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Novel Effector Molecules in Type 2 Inflammation: Lessons Drawn from Helminth Infection and Allergy

Meera G. Nair, Katherine J. Guild, and David Artis

Type 2 cytokine-induced inflammatory responses are critical components of the mucosal immune response required for host defense against helminth infection and are also responsible for the pathogenesis of many debilitating diseases including asthma, allergy, and forms of inflammatory bowel disease. Given the global prevalence of helminth infections, with an estimated two billion individuals infected worldwide, and the pandemic levels of asthma and allergy, with 30% of the population affected in North America, it is essential to define the molecules and pathways that underlie the protective or pathologic consequences of type 2 inflammation. In this review, we will focus on four families of proteins that are highly induced in helminth infection and allergy: 1) the arginases; 2) the resistin-like molecules; 3) the chitinase-like mammalian proteins; and 4) the interleukins. Here, we summarize what is known about their regulation and potential function in protecting against infection and/or exacerbating inflammation. The Journal of Immunology. 2006, 177: 1393–1399.

Type 2 inflammatory responses

Type 2 inflammatory responses are promoted by differentiated CD4+ Th type 2 cells that secrete a panel of cytokines including IL-4, IL-5, IL-9, IL-13, IL-25, IL-31, and IL-33 (1–5), and are characterized by the recruitment of multiple effector cells including B cells producing IgE, mast cells, eosinophils, and basophils. Although significant advances have been made in defining the immune cells, soluble mediators, and signaling pathways that orchestrate type 2 immune responses (reviewed in Refs. 6, 7), the effector molecules that either mediate protective immunity against helminths or provoke destructive inflammatory responses remain poorly defined. Recent gene profiling studies in patients and murine model systems have identified a number of novel molecules that are highly up-regulated during type 2 inflammation at mucosal surfaces. Many of these factors are secreted by macrophages and epithelial cells; cell types that are implicated in influencing the inflammatory response and innate defense to parasitic infection. A better understanding of the factors that regulate expression of these molecules and what functions they have in these disease settings may facilitate the identification of novel therapeutic targets. In this review, we will discuss the properties and putative functions of four families of molecules that are expressed by macrophages and/or epithelial cells in protective and pathologic type 2 inflammatory responses: 1) the arginases; 2) the resistin-like molecules (RELM); 3) the chitinase-like mammalian proteins; and 4) the interleukins.

The arginase family

There are two main macrophage activation pathways. In response to proinflammatory signals such as TLR ligands and IFN-γ, macrophages become “classically activated” and express inducible NO synthase (iNOS), resulting in the breakdown of 1-arginine to NO and reactive oxygen species that are potent microbicidal molecules against intracellular pathogens (8). In contrast, following exposure to IL-4 or IL-13, macrophages adopt an “alternatively activated” phenotype (termed AAMacs; alternatively activated macrophage) and express a number of factors including arginase (9). This enzyme competes with iNOS for the substrate 1-arginine, resulting in the accumulation of 1-ornithine (which can be further metabolized into 1-proline and polyamines) and urea (10). There are two isoforms of arginase in vertebrates that display distinct expression profiles. Arginase-1 is cytosolic and predominantly expressed in the liver of naive mice, where it plays an essential role in the urea cycle, whereas arginase-2 is mitochondrial and expressed at low levels in other tissues such as the brain and the kidney, where its function is less well characterized (11). Although macrophages can express both arginase-1 and arginase-2, their induction is differentially regulated. For example, whereas LPS drives expression of both isoforms, only arginase-1 contains STAT-6 response elements upstream of its promoter region and is induced by Th2 cytokines (12).

Arginase-1 expression by macrophages is a common feature in several models of helminth infections. Loke et al. (13) demonstrated that the accumulation of arginase-1-positive macrophages during infection with the filarial helminth Brugia malayi was an IL-4-dependent phenomenon. Furthermore, in murine...
models of schistosomiasis, arginase-1 expression was correlated with the *Schistosoma mansoni* egg-induced granulomatous pathology in the liver and the lung (14). Although helminth infections appear to specifically induce arginase-1 expression, gene-profiling studies have demonstrated that both arginase isoforms are induced in murine models of airway inflammation and in asthma patients (15–17). In a murine model for asthma, arginase-1 and arginase-2 differed in their regulation and temporal expression; arginase-2 was induced early following challenge and expression was STAT-6 independent, whereas arginase-1 was induced later and was dependent on STAT-6 signaling (15). In addition to their differential expression, it is likely that both arginase isoforms have distinct cellular sources in the lung. For example, in a murine model for pulmonary fibrosis induced by chemical injury with bleomycin, arginase-1 was expressed by macrophages in the lung while arginase-2 expression was restricted to the pulmonary epithelium (18).

