

Complement Activation Pathways

A Bridge between Innate and Adaptive Immune Responses in Asthma

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Although it is widely accepted that allergic asthma is driven by T helper type 2 (Th2)-polarized immune responses to innocuous environmental allergens, the mechanisms driving these aberrant immune responses remain elusive. Recent recognition of the importance of innate immune pathways in regulating adaptive immune responses have fueled investigation into the role of innate immune pathways in the pathogenesis of asthma. The phylogenetically ancient innate immune system, the complement system, is no exception. The emerging paradigm is that C3a production at the airway surface serves as a common pathway for the induction of Th2-mediated inflammatory responses to a variety of environmental triggers of asthma (i.e., allergens, pollutants, viral infections, cigarette smoke). In contrast, C5a plays a dual immunoregulatory role by protecting against the initial development of a Th2-polarized adaptive immune response via its ability to induce tolerogenic dendritic cell subsets. On the other hand, C5a drives type 2-mediated inflammatory responses once inflammation ensues. Thus, alterations in the balance of generation of the various components of the complement pathway either due to environmental exposure changes or genetic alterations in genes of the complement cascade may underlie the recent rise in asthma prevalence in westernized countries.

Keywords: complement pathways; innate immunity; asthma; dendritic cells

The allergic asthma epidemic continues unabated in the developed world. Although asthma is multifactorial in origin, it is generally accepted that it arises as a result of inappropriate immunological responses to common environmental antigens in genetically susceptible individuals (1). Specifically, a multitude of evidence suggests that a T helper type 2 (Th2) polarized immune response mediates this chronic lung disease, marked by airway hyperresponsiveness, eosinophilic inflammation, excessive mucus production, and elevated serum immunoglobulin E (2). However, the mechanisms underlying the generation and the expression of the Th2-biased immune response in asthma remain a mystery.

Recent years have brought growing mechanistic awareness of the profound influence of the innate immune system on the development of adaptive immune responses. The complement system, a phylogenetically ancient part of the innate immune system, is no exception. In addition to its long-recognized role as a lytic effector system that protects against microbial pathogens, it is clear that complement components provide a key link

between innate and specific immune responses (3, 4). First, the complement system is an important regulator of B cell activation, providing an important mechanism for pathogen (or "danger") recognition by B lymphocytes. Second, the engagement of complement receptors on antigen-presenting cells (APC) leads to potent effects on the production of immunoregulatory cytokines such as IL-12. Third, while the anaphylatoxins (the complement activation products C3a and C5a) have long been appreciated for their effects on myeloid cell migration, activation, and effector functions, it has recently become clear that these molecules also regulate the functions of APCs and T cells. In the context of allergic diseases, a substantial amount of evidence is accruing to suggest that complement pathways are indeed activated in patients with asthma and that mediators of this pathway play an important role in both influencing the nature of the adaptive immune responses to inhaled allergens as well as amplifying the Th2 immune response once initiated.

COMPLEMENT PATHWAY COMPONENTS AS INNATE IMMUNE MEDIATORS

The complement system is a sophisticated network of soluble and membrane-bound proteins, which serve as both immune sensors and immune activators. Like the more recently identified Toll-like receptor (TLR) pathway, the complement system, can be activated by "hard-wired" pattern recognition receptors that have evolved to recognize pattern-associated molecular patterns (PAMPs). PRRs in the complement system recognize exogenous as well as endogenous "danger" motifs. Recognition receptors (PRR) in the complement system include: specific antibody, mannan-binding lectin (MBL), ficolins, C-reactive proteins, C1q, and natural IgM (4, 5). These PRRs activate three separate complement pathways referred to as the classical, lectin, and alternative. The Classical pathway is activated by specific antibody released following a humoral immune response or by natural antibody. The lectin pathway is activated after the recognition and binding of PAMPs by lectin proteins including MBL, ficolin H, and ficolin L. MBL recognizes polysaccharide structures on bacteria, fungi, viruses, and parasites, as well as on apoptotic host cells. The alternative pathway is constitutively active and binds to a wide range of suitable acceptor molecules. Several cell types in the lung, including alveolar macrophages, alveolar type II epithelial cells, and fibroblasts, produce the PRMs C1q and M-ficolin as well as the complement factors B, D, and C3. Although each of these pathways is activated by distinct PRRs, they all culminate in activation of C3, the central step in complement activation. Activation of C3 leads to the generation of two pro-inflammatory anaphylatoxins, C3a and C5a, and the membrane attack complex (MAC). A variety of biological outcomes are downstream of C3 activation, including: (1) microbial lysis by the MAC; (2) activation of granulocytes and endothelia by sublytic quantities of attack complexes; (3) deposition of C3 fragments on membranes and/or particles (e.g., antigen-antibody complexes, microbes) leading to phagocytosis and clearance and B-cell activation; (4) recruitment and/or activation of cells of the innate immune response, including dendritic cells and epithelial

