

Allergy Review Series VII: Intracellular signaling and regulation of allergic reactions

Editorial

Signal transduction mechanisms as new targets for allergists

M. L. Kowalski¹, R. Alam²

¹Department of Clinical Immunology and Allergy, Medical University of Łódź, Łódź, Poland; ²Division of Allergy and Immunology, Department of Internal Medicine, Texas University, Galveston, TX, USA

Prof. M. L. Kowalski
Department of Clinical Immunology and Allergy
Medical University of Łódź
251 Pomorska Street
92 213 Łódź
Poland

Accepted for publication 30 October 2000

Introduction

The basic paradigm of cell biology that extracellular signals are recognized by specific receptors on target cells has been widely appreciated by allergists and immunologists. This information is no longer considered purely a basic science, but, on the contrary, it has become the core of applied allergy/immunology with direct relevance to our clinical practice. The increasing understanding of the structure and function of cell-surface receptors is being used to design new receptor agonists (e.g., long acting β_2 -agonists, glucocorticosteroids) and antagonists (e.g., antihistamines, leukotriene LT_1 receptor antagonists) which allow for influencing cell functions and modulating allergic reactions in clinical settings. Interaction of IgE molecules with specific receptors, a crucial event for atopic reaction, has been recently targeted for therapeutic use employing recombinant anti-IgE antibodies (1). At the same time, it is becoming increasingly obvious that the outcome of agonist/receptor interactions is influenced by intracellular events that activate the signaling machinery of the cell. A single agonist elicits different responses in different target cells even though it binds to the same receptor, because the receptor may activate different signaling pathways in different cells.

Basic mechanisms of intracellular signal transduction

Signal transduction means communication between a receptor and molecules that are directly involved in ligand-specific cellular responses. A variety of molecules are involved in this communication, and a brief list of various classes of signaling molecules is shown in Table 1. It should be noted that many structural molecules are involved in signal transduction mechanisms. Signal transduction employs a variety of mechanisms in facilitating protein–protein interactions. Typical examples include phosphorylation/dephosphorylation of proteins; association with modular adapter proteins, scaffolding proteins, and chaperons; interaction with GTP or ions (Ca^{2+}); acylation; and ubiquitination (Fig. 1). The outcome of this protein–protein interaction is the translocation of the messenger to its subcellular destination and activation of the effector mechanism of cellular functions (e.g., chemotaxis, degranulation, gene transcription, proliferation, and apoptosis).

Most signaling proteins are not receptor-specific. Yet the outcome of the signal transduction process is relatively receptor-specific. How a group of nonspecific signaling molecules elicits a specific cellular function is not well understood at the moment. One explanation is that the signaling mechanism functions like a combina-

Table 1. Various classes of signaling molecules

- Enzymes - Transferases (kinases, acylases) - Hydrolases - Phosphatases - Proteases (e.g., caspases) - GTP-binding proteins (heterotrimeric and small GTPases) - Lipid hydrolases - Binding proteins - Adapter proteins (e.g., Grb2) - Motif proteins (e.g., SLAM) - Scaffolding proteins (e.g., Mpl) - Chaperons (e.g., HSP90) - Ions and metabolites - Ca²⁺ - Reactive oxygen species - Cyclic nucleotides (cAMP, cGMP) - Lipids (e.g., DAG) - Cytoskeletal proteins (e.g., actin and its regulatory proteins) - Motor proteins (myosin, kinesin, microtubules) - Transcription factors - Inhibitors (e.g., p21, IκB, SOCS) - Others

tion lock wherein a specific combination of signaling molecules determines the cellular response (Fig. 2). In this model, no single signaling molecule is specific for a given cellular function, but may be essential for it.