Collectively, these studies identify arginase expression as a common feature of both helminth infection and asthma, although its functions in these disease settings are still unclear. Most evidence suggests a role for arginase in driving fibrosis, because it mediates the production of proline and polyamines, which act both as substrates for collagen synthesis and can induce cell proliferation (19). For instance, in *S. mansoni* egg-induced fibrosis in the lung, the effect of increasing arginase activity through the inhibition of iNOS or the use of NOS-2-deficient mice led to larger granulomas (20, 21). In addition to a directly pathogenic role, polyamines have been implicated in the regulation of mast cell activation and secretion (22), suggesting arginase-positive AAMacs can promote airway inflammation via recruitment and retention of other inflammatory cells.

Although detrimental in the context of granulomatous disease and asthma, fibrosis is a necessary process for wound repair and tissue remodeling following injury and may play an important protective function against helminth parasites (19). For example, the fibrogenic properties of arginase that promote granuloma formation could sequester helminth parasites and reduce their motility, facilitating parasite killing by effector cells such as eosinophils or neutrophils that are recruited to the site of tissue remodeling (23). In vitro studies also showed a positive correlation between macrophage killing of *S. mansoni* and arginase activity, suggesting a direct antiparasitic role for arginase through mechanisms that are still unclear (24). Supporting a protective function in the context of infection, macrophage-specific IL-4Ra-/- mice that are deficient in AAMacs developed severe egg-induced intestinal inflammation and died from acute sepsis following exposure to *S. mansoni*, potentially due to a defect in AAMac-dependent tissue repair (25). Because arginase can also suppress T lymphocyte proliferation through limiting the availability of L-arginine (26), arginase-positive AAMacs may also down-regulate proinflammatory responses during helminth infection. Therefore, in both asthma and helminth infection, the tissue remodeling and immunoregulatory properties of arginase are likely to have important consequences for host protection and the magnitude of the inflammatory response.

**The RELM family**

The RELM family, composed of RELM-α, RELM-β, and RELM-γ, is a group of proteins that share sequence homology to resistin, an adipocyte-secreted factor that can regulate responsiveness to insulin (27). All RELM proteins are secreted, contain highly conserved C-terminal cysteine residues (Fig. 1), and are expressed during type 2 inflammation (13, 28, 29). Through the formation of disulfide bonds, the cysteine-rich signature motif shared by RELM proteins dictates their tertiary structure (28, 30), and is hypothesized to play a critical role in signaling or in the binding to a family of, as yet unidentified, receptors. Resistin and RELM-β contain an additional cysteine residue that contributes to their oligomerization and is critical for protein function (Fig. 1) (30). Interestingly, RELM proteins can form multimeric complexes with each other as well as with themselves, expanding their scope of potential functions through the promiscuous use of ligands and receptors (31).

RELM-α has the widest expression pattern of the RELM family and shows greatest pleiotropy in putative functions. First, reports of STAT-6-dependent induction of RELM-α in the alveolar epithelium during pulmonary inflammation and fibrosis have implicated this protein in the asthma pathogenesis (32, 33). Indeed, RELM-α has mitogenic properties and may mediate fibrosis through the stimulation of smooth muscle cell proliferation and the induction of actin and collagen deposition (34).

