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Why is effective treatment of asthma so difficult? An integrated systems biology hypothesis of asthma

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Abstract

A hypothesis is presented that asthma is not only an airway disease, but that the disease involves the entire lung, and that the chronicity of asthma and asthma exacerbations can perhaps be explained if one considers asthma as a systemic disease. Increased lung—not only airway—vascularity may be the result of the action of angiogenesis factors, such as vascular endothelial growth factor (VEGF) and sphingosine-1-phosphate (S1P). A bone-marrow lung axis can be postulated as one element of the systemic nature of the asthma syndrome, in which the inflamed lung emits chemotactic signals, which the bone marrow responds to by releasing cells that contribute to lung angiogenesis. A molecular model of the pathobiology of asthma can be built by connecting hypoxia-inducible transcription factor-1 α , VEGF S1P, and bone-marrow precursor cell mobilization and acknowledging that angiogenesis is part of the inflammatory response.

Keywords

angiogenesis; asthma; bone marrow; HIF-1 α ; systems disease; VEGF

Asthma is a major world-wide health problem; more people are contracting asthma,¹ perhaps because of an increasingly toxic environment² and/or a diminishing exposure of infants to dirt and farm animals.³ Gene hunters have tracked genes that may predispose to atopic reactions and to ‘jittery’ airways—airway hyperreactivity.⁴ The repertoire of treatments for asthma has recently been expanded from the use of β -adrenergic antagonists and inhaled steroids to include antibodies directed against TNF- α and IgE.^{5,6} The list of important mediators of airway smooth muscle contraction and inflammation was initially short: histamine, acetylcholine, and slow-reacting substance of anaphylaxis—eventually shown to be leukotrienes, arachidonic acid metabolites produced by eosinophils, and mast cells that were clearly identified in the airways of asthma patients.⁷

More recently, the term ‘remodeling’ has been added to describe structural changes of the small bronchi in asthma, such as fibrosis, smooth muscle hypertrophy, and epithelial cell hyperplasia,^{8–10} which may explain why conventional bronchodilator drugs are not effective or why the disease is often irreversible. Viral¹¹ and mycoplasma infections^{12,13} are being considered as trigger factors, and obesity has recently captured the attention of asthma researchers.¹⁴ There are different presentations of the asthma syndrome, and different ‘phenotypes’ and exacerbations of eosinophilic asthma have recently been shown to be reduced by treatment of these patients with an anti-interleukin 5 monoclonal antibody.¹⁵

WHERE THE PROBLEM MAY BE

As asthma presents with airway hyperreactivity and bronchospasm, the tacit assumption has been that asthma is overwhelmingly or entirely an airway disease.^{16,17} Although clinicians and researchers used to make a distinction between extrinsic and intrinsic forms of asthma, the bronchial tree is the target of the immune response and the combat zone of inflammatory cells and mediators. Some investigators have recently questioned whether a strict compartmental approach to chronic and progressive lung diseases has narrowed our view and denied access to critical cues and information, which can only be obtained if one considers a more holistic viewpoint, that is the entire lung is involved and asthma is in fact a systemic disease.^{18,19}

HYPOTHESIS

The hypothesis entertained here is that asthma is not only an airway disease, but also a *vascular* disease. A second element of the overall hypothesis is that the *chronicity of the asthma syndrome* is maintained by bone-marrow-derived precursor cells.

Hyperemic bronchial mucosa in asthma has been recognized for many years.^{20–23} Bronchial biopsy studies have repeatedly documented hypervascularity,^{23,24} and this phenomenon has been explained as part of the airway inflammation (calor, rubor). Yet, recent data document that one powerful endothelial cell growth factor and important angiogenesis factor—vascular endothelial growth factor (VEGF)—is also overexpressed in the lung *parenchyma* and the *supernormal* diffusing capacity (DLCO, total lung diffusing capacity, a standardized measure of the diffusion of carbon monoxide across lung capillary membranes) measured in some patients with asthma²⁵ is consistent with the concept that the asthmatic *lung* has more vessels and more capillaries. A hypervascularized lung is likely to produce symptoms of a ‘congested lung’. Anti-inflammatory asthma therapy may work because it affects the vascularity of the bronchi and of the lung parenchyma, and thus reduces swelling (congestion). It is surprising that in the mind of many investigators, hypervascularity is restricted to the airway mucosa.