Significance of signal transduction for understanding the pathogenesis of allergic reactions

Recent studies suggest that aberrant or dysregulated intercellular signaling mechanisms may play a role in the pathogenesis of allergic responses. Since the discovery of IgE over three decades ago, significant progress has been achieved in understanding the mechanism of IgE production, including the molecular events of B-cell IgE switching and IL-4 and IL-13 receptor signaling (2, 3). It has been demonstrated that the isotype switching occurs sequentially from IgM to IgE via IgG4. Several transcription factors contribute to the regulation of the expression of epsilon and gamma-4 germline transcripts (4).

The new data provide a rational explanation for the observation that antigen-specific IgE and IgG4 are competitively generated during the allergic response, and their production may be modulated by specific immunotherapy (5). It is believed that understanding the mechanism that controls IgE production may be essential not only for dissecting the pathogenesis of atopic diseases, but also for intervention at the early stage of their development. The signaling through the FcεRI receptor in mast cells is the central event in the induction and maintenance of the allergic response. It is initiated by cross-linking of receptor-bound IgE molecules with multivalent allergens. This activates multiple signaling pathways, leading to the release of

mast-cell mediators and acute symptoms of allergy. Triggering the mast cell also leads to the induction of cytokine gene transcription with subsequent development of chronic allergic inflammation. Several polymorphisms of the β chain of the FcεRI receptor have been reported to be associated with allergic phenotypes in various populations (6). The intracellular signaling mechanism of mast-cell activation has been elucidated, and possible targets within signaling pathways are currently under investigation (7).

Allergic inflammation is the outcome of a Th2-type immune response. Investigation of the difference in signaling pathways that leads to differentiation into opposing T helper cells, Th1 and Th2, has remained a very active area of research. GATA-3, a member of the GATA family of transcription factors, is implicated in the differentiation of Th2 cytokines into T cells (8). More recently, a significant increase in GATA-3 expression has been demonstrated in asthmatic airways and in the nasal mucosa of patients with allergic rhinitis, suggesting a potential role for this transcription factor in the pathogenesis of allergic inflammation in the lower and upper airways (9, 10).

IL-4, which is essential for the induction of IgE synthesis, is capable of directing lymphocyte differentiation toward the Th2 type. It also participates in the development of eosinophilic inflammation by augmenting the expression of the adhesion molecule VCAM. The interaction of IL-4 with its receptor induces the activation of Janus kinase-1 (Jak-1) and Jak-3, which, in turn, phosphorylate the signal transducer and activator of transcription factor 6 (STAT6). Animal studies demonstrate that STAT6 knockout mice are not capable of generating Th2 cells and fail to mount allergen-specific IgE responses after an allergen challenge (11). In man, a genetic variant of IL-4Rα (a subunit of the IL-4 receptor) is strongly associated with augmented STAT6 activation, which is correlated with increased IgE synthesis and atopy (12). More recent studies provide evidence of increased STAT6 expression in human allergic mucosa, an expression which is downregulated by topical corticosteroids (13).

Other transcription factors have also been thought to play a role in the development of allergic inflammation. The synthesis of many mediators of allergic inflammation is under the control of the transcription factor NF-κB. Downregulation of NF-κB could lead to significant inhibition of inflammatory responses regardless of the nature of the triggers. In fact, one of the mechanisms of the anti-inflammatory effects of corticosteroids seems to involve their interaction with this transcription factor (14). It has been demonstrated that proteasome inhibitors, which block IκB degradation and thereby prevent NF-κB translocation to the nucleus, have anti-inflammatory actions and effectively decrease delayed-type hypersensitivity in animals (15).

