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Mechanistic Systems Biology of Inflammatory Gene Expression in Airway Smooth Muscle as Tool for Asthma Drug Development

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Abstract

There is compelling evidence that airway smooth muscle cells may function as inflammatory cells in the airway system by producing multiple inflammatory cytokines in response to a large array of external stimuli such as acetylcholine, bradykinin, inflammatory cytokines, and toll-like receptor activators. However, how multiple extracellular stimuli interact in the regulation of inflammatory gene expression in an airway smooth muscle cell remains poorly understood. This review addresses the mechanistic systems biology of inflammatory gene expression in airway smooth muscle by discussing: a) redundancy underlying multiple stimulus-product relations in receptor-mediated inflammatory gene expression, and their regulation by convergent activation of Erk1/2 mitogen-activated protein kinase (MAPK), b) Erk1/2 MAPK-dependent induction of phosphatase expression as a negative feedback mechanism in the robust maintenance of inflammatory gene expression, and c) cyclooxygenase 2-dependent regulation of the differential temporal dynamics of early and late inflammatory gene expression. It is becoming recognized that a single-target approach is unlikely to be effective for the treatment of inflammatory airway diseases because airway inflammation is a result of complex interactions among multiple inflammatory mediators and cells types in the airway system. Understanding the mechanistic systems biology of inflammatory gene expression in airway smooth muscle and other cell types in the airway system may lead to the development of multi-target drug regimens for the treatment of inflammatory airway diseases such as asthma.

Keywords

ASM; asthma; cyclooxygenase; cytokine; inflammation; MAPK; mathematical; model

1. INTRODUCTION

Airway inflammation, a central problem in asthma, is mediated by a complex network of interactions among multiple inflammatory mediators acting on multiple cell types in the airway system [1, 2]. Given the tremendous redundancy in this inflammatory network, It is becoming recognized that a single-target approach is unlikely to be effective for the

treatment of inflammatory airway diseases [3-6]. Furthermore, complete inhibition of major signaling pathways that regulate both physiological functions and inflammatory gene expression can lead to unacceptable side effects [7]. The alternative approach of targeting multiple inflammatory mediators and cell types requires understanding of the systems biology of the airway system and the resident cells. Such approach has been taken for understanding cell cycle regulation and targeting diseases of abnormal cell proliferation such as cancer [8]. Several reviews have been published recently on the general principle of utilizing systems biology for drug discovery [9-11]. This brief review discusses some principles of mechanistic systems biology that are relevant to understanding the regulation of inflammatory gene expression in airway smooth muscle. The term “mechanistic” is added here to emphasize the explicit identification of molecular mechanisms in the models presented in this review.

Airway smooth muscle cells serve the dual function of contraction and inflammation in the airway system. Excessive contractility of airway smooth muscle contributes to the narrowing of airways in inflammatory airway diseases such as asthma. At the same time, airway smooth muscle cells are capable of secreting inflammatory mediators in response to inflammatory stimuli, thereby participating in the inflammatory network of the airway system [2, 12-15]. Further-more, recent reports indicate the existence of functional high affinity IgE (Fc ϵ RI) and toll-like receptors on airway smooth muscle cells. Sensitization with IgE, followed by anti-IgE antibody cross-linking, lead to the release of IL-4, IL-13, IL-5, and eotaxin by airway smooth muscle cells [13, 16]. Similarly, toll-like receptor activation by dsRNA and LPS also stimulates the release of IL-6, IL-8, and eotaxin by airway smooth muscle cells [17-19]. These findings together strongly suggest that airway smooth muscle cells can participate in the inflammatory process in the airway system.

Much has been published on the stimulatory effect of individual receptor agonists on the release of inflammatory cytokines by airway smooth muscle cells [2, 15]. As depicted in Fig. (1), compilation of the literature reveals a high degree of redundancy in multiple inflammatory stimulus-product relations in airway smooth muscle cells. For example, as shown in Fig. (1), a single inflammatory stimulus, such as IL- β , may induce the release of multiple inflammatory products, such as IL-8, by airway smooth muscle cells. Conversely, multiple inflammatory stimuli may induce the release of the same inflammatory product by airway smooth muscle cells. The cross-mapping between multiple stimuli and products suggests redundancy in the system of inflammatory gene expression in airway smooth muscle cells. An additional complexity in multiple inflammatory stimulus-product relations, as shown in Fig. (1), is the induction of COX-2, a critical enzyme for the synthesis of prostanoids, which function as autocrines/paracrines in stimulating the production of inflammatory and anti-inflammatory cytokines. Accordingly, this brief review addresses some basic principles of mechanistic systems biology for understanding redundancy, robustness, and autocrine/paracrine function of inflammatory gene expression in airway smooth muscle. These general principles are applicable to other inflammatory cell types in the airway system, and may serve as a conceptual framework for developing multi-target drug regimens for the treatment of inflammatory airway diseases such as asthma.

