Molecular mechanisms in allergy and clinical immunology

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Signaling molecules as therapeutic targets in allergic diseases

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A molecular understanding of physiologic and pathologic processes requires complete knowledge about the signal transduction mechanism of involved cells. Signal transduction research is a rapidly growing field in basic science. Unlike intercellular inflammatory mediators, signaling molecules show less functional redundancy. This allows inhibition of multiple cytokines/mediators by blocking one common signaling molecule. Interference with signaling pathways has shown significant potential for inhibition of fundamental processes as well as clinical phenotype of allergic diseases. The purpose of this review was to provide a theoretical classification of signaling molecules based on their function and to analyze various strategies for developing effective signaling inhibitors for allergic diseases. (J Allergy Clin Immunol 2003;112:241-50.)

Key words: *Signal transduction, therapy, allergy, asthma*

Signaling processes are fundamental to functioning of cells. As a consequence, they are responsible for development of both normal and abnormal phenotypes. For this reason signaling molecules are logical targets for therapeutic intervention in allergic diseases. Many signaling molecules have already been studied for their role in the pathogenesis of allergic diseases. For a detailed review of specific signaling pathways we would like to refer readers to some outstanding review articles that have been published in this journal in recent years.[1-5] In the following review we provide a theoretical framework to develop strategies for signaling inhibition and analyze existing studies involving signaling inhibition of allergic phenotype.

Abbreviations used

Blk:	B-cell lymphocyte kinase
BLNK:	B-cell linker protein
dsRNA:	Double-stranded RNA
ESE:	Epithelium-specific Ets factor
Ets:	Erythroblastosis virus E26 oncogene homologue
GTP:	Guanosine triphosphate
Id:	Inhibitor of differentiation
IκB:	Inhibitor of kappa B
JAK:	Janus kinase
LAT:	Linker for activation of T cells
Lck:	Lymphocyte-specific protein tyrosine kinase
MAPK:	Mitogen-activated protein kinase
MyD:	Myeloid differentiation factor
NEDD:	Neural precursor cell expressed developmentally downregulated
NF-κB:	Nuclear factor kappa B
ODN:	Oligodeoxynucleotide
PIAS:	Protein inhibitor of activated STAT
PKC:	Protein kinase C
RISC:	RNA-induced silencing complex
SHIP:	SH2-containing inositol 5-phosphatase
SHP:	SH2 domain-containing phosphatase
siRNA:	Small interfering RNA
SLP-76:	SH2 domain-containing leukocyte protein of 76 kd
SOCS:	Suppressor of cytokine signaling
Src:	Rous sarcoma oncogene homologue
STAT:	Signal transducer and activator of transcription
SUMO:	Small ubiquitin-related modifier
Syk:	Spleen tyrosine kinase
T-bet:	T-cell–specific T-box transcription factor
ZAP-70:	Zeta-chain associated protein kinase 70 kd

CLASSIFICATION OF SIGNALING MOLECULES

Functional classification

Stimulatory vs inhibitory signaling molecules. Signaling molecules might belong to proteins, lipids, lipoproteins, glycoproteins, nucleotides (cyclic and noncyclic), and ions. Although of structural importance the chemical nature of signaling molecules does not predict their functions. For this reason, signaling molecules are best classified on the basis of their functions. Most signaling molecules are stimulatory, ie, the signal results in elicitation of a cellular function. Examples include most kinases, guanosine triphosphate (GTP)–binding proteins, adapter proteins, cyclic nucleotides, and others. On the other hand, there are signaling molecules that primarily function as inhibitors of cellular functions. The examples of the latter include suppressor of cytokine signaling (SOCS), some phosphatases (eg, SH2 domain-containing phosphatase [SHP-1], SH2-

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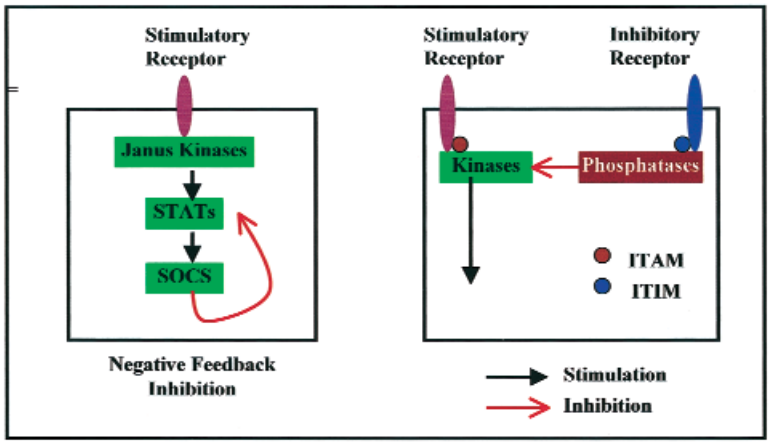