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cells; and (5) recruitment and/or activation of a number of cells types relevant to the allergic response (i.e., eosinophils, mast cells, smooth muscle cells). Most of these activities depend upon the engagement of specific complement receptors, including the RCA family members, the β -integrins CR3 and CR4, the anaphylatoxin receptors C3aR and C5aR, and receptors for C1q and factor H.

MECHANISMS OF COMPLEMENT ACTIVATION IN ASTHMA

There is substantial evidence that complement pathways are activated in the lungs of individuals with asthma (6–8). Specifically, it has been shown that C3a and C5a levels are higher in the BAL of individuals with asthma compared with healthy control subjects at baseline (8). Moreover, Krug and coworkers (7) demonstrated elevations in BAL levels of both C3a and C5a following segmental allergen challenge of individuals with asthma, whereas no elevations were observed in normal individuals. In addition to allergens, other triggers of asthma have been shown to activate complement pathways both in humans and in animal models. For example, C3 levels are elevated in the BAL of both mice and humans exposed to ozone (9, 10). RSV infection in immunized humans and mice has been shown to result in activation of the classical pathway of complement. In mice, RSV immunization-driven AHR is C3 dependent (11). Likewise, airborne particulate matter exposure of mice has been shown to lead to C3 deposition that mediates the ensuing AHR. Plasma levels of C3a and C4a are also higher in aspirin-sensitive individuals, and these levels correlate with changes in FEV₁ in aspirin-sensitive individuals after aspirin challenge (12). Finally, elevated serum C3 levels are found in children living in homes with smokers compared with those from nonsmoking homes (13). Taken together, these findings suggest that complement pathways are clearly activated at the airway surface after exposure to many distinct environmental triggers of asthma, suggesting that complement activation may be a common step in the development of airway dysfunction associated with asthma.

Although the exact mechanisms of complement activation are unknown, generation of anaphylatoxins in the airways may occur via multiple mechanisms. First, C3 and C5 can be cleaved through the generation of the classical pathway convertases as a result of antibody formation; however, IgE antibodies do not fix complement, and thus activation of the classical pathway via antibody–antigen complexes is less likely in allergen-driven immune responses. Alternatively, C5 and C3 could be cleaved by proteases released from mast cells and other inflammatory cells (i.e., trypsin, thrombin, elastase) in the airway wall as a secondary consequence of IgE-mediated processes. Second, pattern recognition of carbohydrate structures on allergens by the mannose-binding lectin pathway is also conceivable. Likewise, some macromolecular structures from the dust mite, fungi, or ragweed allergen may be recognized by the alternative pathway and fix complement directly. Lastly, anaphylatoxins may be generated by mechanisms independent of the classical and alternative pathways of complement activation. Specifically, exogenous proteases derived from allergens such as *Der p3* and *Der f3* have been shown to directly cleave C3 and C5 into their active fragments (14). Nonallergenic environmental triggers of asthma, such as RSV infection and cigarette smoke, activate complement pathways in the airways, through distinct pathways. Cigarette smoke activates complement directly through cleavage of an internal thiol ester bond in C3 (15), whereas RSV immunization activates complement through the classical pathway (11). C3 has also been shown to be produced by the airway epithelium by the Th2 cytokines IL-4 and IL-13 in the absence of traditional