2. EFFICACY AND POTENCY IN MULTIPLE STIMULUS-PRODUCT RELATIONS

As shown in Fig. (1), the cross-mapping between multiple inflammatory stimuli and multiple inflammatory products in airway smooth muscle indicates that a single agonist may stimulate the release of multiple inflammatory products, and vice versa. For example, IL-1 β stimulates the production of CCL2, eotaxin, IL-6, IL-8, and RANTES in airway smooth muscle cells (Fig. (1)). Conversely, IL-1 β , IL-4, IL-9, IL-13, and TNF- α are all capable of stimulating eotaxin expression in airway smooth muscle (Fig. (1)). An important first step in understanding the systems biology of inflammatory gene expression in airway smooth muscle is the identification of the relative efficacy and potency of the multiple inflammatory stimulus-multiple product relations, because potency and efficacy are important criteria for determining the physiological significance of a given inflammatory stimulus-product relation in airway smooth muscle. Potency of a stimulus-product relation may be defined in terms of the half-maximal concentration of an agonist necessary for stimulating the expression of an inflammatory product. Efficacy of a stimulus-product relation may be defined in terms of the maximum expression of an inflammatory product that can be induced by a given agonist.

Since a single stimulus may stimulate the gene expression of multiple products, values of relative potency and efficacy are specific for the relationship between one stimulus and one product. Accordingly, multiple inflammatory stimuli (S_i ; $i = 1$ to m) may induce the expression of multiple inflammatory product (P_j ; $j = 1$ to n), but each specific stimulus-product relation, S_i - P_j , is characterized by a specific half-maximum concentration, $K(i,j)$, and a specific maximum response, $PMAX(i,j)$, relative to an internal control. For example, IL-1 β -stimulated production of CCL2, eotaxin, IL-6, IL-8, and RANTES in airway smooth muscle, as shown in Fig. (1), should have distinct values of $K(i,j)$ and $PMAX(i,j)$ for each one of the inflammatory products. Similarly, IL-13-stimulated production of CCL2, CCL26, CCL27, and eotaxin in airway smooth muscle, as shown in Fig. (1), should have distinct values of $K(i,j)$ and $PMAX(i,j)$ for each one of the inflammatory products. Furthermore, in these two examples, although IL-1 β and IL-13 both stimulate eotaxin expression, the relative potency and efficacy for IL-1 β -eotaxin and IL-13-eotaxin relations are expected to be different. In the comparison of multiple stimulus-product relations, relative potency may be represented by the reciprocal of $K(i,j)$, and relative efficacy may be represented by $PMAX(i,j)$. Thus, the two-dimensional matrices of $K(i,j)$ and $PMAX(i,j)$ values for multiple inflammatory stimulus(S_i)-product ($P(i,j)$) relations map the relative potency and efficacy in these relations. However, although values of relative potency and efficacy are necessary for understanding redundancy and robustness in multiple stimulus-product relations, these values alone are insufficient for predicting the combined effect of multiple stimuli on inflammatory gene expression. This is because the effects of multiple stimuli are not necessarily additive in inducing the expression of a given product. Understanding redundancy in multiple stimulus-product relations requires a mechanistic model for integrating the convergence of multiple stimuli on inflammatory gene expression.