FIG 1. Homeostatic regulation of signaling mechanism. Two different regulatory mechanisms are shown. The *left panel* is an example of negative feedback mechanism, whereas the *right panel* illustrates a counter-regulatory mechanism.

containing inositol 5-phosphatase [SHIP], phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase), and protein inhibitor of activated STAT (PIAS). The inhibitory signaling molecules are frequently induced as a result of activation of stimulatory molecules (Fig 1). For example, the activation of the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway leads to the induction of SOCS,[6-8] which then inhibits signal transduction via the JAK-STAT pathway (negative feedback mechanism). SOCS block the JAK-STAT signaling pathway by interfering with target binding sites and by targeting the signaling proteins to proteosomal degradation.[9] In other instances, inhibitory signaling molecules are recruited to an inhibitory receptor. Stimulatory receptors frequently have the so-called immunoreceptor tyrosine-based activation motif sequences that, when phosphorylated (Fig 1), serve as the docking site for other cytosolic signaling molecules.[10] In contrast, inhibitory receptors have immunoreceptor tyrosine-based inhibitory motif sequences.[11] On coligation with a stimulatory receptor the ITIM is phosphorylated, which results in the recruitment of phosphatases such as SHIP-1.[12] The foregoing then abrogates further signal transduction by the stimulatory receptors. An example of this inhibition is co-ligation of FcγRII with FcεRI, which results in inhibition of mast cell degranulation. The activation of this type of inhibitory receptors is one postulated mechanism of action of intravenous immunoglobulin therapy in many diseases.[13] Therefore, one approach to therapeutic intervention of signaling pathways is to activate an inhibitory receptor.

Specific regulators vs master regulators. Many signaling molecules have specific and restricted functions in the cell. Function-specific signaling molecules are typically found at the proximal and distal ends of a signaling pathway. If a receptor regulates a specific function of a cell, usually the receptor-proximal signaling molecules are likely to be specific for that particular cell function (Fig 2). An example is T-cell signaling leading to IL-2 production and cell proliferation. One of the receptor-proximal signaling molecules that are specific for the foregoing functions is the lymphocyte-specific protein tyrosine kinase (Lck).[14] Although Lck is present in lower quantities in other cells, its absence primarily affects T-cell growth and function. An example of a function-specific distal signaling molecule is the myosin light chain kinase, which controls cell motility.[15] Myosin light chain kinase is not specific for any particular receptor or cell type. Therefore, its inhibition blocks migration of most cell types.

Some signaling molecules control many cellular functions by regulating multiple signaling pathways. These signaling molecules can be called master regulators (Fig 3). Typically, signaling molecules that function upstream of multiple signaling pathways function as master regulators. Examples of such upstream regulators are heterotrimeric GTP-binding proteins (G proteins[16]) and receptor-associated kinases (eg, JAKs, Rous sarcoma oncogene homologue [Src] kinases, and spleen tyrosine kinases [Syks]).[17-19] Some of these upstream regulators do not have any enzymatic activities and yet function as key signaling regulators. Myeloid differentiation factor 88 (MyD88) is such a regulator, which controls activation of nuclear factor kappa B (NF-κB) and innate immune response.[20] Mitogen-activated protein kinases (MAPKs) represent a convergence point of many upstream signaling pathways.[21] They control many downstream signaling pathways, transcription factors, and histone remodeling. As such, MAPKs are master signaling regulators. There are other master regulators that control one or more cellular processes such as differentiation. An example is GATA-3, which when activated initiates and sustains the differentiation of T_H2 cells.[22]

Expression-based classification

Most signaling molecules are nonspecific. However, there are a few signaling molecules that are relatively specific for certain cell types.

