests an evolutionary protection mediated by lung DCs to prevent unwarranted immune responses and help maintain a low IL-12 environment in the lung. A prior publication highlighted immunomodulatory functions of c-kit where blocking c-kit function by Gleevec reversed established tolerance to tumors by significantly elevating IL-12 levels in DCs, thereby promoting IFN- γ production from T cells in a vaccination strategy.¹³¹ That study suggested a possible role for c-kit-triggered PI3-kinase activation in inhibiting TLR-mediated IL-12 production.¹³¹

The activation of PI3 kinase is an important event in c-kit signaling in DCs that helps promote Th2 and Th17 responses. Activation of PI3 kinase serves the dual purpose of blocking IL-12 production and concomitantly promoting IL-6 production.¹³² This positive-feedback loop is amplified because IL-6 is also known to antagonize IL-12 production^{133,134} and mice deficient in IL-6 have increased IFN- γ secretion from activated T cells.¹³⁴ The reduced levels of AKT phosphorylation seen in DCs from c-kit mutant mice³⁰ and the established importance of the PI3-kinase pathway in promoting Notch-mediated signaling in the setting of various tumors¹³⁵ leads us to speculate that the PI3-kinase pathway is an important regulator of the expression of Notch ligands on DCs. It is possible that activation of PI3 kinase promotes expression of Jagged-2 favoring a Th2 response and the inhibition of this pathway facilitates expression of the Th1-promoting Notch ligand Delta-4.^{43,81,135}

In a pattern similar to the TLR cascades, adaptor molecules also regulate c-kit signaling. Gab2, one such adaptor downstream of c-kit, is an important activator of PI3 kinase. Underscoring the importance of Gab2 signaling, Gab2-deficient mice show reduced airway

inflammation, decreased IL-6 production, and reduced mast T-cell numbers.¹³⁶ Additionally, Gab-2 signaling is a limiting component of MAP kinase signaling, highlighting its role in the fine tuning of IL-6 gene expression.¹³⁷

A comparison of different immune responses triggered via the activation of CD40 and c-kit highlights the diversity in the antigen recognition armamentarium of the innate immune system. The activation of CD40 on DCs by Th1-inducing agents, like LPS or CpG ODN, specifically promotes the production of IL-12.¹³⁸ Mice deficient in CD40 succumb to infection from different pathogens as a result of the inability to mount a robust Th1 response. The deficiency in Th1 results from decreased IL-12 production, which in turn cripples the production of IFN- γ , and high levels of both these cytokines are a prerequisite for a dominant Th1 response.¹³⁹ It is important to note that the signaling events downstream of CD40 remain to be better elucidated, although studies have dissected the key molecules involved in IL-12 production. For example, dampening of PI3 kinase coupled with low levels of AKT phosphorylation promotes IL-12 production.¹³² Mice deficient in PI3-kinase activation have increased IL-12 production, which leads to deleterious effects, like the development of inflammatory bowel disorder.^{140,141} Hence, it is possible that a mechanism by which CD40 mediates a Th1 response is by negatively regulating PI3 kinase, thereby promoting IL-12 production. A role for IL-6 in inhibiting IL-12 production is also evident in other studies.^{133,142,143} IL-12^{-/-} mice are prone to formation of several tumors, and administration of IL-12 mitigates tumor growth.^{144,145} The ratio of the signaling molecules downstream of IL-6 and IL-12, STAT3 and STAT4, respectively, in tumor cells during their genesis can influence tumor progression.¹⁴⁶ Collectively, data from our studies and those of others indicate a central role for c-kit signaling in DCs as a switch in the adaptive immune response that regulates the IL-6/IL-12 cytokine balance. Activation of c-kit in DCs by an allergen or CT promotes the activation of the PI3-kinase pathway, and this inhibits IL-12 production.³⁰ However, concomitant with suppression of IL-12 production, PI3-kinase activation promotes IL-6 production.^{30,132,147,148}