RELM-α is also a prominent gene induced following exposure to variety of helminths (13, 26, 29, 35). In murine infection with *B. malayi*, RELM-α was expressed by macrophages recruited to the site of infection, and by B cells and dendritic cells in the draining lymph nodes (13, 35). Additionally, RELM-α was induced at the sites of infection with several gastrointestinal (GI) helminths, where the cell types responsible for gene expression may be different (29, 35, 36). For example, Voehringer et al. (36) reported that eosinophils from the lungs of mice infected with *Nippostrongylus brasiliensis* could express RELM-α, whereas immunohistochemical analysis of the colonic tissues of mice infected with *Trichuris muris* showed that RELM-α was expressed predominantly by infiltrating cells (likely macrophages) in the lamina propria (Fig. 2, A and B) and also by a subset of goblet cells in the colonic epithelium (29). Although the functions of RELM-α in the context of helminth infection are still unclear, they may depend on the site of induction and the cellular source of the protein. For example, RELM-α expression by B cells and dendritic cells suggests an immunoregulatory role, potentially through influencing cell proliferation or through the regulation of nerve growth factor, which can influence the recruitment and activation of inflammatory cells (28, 34, 37). However, at the site of infection, the fibrotic properties of RELM-α could, like arginase, provide protection against infection by contributing to sequestration of parasites in tissues. Additionally, through its ability to regulate nerve growth factor action, RELM-α could promote immunity to infection by influencing enteric nerve cell function, a pathway previously suggested to contribute to expulsion of helminth parasites from the GI tract (38). Although a RELM-α ortholog has not been identified in the human genome, human resistin shows a greater similarity in expression pattern to murine RELM-α than murine resistin, and is expressed by leukocytes and myeloid cells (39, 40). Thus, the putative functions for murine RELM-α may be shared with resistin in humans.

In contrast, RELM-β is present in both the human and mouse genomes, where it displays a similar expression profile and is present in the GI tract and the lungs (27, 28). Similar to
RELM-α, RELM-β is induced in a Th2 cytokine-dependent manner in murine models of asthma and in response to multiple GI helminth infections (35, 41, 42). However, in the GI tract, RELM-β displays a more restricted expression pattern and is uniquely expressed by goblet cells (see Fig. 2, C and D), implying that despite structural similarities, both proteins may differ in function. RELM-β was also up-regulated during bacterial colonization of the gut, suggesting a broader function in response to diverse microorganisms in the GI tract (43). Intriguingly, the transcription factor Cdx2 was implicated in RELM-β induction in response to bacterial colonization but not helminth infection, implying that transcriptional regulation of RELM-β may be stimulus-dependent (29). Although a function for RELM-β in the context of bacterial colonization has not been determined, studies have implicated a protective function against helminths. For example, in multiple GI helminth infection models, maximal RELM-β secretion was correlated with Th2 cytokine expression and worm expulsion (41). Furthermore, in vitro studies with *T. muris* and *Strongyloides stercoralis* demonstrated that recombinant RELM-β could bind to the helminth sensory apparatus and inhibit chemotactic migration toward host tissue extract (41).

The least well-characterized RELM protein is RELM-γ, which shares the greatest sequence identity with RELM-α (Fig. 1). Similar to RELM-α, murine RELM-γ is expressed in the lung and by hemopoietic cells (44) and in the GI tract following exposure to several helminth parasites (29), although whether it has overlapping or distinct functions with other RELMs is unknown at present. In contrast to the RELM family members, there are no reports of adipocyte-derived resistin expression in murine models of allergy or helminth infection. Although beyond the scope of this review, it is important to consider that adipocytes may influence the immune response through various mechanisms. For instance, recent studies demonstrated that recombinant resistin can stimulate cytokine production in human macrophages (45), and can also activate suppressor of cytokine signaling-3 (46), a transcription factor that regulates inflammation in multiple disease settings. Thus, we cannot rule out a role for resistin in the regulation of type 2 inflammatory responses.

Interestingly, comparative phylogenomic studies in mice and humans have revealed that although the RELMs are conserved, the expression patterns and functions of this family of proteins may be species-specific (40). Functional studies of the human RELM family will allow a better understanding of what role they play in type 2 inflammation and will facilitate comparative analysis of shared and non-overlapping functions of RELM proteins in different species. Notwithstanding this, the reports
were wax-embedded, and 5-μm sections were prepared, as previously described (41). All sections were stained with nuclei-specific 4',6'-diamidino-2-phenylindole (blue). Standard immunofluorescence staining was performed with monoclonal rabbit anti-RELM-α (red; A and B), monoclonal rabbit anti-RELM-β (green; C and D) (both obtained from PeproTech), and polyclonal chicken anti-intelectin 1/2 (E and F) (a gift from H. Miller, University of Edinburgh, U.K.). Blocking peptides confirmed specificity of staining.

of RELM expression in multiple inflammatory diseases implicates members of this family as targets for manipulating infection and disease outcome.