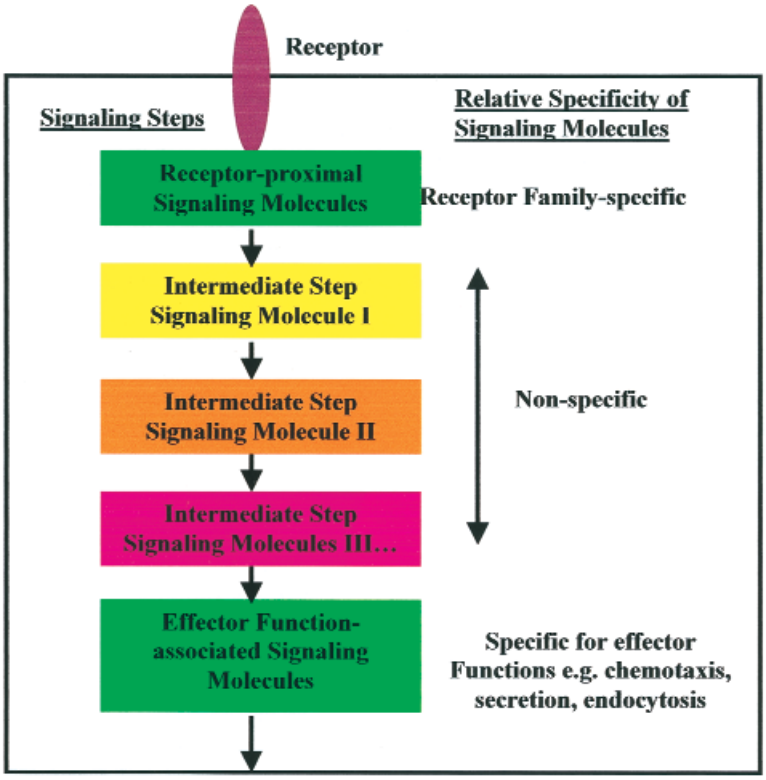

FIG 2. Specificity of signaling molecules. Specificity is usually determined by the position of the signaling molecule in the hierarchy of the signaling cascade. Receptor-proximal molecules are usually specific for the receptor family, whereas the effector function–associated molecules are relatively specific for the function. The intermediate signaling molecules are usually nonspecific. A right combination (quality and quantity) of nonspecific signals elicits a specific cellular function.

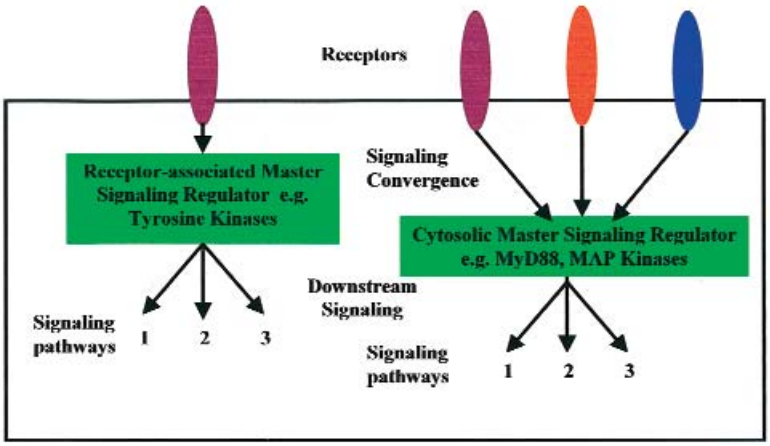

FIG 3. Examples of master signaling regulators. The *left panel* shows a master regulatory molecule that is receptor-associated and transduces signals through multiple downstream pathways. The *right panel* shows an example of a master regulatory molecule, which is not receptor-associated and functions downstream of many receptors. Signals from multiple surface receptors converge on this signaling molecule, which then transduces signals through multiple pathways.

Immune-specific signaling molecules. Many signaling molecules are predominantly expressed in lymphoid and myeloid cells. Examples include protein kinase C (PKC)ϕ,[23] Lck,[14] linker for activation of T cells (LAT),[24] B-cell linker protein (BLNK),[25] B-cell lymphocyte kinase (Blk),[26] and others. Lck is an Src family tyrosine kinase that initiates TCR signal transduction.[19] As discussed before, it is considered a master regulator of TCR signaling. Mice deficient in Lck have severely impaired thymocyte development.[27] Lck deficiency in humans results in

severe combined immunodeficiency.[28] For that reason pharmacologic targeting of Lck should never be complete. Lck recruits another tyrosine kinase, zeta-chain associated protein kinase 70 kd (ZAP-70), to TCR, which in turn phosphorylates adaptor proteins LAT and SH2 domain-containing leukocyte protein of 76 kd (SLP-76). Phosphorylated LAT and SLP-76 are essential for formation of macromolecular signaling complexes at the membrane and, as a result, for downstream signaling.[29,30] Similarly to Lck, LAT or SLP-76 deficiency in mice causes severe impairment of lymphopoiesis. PKCϕ is expressed mainly in T cells and activates NF-κB in response to TCR signals. Inhibition of PKCϕ leads to abnormal T-cell activation.[23] BLNK is a B-cell counterpart of SLP-76.[25] Blk is Src type tyrosine kinase, which, together with Lyn and Fyn, is activated after B-cell receptor cross-linking. The mice deficient in Blk do not demonstrate any B-cell defects, suggesting that Blk functions can be easily taken over by Lyn and Fyn.[31] For that reason Blk alone is not a good pharmacologic target. Targeting of molecules discussed in this paragraph is less likely to affect functions of other cells and tissues. However, most of them are critical for immune cell function, and complete inhibition might result in immunodeficiency.

Airway-specific signaling molecules. Very few signaling molecules have been identified that are specific for airway epithelial cells. nPKCη, a member of the novel PKC family, is primarily present in epithelial cells.[32] Recently it was shown that epithelium-specific Ets factor (ESE-3), a member of the erythroblastosis virus E26 oncogene homologue (Ets) domain transcription factor family, is specifically expressed in airway epithelial cells.[33] ESE-3 acts as a transcriptional repressor of gene expression. Targeting the foregoing molecules might allow modification of epithelial function in a specific manner.