The immunoregulatory effects exerted by CD40 and c-kit signaling cannot be explained by differential PI3-kinase activation alone. Recent evidence suggests that strong antigenic activation of CD40 provided by high doses of microbial stimuli promotes IL-6 production from DCs, which skews the T cell toward a Th17 response and, in fact, blunts the Th1 response.¹⁴⁹ Along the same lines, it is plausible that c-kit activation may be modulated by the strength of TCR activation. From our study it is evident that DCs stimulated by low doses of CT are unable to promote c-kit expression or IL-6 production, and these DCs are not efficient polarizers of Th2 or Th17 responses in spite of the high expression of other costimulatory molecules, like CD86, and major histocompatibility complex II expression.³⁰ A relatively high dose of 1 μ g of CT simultaneously promotes the expression of c-kit and IL-6 and endows the DCs with the capacity to prime a robust Th2 and Th17 response.^{30,150} Increased Jagged-2 expression mediated by c-kit, as observed in our study, would also reinforce IL-6 production on the basis of Jagged-2-induced increased IL-6 production from malignant plasma cells isolated from MM patients.^{89,90} Figure 1 illustrates our current understanding about the role of c-kit in adaptive immunity.

All of these immune functions attributed to c-kit signaling warrant stringent molecular surveillance to prevent chronic c-kit activation, which can result in inflammation and tissue damage. RabGEF1 is a negative regulator of c-kit signaling, and mast cells from mice deficient in this regulator exhibit prolonged PI3-kinase activation, increased IL-6 production, and display the inability to downregulate c-kit expression even upon exposure to high levels of sSCF. This sustained c-kit activation is indeed deleterious, and RabGEF1 mice exhibit severe skin inflammation.^{151,152} It is intriguing to note that sustained c-kit signaling is triggered in CT- or HDM-stimulated DCs, which is critical for promoting Th2

and Th17 response. The simultaneous upregulation of c-kit and mSCF in these DCs mimics the phenotype displayed by RabGEF1-deficient mast cells with prolonged expression of c-kit coupled with AKT phosphorylation, resulting in elevated IL-6 but lower IL-12 production. This cytokine milieu is conducive to the development of Th2 and Th17 responses.

c-kit in disease pathogenesis and a target in therapy?

A great deal has been written regarding the involvement of c-kit in a number of disease conditions. Not surprisingly, given the importance of c-kit for mast cell function, much of this has focused on the role(s) these cells play and the efficacy of interventions targeting c-kit signaling pathways. The c-kit–SCF axis in mast cells is known to be active in wound healing, and the aforementioned condition of mastocytosis represents an abnormal manifestation of this normal process.²⁹ Other conditions characterized by mast cell accumulation, such as allergic rhinitis, asthma, and rheumatoid arthritis, respond favorably to a blockade of c-kit/SCF via agents such as Gleevec or neutralizing antibodies.¹⁸ Another area that has garnered tremendous attention with regard to direct activation of cells via c-kit is tumor biology. In particular, the intense study of GISTs, many of which harbor gain-of-function mutations in c-kit, has resulted in development of the highly effective tyrosine kinase-blocking therapies, such as Gleevec.^{153–155} As discussed above, the efficacy of Gleevec in promoting antitumor effects involving DC-mediated NK cell activation illustrates yet another scenario where c-kit PI3-kinase-regulated IL-6/IL-12 balance in DCs controls immune outcome. Gleevec is an allosteric inhibitor of c-kit, which is being effectively used in the treatment of different cancers, such as GIST and chronic myeloid leukemia. Gleevec's mode of action involves inhibition of tyrosine kinase activity of specific receptors, such as c-kit and the platelet-derived growth factor receptor.¹⁵⁶ Since Gleevec also has associated cardiotoxicity owing to its inhibition of Bcr-Abl function, a modified form of Gleevec was recently designed that maintains its anticancer effects in an animal model of GIST but prevents its effects on Bcr-Abl, reducing cardiotoxic effects.¹⁵⁷