Chitinase-like mammalian proteins

Chitinases are enzymes that degrade chitin, an abundant polysaccharide present in fungal walls, the cuticles of helminths, and the exoskeletons of arthropods, but strikingly absent from higher organisms. These enzymes are expressed by most lower organisms including plants and fish, where they have a known protective function against chitin-bearing pathogens (47). Chitinase genes have also been discovered within the mammalian genome (48) and are collectively known as the chitinase-like mammalian protein family. This family contains two functional chitinases: chitotriosidase, which was discovered in lipid-laden macrophages recruited in Gaucher disease (48), and acid mammalian chitinase (AMCase), which is expressed in the lung tissue and stomach of rodents and humans (49). Other members of this family include murine Ym1 (50), human HCgp-39 (51), YKL-39 (52), oviductin (53), and recently identified human stabilin-1-interacting chitinase-like protein (Si-CLP) (54). However, none of these family members display chitinase activity due to mutations within the catalytic site (50).

Induction of chitinase-like mammalian proteins is a consistent feature of several helminth infection models (35, 55). Because most parasitic helminths synthesize chitin during several stages of their life cycle (56), chitinases may perform an effector role against parasites by binding to the cuticle and mediating its breakdown. Consistent with this hypothesis, a study of the genetic polymorphisms of human chitotriosidase showed a correlation between chitotriosidase deficiency and susceptibility to filarial infection with Wuchereria bancrofti (57). However, the high expression levels of chitinases in asthma and other inflammatory diseases implicate pathologic roles beyond host protection (15, 48, 58). Zhu et al. (58) reported AMCase induction in the airway epithelium and pulmonary macrophages in a murine model of asthma. Furthermore, through the use of recombinant protein and in vivo blocking studies, it was demonstrated that AMCase mediated pulmonary inflammation, partly through the induction of eotaxin and MCP-1 (58). In support of a role for AMCase in asthma, a genetic study of human AMCase polymorphisms showed a strong correlation between a newly identified variant of AMCase and asthma severity (59).

Although the expression of AMCase during type 2 inflammation has only been reported in the lung, Ym1, a secreted chitinase-like lectin in mice, has a wider expression pattern. In addition to Th2 cytokine-dependent up-regulation in the bronchoalveolar lavage fluid and lung tissue in murine models of asthma (60), Ym1 is also induced at multiple sites of helminth infection including the thoracic cavity, the peritoneum, and the GI tract (35, 61). Macrophages and neutrophils are the dominant cellular source of Ym1 in these settings, although expression by Th2 cytokine-activated dendritic cells and B cells has also been reported (35, 61, 62). Although Ym1 can bind chitin, it is unlikely that it plays a direct defensive role against chitin-bearing pathogens because it displays no chitinase activity. Nevertheless, Ym1 has eosinophil chemotactic properties, and thus could play an indirect role in parasite killing through binding the helminth and mediating recruitment of effector eosinophils (63, 64). In addition to binding chitin, Ym1 can bind the glycan heparin (61), which is abundant on the cell surface and extracellular matrix, suggesting that Ym1 might mediate cell-to-cell and cell-to-matrix interactions. Thus, Ym1 could participate in extracellular matrix deposition during tissue remodeling and thus contribute, like arginase and RELM-α, to the tissue remodeling and fibrosis observed in murine models for asthma or in S. mansoni egg-induced granulomas. In support of a pathogenic role, Ym1 can spontaneously form crystals in murine lungs, structures that are implicated in airway blockade, similar to the Charcot-Leyden crystals that develop in several eosinophil-rich inflammatory diseases in humans (62, 65).