3. MECHANISTIC SYSTEMS MODELS OF INFLAMMATORY GENE EXPRESSION

3.1. Erk1/2 MAPK Kinase/Phosphatase Model of Redundancy

Redundancy in inflammatory gene expression may be defined as the coexistence of multiple stimuli for inducing the expression of the same gene product. For example, as shown in Fig. (1), there is redundancy among IL-1 β , IL-4, IL-9, IL-13, and TNF- α in stimulating eotaxin expression in airway smooth muscle [20-27]. Similarly, there is redundancy among IL-1 α , IL-1 β , IL-17, TGF- β , TNF- α , bradykinin, and carbachol in stimulating IL-8 expression in airway smooth muscle [24, 28-36]. The existence of redundant stimuli for inducing the expression of the same product suggests that changing the number of stimuli may not necessarily affect the expression of a product due to compensation by other stimuli [37]. Inflammatory stimuli are extracellular mediators that induce inflammatory gene expression by activating signaling mechanisms in airway smooth muscle cells. Therefore, a common approach to modeling redundancy is to consider the convergence of multiple stimuli onto the activation of a few common signaling mechanisms [38]. Accordingly, this section discusses the Erk1/2 MAPK signaling systems as the convergent mechanism that integrates multiple stimuli in the regulation of inflammatory gene expression in airway smooth muscle.

There is compelling evidence that Erk1/2, p38, and JNK MAPK signaling systems regulate inflammation gene expression in many cell types [39]. Table 1 summarizes the literature for inflammatory gene expression in airway smooth muscle cells. As shown in Table 1, there is substantial overlap among Erk1/2, p38, and JNK MAPK's in the regulation of inflammatory gene expression in airway smooth muscle. For example, both Erk1/2 and p38 MAPK's have been implicated in the following six stimulus-product relations: bradykinin-induced IL-6 expression, IL-1 β -induced eotaxin expression, IL-13-induced eotaxin expression, IL-17-induced IL-8 expression, IL-17A-induced eotaxin expression, and PAF-induced RANTES expression. JNK has also been implicated in three of these six stimulus-product relations.

The simultaneous involvement of Erk1/2, p38, and JNK MAPK in the regulation of inflammatory gene expression may be explained by the distinct but complementary mechanisms of these MAPK systems. The Erk1/2 MAPK system is activated by the small G-protein Ras, and coupled to the phosphorylation of the transcription factors such as cyclic AMP-responsive element binding protein [40, 41]. In contrast, the p38 MAPK system is activated by the MAPK kinase, MKK3/6, which can be activated by TGF β -activated kinase 1 (TAK1) or apoptosis signal-regulating kinase 1 (ASK1). An important regulatory function of p38 MAPK is the stabilization of mRNA against de-adenylation [42, 43]. JNK represents a family of kinases that phosphorylates a large number of transcription factors [44]. Erk1/2, p38, and JNK MAPK's are similar in their regulation by a three-kinase cascade, a structure that enhances the sensitivity of stimulus-product relations [45-47]. The Erk1/2 MAPK redundancy model, as discussed in this section, is applicable to each kinase within the three-kinase cascade, as well as the p38 and JNK MAPK systems. Alternatively, Erk1/2, p38, and JNK MAPK's may function as modular switches in the regulation of inflammatory gene expression [48, 49].

Protein kinases and phosphatases are major regulatory enzymes of signaling in cells [50]. Accordingly, the Erk1/2 MAPK redundancy model, as shown in Fig. (2A), proposes that Erk1/2 MAPK kinase (KK) is the common signaling enzyme that is activated by multiple inflammatory stimuli (S_1 to S_n) in the regulation of inflammatory gene expression. For example, convergent activation of Erk1/2 MAPK may explain the redundancy among IL-1 α , IL-1 β , IL-17, TGF- β , TNF- α , bradykinin in the stimulation of IL-8 expression in airway smooth muscle. Using three stimuli as an example, Fig. (2B) shows the additive effect of multiple stimuli on Erk1/2 MAPK kinase activity (KK). In this example, the three stimuli have the same efficacy, but different potencies in activating Erk1/2 MAPK kinase activity, as represented by the three stimulus-kinase relations, labeled as KK1, KK2, KK3 in Fig. (2B). The additive effect of these stimuli on total Erk1/2 MAPK kinase activity is represented by the graph, labeled as KK123. As shown in Fig. (2B), the rate constant KP represents MAPK phosphatase activity for Erk1/2 MAPK dephosphorylation. The essential role of MAPK phosphatases in the regulation of cytokine production has been demonstrated by several knockout animal studies [51-57]. It is noteworthy that at least ten MAPK-specific phosphatases have been identified within the human genome, [58-62]. The model assumes that the Erk1/2 MAPK kinase/phosphatase (KK/KP) ratio determines the level of Erk1/2 MAPK phosphorylation and inflammatory gene expression. As discussed later in this section, the MAPK redundancy model predicts that MAPK phosphatase activity is a critical determinant of the degree of redundancy in multiple stimulus-product relations.