ASTHMA AND ANGIOGENESIS

The hypoxia-inducible transcription factor-1 alpha (HIF-1 α) binds to hypoxia-response elements in the promoters of many genes involved in the adaptation to an environment of insufficient oxygen or hypoxia. Lee *et al.*²⁶ examined the levels of VEGF and HIF-1 α in the bronchial mucosa of patients with asthma by immunohistochemistry and found increased expression of both proteins. Mast cells, which are known to be a source of increased airway production of VEGF,²⁷ can mediate an increase in vascular permeability through HIF-1 α /VEGF,²⁸ but mechanical strain of airway smooth muscle can also increase expression of HIF-1 α and VEGF.²⁹ The relationship between VEGF-dependent airway angiogenesis and its regression and airway remodeling in the mouse has been investigated by McDonald and co-workers.^{30,31} Airway smooth muscle cells from asthmatic patients promote *in vitro* angiogenesis and BALF from asthmatic patients promotes angiogenesis.³² VEGF serum levels were found to be elevated in patients with Churg–Strauss syndrome³³ and in patients with toluene diisocyanate-induced asthma.³⁴ Elias and his group have shown that VEGF induces an asthma-like phenotype and that nitric oxide (NO) mediates VEGF-induced pulmonary angiogenesis and airway hyperreactivity.^{35,36} Asthma is also a syndrome that is associated with enhanced lymphoangiogenesis mediated by VEGF-C and VEGF-D through the VEGFR3.³⁷

HYPOXIA-INDUCIBLE TRANSCRIPTION FACTOR-1 ALPHA

Lee *et al.*²⁶ reported increased expression of HIF-1 α protein in the airway mucosa of patients with asthma; this is to our knowledge the only published report to date, which has attempted

to link HIF-1 α and clinical asthma. Of interest in regards to a 'viral triggers of asthma' hypothesis^{38–40} are the observations that human rhinovirus,⁴¹ RSV,^{42,43} and EBV⁴⁴ all induce stabilization of HIF-1 α . House dust mite exposure causes airway remodeling in mice⁴⁵ and non-human primates is associated with increased airway and lung tissue VEGF expression;⁴⁶ however, HIF-1 α expression was not measured in these experiments.

Although HIF-1 α has been examined in the context of cancer/malignancy and hypoxia, there is a growing appreciation of its central role in inflammation, and that NF- κ B, a transcription factor that promotes cytokine expression, links innate immunity to the hypoxic response through transcriptional control of HIF-1 α .⁴⁷ In tissue foci of inflammation, to maintain homeostasis, phagocytic cells (as part of the inflammation paradigm) generate ATP through glycolysis, and virtually every enzyme of the glycolytic pathway is transcriptionally controlled by HIF-1 α .⁴⁸ HIF-1 α may contribute to increased airway smooth muscle contractility.⁴⁹ Voelkel and co-workers have found that inhibition of VEGFRs inhibits phagocytic uptake of apoptosed cells. In this case, the inhibited VEGF receptors are on macrophages, illustrating one of the roles of VEGF signaling in the context of resolution of inflammation. Indeed, hypoxia causes an increase in phagocytosis by macrophages in an HIF-1 α -dependent manner,⁵⁰ and induces endothelial cell proliferation through Akt.⁵¹ HIF-1 α myeloid-null mice have greatly diminished joint swelling and cartilage destruction in an arthritis model.⁵²

Loss of HIF-1 α expression in fibroblasts accelerates the onset of cellular senescence.⁵³ Bone-marrow-derived mesenchymal cells depend on HIF-1 α expression to express VEGFR1 and migrate in response to VEGF.⁵⁴ In the absence of HIF-1 α , fewer endothelial cell precursors are recruited to tumors,⁵⁵ whereas HIF-1 α overexpression mobilizes circulating angiogenic cells.⁵⁶ Copper is involved in activation of HIF-1 α by regulating its binding to the hypoxia-response element of target genes.⁵⁷ This fact is of interest in the context of particulate air pollution and sensitivity of asthmatic individuals to inhaled particles. Kennedy *et al.*⁵⁸ detected Cu⁺⁺ in air pollution particulates and showed Cu⁺⁺-dependent IL-6 and IL-8 stimulation in bronchial epithelial cells. Copper can stimulate cell proliferation through HIF-1 α and facilitate epithelial-to-mesenchymal transition and fibrosis.^{59,60} Thus, highly active HIF-1 α could contribute both to angiogenesis (hypervascularity) and fibrotic airway remodeling in asthma. One study indeed reported elevated serum copper levels in patients with asthma.⁶¹

• NO

Numerous reports have described increased exhaled •NO in patients with asthma,⁶² although standardization of the methodology and confounding factors have not led to the establishment of exhaled •NO as a reliable biomarker of asthma disease activity.⁶³ There are also complicated interactions between •NO and VEGF. For example, incubation of endothelial cells with VEGF causes eNOS activation and •NO production,⁶⁴ and VEGF upregulates eNOS in lung epithelium.⁶⁵ Bhandari *et al.*³⁵ pointed out that •NO plays a critical role in VEGF-induced asthma-like lung responses. On the other hand, •NO modulates the HIF-1 α -dependent induction of prolyl hydroxylase 2, the enzyme responsible for HIF-1 α protein stabilization.⁶⁴

