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Arginase: marker, effector, or candidate gene for asthma?

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Commentary

Microarray analysis of the expression profiles of lung tissue in two murine models of asthma revealed high levels of arginase I and arginase II activity, in association with IL-4 and IL-13 overexpression, suggesting that arginine pathways are critical in the pathogenesis of asthma.

molecular bases of the TNF signaling. Given its important contribution to TNF-induced JNK activation, AIP1 may represent a suitable target for possible therapeutic applications in human diseases characterized by increased TNF-α–mediated apoptosis.

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Microarray analysis of the expression profiles of lung tissue in two murine models of asthma revealed high levels of arginase I and arginase II activity, in association with IL-4 and IL-13 overexpression (see the related article beginning on page 1863), suggesting that arginine pathways are critical in the pathogenesis of asthma.

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Despite intense research efforts, asthma remains a major medical and scientific challenge. Prevalence of this disease increased 75% between 1980 and 1998. Although this rate may now be stabilizing, the 2001 National Health Interview Survey estimated that 6.9% of adults, and 8.9% of children under the age of 18 in the United States, suffered from asthma (1). The reasons why asthma prevalence has been on the rise for so long remains a matter of intense speculation. The pathogenetic mechanisms of the disease, and the contributing genetic factors, also remain elusive. This state of affairs probably reflects the inherent complexity of the disease, and the difficulty associated with stringently defining asthmatic phenotypes so that homogenous subject groups can be identified for mechanistic studies.

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Nonstandard abbreviations used: protein inhibitor of activated STAT1 (PIAS1).

Microarrays: a powerful tool to dissect asthma

An aggressive approach to the identification of new asthma genes is discussed in this issue of the *JCI* by Zimmermann and collaborators (2), who determined transcript expression profiles in lung tissue from mice with an asthma-like phenotype induced by sensitization with OVA or *Aspergillus fumigatus*. The recognized strength of microarray experiments lies in their ability to address an issue globally, and highlight the unexpected. The results of this study are no exception. An important quantitative finding was that 6.5% of the 12,422 genes analyzed showed a greater than twofold change in expression in challenged mice. These data show that, although asthma remains confined to the lung, the mechanistic dysregulation underlying the disease — whatever that may be — mobilizes a vast genetic program. Even more importantly, among the 496 and 527 genes identified in the OVA and *Aspergillus* models, respectively, only 291 were common to both. Since all mice had the same genetic background, this pattern is likely to result from differences in pathogenetic mechanisms, possibly related to the nature of the allergen and/or the immunization route. Such data should provide molecular epidemiologists and clinicians interested in asthma with spicy food for thought.

Enter arginine and its pathways

Intriguing findings also came from the qualitative analysis of lung transcript

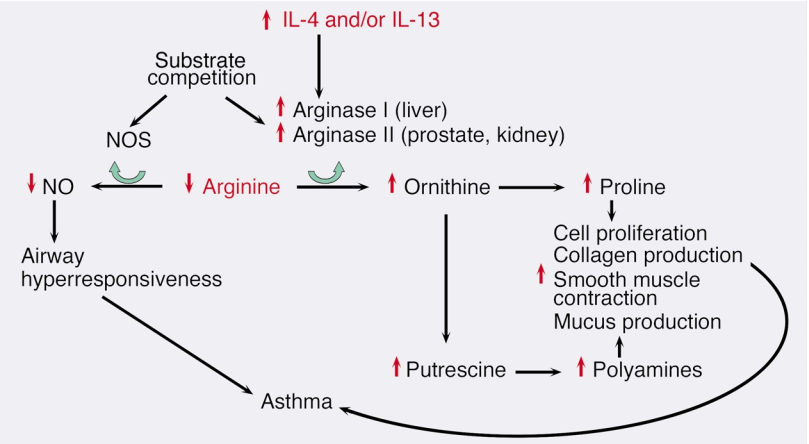

Figure 1
Arginine, arginase, and asthma. Arginase I and arginase II control the transformation of arginine into ornithine, which in turn gives rise to proline and polyamines. These products have multiple effects on connective tissue, smooth muscle, and mucus synthesis. Arginine also serves as a substrate for NO synthase (NOS), which generates NO, a critical regulator of airway physiology. The NOS and arginase pathways interfere with each other through substrate competition. Th2 cytokines induce arginase expression. During allergic inflammation, increased IL-4 and/or IL-13 expression results in increased expression of arginase and amplification of the arginase-dependent pathway, with concomitant suppression of NO generation. This leads to airway hyperresponsiveness and increased generation of mucus and collagen, all of which may contribute to the pathogenesis of asthma. The red arrows mark the upregulatory or downregulatory events that occur in arginine metabolism following increased expression of Th2 cytokines.

profiles. The genes differentially expressed in challenged mice included arginase I, arginase II, and the L-arginine transporter cationic amino acid transporter-2. All of these molecules are involved in arginine metabolism (Figure 1). In particular, arginase catalyzes the hydrolysis of arginine to ornithine and urea, and exists in two isoforms. Arginase I participates in the urea cycle, and is expressed at high levels in the liver. Arginase I deficiency results in argininemia, a disorder characterized by mental impairment, growth retardation, spasticity, and sometimes fatal episodes of hyperammonemia. Arginase II is most highly expressed in the prostate and kidney, and poorly expressed in the liver. Arginase II is thought to be involved in the synthesis of proline and/or polyamines (e.g., putrescine, spermidine, and spermine), which control cell proliferation and collagen production. To date, no human disease has been associated with a deficiency in arginase II (3).