Nonspecific signaling molecules and biologic outcome. Although many signaling molecules are relatively nonspecific, ie, most cells express them, the effect of their inhibition might sometimes be fairly tissue- or organ-specific. Examples include cyclophilins,[34] FK506-binding protein,[35] and phosphodiesterases.[36] Cyclophilins, on binding with cyclosporine A, activate calcineurin. The latter has phosphatase activity, which is turned on by calcium signaling. Activated calcineurin dephosphorylates the transcriptional factor NF-AT. Dephosphorylated NF-AT translocates to the nucleus and initiates transcription of many genes.[37] Phosphodiesterases break down cyclic nucleotides such as cyclic adenosine monophosphate or cyclic guanosine monophosphate. Despite widespread tissue distribution of cyclophilins or phosphodiesterases, cyclosporines and phosphodiesterase inhibitors have fairly restricted tissue-specific actions. It is likely that this tissue-specific action is the result of inhibition of specific isoforms of the signaling molecule in a given tissue. However, it is also possible that some cells/tissues use alternative signaling pathways in the presence of an inhibitor and are less affected by the inhibitor. Thus, it is very important that the effect of signaling inhibitors is tested on multiple organs in an animal model.

STRATEGIES FOR SIGNALING INTERFERENCE

Inhibition of pro-allergic molecules

Inhibition of pro-allergic signaling molecules is a straightforward strategy. The development of inhibitors for many pro-allergic signaling molecules and early human trials with some of them are now underway. Examples include Syk,[38] JAK-STATs,[39] and MAPKs.[40]

Stimulation of anti-allergic molecules

Counter-regulatory molecules. Counter-regulatory molecules are those that are physiologically induced by stimulatory signaling pathways and represent the normal homeostatic mechanism. These counter-regulatory molecules inhibit further transduction of the signal by directly antagonizing one of the upstream molecules. SOCS and PIAS are induced by the JAK-STAT pathway and block the activation of STAT transcription factors. Overexpression of SOCS blocks arthritis-associated inflammation[41] and viral immune response.[42]

Inhibitory signaling molecules. Inhibitory signaling molecules are not induced by stimulatory receptors. Their activation might require engagement of an inhibitory receptor. The inhibitory signaling molecules are often phosphatases, and examples include SHP-1, SHIP, CD45, and MKP. SHP-1 physiologically downregulates airway inflammation. A natural deficiency of SHP-1 in moth-eaten mice causes enhanced airway eosinophilic inflammation in an asthma model.[43] Another type of inhibitory signaling molecule is a binding protein that associates with a stimulatory signaling molecule and neutralizes or sequesters it from the signaling action. Inhibitor of kappa B (IκB) is such an example. It binds to NF-κB and prevents its translocation to the nucleus. Interference with IκB augments NF-κB function.[38]

Anti-allergic molecules. Some signaling molecules and transcriptional factors exhibit anti-allergic properties through their effect on immunodeviation and immunomodulation. An example is T-cell–specific T-box transcription (T-bet), a transcriptional factor that promotes T_H1 and antagonizes T_H2 differentiation. In addition to its effect on immunodeviation, T-bet might control airway responsiveness. Mice deficient in T-bet have increased baseline airway hyperreactivity.[44]

SIGNALING MOLECULES AND ALLERGIC PHENOTYPE

Strengths and weaknesses of knockout models

The biologic relevance of signaling molecules is frequently studied in the model of null mutation. With this approach many signaling molecules have been shown to contribute to T_H2 inflammation, airway hyperreactivity, and increased mucus production. A list of signaling molecules that have been shown to regulate various aspects of allergic phenotype is shown in Table I. The strength of this approach is that it is a definitive approach. The weak-

TABLE I. Interference with signaling molecules and allergic phenotype

Inhibition or deficiency of	Eosinophilic inflammation	IgE	Airway reactivity	Mucus production
Fgr tyrosine kinase	↓			
Tyk2	↑	↑		
Syk tyrosine kinase	↓			
SHP-1	↑		↑	↑
Ras	↓		↓	
ERK MAPK	↓			
P38 MAPK	↓			
STAT4	↑	↑	↑	↑
STAT6	↓	↓	↓	↓
p50 Rel (NF-κB)	↓			
c-Rel	↓	↓	↓	
GATA-3	↓	↓		↓
NFATp	↑			
T-bet	↑	↑	↑	
REF1	↓			
Bcl-6	↑			
Id2		↑		

nesses include the fact that the importance of the signaling molecule during embryonic and postembryonic development might influence the subsequent expression of the allergic phenotype. In this regard conditional knockout models are preferred.[45]

Advantages and disadvantages of pharmacologic inhibitors

There are many low molecular weight inhibitors for various signaling molecules. The inhibitors are mostly cell permeable and are easy to use. The major disadvantages include relative lack of specificity. Many inhibitors block additional signaling pathways nonspecifically at higher concentrations.