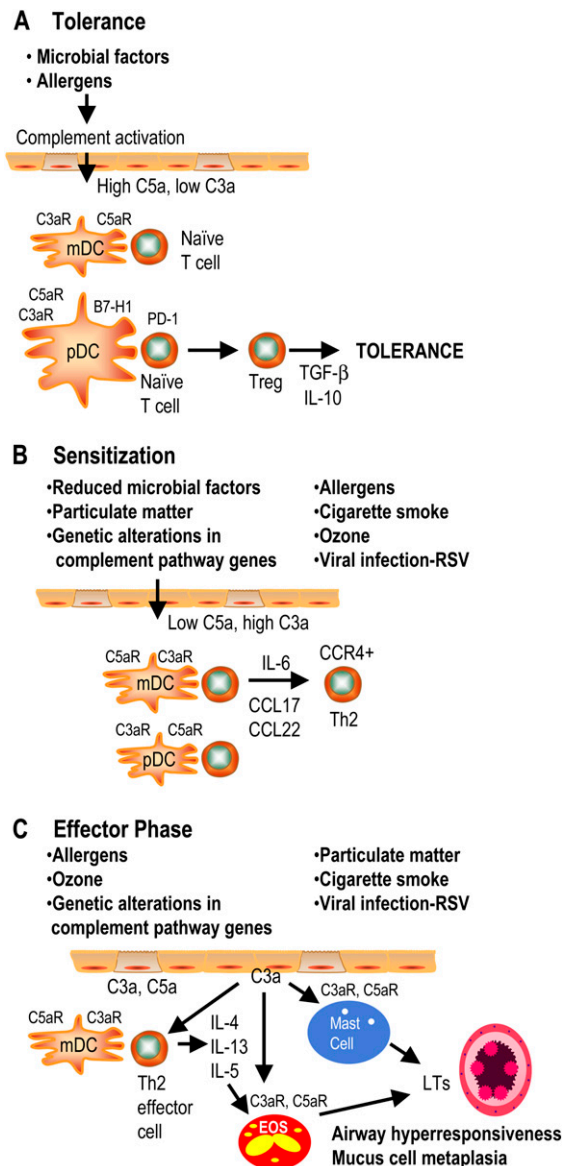


Figure 1. Immunoregulatory role of complement activation pathways in asthma. (A) C5/C5a production driven either by microbial infections or their by-products or allergens during allergen sensitization leads to development of immune tolerance to inhaled antigens through the preferential recruitment, survival, or activation of plasmacytoid dendritic cells (pDCs). pDCs suppress the generation of effector cells by myeloid dendritic cells (mDCs) by sending an unknown inhibitory signal to mDCs and to T lymphocytes (perhaps in the form of B7-H1:PD-1 [programmed death ligand-1] ligation). Alternatively, pDCs may directly stimulate the differentiation of Tregs (CD4⁺ CD25⁺ T lymphocytes), leading to the development of tolerance to inhaled antigens. (B) C5 deficiency or C5aR blockade during initial exposure to allergens leads to an increase in the numbers of mDCs compared with pDCs, leading to sensitization to inhaled antigens, thus releasing the inhibitory “brake” on T cell stimulation. mDCs also produce the Th2-selective chemokines CCL17 and CCL22, further intensifying Th2 effector cytokine production. Direct production of C3a or as a result of C5a blockade may also result in enhanced Th2 cytokine production through as yet unknown mechanisms. (C) During the effector phase, both C3a and C5a are generated through activation of the classical pathway, and through proteases released from inflammatory cells. C3 is further amplified through the direct induction by IL-4 and IL-13 in the airway epithelium. Both C5a and C3a are pro-allergic and play an important role in the recruitment and activation of inflammatory cells (i.e., eosinophils, mast cells) known to be important in the induction of the cardinal features of asthma, including airway hyperresponsiveness, mucus metaplasia, and airway remodeling.

complement activation pathways (16). Although complement pathways are likely activated through multiple pathways in the context of allergic diseases, the exact mechanisms remain to be fully elucidated.