Fig. (2C) shows the behavior of the model when Erk1/2 MAPK phosphatase activity (KP) is relatively low ($KP = 0.01$), relative to the Erk1/2 MAPK kinase rate constants. The three broken lines in Fig. (2C), labeled as P1, P2, and P3, represent the model prediction of individual stimulus-product relations, that has been generated from using the three stimulus-kinase rate constant relations, KK1, KK2, KK3, from Fig. 2B, and a KP value of 0.01. The solid line in Fig. (2C), labeled as P123, represents the model prediction of total product as function of [stimulus] when the three stimuli are applied to the system simultaneously. The graph P123 was obtained by running the model using the stimulus-total kinase rate constant relation KK123 from Fig. (2B), and a KP value of 0.01. Comparison of the total product graph, P123, to the individual product graphs, P1, P2, and P3, indicates different half-maximal concentrations, but identical maximum [product]. This model prediction implies that, when Erk1/2 MAPK phosphatase activity is relatively low, the system exhibits complete redundancy in the sense that changing the number of stimuli will have insignificant effect on maximal inflammatory gene expression. As discussed in the next section, this is not the case when the MAPK phosphatase activity is relatively high.

Fig. (2D) shows the behavior of the model when the MAPK phosphatase activity (KP) is relatively high ($KP = 1$), relative to the Erk1/2 MAPK kinase rate constants. The three broken lines in Fig. (2D), labeled as P1, P2, and P3, represent the model prediction of individual stimulus-product relations, using the three stimulus-kinase rate constant relations, KK1, KK2, KK3, from Fig. (2B), and a KP value of 1. The solid line in Fig. (2D), labeled as P123, represents the model prediction of total product as a function of [stimulus] when the three stimuli are applied to the system simultaneously. The graph P123 was obtained by simulating the model using the stimulus-total kinase rate constant relation KK123 from Fig.

(2B) and a KP value of 1. Comparison of the total product graph P123 to the individual product graphs, P1, P2, and P3, indicates different half-maximal concentrations and different maximum [product]. This model prediction implies that, when MAPK phosphatase activity is relatively high, the system exhibits incomplete redundancy in the sense that changing the number of stimuli may significantly alter maximal inflammatory gene expression.

MAPK phosphatase plays an important role in the regulation of inflammatory cytokine production in various cell types [54, 63-66]. The model predictions, as presented in Figs. (2C and 2D), suggest a novel function of MAPK phosphatase activity in determining the degree of redundancy in a system of multiple inflammatory stimulus-product relations. The model predicts that a lower MAPK phosphatase activity is not only associated with a higher sensitivity of inflammatory stimulus-product relations, but is also associated with greater degree of redundancy, such that complete inhibition of all inflammatory stimuli will be necessary for attenuating the maximal level of inflammatory gene expression. In contrast, the model predicts that a higher MAPK phosphatase activity is associated with lesser degree of redundancy, such that inhibition of some inflammatory stimuli may be effective for attenuating the maximal level of inflammatory gene expression. These model predictions suggest a novel approach to controlling airway inflammation by inducing the expression of MAPK phosphatase expression, in combination with the inhibition of inflammatory stimuli.