Emerging and novel RNA targeting approaches (antisense, small interfering RNA)

The synthesis of chemical inhibitors typically requires a complete understanding of the three-dimensional structure of the target signaling molecules. This information is not available for many signaling molecules. An alternative approach is to specifically block the synthesis of the signaling molecule. The latter can be achieved by specifically targeting the mRNA for the protein. There are 2 different approaches to block mRNA in a specific manner. Antisense oligodeoxynucleotides (ODNs) have been used for many years; they take advantage of the destruction of RNA-DNA duplex by RNAse H.[46] Various modifications of the nucleotide backbone improve the half-life and duration of action.[47] Specificity of action can be improved by screening the GeneBank database (www.ncbi.nlm.nih.gov) for cross-reactivity. Appropriately selected antisense ODNs are very effective in blocking protein synthesis. The disadvantages include short half-life and low level of cell permeability. The antisense ODN approach has previously been used to study allergic inflammation. Antisense ODN for GATA-3 has been shown to block T_H2-type eosinophilic inflammation in the airways.[48]

The newer approach takes advantage of a natural phenomenon in prokaryotes, in which small interfering RNA (siRNA) specifically blocks the synthesis of mRNA. Long double-stranded RNAs (dsRNAs; typically greater than 200 nucleotide) can be used to silence the expression of target genes in a variety of organisms and cell types (eg, worms, fruit flies, and plants). On introduction, the long dsRNAs enter a cellular pathway that is commonly referred to as the RNA interference pathway.[49,50] First, the dsRNAs get processed into 20 to 25 nucleotide siRNAs by an RNase III–like enzyme called Dicer (initiation step). Then, the siRNAs assemble into endoribonuclease-containing complexes known as RNA-induced silencing complexes (RISCs). The siRNA strands are then unwound to form activated RISCs. The siRNA strands subsequently guide the RISCs to complementary RNA molecules, in which they cleave and destroy the cognate mRNA (effector step). This sequence-specific degradation of mRNA results in gene silencing.

STRATEGIES TO DEVELOPING SIGNALING INHIBITORS

Blockade of synthesis

There are no pharmacologic inhibitors to specifically block the synthesis of a single signaling molecule. The antisense and siRNA approach has been discussed in the previous section.

Inhibition of enzymatic activity

There are a wide variety of pharmacologic inhibitors that affect the enzymatic action of many signaling molecules. Many inhibitors have been developed that block both receptor and nonreceptor tyrosine kinases. Examples include inhibitors for Src family kinases, JAKs, and

epidermal growth factor receptor kinase. The mechanism of inhibition of Src-type kinases by the inhibitor PP1 has been by crystallography.[51] PP1 binds to the adenosine triphosphate–binding pocket of Src-type kinases. Examples of other enzymatic inhibitors include PD98059 for MEK1/2[52] and pyridinylimidazole (diaryl imidazole) inhibitor (eg, SB203580) for p38 MAPK.[53]

Interference with modular domain-based protein-protein interactions: Inhibition of dimerization

Many signaling molecules undergo dimerization after activation. One of the effects of dimerization is the specific translocation of the signaling molecule to the target intracellular compartment. An example of dimerization inhibitors is N-[(1,3-benzodioxol-5-yl)methyl]-1-[2-(1H-imidazole-1-yl)pyrimidin-4-yl]-4-(methoxycarbonyl)-piperazine-2-acetamide (BBS-1). BBS-1 blocks dimerization of the inducible nitric oxide synthase and thereby inhibits its activity.[54]

Blockade of post-translational modifications

Post-translational modification is a major mechanism by which signaling molecules transduce signal downstream. Frequent post-translational modifications include phosphorylation, N-terminal acylation, C-terminal prenylation, ubiquitination (addition of ubiquitin), sumoylation (addition of ubiquitin-related protein, small ubiquitin-related modifier [SUMO]), neddylation (addition of ubiquitin-related protein, neural precursor cell expressed developmentally downregulated [NEDD]), nitrosylation, methylation, acetylation, and adenosine diphosphate–ribosylation. N-acylation and C-prenylation allow signaling molecules to interact with the membrane cell and specific lipid-rich structures within the cell membrane such as rafts.[55] It is believed that most of signaling starts in rafts. Therefore, the interference with membrane/raft localization of signaling molecules blocks signal transduction at the very early phase. N-acylation occurs in the form of myristoylation or palmitoylation.[55] Many critical signaling molecules undergo this modification (eg, Src tyrosine kinases Lck and Fyn in T cells, adaptor LAT or G proteins, and regulators of G protein signaling).[55-57] Inhibitors of polyunsaturated fatty acids include statins (eg, lovastatin) and 2-bromopalmitate.[56,57] The treatment of T cells with 2-bromopalmitate results in redistribution of Src kinases from the cytoplasmic membrane to the perinuclear region. As a result, Src kinases can no longer be activated by TCR and cannot phosphorylate key membrane-localized signaling substrates like ZAP-70, LAT, phospholipase Cγ1, and phosphoinositide 3-kinase. Impaired activity of these molecules results in the inhibition of T-cell receptor signaling.