COMPLEMENT PATHWAY REGULATION OF ALLERGIC EFFECTOR PATHWAYS

The anaphylatoxins, C3a and C5a, have long been recognized as potent mediators of the effector phase of the allergic response. Through binding their receptors on inflammatory cells and resident airway cells, C5a and C3a have been shown to induce many of the pathophysiologic features of allergic asthma, such as smooth muscle contraction, increased vascular permeability, mucus secretion, and recruitment of inflammatory cells. In support of its pro-allergic role, studies in which C5a has been inhibited pharmacologically after initial allergen sensitization have shown that C5a plays an important role in driving allergen-driven AHR and airway inflammation in mouse models of asthma (17–19). However, C5a does not directly induce smooth muscle contraction in the mouse airway, suggesting that C5aR ligation of resident airway cells is not sufficient to induce ASM contraction (20). In contrast, C5a administration to a previously allergen-sensitized mouse induces a contractile response, suggesting that C5aR ligation on infiltrating inflammatory cells is a prerequisite for the contractile response. Similar effects have been shown for C3a. Specifically, several groups have now shown that deficiencies in C3 (21, 22) or the C3aR (7, 23, 24) protect against the development of allergen-driven AHR. Likewise, the AHR induced by exposure to several environmental stimuli, including RSV (11), particulate matter (22), and ozone (9), has been shown to be C3-dependent. Collectively, these studies suggest that both C5 and C3 are important mediators of the AHR induced by a variety of stimuli. However, a head-to-head comparison of C3aR and C5aR blockade during the effector phase of the allergic response in mice suggests that these two mediators may have distinct actions on inflammatory cell recruitment and activation. Further studies are required to more fully elucidate their distinct roles.

ROLE OF C5/C5A IN REGULATING ADAPTIVE IMMUNE RESPONSE TO ALLERGENS

The first hint that complement pathways also played a role in regulation of the initiation of the adaptive immune response to inhaled allergens came from a genetic/genomic study aimed at identifying asthma susceptibility genes in mice (25). Contrary to the previously known pro-allergic role of C5, these studies showed that susceptibility to allergen-driven AHR was linked to a loss-of-function mutation in C5. In other words, C5 generation during exposure to allergens is protective against the development of allergen-driven AHR. These original observations have been corroborated by studies in C5 congenic mice (26), C5aR-deficient mice (20), and after pharmacologic targeting of the C5a receptor during allergen sensitization (20). As would be predicted, C5a blockade markedly enhances Th2 adaptive immune response, airway inflammation, and pathological changes in lung physiology. The fact that C5a plays a protective role at the time of initial allergen exposure and a pro-allergic role in the effector phase of the response suggests that C5a plays a dual role in regulation of allergic inflammation depending upon the timing of activation and the inflammatory cell milieu present during its activation. This duality of function is an emerging paradigm for many innate immune mediators. For example, TLR4 agonists have opposing actions on lung function depending on the dose and timing of their administration (27).

Although the mechanism(s) by which C5a production in the airways protect(s) against allergic sensitization are not well understood, several lines of evidence suggest that C5a modulates the adaptive immune response through altering the phenotype and function of antigen-presenting dendritic cells (DCs) (20). DCs in the respiratory tract form a network in the upper layers of the airway epithelium. In their immature state, they take up antigen, and in the context of a danger signal (PAMP) migrate to the draining lymph node, where they become fully mature and provide costimulatory molecules and cytokine signals for initiating and polarizing the T helper response. To further complicate matters, at least two different subsets of DCs have been shown to be important in respiratory tract responses to antigens, namely myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) (28, 29). Myeloid DCs produce little IL-12, a prototype Th1-skewing cytokine, yet secrete the Th2-prone cytokine IL-6, while pDCs predominantly secrete IFN- α , but also produce IL-12. De Heer and colleagues have elegantly shown that myeloid DCs appear to drive Th2 sensitization to inhaled antigens, while pDCs mediate the development of tolerance to inhaled antigens (28). Interestingly, a deficiency in C5, either due to a genetic deletion or to pharmacologic blockade of the C5aR during priming to inhaled OVA or HDM, leads to an early and persistent shift of the proportion of the mDC to pDCs in the lungs and to the development of an effector Th2 response (20). These studies suggest that C5a may mediate inhalational tolerance to allergens via its ability to alter the ratio of immunogenic to tolerogenic DC populations at the airway interface with the environment.