The beneficial effects of Gleevec or neutralizing antibodies are likely mediated by inhibition of inflammatory mediators produced by mast cells as well as by eosinophils, which are also important in the pathogenesis of asthma, for example.^{158–163} Furthermore, experimental models have demonstrated exacerbation of disease by c-kit activation, including intratracheal administration of SCF to mice, which was reported to induce mast cell-dependent airway hyper-responsiveness.¹⁶⁰ Another interesting mechanism of action can be found in rheumatoid arthritis (RA) where the deleterious effects of TNF- α production by mast cells can be mitigated by c-kit inhibition via Gleevec administration.¹⁶⁴ It has been suggested that modulation of c-kit signaling might therefore be a critical pathway in RA, given the known beneficial effects of anti-TNF- α therapy.¹⁶⁵

Whereas mast cell/eosinophil-mediated effects in various conditions are well described, additional contributing immunologic pathways may also be critically important under certain circumstances. We and others have reported potential roles for c-kit expression on DCs in airway disease and tumor biology.^{30,52} Identification of c-kit as a regulator of adaptive immune response in response to specific environmental or endogenous stimuli has opened up a new avenue of investigation in understanding disease pathogenesis. This is especially significant considering the potential value of DCs as disease-modifying biologic agents.¹⁶⁶

We have described a pivotal role for c-kit-expressing DCs in the generation of Th2 and Th17 cells, with subsequent development of allergic airway inflammation.³⁰ Therefore, inhibition of c-kit signaling could have long-term effects beyond beneficial action on the

acute effects of mast cell/eosinophil degranulation and production of inflammatory mediators. An interesting question is whether therapy using adoptive transfer of DCs could permanently redirect the T-cell immune response away from Th2/Th17 toward Th1 or, even more ideally, toward a Treg response. DCs in which c-kit was disabled might provide such a candidate population. Our work suggests that c-kit-deficient DCs produce little IL-6, do not express the Th2-promoting Jagged-2, and tend to drive T-cell production of IFN- γ , presumably via IL-12 production, therefore hypothetically possessing the qualities needed to redirect the immune response in the manner we describe.³⁰ However, as mentioned in a previous section, Jagged-2 expression in Lin-Sca-1⁺c-kit⁺ HPCs has also been associated with Treg cell development.⁹⁷ The questions arise: Do DCs express high levels of Jagged-2/Jagged-1 without c-kit upregulation under any condition? Would this DC phenotype favor immunosuppression rather than Th2 development? In future studies it will be important to learn more about the relative importance of c-kit and Notch signaling in different diseased states to be able to develop better therapeutics.

Although upon initial examination a different pathway may be involved, could the results of Zitvogel and colleagues be explained by a similar mechanism(s) to that we describe? They found that DCs were involved in the activation of NK cells in the setting of non-c-kit-expressing GISTs and that ckit signaling appeared to inhibit that activation.⁵² In these particular cases, Gleevec, which was ineffective on the tumors because they did not express c-kit, was able to reverse the inhibitory effect of c-kit signaling on NK cell activation *in vitro*. Importantly, this effect appeared to be operative *in vivo* because NK cell activation could be demonstrated in GIST patients in the absence of c-kit mutation. In these studies, IFN- γ was produced by a higher percentage of NK cells, from Gleevec-treated subjects versus nontreated controls, when cultured with DCs in the presence of bacterial LPS.⁵² It is possible that inhibition of IL-6 production via the mechanism we have described could have been responsible for the observed effects. Although the literature is somewhat contradictory, there are reports that IL-6 can inhibit NK cell activation.¹⁶⁷ Therefore, it is possible that blocking c-kit-mediated IL-6 production might enhance NK cell activation. Such a mechanism would be directly in line with what we have shown in the setting of airway disease.