The development of allergic inflammation depends

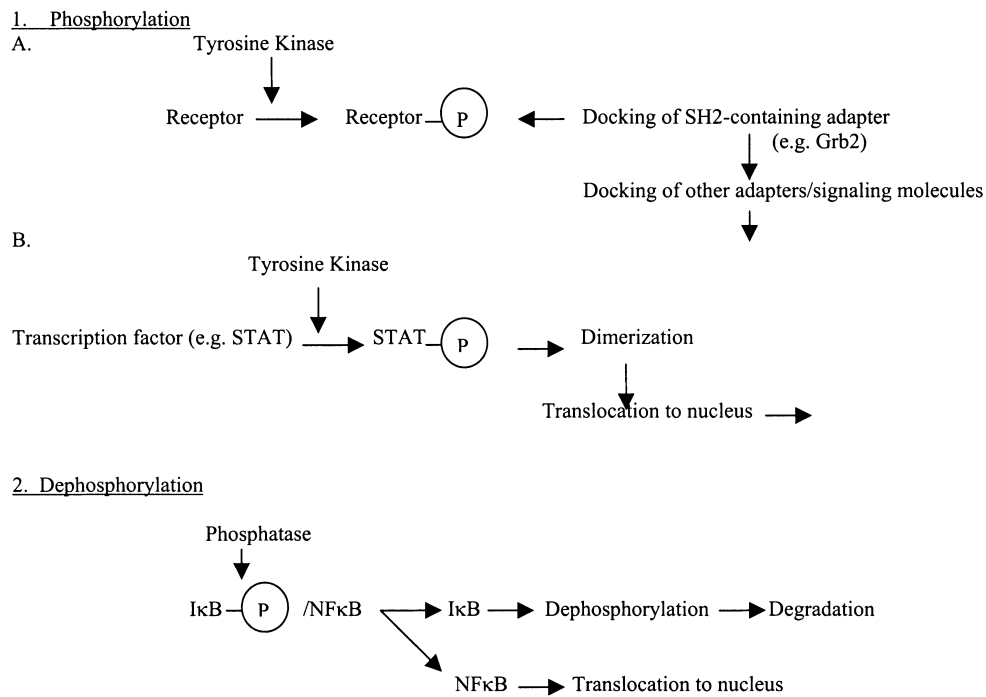


Figure 1. Examples of signaling mechanism.

on recruitment of inflammatory cells such as eosinophils into the tissues and their subsequent activation. Although many cytokines are known to regulate eosinophil differentiation, activation, and survival, IL-5 seems to play a dominant role in this process. The signal transduction mechanism of IL-5 has been partially delineated. The Lyn and Jak2 tyrosine kinases and p38 MAP kinase play a critical role in eosinophil differentiation (16, 17). Lyn appears to be relatively specific for eosinophil differentiation whereas Jak2 is important for hematopoiesis in general. Raf-1 kinase, which is a MAP kinase, is important for eosinophil survival, degranulation, and adhesion (16). MAP kinases (ERK 1/2 and p38) are critical for eosinophil degranulation and locomotion (18). These signaling

studies have demonstrated that various signaling molecules differentially regulate eosinophil functions. Thus, it is possible to interfere with a specific function of eosinophils by blocking one particular signaling pathway.

Practical aspects of understanding intracellular signaling mechanisms

The elucidation of the mechanism of action of proallergic molecules opens new prospects for development of treatment modalities based upon interference with their extra- and intracellular pathways. The understanding of the interaction of IgE with the FcεRI receptor allowed development of a humanized

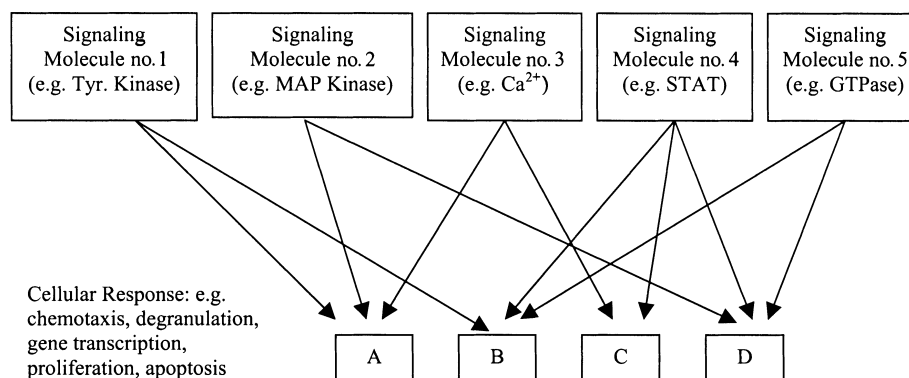


Figure 2. Hypothetical model of signal transduction leading to specific cellular responses. The model proposes that a specific function requires a combination of signals for optimal activation. The signals themselves are not specific for a given cellular function but individually may play an essential role in its activation. The principle of this model is that a combination of nonspecific signals constitutes a specific signal for a cellular function.