3.2. Negative Feedback Model of Robustness

Robustness of a biological system may be defined as the maintenance of function despite external perturbations to the system [11, 38, 67]. Within the context of this review, robustness may be considered as the maintenance of inflammatory gene expression despite changes in the number and/or strength of inflammatory stimuli. As discussed in the previous section, the convergence of multiple stimuli onto the activation of Erk1/2 MAPK kinase, together with a low MAPK phosphatase activity, results in robustness in inflammatory gene expression, relatively independent of the number of stimuli. This section discusses the induction of MAPK phosphatase expression by negative feedback as an alternative mechanism in mediating robustness in inflammatory gene expression, as shown in Fig. (3A). It is noteworthy that induction of MAPK phosphatase expression has been proposed as a mechanism of glucocorticoid-mediated inhibition of inflammation [68, 69]. Furthermore, prolonged Erk1/2 MAPK activation has been shown to induce the expression of MAPK phosphatases such as MKP-1 and MKP-3 in some cells [70-72]. Erk1/2 MAPK-mediated induction of MAPK phosphatase expression is considered a negative feedback mechanism because MAPK phosphatase attenuates Erk1/2 MAPK activation by dephosphorylation [73]. Therefore, overtime, when a relatively high Erk1/2 MAPK kinase activity is accompanied by a relatively high MAPK phosphatase activity by gene induction, the Erk1/2 MAPK kinase/phosphatase ratio may remain relatively constant. As a result, steady-state inflammatory gene expression is maintained at a robust level, relatively independent of the strength of the initial stimulus. Fig. (3A) shows a schematic diagram of a negative feedback model of robustness in which prolonged Erk1/2 MAPK activation induces the expression of MAPK phosphatase (MKP), as represented by the MAPK phosphatase rate constant, KP. In this model, multiple stimuli converge onto the activation of Erk1/2 MAPK kinase (KK) in a concentration-dependent manner, and the level of inflammatory gene expression ([product])

is determined by the Erk1/2 MAPK kinase/phosphatase ratio. As shown in Fig. (3B), the model predicts that increasing MAPK phosphatase activity by gene induction will attenuate the sensitivity and maximum of inflammatory gene expression, as indicated by comparing the graphs labeled as “KP = 0.01” and “KP = 1”. Furthermore, when an increase in Erk1/2 MAPK kinase activity is matched by a proportional increase in MAPK phosphatase activity, then [product] becomes relatively constant, independent of [stimulus], as shown by the broken lines in Fig. (3B). These model predictions suggest the intriguing possibility that a dysfunction in negative feedback regulation of MAPK phosphatase expression may contribute to airway inflammation.

3.3. Autocrine/Paracrine Model of COX-2-Mediated Inflammatory Gene Expression

In response to inflammatory stimuli, airway smooth muscle cells secrete autocrines and/or paracrines that can result in the amplification or attenuation of the initial inflammatory response [74-76]. An important paracrine/autocrine system in airway smooth muscle is the cyclooxygenase (COX) enzymes, which catalyze the conversion of arachidonic acid to prostaglandin H₂, a precursor for the production of prostanoids and thromboxane. The COX-1 isozyme is constitutive, whereas the COX-2 isozyme is inducible [77]. It is noteworthy that COX-knockout mice typically exhibit increased allergic lung responses [78-80]. Similarly, pharmacologic inhibition of COX enzymes also increases allergic inflammation and airway hyperresponsiveness in mice [81]. These findings suggest that COX inhibitors alone are unlikely to be effective for the treatment of airway inflammation. The inflammatory function of COX enzymes is complex because some COX-derived prostanoids are inflammatory, whereas other COX-derived prostanoids are anti-inflammatory [82-85]. For example, the prostaglandin (PG) molecule, PGD₂, and its receptors have been implicated in allergic inflammation and asthma [86-91]. However, at least one study suggests that PGD₂ may also exert anti-inflammatory effects on the airway system [87]. In contrast, PGE₂ has been shown to inhibit allergen-induced airway inflammation in human subjects and animal models of allergic asthma [92-94]. Airway smooth muscle cells are capable of expressing COX-2 and releasing PGE₂ in response to inflammatory stimuli such as bradykinin and interleukin-1 β [95-102]. Interestingly, COX-2-derived PGE₂ enhances IL-1 β -induced COX-2 expression in airway smooth muscle cells [103]. The cycle of self-amplification between COX-2 and PGE₂ has been considered as anti-inflammatory for the airway system because PGE₂ inhibits allergen-induced airway inflammation [92-94, 103, 104]. However, PGE₂ also stimulates IL-6 production, potentiates IL-1 β -stimulated granulocyte colony-stimulating factor, and enhances bradykinin-stimulated IL-6 and IL-8 production in airway smooth muscle cells [31, 36, 105, 106]. Since IL-1 β , IL-6, and IL-8 are inflammatory mediators [107-109], these results suggest that PGE₂ also exerts pro-inflammatory effects on the airway system. A recent study indicates that PGD₂ promotes the migration of airway smooth muscle cells [110], but relatively little is known about the function of PGD₂ on the inflammatory function of airway smooth muscle.