Although the exact consequences of C5-driven alterations in mDC and pDC populations in the lung are unknown, several possibilities exist. First, mDCs may enhance Th2 effector recruitment to the airways via the profile of chemokines they produce. In this regard, Köhl and colleagues demonstrated that C5a suppressed homing of Th2 cells through suppression of the production of the Th2-specific chemokines, CC chemokine ligand 17 (CCL17) and CCL22 (a macrophage-derived chemokine), by pulmonary mDCs (20). This may explain the higher Th2 cytokine levels in C5aR-targeted mice. This finding is consistent with the fact that Hammad and coworkers have shown that the production of CCL17 and CCL22 by human monocyte-derived DCs in response to *Der p 1* exposure is preferentially in mDCs derived from HDM-allergic donors, whereas in non-HDM-allergic healthy control subjects, *Der p 1* induced mainly CxCL10 (30).

Second, the shift toward Th2 cytokine polarization in the lungs under conditions in which C5 is absent may be the result of a lack in the production of the Th1-skewing cytokine, IL-12, as mDCs are poor producers of IL-12. This contention is consistent with previous studies showing that C5a can induce Th1 responses through its ability to enhance IL-12 production in APCs (25, 31). Although there is no direct evidence that C5aR blockade leads to an enhancement of Th2 priming by a lack of counterbalancing IL-12 production by mDCs *in vivo*, Gavett and colleagues (32) have previously shown that administration of rIL-12 to C5-deficient A/J mice suppressed allergen-induced AHR concomitant with a reduction in Th2 cytokine production.

Finally, C5 may mediate inhalational tolerance via recruitment and/or activation of pDCs as they have been shown to induce Tregs (CD4+CD25+ T cells). In this regard, Lewkowich and coworkers have shown that Treg blockade in C5 sufficient C3H/HeJ mice exposed to HDM abolished their protection from the development of AHR concomitant with a reduction in the numbers of pulmonary pDCs and an increase in mDCs (33). Moreover, DCs from Treg cell-depleted mice demonstrated an increased capacity to stimulate T cell proliferation and Th2 cytokine production. In contrast, blockade of Tregs in C5-deficient A/J mice had no effect on AHR or DC subsets. pDC selective

induction of Tregs may occur through inhibitory signals conveyed to Teffectors and/or DCs through indoleamine 2,3-dioxygenase (IDO) or through programmed death receptor ligation by B7-H1. These data suggest that C5 may regulate resistance to allergen-driven AHR by enhancing the recruitment or activation of pDCs, which drive CD4+CD25+ Treg cell-induced tolerance. Moreover, the absence of this regulatory pathway under conditions in which C5 is absent results in a loss of tolerance and contributes to susceptibility to asthma.

ROLE OF C3A IN ALLERGEN SENSITIZATION

The effects of C3a on processes occurring during allergen sensitization have not been directly evaluated. Studies in C3aR KO mice show that C3a regulates Th2 cytokine production, suggesting that either C3a regulates recruitment and activation of Th2 cells or regulates processes occurring during sensitization or during the effector phase of the allergic response. Although this has not been evaluated directly, studies in which complement activation was blocked at the level of C3 either at the time of sensitization or after sensitization showed that complement blockade effectively suppressed Th2 cytokine production and associated effector functions after priming, but not at the time of antigen sensitization (34). Although this approach is not specific for C3a, the results suggest that C3a does not influence either the nature or magnitude of the T cell response at the sensitization phase, but may regulate Th2 cytokine production and Th2-mediated effector functions through its ability to recruit and/or activate both Th2 effector cells and non-T cells capable of producing Th2 cytokines. Thus, determination of the exact mechanism(s) by which C3a mediates Th2-driven immune responses in the lung await studies examining the role of C3aR signaling at the time of initial sensitization.

C3A AND C5A: YING AND YANG

Based on the knowledge at hand, the balance of C5a to C3a in the airways during early life exposures to allergens may be a major determinant of the development of tolerance or immunity to inhaled antigens. Although the exact levels of each of these mediators in early life are unknown, we propose the following scenario: that high levels of C3a, induced locally through exposure to environmental triggers of asthma such as ozone, cigarette smoke, RSV, or particulate matter, would drive sensitization to inhaled allergens at the airway surface. This level of C3 would presumably set up a Th2 environment in the lungs, which is consistent with data suggesting that the lungs have a Th2 skew at birth and shortly thereafter. If C3 levels persist unopposed by C5a during early allergen exposures, the ensuing Th2 inflammatory response would perpetuate the process by inducing additional C3 production by the airway epithelium. This scenario is consistent with the relatively higher levels of C3a over C5a found in asthmatic airways as compared with those of control subjects after allergen challenge (7). The conditions under which C5a would dominate are unknown, but may include frequent microbial exposure and/or lack of exposure to environmental triggers such as pollution and smoke. Nonetheless, the balance between these two anaphylatoxins is clearly an important determinant of protection or sensitization to inhaled antigens; thus, additional studies aimed at determining the mechanisms driving differential production of C3a and C5a in the airways are clearly warranted.