anti-IgE antibody, which is currently in clinical trials. Initial results demonstrate the capacity of humanized anti-IgE antibodies to downregulate IgE responses *in vivo* with concomitant amelioration of asthma (1, 19). Further research defining the pathways of mast-cell/basophil activation could lead to development of specific inhibitors to block the release of inflammatory mediators (20).

Several studies strongly suggest an important role for GATA-3, STAT6, NF κ B, c-Maf, and some species of NFAT in selective enhancement of TH2 responses, promising future therapeutic manipulation at this level (21). It is tempting to speculate that targeting Th2-specific transcription factors, such as GATA-3, by antisense approaches may shift a T-cell population toward the Th1 profile with concomitant clinically relevant modulation of inflammation.

Another approach to modulating the T-cell cytokine profile is the application of CpG oligonucleotides,

which are potent inducers of Th1 cells. In ragweed-sensitized mice, administration of CpG oligonucleotides resulted in the inhibition of IgE production, eosinophilic inflammation, and airway hyperreactivity (22). Clinical trials aimed at delineating the efficacy of CpG oligonucleotides in allergic diseases are currently being preformed.

Signal specific inhibition of cellular functions

Receptors are the primary target for pharmacologic intervention in modern medicine, and receptor antagonists are the mainstay of therapy for many diseases. Receptors are frequently coupled to multiple signaling pathways. Receptor antagonists block all these pathways, but, as a result, are prone to developing unwanted side-effects. In order to circumvent this problem, the concept of signal-specific