Prenylation is a covalent addition of hydrophobic polymers of hydrocarbon isoprene. C-terminal prenylation occurs through the involvement of 2 enzymes, farnesyl and geranyl transferases. Allyl and vinyl forms of farnesols and geraniols block prenylation of signaling molecules.[58-61] Manumycin A is a natural inhibitor of protein farnesylation.[62] Geranylgeranyl transferase inhibitor 2147 (GGTI-2147) is a peptidomimetic inhibitor of protein geranylgeranylation. SCH 66336 is an example of tricyclic farnesyl transferase inhibitors. Ras GTP-ase is one of the molecules that undergoes aforementioned modifications.[63] The farnesylation is essential for membrane translocation of Ras. At the membrane Ras meets its activator Sos. Activated by receptor signaling, Sos induces exchange of nucleotide guanosine diphosphate to GTP, which activates Ras. Membrane localization of Ras is also critical for activation of membrane-bound Ras targets such as Raf-1. The inhibitors of prenylation block Ras-dependent cellular functions such as cell survival and secretion.[58,62]

Inhibition of intracellular translocation

Intracellular translocation is a critical element for proper signal transduction and cellular function. As described above, N-acylation and C-prenylation inhibitors block movement of signaling molecules (eg, Ras, Rho) to the cell membrane. Endocytosis of receptors and receptor-associated signaling molecules is another form of regulation of signal transduction. Inhibition of endocytosis interferes with signal transduction of the T-cell receptor.[64] Translocation of many signaling molecules and transcription factors into the nucleus is important for transcriptional regulation of cell function. Inhibition of nuclear transport of NF-κB by dimethylfumarate blocks responsive gene transcription, which might explain its clinical efficacy in psoriasis.[65]

Modulation of signaling by protein degradation

One important homeostatic mechanism of regulation of signaling processes is the proteosomal degradation of signaling molecules. Proteosomal degradation of proteins might activate but more commonly downregulate a signaling process.[66] An example of activation is the proteosomal degradation of IκB, which then allows NF-κB to migrate into the nucleus and initiate transcriptional processes.[67] Many more signaling molecules/pathways are actually inhibited as a result of proteosomal degradation. Examples include Src and Syk family kinases.[68] There are a number of mechanisms that target proteins for proteosomal degradation—ubiquitination, neddylation, and sumoylation (sentrination). Ubiquitination is a more thoroughly studied mechanism.[69] Inhibition of ubiquitination of IκBα by the pharmacologic agent Ro106-9920 blocks the NF-κB pathway.[70]

INTERVENTION WITH SIGNALING MOLECULES IN ALLERGIC DISEASES

Theoretically, master regulators are very attractive targets for pharmacologic intervention. Inhibition of a master regulatory molecule involved in the induction of the pathologic phenotype should block the disease process. Unfortunately, many master regulators are not cell- or