Another complexity of the COX-2 enzyme system is the temporal dynamics of COX-2 expression and prostanoid synthesis, as it is becoming recognized that COX-2 may preferentially produce pro-inflammatory prostanoids at the beginning of inflammation, but anti-inflammatory prostanoids towards the end of inflammation [111-113]. Therefore,

understanding the temporal dynamics and regulation of COX-2 expression is important for elucidating the inflammatory and anti-inflammatory functions of COX-2 enzymes in the airway system. Accordingly, Fig. (4) shows an autocrine/paracrine model of COX-2-mediated inflammatory gene expression in airway smooth muscle. In this model, a primary stimulus induces the protein expression of multiple early genes, $EG1(t)$, $EG2(t)$, ..., $EGn(t)$, some of which may serve as an autocrine/paracrine in inducing the protein expression of COX-2. The first time delay function, $DT1$, indicates the time delay that is necessary for COX-2 expression. For example, during airway inflammation, a primary allergic stimulus may stimulate the expression of IL-1 β as an early gene product in airway smooth muscle. IL-1 β then acts as an autocrine and/or paracrine to induce COX-2 expression in airway smooth muscle. COX-2 then catalyzes the production of prostanoids, which stimulate the protein expression of late genes, $LG1(t)$, $LG2(t)$, $LG3(t)$, ..., $LGn(t)$, as shown in Fig. (4). The second time delay function, $DT2$, indicates the time delay that is necessary for prostanoid synthesis and inflammatory gene expression. For example, COX-2-derived prostanoid(s) may serve as autocrine/paracrine that mediate bradykinin and IL-1 β -stimulated expression of IL-6 and IL-8 [96, 97]. Time is necessary for the sequential synthesis of autocrines, paracrine, and inflammatory gene products; therefore, time delay is an implicit characteristic of an autocrine/paracrine system. One approach to modeling the time delay between early and late gene expression is the sequential processing of a signal through a series of kinetic compartments [114], as shown in Fig. (5A). In this model, $INPUT(t)$ represents a primary stimulus as a ramp function of time (t). $X1(t)$ to $X6(t)$ represent the transient signal, or product, in the six compartments. $OUTPUT(t)$ represents the emerging signal, or product, after the transition of the input signal through the six compartments. Turnover of the $OUTPUT$ compartment is represented by the rate constant $K2$. Fig. (5B) shows the transformation of the linear input signal through the six kinetic compartments. As shown in Fig. (5B), the linear $INPUT(t)$ is progressively transformed to the non-linear $OUTPUT(t)$ with a time delay. The temporal relationship between the primary input and the final output is determined by the number of processing steps (compartments) and the kinetics of the processes (rate constants). Accordingly, an important implication of these results is that differential compartmentalization and kinetics of COX-2 and downstream enzymes will determine the differential temporal dynamics of COX-2-derived prostanoids and expression of COX-2-mediated inflammatory and anti-inflammatory gene products during inflammation [111-113].

4. MECHANISTIC SYSTEMS BIOLOGY AS TOOL FOR ASTHMA DRUG DEVELOPMENT

It may be useful to consider airway inflammation as resetting of a physiological system to a pathophysiological system with changes in multiple variables. Accordingly, returning the pathophysiological state to the physiological state may necessitate pharmacologic targeting of multiple molecular sites. The development of multi-target drug regimens requires understanding the system as a whole. Systems biology is becoming recognized as an important tool for understanding the complexity of inflammatory diseases including asthma [115-121]. This review illustrates the utility of mechanistic systems biology as a tool, using airway smooth muscle as an example, for understanding inflammatory gene expression. A