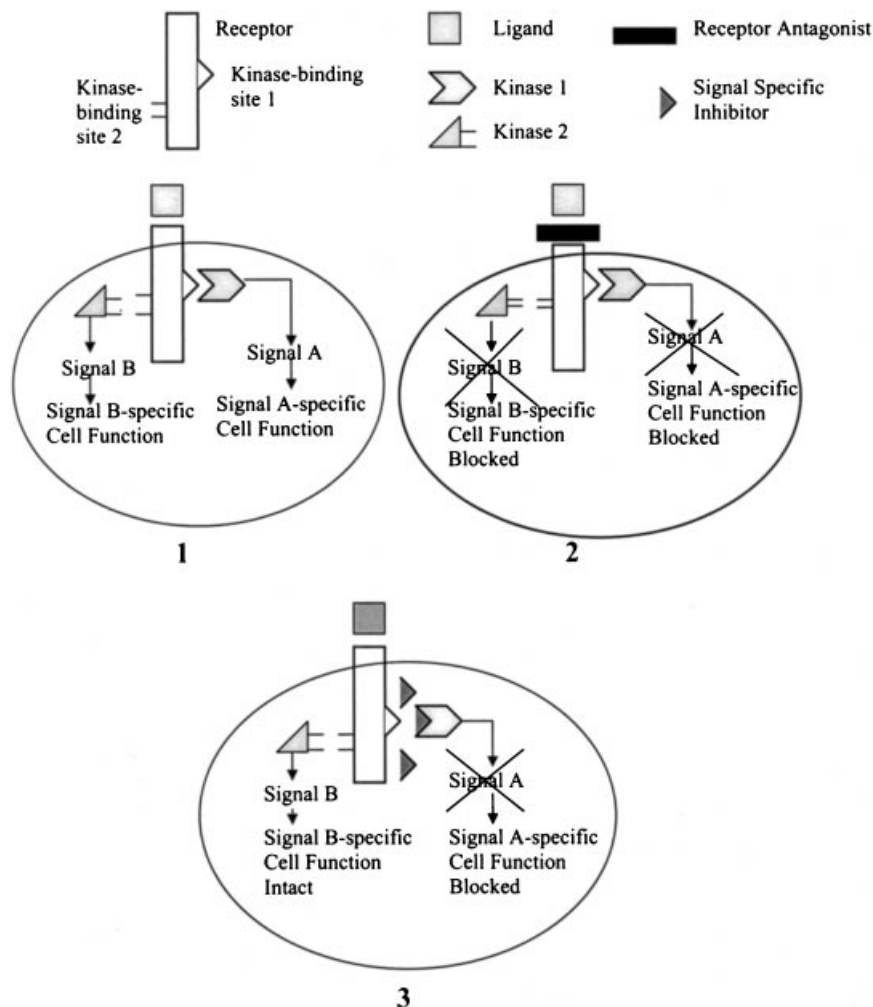


Figure 3. Rationale for signal specific inhibition of receptors. 1) Binding of ligand with receptor induces multiple signaling pathways (e.g., A and B) through distinct signal-generating sites (kinase-binding sites 1 and 2) within intracellular region of receptor. 2) Receptor antagonists block all cytosolic signals indiscriminately. 3) Signal-specific inhibitor is designed to mimic one kinase-binding site. This inhibitor competitively binds to kinase and prevents its access to receptor. Therefore, it blocks single signaling pathway, leaving other pathways intact (Ref. 23) (with permission of *Journal of Immunology*).

inhibition has been recently introduced (Fig. 3) (23). The rationale is that the intracellular domain of a receptor interacts with diverse signaling molecules and thereby activates multiple signaling pathways. If specific signal-generating sites within the intracellular domain of the receptor can be mapped and identified, it is theoretically possible to inhibit a single signaling pathway while sparing the others. One important aspect of this concept is that, unlike signaling molecules, which are nonspecific, the sequence of the signal-generating site of a receptor is relatively specific. Therefore, interference with the signal-generating site may confer some degree of specificity. The concept has successfully been tested in the model of IL-5 signaling via Lyn kinase in eosinophils. Based upon the sequence information of the receptor-binding site of Lyn, a cell-permeable Lyn-specific inhibitor has been developed. This inhibitor blocks Lyn, but not Jak2, activation by IL-5. Biologically, it

blocks eosinophil differentiation and survival. Furthermore, it prevents allergen-induced eosinophilic inflammation of the airways in a mouse model of asthma.

Conclusions

Signal transduction is a rapidly expanding field of sophisticated molecular research, which until recently seemed to be distant from the interest of allergist. The emerging information not only helps us to understand the pathophysiology of asthma and allergy but also raises a realistic hope of designing new therapeutic approaches to allergic diseases. Recent advances in this area justify the presentation of a series of review articles that will address various aspects of intracellular signaling relevant to the pathophysiology of allergic diseases.