GENETIC DETERMINANTS OF COMPLEMENT PATHWAY ACTIVATION

The differential production of complement components in response to various environmental stimuli between individuals

with asthma and those without asthma suggest that there may be alterations in the genetic control of the production of, the activation of, or the response to various complement components which may underlie susceptibility to asthma. Indeed, there are several reports suggesting an association between chromosomal regions containing complement genes and asthma susceptibility (35, 36). Moreover, in support of the opposing roles of C3 and C5 in animal studies, Hasegawa and coworkers have recently reported an association between a single nucleotide polymorphism (SNP, 4896C/T) in the C3 gene and atopic asthma in both children and adults in a Japanese population, whereas SNPs in the human C5 gene are associated with protection against both childhood and adult asthma (37). Similarly, Barnes and colleagues have found a significant association between a 3-SNP haplotype in the C3 gene and asthma, IgE levels and the ratio of IFN- γ /IL-13 levels in serum in an Afro-Caribbean population (38). Interestingly, the frequency of these SNPs is high, suggesting that these polymorphisms may have conferred evolutionary advantage in the past and perhaps in protection from parasitic infections. Moreover, as these SNPs are not in the promoters of these genes, they may reflect structural changes in the proteins that either alter their ability to recognize exogenous and endogenous danger signals or alter their ability to be activated by other molecules in the complement pathway or by exogenous proteases (i.e., allergens, inflammatory cell-derived proteases). Clearly, further studies in additional populations are needed to determine the general importance of variants in complement genes to asthma susceptibility and to elucidate the functional consequences of the known SNPs in these genes.

INTERACTIONS BETWEEN COMPLEMENT PATHWAYS AND OTHER INNATE IMMUNE PATHWAYS

The complement system and the family of TLRs are two central arms of innate immunity that are critical to host defense as well as the development of adaptive immunity. Most pathogens and presumably allergens activate both complement and TLRs, suggesting that coordinated interactions between these two systems may be important in the development of disease. Indeed, Hawlisch and Köhl have recently shown that C5a negatively regulates TLR4-induced synthesis of IL-12 family members in macrophages through extracellular signal-regulated kinase and phosphoinositide 3 kinase-dependent pathways (39). Interactions between PRRs can increase and diversify the recognition and overall handling of pathogens by the innate immune system that are otherwise limited by the genetic bottleneck. Although these interactions have not been formally explored in the pathogenesis of asthma, studies that explore the interaction of the complement system with other innate immune pathways are clearly warranted.

CONCLUSIONS

It is becoming increasingly clear that immunoregulatory events occurring at the interface of innate and adaptive immunity play an important role in asthma pathogenesis. Complement stands at this interface. The data reviewed here suggest that the complement activation pathway serves as a central regulator of adaptive immune responses to a variety of inhaled substances. The emerging paradigm suggests that C3/C3a generation at the airway surface serves as a common pathway for induction of AHR by a variety of environmental triggers. On the other hand, C5/C5a plays a dual immunoregulatory role by protecting against the initiation of Th2-mediated immune responses during initial contact with allergens through its ability to influence DC/T cell interactions and a more traditional pro-inflammatory role once immune responses are established. Thus, factors regulating the

balance between C5a and C3a generation may determine the tendency to develop tolerance or immunity to inhaled allergens, respectively. Although we are in the initial stages of understanding the complex interactions between various components of the complement pathway as well as their interactions with other innate immune pathways, one may postulate that changes in the activation of specific complement components due to differences in exposure to microbes or to genetic alterations in complement family genes or the convergence of both of these factors (gene–environment) may explain the recent rise in allergic diseases in the modern world (the Hygiene Hypothesis).

Conflict of Interest Statement: M.W.-K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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