Although there is no human ortholog of Ym1, the macrophage-expressed human genes HCgp-39 and Si-CLP show similar properties to this murine lectin. For instance, HCgp-39 is overexpressed in many pathologic conditions involving tissue remodeling and collagen deposition including liver fibrosis (66). Although Si-CLP levels have not been correlated with pathogenesis, this human lectin was detected in bronchoalveolar lavage fluid and is secreted by AAMacs, drawing parallels to murine Ym1 (54). Taken together, the presence of chitinase-like proteins in mammalian genomes suggests that they may have been conserved to provide some protective benefit against helminths or other chitin-bearing pathogens. However, the existence of family members that do not exhibit or have lost chitinase activity, coupled with the prominence of this family in multiple inflammatory disease settings, implies that their expression may also have pathogenic consequences.
Intelectins

Of all the molecules discussed in this review, the human and murine intelectin family, composed of two calcium-dependent galactose-binding lectins (intelectin-1 and intelectin-2) is the least well characterized, with relatively little known on the regulation of their expression and putative functions (67, 68). However, gene profiling analysis identified intelectin expression as a hallmark of parasitic helminth infection in the murine GI tract (69, 70). Indeed, proteomic analysis revealed that up-regulation of intelectin-2 was the most marked change in protein expression in the intestinal epithelium following infection with *Trichinella spiralis* (71). Intelectin-1 and intelectin-2 mRNA transcription was also up-regulated in the GI tract of genetically resistant mice following exposure to *T. muris* (our unpublished observation). Similar to RELM-β, maximal intelectin expression was correlated with expression of type 2 cytokines and worm expulsion, suggesting a function in host-protective immunity. Supporting this hypothesis, immunohistochemical analysis demonstrated that the cellular sources of intelectins were either goblet cells or paneth cells, both populations that are specialized for the luminal secretion of antimicrobial factors (70) (Fig. 2, E and F). Previous studies have shown that intelectins bind galactofuranosyl-containing residues in bacterial cell walls (68), thus intelectins may bind similar carbohydrate residues in parasitic helminths and impair attachment, feeding, or other essential biological processes that would render the worms more susceptible to immune-mediated expulsion. Alternatively, analogous to the *Xenopus* ortholog, mammalian intelectins may contribute to host defense indirectly, through binding to and altering the properties of other mucin glycoproteins secreted by intestinal goblet cells (72).

Consistent with the other families of molecules discussed above, recent studies have reported expression of intelectins in murine models of airway inflammation (73), suggesting a broader function for this family of molecules than defense

![Table I. Putative functions for novel molecules in helminth infection and allergy](http://www.jimmunol.org/)

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Denotes likely function; *, denotes unknown functions at present.

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**FIGURE 3.** Novel type 2 cytokine-induced molecules in helminth infection and allergy. Schematic diagram of the mucosal sites (lung and intestine) and the cell types (epithelial cells, red; macrophage, blue) where these novel molecules are expressed.
against helminth parasites. However, at present, it is unclear whether invertebrate expression in the inflamed lung can influence the magnitude of Th2 responses and/or contribute to the inflammatory response. Certainly, it is possible that expression of invertebrates is simply a by-product of the type 2 cytokine-induced inflammatory milieu of the tissue. The possibility that invertebrates perform overlapping or unique functions with RELM or chitinase-like family members will be an area for future studies.

Conclusions

In this review, we have discussed the expression profile and putative functions of several families of proteins that are highly expressed during helminth infection and allergy. These molecules all share similar features including regulation by Th2 cytokines and expression by macrophages and/or epithelial cells at mucosal sites of inflammation (Fig. 3). Although space constraints limit the scope of this review, it is important to acknowledge that additional Th2 cytokine-regulated protein families may play equally important roles in these disease settings, including chemokines, small proline-rich proteins, mucins, metalloproteinases, and their inhibitors. Although all of these proteins share properties that imply a protective role against helminths and in mediating tissue repair following infection-induced injury, several studies suggest an additional pathogenic role in type 2 allergic diseases (Table 1). These conflicting roles (protective and pathologic) are consistent with the hygiene hypothesis, which proposes that coevolution of humans and parasites has led to an immune system that provides a protective function against parasites in the past, but has become obsolete or pathologic due to the lower prevalence of parasitic infections in industrialized nations (74). Regardless, further studies on these protein families will offer the prospects of novel therapeutics in promoting resistance against parasitic infections or in limiting type 2 cytokine-mediated chronic inflammation.

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Disclosures

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