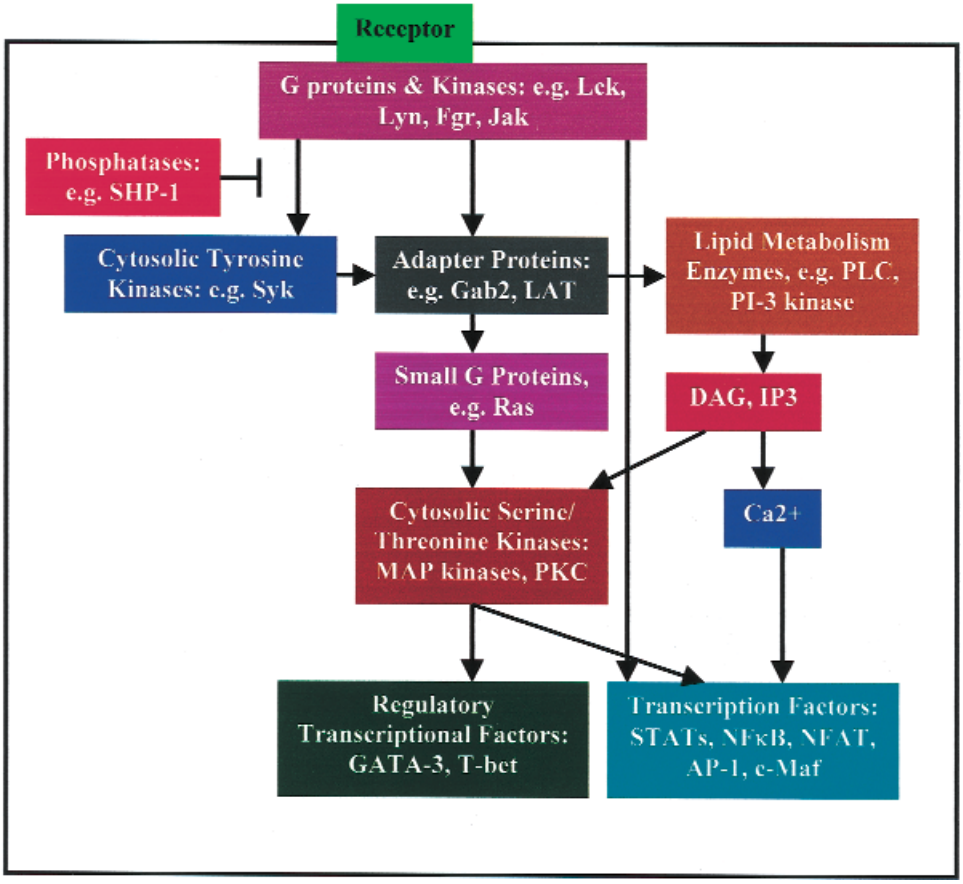

FIG 4. A schematic model of signal transduction from the receptor. Binding of the ligand to the receptor activates receptor-associated signaling molecules, eg, G proteins or tyrosine or serine/threonine protein kinases. The foregoing signaling molecules might activate cytosolic tyrosine kinases, eg, Syk, which leads to the recruitment of adapter protein. Some receptor-associated tyrosine kinases (eg, JAKs) directly activate transcriptional factors of the STAT family. Many phosphatases (eg, SHP-1) downregulate signaling processes by inactivating kinases at this stage. Adapter proteins, in turn, recruit downstream molecules such as lipid metabolism enzymes or small G proteins. The activation of these molecules results in the stimulation of cytosolic serine-threonine kinase cascade (eg, MAPKs) or calcium signaling. These pathways lead to the activation of various transcriptional factors.

disease-specific. On the other hand, the pharmacologic control of individual specific regulators might not be sufficient to reverse the pathologic phenotype.

Intervention with master regulators of allergic diseases

Inhibition. The master or specific role of many signaling molecules in allergic inflammation as well as their specificity for the diseases has been defined in knockout mice. Mice deficient in STAT6,[71] c-Rel,[72] p50 subunit of NF-κB,[73] have decreased eosinophilic airway inflammation or airway hyperreactivity when challenged with allergen (for the position of the molecule in the signaling cascade, refer to Fig 4). STAT6[71] and NF-κB[73] regulate the differentiation of T_H2 cells. The decrease of T_H2 cytokine production is believed to be responsible for the phenotype of the foregoing knockout animals. c-Rel regulates the expression of pro-inflammatory and, interestingly, T_H1 but not T_H2 cytokines.[74] Because the inhibition of the abovementioned molecules abrogates the entire inflammatory process, these proteins should be considered master regulators. STAT6 is an especially promising candidate for drug development because it is relatively specific for IL-4 and IL-13 signaling and regulation of T_H2 response. Mice deficient in STAT6 do not demonstrate any other major abnormalities. However, the blockade of the STAT6 pathway must be done with caution, because it might produce excessive T_H1 response and contribute to autoimmunity. Although STAT6 deficiency does not result in spontaneous autoimmune diseases, the course of experimentally induced T_H1 diseases like experimental autoimmune encephalomyelitis is more severe in these mice.[75] Mice deficient in c-Rel have impairment of B- and T-lymphocyte survival, differentiation, and function. In addition to abrogation of eosinophilic inflammation, these mice are resistant to autoimmune encephalitis.[74] We do not believe that c-Rel would be a good pharmacologic target because the systemic inhibition of this molecule would most likely evoke severe immunodeficiency. It is, however, possible that local organ-specific inhibition of this transcriptional factor will have limited immunosup-

pressive effects. Mice deficient in NF-κB (p50 Rel) have dysfunctional B cells. The inhibition of NF-κB might therefore produce immunodeficiency as well. Some master regulators (eg, GATA-3, ERK, p38, JunB) cannot be studied in knockout models because their deficiency is embryonic lethal.[76-78] To circumvent this problem alternative approaches have been developed. Examples include pharmacologic inhibition of p38 MAPK with pyridinyl imidazole inhibitors,[40] overexpression of dominant negative GATA-3 under the T-cell promoter,[79] and selective inhibition of JunB in the thymus.[71] Postembryonic inhibition of the foregoing signaling molecules shows marked reduction in airway inflammation, especially cytokine expression. GATA-3 is a master regulator of T_H2 differentiation.[80] GATA-3 is also essential for naïve T cells, hematopoiesis, and nervous system development. Mouse embryos deficient in GATA-3 show retarded growth, severe defects of brain and spinal cord, massive internal bleeding, and abnormalities in hematopoiesis.[76] We believe that in adults, the blockade of GATA-3 might result in bone marrow suppression and severe immunodeficiency unless done locally in a strictly regulated manner. T_H2 cytokine secretion is also affected in mice deficient in the tyrosine kinase Itk.[81] This kinase has not been examined yet in an animal model of airway inflammation.