Arginine, a molecule of many trades

Of note, arginine serves as a substrate for both arginase and NO synthase (Figure 1). The arginase and NO synthase pathways can therefore interfere with one another through substrate competition (3). NO is a ubiquitous gaseous molecule that regulates many aspects of human airway biology, including airway and vascular smooth muscle tone (4). An increased concentration of NO in exhaled air is now recognized as a critical component of the asthmatic phenotype (5). The links with the NO pathway and collagen generation make arginine metabolism a rising star in the asthma firmament. Arginase I and arginase II were recently shown to contribute to the development of mouse lung fibrosis (6). Increased arginase activity underlies allergen-induced deficiency of NO and airway hyperresponsiveness in a guinea pig model of allergic asthma (7). Perhaps even more importantly, arginase expression appears to be controlled by Th2 cytokines, central mediators of allergic asthma (8). IL-13–mediated induction of arginase I in macrophages has been implicated in IL-13–dependent inhibition of NO production (9), which in turn may contribute to asthma pathogenesis. We may infer that NO inhibition resulted from substrate competition, because expression of arginase, but not NO synthase, was altered in the lungs of the allergen-challenged mice (2). Last, but not least, at the molecular level, arginine appears to be a key regulator of signaling through the JAK/STAT pathway. Indeed, post-translational modification (methylation) of a highly conserved arginine residue in the N-terminal domain of STAT1 is a requirement for IFN-α– and IFN-β–induced transcription (10). In the absence of arginine methylation, STAT1-DNA binding is impaired due to an increased association of the protein inhibitor of activated STAT1 (PIAS1) (11) with phosphorylated STAT1 dimers. No STAT6-specific PIAS has been identified to date, but the negative regulation of STAT signaling is expected to involve more than one member of the PIAS family. The search is on, and may well be successful.

A novel asthma gene?

The study by Zimmermann et al. (2) confirms that expression of arginase is increased in the asthmatic lung through a Th2-induced, STAT6-dependent mechanism, and most importantly extends these findings to humans. Interestingly, in situ hybridization in the lung of asthmatic patients revealed expression of arginase not only in submucosal inflammatory cells (most likely macrophages, as observed in the murine model) but also in airway epithelium, suggesting an even broader pattern of dysregulation. In the scenario proposed by Zimmermann and collaborators, a Th2 cytokine-dependent increase of arginase expression in the lung would affect arginine metabolism, and contribute to asthma pathogenesis through inhibition of NO generation and alterations of cell growth and collagen deposition. Thus, arginase would act as an effector of Th2 activation. The available arginase I and arginase II knock-out mice (12, 13), and conditional and/or tissue-specific knockouts generated ad hoc, may serve to highlight the effector role of arginase. The real question, however, is whether arginase will make it onto the list of asthma genes. The attribution of a

causative role to arginase will depend on the results of the genetic studies that Zimmermann and colleagues' work warrants. Do single nucleotide polymorphisms in human arginase dysregulate expression and/or function so as to contribute to asthma pathogenesis? The role of arginase in the realm of asthma will ultimately be dictated by the answer to this question.

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An eye on insulin

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Diabetic retinopathy, the most frequent complication of diabetes and leading cause of vision loss, involves vascular and neural damage in the retina. Insulin and IGF-1 signaling are now shown (see the related article beginning on page 1835) to contribute to retinal neovascularization, in part, by modulating the expression of various vascular mediators.

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Physiologic and pathologic blood vessel growth patterns are stimulated by local and systemic influences. The hope of angiogenesis research is to understand these complex interactions in order to provide better means to control pathologic vessel formation, or perhaps stimulate appropriate vessel growth, to reduce maladaptive consequences. In the retina, normal vessel growth occurs in the plane of the retina from the optic nerve toward the periphery in a radial pattern and is guided by cues from astrocytes in the inner retina (1). This growth is mediated by VEGF and other ligands (2), while angioblasts from the circulation can provide endothelial progenitors (3).

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Nonstandard abbreviations used: retinopathy of prematurity (ROP); diabetic retinopathy (DR); hypoxia-inducible factor-1 (HIF-1); endothelin-1 (ET-1); insulin receptor (IR); insulin-like growth factor-1 receptor (IGF-1R), vascular endothelial insulin receptor knockout (VENIRKO); vascular endothelial insulin-like growth factor receptor knockout (VENIFARKO).

The problem of retinal neovascularization

Pathologic neovascularization of the retina is a common and serious complication of retinopathy of prematurity (ROP) and diabetic retinopathy (DR). The treatment of DR, ablation of the diseased retina with laser photocoagulation or cryotherapy to cause involution of the new vessels, has remained fundamentally unchanged for almost 50 years. The nature of the growth promoting stimuli is not well understood, but in ROP the stimulus is assumed to be in part due to perinatal retinal hyperoxia followed by hypoxia. The classic response to hypoxia includes hypoxia-inducing factor-1 (HIF-1) translocation to the nucleus and subsequent downstream events such as the upregulation of VEGF, eNOS, and endothelin-1 (ET-1). The fact that VEGF is increased in the vitreous of diabetic patients makes it tempting to speculate that diabetes induces a hypoxic, or HIF-1–driven response that is similar to that observed in ROP. Current animal models of diabetes do not develop proliferative retinopathy and the only model that simulates the neovascularization seen clinically is that induced by relative hypoxia in developing retinas. Postnatal mice are placed in hyperoxic conditions for several days, at a time when their vessels have not yet reached the peripheral retina, which causes vasoconstriction; when they are returned to room air, the vasoconstriction is relieved and neovascularization develops when the retina perceives relative hypoxia (4). This model provides a rodent model of neovascularization in the absence of systemic metabolic defects due to insulin depletion or resistance. Smith et al. (5) previously showed that an IGF-1 inhibitor blocked neovascularization in this model but no studies have examined the effect of the insulin receptor.