References

1. PATALANO F. Injection of anti-IgE antibodies will suppress IgE and allergic symptoms. *Allergy* 1999;**54**:103–110.
2. CORRY DB, KHERADMAND F. Induction and regulation of the IgE response. *Nature* 1999;**401**:B18–23.
3. NELMS K, KEGAN AD, ZAMORANO J, RYAN JJ, PAUL WE. The IL-4 receptor: signaling mechanisms and biologic functions. *Annu Rev Immunol* 1999;**17**:701–738.
4. JABARA HH, LOH R, RAMESH N, VERCELLI D, GEHA RS. Sequential switching from μ to ϵ via $\gamma 4$ in human B cells stimulated with IL-4 and hydrocortisone. *J Immunol* 1993;**151**:4528–4533.
5. VERCELLI D, DEMONTE L, MONTICELLI S, DI BARTOLO C, AGRESTI A. To E or not to E. Can an IgE-induced B-cell choose between IgE and IgG4? *Int Arch Allergy Immunol* 1998;**116**:1–4.
6. SANDFORD AJ, SHIRAKAWA T, MOFFAT MF, et al. Localisation of atopy and beta subunit of high affinity IgE receptor (Fc ϵ RI) on chromosome 11q. *Lancet* 1993;**341**:332–334.
7. TURNER H, KINET J-P. Signaling through the high-affinity IgE receptors Fc ϵ RI. *Nature* 1999;**402**:B24–B30.
8. ZHANG DH, COHN L, RAY P, BOTTOMLY K, RAY A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2 specific expression of the interleukin-5 gene. *J Biol Chem* 1997;**272**:21597–21603.
9. NAKAMURA Y, GHAFAR O, OLIVENSTEIN R, et al. Gene expression of GATA-3 transcription factor is increased in atopic asthma. *J Allergy Clin Immunol* 1999;**103**:215–222.
10. NAKAMURA Y, CHRISTODOULOUPOULOS P, CAMERON L, et al. Upregulation of the transcription factor GATA-3 in upper airway mucosa after *in vivo* and *in vitro* allergen challenge. *J Allergy Clin Immunol* 2000;**105**:1146–1152.
11. SHIMODA K, VAN DEURSON J, SANGSTER MY, et al. Lack of IL-4 induced TH2 response and IgE switching in mice with disrupted Stat6 gene. *Nature* 1996;**380**:630–633.
12. MITSUYASU H, IZUHARA K, MAO X-Q, GAO PS, ARINOBU Y, ENOMOTO T. Ile5-Val variant of IL4R α upregulates IgE synthesis and associates with atopic asthma. *Nat Genet* 1998;**19**:119–120.
13. GHAFAR O, CHRISTODOULOUPOULOS P, LAMKHOUED B, et al. *In vivo* expression of signal transducer and activator of transcription factor 6(STAT6) in nasal mucosa from atopic allergic rhinitis: effect of topical steroids. *Clin Exp Allergy* 2000;**30**:86–93.
14. BARNES PJ, ADCOCK IM. NF- κ B: a pivotal role in asthma and a new target for therapy. *Trends Pharmacol Sci* 1997;**18**:46–50.
15. ELLIOT PJ, PIEN CS, MCCORMACK T, CHAPMAN ID, ADAMS J. Proteasome inhibition: a novel mechanism to combat asthma. *J Allergy Clin Immunol* 1999;**104**:294–300.
16. PAZDRAK P, OLSZEWSKA-PAZDRAK B, STAFFORD S, ALAM R. Lyn, Jak2 and Raf-1 kinases are critical for the anti-apoptotic effect of interleukin-5 whereas only Raf-1 kinase is essential for eosinophil activation and degranulation. *J Exp Med* 1998;**188**:421–429.
17. ADACHI T, CHOUDHURY BK, STAFFORD S, SUR S, ALAM R. The differential role of extracellular signal-regulated kinases and p38 mitogen-activated protein kinase in eosinophil functions. *J Immunol* 2000;**165**:2198–2204.
18. KAMPEN G, STAFFORD S, ADACHI T, et al. Eotaxin induces degranulation and chemotaxis of eosinophils through the activation of ERK2 and p38 mitogen-activated protein kinases. *Blood* 2000;**95**:1911–1917.
19. MILGROM H, FICK RB Jr, SU JQ, et al. Treatment of allergic asthma with monoclonal anti-IgE antibody. *N Engl J Med* 1999;**341**:1966–1973.
20. OKAZAKI H, SIRAGANIAN R. Signal transduction for secretion of basophils. *ACI Int* 1999;**11**:86–92.
21. GLIMCHER LH, SINGH H. Transcription factors in lymphocyte development – T and B cells get together. *Cell* 1999;**96**:13–23.
22. SUR S, WILD JS, CHOUDHURY BK, et al. Long term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligonucleotides. *J Immunol* 1999;**162**:6284–6293.
23. ADACHI T, STAFFORD S, SUR S, ALAM R. A novel Lyn-binding peptide inhibitor blocks eosinophil differentiation, survival, and airway eosinophilic inflammation. *J Immunol* 1999;**163**:939–946.