major point of this review is to suggest that elucidating the cause of asthma, a multi-factorial disease of airway inflammation, may require the use of mechanistic systems biology for understanding the mechanistic interactions among multiple cell types in the release of multiple cytokines. The three topics in this review are connected conceptually as successive steps in understanding a biological system, that is, from characterization of the input-output relations (multiple stimulus-product relations), to mechanistic understanding of the steady-state behavior of the system (MAPK/MKP system), to mechanistic understanding of the kinetic behavior of the system (temporal dynamics). As discussed in this review, redundancy, negative feedback, and autocrine/paracrine together contribute to the complexity and fluidity of the system of inflammatory gene expression. The following two studies exemplify redundancies in the regulation of gene expression by transcription factors and receptor signaling systems. Hu *et al.* [129] showed that ectopic expression of any one of the C/EBP- α , - β , and - δ transcription factors in C/EBP-deficient lymphoblasts was sufficient to induce IL-6 expression, suggesting that activities of C/EBP α , - β , and - γ transcription factors are redundant for regulating the expression of IL-6. Similarly, Albrektzen *et al.* [130] showed that a substantial fraction of cytokine genes regulated by Factor VIIa/PAR2 signaling was also regulated by thrombin/PAR1 signaling in breast carcinoma cells, suggesting extensive redundancy between the two receptor signaling systems. Protein kinase/phosphatase system is a major mechanism in cell signaling. This review focuses on the MAPK/MKP system for two reasons. First, as an example of evidence-based modeling [131], the MAPK/MKP system was selected based on substantial data (Table 1) that this system regulates inflammatory gene expression in airway smooth muscle cells. Second, the MAPK/MKP system illustrates the general principle of how a protein kinase/phosphatase system may explain potency and efficacy of stimulus-product relations. Using the MAPK/MKP system as an example, this review illustrates the general principle of how a protein kinase/phosphatase system may explain potency and efficacy of stimulus-product relations. The same principle may be utilized to develop a comprehensive model of inflammatory gene expression, which could include additional mechanisms, as guided by experimental data. As emphasized in this review, model structure and rate constants are equally important in determining the behavior of a system. For example, the level of MAPK phosphatase activity determines the degree of redundancy in multiple stimulus-product relations. However, in order to realize the power of systems biology in solving the problem of airway inflammation, it is essential to apply the concepts of systems biology at both experimental and modeling studies. For example, it is important to design experiments that employ multiple stimuli, monitor multiple products, and address multiple mechanisms, as directed by model predictions. Similarly, it is essential to revise the systems model as directed by experimental data. Mechanistic systems biology, as discussed in this review, consists of two essential components, that is, experimentation and modeling, both of which should be guided by a mechanistic hypothesis in the form of a system of multiple variables connected by mechanistic interactions. The iteration between experimental and modeling studies should lead to the development of a working model that is capable of predicting multiple targets for the treatment of airway inflammation.

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Fig. (1).

Multiple stimulus-product relations in inflammatory gene expression in airway smooth muscle. Each line connecting a stimulus and a product indicates a causal relationship, as reported in the literature. Abbreviations: CCL, chemokine C-C motif ligand; COX, cyclooxygenase; CXCL, CX-C ligand; IFN, interferon; IL, interleukin; RANTES, regulated on activation, normal T cell expressed and secreted; TGF, transforming growth factor; TNF, tumor necrosis factor.

Fig. (2). Erk1/2 MAPK kinase model of redundancy

(A) A schematic diagram of the model, showing the convergence of multiple inflammatory stimuli, S1 to Sn, onto the kinase rate constant, KK, that governs the phosphorylation of Erk1/2 MAPK. KP represents the rate constant that governs dephosphorylation of Erk1/2 MAPK. Phospho-Erk (ERK-P) then activates inflammatory gene expression ([product]). (B) Model prediction of the additive effect of multiple stimuli on the Erk1/2 MAPK kinase rate constant (KK123). The three graphs, labeled as KK1, KK2, KK3, represent three [stimulus]-kinase relations having the same maximum value, which is set at 1, but different half-maximal concentrations ranging from 0.5 to 2×10^{-6} . The graph labeled as KK123 represents the sum of KK1, KK2, to KK6, when all three stimuli are applied simultaneously at the same concentration. (C) Model prediction of stimulus-product relations at a relatively low MAPK phosphatase activity ($KP = 0.01$). The three broken-line graphs represent the predicted stimulus-product relations (P1, P2, and P3) for the three individual stimuli, as predicted by the three stimulus-kinase rate constant relations, KK1, KK2, KK3, in panel B. The solid line graph represents the predicted stimulus-total product relation (P123) when the three stimuli are applied simultaneously, as predicted by the stimulus-total kinase rate constant relation KK123, and the rate constant for MAPK phosphatase activity (KP). (D) Model prediction of stimulus-production relations at a relatively high phosphatase activity ($KP = 1$). The labeling of graphs in this panel is the same as that in panel C.