Furthermore, although Zitvogel and co-workers did not examine this particular aspect, c-kit-negative DCs might promote a Th1 response in the tumor microenvironment, which could have obvious benefits. Presumably in the absence of c-kit, Jagged-2 would also not be expressed, potentially allowing the interaction of Delta-like ligands 1 and 4 with Notch 1/3 on T cells, resulting in the combined effect of downregulating IL-4 receptor signaling as well as transactivation of the *Tbx21* gene and subsequent T-bet expression.^{42,43,168,169} Again, this would be compatible with our observation that c-kit-negative DCs enhance IFN- γ production by T cells. Carrying this idea one step further, as discussed above, Wang and colleagues, reported that T cells tolerized to an experimental tumor antigen are able to produce Th1-associated cytokines when stimulated *in vitro* with bone marrow-derived DCs treated with Gleevec.¹³¹

It is interesting to speculate what it might mean for the possible efficacy of DC-based biologic therapies involving cells that do or do not express c-kit to redirect the immune response in a manner tailored to the particular disease. Steinman has recently written about the multifaceted multifunctional nature of DCs being essentially a largely untapped reserve of promise for development of vaccines and other DC-based biologic disease-modifying strategies.¹⁶⁶ Clearly though, many questions remain. For example, blocking c-kit with the intention of inhibiting Th2 might have undesirable consequences if T-cell tolerance were broken and/or a robust Th1 response ensued. With the development of modified forms of

Gleevec with fewer deleterious side effects, such studies could be developed in animal models and clinical trials.

Conclusions

The receptor tyrosine kinase that has emerged as the most prominent regulator of diverse functions in the immune system is clearly c-kit. Recent studies show a previously unrecognized role of this receptor in DCs in regulating the adaptive immune response.^{30,52} The cardinal rule for tyrosine kinase receptor activation is that phosphorylation and dephosphorylation should be stringently regulated. Dysregulation in either of these key events leads to altered function of the receptor. While c-kit function has been best studied in the context of mast cell biology, recent studies, including our own, highlight additional functions of this receptor in the immune system, particularly with respect to DC function.^{30,52} Harnessing the potential of these DCs in immunotherapy could lead to treatments for many tumors as well as for combating viral infections, both of which require significantly high levels of IFN- γ to activate the cytolytic CD8 T cells. If c-kit is a key player in promoting allergic disease/asthma in humans, it provides an opportunity to better control this disease using c-kit-directed therapies, such as improved versions of Gleevec with attenuated cardiotoxicity. Future studies are needed to determine whether c-kit signaling also promotes memory responses to allergens. Expression of c-kit in microglia raises the role of this molecule in promoting Th17 responses in diseases such as multiple sclerosis. It is clear that the c-kit ligand SCF exerts different effects in soluble versus membrane-bound form that arise from differential splicing events. Similarly, different isoforms of c-kit are also known. Beyond this knowledge of existence of alternate forms of c-kit and SCF, not much is known about how these different forms are generated. A better understanding of the generation of these splice forms and their signaling abilities will be important in the design of more efficient therapeutic targets targeting this receptor/ligand system. Another exciting area for future development is interactions between c-kit and Notch in regulating immune responses to allergens, pathogens, and various danger signals within the host.

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Figure 1.

Dual upregulation of c-kit and membrane-bound stem cell factor (mSCF) on dendritic cells by allergens and allergy-inducing adjuvants promotes IL-6 and Jagged-2 expression. Dendritic cells stimulated with cholera toxin, house dust mite, or prostaglandins upregulate c-kit and mSCF expression that is mediated by cyclic adenosine monophosphate (cAMP).³⁰ c-kit activation by mSCF via cell–cell interactions triggers sustained downstream activation of the PI3-kinase/ protein kinase B (AKT) pathway³⁰ and possibly the mitogen-activated protein kinase and Janus kinase/signal transducers and activators of transcription pathways,¹⁸ resulting in increased production of IL-6, whose transcription is known to be dependent on nuclear factor and NF-IL-6¹⁷⁰ and may also involve Notch–Notch ligand interactions. Activation of c-kit also upregulates expression of Jagged-2³⁰ and Notch (Notch).⁸⁰ The red and blue arrows indicate expression of molecules upregulated and downregulated, respectively, by activation of the c-kit–PI3 kinase axis.