ASTHMA AND BONE MARROW

A lung/bone-marrow functional interaction is now apparent to a number of lung researchers. Mobilization of hematopoietic progenitor cells from the bone marrow is a feature of the inflammatory asthmatic responses.^{66–68} We postulate that both the chronicity of the asthma syndrome and also part of the angiogenesis of the asthma lung can be explained by actions of cells developed from bone-marrow-derived progenitors. A few investigations have examined the response of the bone marrow and precursor cell release into the circulation in patients with asthma after antigen inhalation challenge,^{66–68} and Asosingh *et al.*⁶⁹ reported increased endothelial cell precursor cells in the circulation of asthma patients. Anti-chemokine receptor

3 antibodies inhibited migration of bone-marrow-derived CD34⁺ progenitor cells,⁷⁰ and IL-5 inhalation was shown to increase eosinophils in the bone marrow and in the bronchi of asthma patients.⁷¹ Nakae *et al.*⁷² proposed that bone-marrow-derived mast cells are not required for the induction of ovalbumin (OVA)-specific memory T lymphocytes, but are required for increased lymphocyte recruitment in the lung during the challenge phase. Reuter *et al.*⁷³ used a more acute OVA sensitization (without adjuvant) and challenge, confirming that bone-marrow-derived mast cells from wild-type (WT) mice, but not from TNF^{-/-} mice, reconstituted the lung inflammation in sensitized and challenged mast cell deficient mice. Although these experiments document the mast cell dependency of the OVA-induced asthma model, they do not rigorously test the bone-marrow dependency. We now suggest an experimental approach that will focus on the *bone-marrow* dependency of lung inflammation, and will answer the question of whether the OVA-induced response in the lung is reduced in HIF-1 α ^{-/+} mice, but can be reconstituted by transplant of WT bone marrow. Finally, Gomperts and Strieter⁷⁴ make a case for fibrocytes (which are also antigen presenting cells and have angiogenic properties) as bone-marrow-derived cells. Nihlberg *et al.*⁷⁵ identified fibrocytes in the airways of patients with asthma. Taken together, in our opinion it is plausible that the bone marrow can deliver cells that directly or indirectly drive angiogenesis in the 'asthma lung'. CD34^{-/-} mice show a mitigated asthma lung phenotype, which is due to a bone-marrow cell mobilization defect.⁷⁶ Interestingly, the role of VEGF produced in the inflamed lung and spilling over into the systemic circulation has not been considered as playing a role in the release of bone-marrow progenitors in asthma. However, it has been reported that allergic lung inflammation promotes the recruitment of circulating tumor cells to the lung.⁷⁷ It will not be difficult for readers to accept such a concept of an important bone-marrow contribution to the pathobiology of asthma, which is neither taught in medical school nor is the basis of particular treatment strategies.

ASTHMA AND SPHINGOSINE-1-PHOSPHATE

Recent data place sphingosine-1-phosphate (S1P) into the asthma paradigm of inflammation and immune response.^{78–80} S1P levels are mainly regulated by a particular cell's enzymatic activity—sphingosine kinases and sphingosine lysase and phosphatases.⁸⁰ Many cells have the capacity to produce and respond to S1P, yet S1P levels are low in tissue and much higher in blood and lymph. Most of the actions of S1P are mediated by binding to five specific G protein-coupled receptors, designated S1P_{1–5}. S1P₁, S1P₂, and S1P₃ receptors play a role in vascular tone regulation, control of endothelial cell barrier function,^{81,82} and endothelial cell proliferation.^{83,84} Tanimoto *et al.*⁸⁵ showed that S1P activates eNOS through VEGFR2 (KDR). Thus, S1P has not only been shown to cause airway hyperreactivity⁸⁶ and OVA-induced airway inflammation,^{87,88} but also plays a role in lung capillary tube formation⁸⁹ and closely interacts with VEGF and eNOS. This suggests that S1P may be important for asthma angiogenesis,^{90,91} in particular as sphingosine kinase-1 is an HIF-1 α regulated gene and S1P stimulates endothelial cell migration through S1P₁.^{92–94} Moreover, Venkataraman *et al.*⁹⁵ provided evidence that the endothelium is a major contributor to the plasma S1P levels. Earlier studies suggest that increases in levels of ceramide, the precursor of S1P, cause pulmonary cell apoptosis and emphysema-like disease in mice.⁹⁶ Inhibition of enzymes controlling *de novo* ceramide synthesis prevented alveolar cell apoptosis, oxidative stress, and emphysema caused by blockade of the VEGF receptors. Interestingly, concomitant addition of S1P prevented lung apoptosis, implying that a balance between ceramide and S1P is important for maintenance of alveolar septal integrity. Taken together, we postulate a lung structure homeostatic balance between ceramide, which is increased in pulmonary emphysema, and S1P, which is increased in asthma. With some degree of over-simplification ceramide is proapoptotic and anti-angiogenic, whereas S1P is anti-apoptotic and pro-angiogenic (Figure 1).