Stimulation. T_H2 development and allergic inflammation are enhanced in animals with selective deletion of the transcription factors T-bet,[44] STAT4,[82] and Bcl-6[83] and the tyrosine kinase Tyk2.[84] T-bet and STAT4 are essential for T_H1 development.[80] Interestingly, mice deficient in T-bet demonstrate spontaneous (not allergen-induced) airway hyperreactivity. Overexpression of exogenous T-bet by retrovirus in T_H2 cells reverses the phenotype and converts these cells into IFN-γ–producing cells.[44] From the therapeutic viewpoint the elevation of T-bet leading to excessive T_H1 activity is potentially dangerous, because it might lead to autoimmunity. Accordingly, mice overexpressing another T_H1 master regulator STAT4 suffer from autoimmune colitis.[82] Bcl-6 is a transcriptional repressor, which negatively regulates genes involved in lymphocyte activation, differentiation, proliferation, and migration. Although the overactivation of this proto-oncogene might be useful in prevention of T_H2-type inflammation, it will result in marked toxicity. Overexpression of Bcl-6 in U2OS cell line induces cell growth arrest and apoptosis.[85]

Intervention with specific regulators of allergic diseases

Inhibition. Pharmacologic inhibition of specific signaling regulators results in blocking some but not all components of the disease. Selective impairment of IL-4 production is seen in c-Maf knockout animals.[86] Because differentiation of T_H2 cells and production of other T_H2 cytokines are not affected and the IgE level is normal, c-Maf does not seem to be a good therapeutic target. In mice deficient in Lyn kinase the differentiation of eosinophils (IL-5–mediated) and degranulation of mast cells are inhibited.[87] The basal eosinopoiesis in these mice, however, is not affected. This observation has obvious therapeutic implications. A peptide inhibitor of Lyn–IL-5 receptor interaction, when administered intrabronchially, inhibits eosinophilic inflammation in the mouse model of asthma.[88] However, a complete systemic blockade of Lyn might not be safe because the Lyn knockout mice suffer from autoimmunity as a result of the B-cell hyperactivity and autoantibody formation.[89] The deletion of another Src kinase, Fgr, in mice also results in the abrogation of eosinophil influx into the lungs.[90] Fgr is linked to CCR3 signaling. Although Fgr kinase is expressed in all myeloid cells and B cells, impaired eosinophil recruitment is the only defect in knockout mice. Therefore, pharmacologic inhibition of Fgr in humans should be relatively safe. Mast cell function is abrogated in abovementioned Lyn knockout[89] as well as Gab2-deficient mice.[91] Gab2 is an adapter protein, which plays the role in PI3K signaling. The gene targeting data are supported by experiments with PI3K inhibitors (Wortmannin), which block mast cell degranulation and leukotriene synthesis.[92] Therefore, inhibitors of the PI3K pathway are likely to prevent allergic inflammation. Gab2 is also a promising therapeutic target because the impairment of mast cell function is the only defect observed in knockout mice.

Stimulation. Some signaling molecules inhibit only specific aspects of allergic inflammation. GADD45 regulates TCR-induced and p38/JNK-mediated production of IFN-γ. It is also responsible for the activation-dependent cell death in T cells. Therefore, pharmacologic enhancement of GADD45 signaling might not be completely safe because it might result in excessive T-cell death and, similarly to T-bet and STAT4, evoke T_H1 autoimmunity. The IgE production is greatly enhanced in mice lacking inhibitor of differentiation (Id)2.[94] Id2 negatively regulates E2A (transcriptional factor)–mediated immunoglobulin class switching. Id2 by the inhibition of Rb protein is also involved in the inhibition of cellular differentiation and stimulation of the cell cycle. Id2 overexpression in thymocytes blocks T-cell development at the $CD4^-$, $CD8^+$, TCR^- stage and triggers lymphomas.[95] This potential oncogenic activity of Id2 disqualifies this protein as a potential therapeutic target for allergy prevention. The interference with the molecules of innate immune system can modulate the adaptive response. MyD88 knockout mice show impairment of T_H1 but not T_H2 responses. Because MyD88 is essential in Toll receptor as well as pro-inflammatory cytokine (eg, IL-1) signaling, enhancement of MyD88 function can lead to autoimmunity.[96]

In summary, many promising signaling molecules have been identified that modulate various aspects of allergic phenotype including eosinophilic inflammation, IgE production, airway hyperreactivity and remodeling. Because multiple etiologic factors are involved in the manifestation of asthma, it would make sense to initially target master signaling regulators that affect multiple elements of allergic phenotype. Experience with therapies targeting IgE or IL-5 suggests that targeting very narrow, "allergy-specific" molecules will produce some beneficial effects, but they are unlikely to impact on all cardinal aspects of

asthma. For this reason strategies to inhibit master regulators are highly desirable. The caveat of this approach is that such a "broad spectrum" signaling inhibition might have untoward effects, which have to be carefully studied and weighed against the beneficial effects.

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