Fig. (3). Negative feedback model of robustness

(A) A schematic diagram of the model showing a negative feedback mechanism by which Erk1/2 MAPK activation induces the expression of MAPK phosphatase (KP) that catalyzes Erk1/2 MAPK dephosphorylation. This model is similar to the redundancy model (Fig. 2A) in having the convergence of multiple inflammatory stimuli, S1 to Sn, onto the activation of Erk1/2 MAPK kinase (KK), which governs phosphorylation of Erk1/2 MAPK. Phospho-Erk (ERK-P) then activates inflammatory gene expression. (B) Model prediction that prolonged Erk1/2 MAPK activation induces the expression of MAPK phosphatase, which results in shifting of stimulus-product relations. The three stimulus-product relations have been generated using different values of MAPK phosphatase rate constant (KP), ranging from 0.01 to 1. As indicated by the broken lines, when both Erk1/2 MAPK kinase and phosphatase activities increase proportionally, steady-state Erk1/2 MAPK phosphorylation, and therefore [product], becomes relatively independent of [stimulus].

Fig. (4). Autocrine/paracrine model of COX-2-mediated inflammatory gene expression

In this model, a primary stimulus induces the protein expression of multiple early genes, $EG1(t)$, $EG2(t)$, ..., $EGn(t)$, some of which may serve as an autocrine/paracrine in stimulating COX-2 protein expression. The first time delay function, DT1, represents the time delay that is necessary for COX-2 expression. COX-2 then catalyzes the synthesis of prostanoids, which stimulates the protein expression of late genes, $LG1(t)$, $LG2(t)$, $LG3(t)$, ..., $LGn(t)$. The second time delay function, DT2, represents the time delay that is necessary for prostanoid synthesis and inflammatory gene expression.

Fig. (5).

(A) A kinetic model of time delay In this model, INPUT(t) represents the primary stimulus in the form of a ramp function that first increases linearly with time, from 0 to 1000 arbitrary units over a duration of 100 time units, and then stays constant beyond 100 time units. X1(t) to X6(t) represent the signals, or products, in the six compartments that are connected kinetically by a rate constant (K1). OUTPUT(t) represents the emerging signal, or product, after transition of the primary stimulus through the six compartments. The rate constant K2 governs the turnover of OUTPUT (t). In this example, the rate constants, K1 and K2, both have been assigned a value of 0.05. **(B)** Model predictions of OUTPUT(t), and X(1) to X(t), in comparison with INPUT(t).

Table 1

Involvement of Erk1/2, p38, and JNK MAPK's in the Regulation of Inflammatory Gene Expression in Airway Smooth Muscle Cells

	Stimulus	Product	References
Erk1/2	Bradykinin	IL-6	[106]
	IL-1 β	Eotaxin, RANTES	[122, 123]
	IL-4	Eotaxin	[25]
	IL-13	Eotaxin	[25, 26]
	IL-17	IL-8	[35]
	IL-17A	Eotaxin	[124]
	IL-1 β + TNF α + INF γ	IL-6, IL-8	[29]
	IL-1 β + TNF α + INF γ	IL-1 β	[29]
	PAF	RANTES	[125]
	Cyclic Stretch	IL-8	[126]
p38	Bradykinin	IL-6	[106]
	IL-1 β	Eotaxin	[122, 123]
	IL-13	Eotaxin	[26]
	IL-17	IL-8	[35]
	IL-17A	Eotaxin	[124]
	TNF α	IL-6, RANTES	[127]
	IL-1 β + TNF α + INF γ	IL-6, IL-8	[29]
	PAF	RANTES	[125]
	Cyclic Stretch	IL-8	[126]
JNK	IL-1 β	Eotaxin	[122]
	IL-17	IL-8	[35]
	IL-17A	Eotaxin	[124]
	TNF α	IL-8, RANTES	[128]

Abbreviations: CCL, chemokine C-C motif ligand; COX, cyclooxygenase; CXCL, C-X-C ligand; IFN, interferon; IL, interleukin; RANTES, regulated on activation, normal T cell expressed and secreted; TGF, transforming growth factor; TNF, tumor necrosis factor.