HOW THE HYPOTHESIS COULD BE TESTED

A few scenarios are sketched here to illustrate how one might experimentally approach our hypothesis. The OVA mouse model of airway hyperreactivity and lung inflammation could be the model of choice simply because of the availability of KO mice, which can be used to take the system apart and to connect the dots. For example, the HIF-1 α ^{+/-} mouse should not develop airway hyperreactivity and the characteristic lung inflammation if HIF-1 α -dependent VEGF upregulation is of importance. VEGF receptor blockade should be similarly effective and S1P–S1P receptor interactions can be specifically probed. To investigate the mobilization of bone-marrow-derived precursor cells, mice with labeled bone-marrow cells can be used and bone marrow from OVA challenged mice—both WT and KO mice—can be transplanted into OVA naïve animals to explore whether any part of the asthma syndrome can be transferred. There are a number of additional experimental strategies to affect HIF-1 α protein stability⁹² and pharmacological inhibitors of S1P receptors are also available.⁹³

SUMMARY AND SIGNIFICANCE

In spite of numerous attempts to control asthma by treatment with bronchodilators, steroids, antigen-directed desensitization, and IgE-directed therapy, use of leukotriene receptor blockers and mast cell release inhibitors, a true control of the asthma syndrome with its multiple manifestations (exercise-induced, nocturnal, steroid-resistant, etc.), has so far eluded us. Perhaps the reason is that neither eosinophils nor mast cells nor leukotrienes are central enough in a disease that is perhaps much more an integrated system problem than only a bronchial problem. Work in recent years has confirmed that the bone-marrow participates,^{66–69} whereas the evidence supporting that asthma is a systemic disease is not yet particularly strong.^{18,19,94} We can say that there is a bone-marrow lung axis. We ask whether there are factors that tie together the actions of all or most of the asthma pathobiology players? If we connect all (or most) of the dots, do we find a superstructure or organizing principle, which allows us to understand how inflammation, airway reactivity, immune response, angiogenesis and airway remodeling⁹⁷ hang together? We are not proposing a theory of ‘everything’, but suggest that HIF-1 α -dependent cellular deregulation may be a large part of this superstructure or organizing principle, which transcends the activation of one particular cell type.

Our hypothesis and the proposed research are based not on a reductionist’s approach, they are rather based on a synthesis of facts and findings. As we connect the dots, we see two principles emerging: (1) angiogenesis is part of the pathogenesis of asthma and (2) the bone marrow delivers cells, which maintain the chronicity of asthma and the angiogenic state of the asthma lung. Successful modulation of HIF-1 α , a master controller of events,^{98–101} may lead us to a very different new treatment strategy of asthma.¹⁰²

The acceptance of a significant contribution of the angiogenic tissue response to the pathobiology of asthma will require (a) further documentation and mechanistic explanation of angiogenesis in the asthmatic lung, (b) experimental evidence that anti-angiogenesis is overall beneficial, that is improves asthma, and^{103,104} (c) a detailed investigation of the interactions between the lung and the bone marrow that drives and maintains lung angiogenesis in asthma.

Bronchospasm and hyperreactive airways naturally focus the attentions of the asthma researchers on the airways to the exclusion of the lung at large and the involvement of other parts of the body. When an asthma patient states that he or she feels ‘congested’, then this is a symptom identical to that described by the patient with ‘congestive’ heart failure. Patients with congestive heart failure can wheeze and the clinicians of past decades had coined the term ‘cardiac asthma’. It is, therefore, peculiar that the non-*cardiac* asthma symptom of congestion is not understood as caused by the lung being overfilled with blood, whereas the cardiologist

visualizes how the blood backs up and congests the lung in mitral valve stenosis. One step in the direction toward a better understanding of asthma is to consider that asthma is a disease of the entire lung, and that it is not restricted to the airways. A too narrow definition or classification of the asthma syndrome may hinder the discovery of causative factors and delay the acceptance of such factors. Likewise, to assume that allergic or non-allergic asthma begins and ends in the airways may blind us to the existence of systemic factors, which may drive the chronicity and exacerbations of asthma.

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Figure 1.

This schematic depicts known interactions between VEGF and S1P. These interactions are both on the ligand as well as the receptor level. The transcription factor HIF-1 α is upstream of both VEGF and SphK1.