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Review Article

Is asthma curable?

Asma tem cura?

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ABSTRACT

Asthma is the product of coordinated, interconnected and complex processes that originate in genes/epigenetics, microbiome, and environment/lifestyle. Currently available drugs are not able to interfere with the insertion of asthma into the body. The current therapeutic approach involves drugs that aim to control symptoms and antagonize part of the effects of some of the cytokines involved. Thus, the current treatment is aimed at controlling asthma and not curing it. Epigenetic mechanisms translate the microbiological and environmental stimuli into altered cellular behavior. For this reason, the identification of epigenetic markers will certainly point out to new therapeutic targets and, ideally, strategies to reverse the altered cellular behavior in the respiratory tract. Then, yes, we could say that asthma is curable.

Keywords: Asthma, epigenomics, biological treatment.

RESUMO

A asma é o produto de processos coordenados, interligados e complexos que têm origem nos genes/epigenética, microbioma e ambiente/estilo de vida. Os medicamentos atualmente disponíveis não são capazes de interferir com a inserção da asma no organismo. A abordagem terapêutica atual envolve fármacos que visam controlar os sintomas e antagonizar parte dos efeitos de algumas das citocinas envolvidas. Dessa forma, o tratamento atual visa o controle da asma e não a sua cura. Mecanismos epigenéticos traduzem os estímulos microbiômicos e ambientais em comportamento celular alterado. Por essa razão, a identificação de marcadores epigenéticos certamente apontará novos alvos terapêuticos e, idealmente, estratégias para reverter o comportamento celular alterado no trato respiratório. Aí, sim, poderíamos dizer que a asma tem cura.

Descritores: Asma, epigenômica, tratamento biológico.

Introduction

One of the phrases attributed to Hippocrates (460-370 BC) has space in a discussion of the cure for asthma: “the cure is linked to time and sometimes to circumstances.” It is known that asthma is the product of the variable interaction of genetic, epigenetic, microbiomic and environmental factors. With different weights, at different times and in different ways, these factors interact and influence each other. Basically, the available therapeutic options reduce inflammation, relax peribronchial smooth muscle, or antagonize certain cytokines. In this way, we achieve asthma control and not its cure. If asthma is only manageable with current resources, treatment is palliative; treats symptoms without removing the cause.

The term *cure* implies discovering and solving the cause of the disease: destroying the responsible microorganism, removing the tumor, restoring the normality of physiological indicators.[1] A Latin term used to define healing is *Restitutio ad integrum*, which means 'to restore to the original condition'. We have, then, a semantic problem if we want to cure asthma. Although the mechanisms involved in the genesis of asthma are still unclear, we know that genes, microbiome, environmental factors, diet and other characteristics are directly involved in the insertion of this disease in the human organism through epigenetic mechanisms, mainly. Furthermore, it is known that part of the construction of asthma begins in the prenatal

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phase. So, how to talk about healing if the original condition is already predisposing?

As new technologies are being used to understand the mechanisms involved in the determination of diseases, we are discovering that cellular and molecular processes, governed by genes, microbes and factors associated with the environment and daily life, generate the insertion of diseases in our organism, modulate its progression and resulting dysfunctions, define its outcomes. Probably the moment in life when each of these processes is initiated matters. In asthma, as in other diseases, there are two fundamental moments. The first, when the causal factor alters the normal (healthy) cellular functioning – insertion of the disease in the organism. The second is represented by the progression of altered functioning and its functional and anatomopathological consequences. If technology is developed that can reverse the processes involved with insertion, the disease could be cured. If therapeutic actions can only control the dysfunctions resulting from the progression of pathological mechanisms, they are only controlling the disease.

The growing understanding of the pathogenesis of asthma makes it increasingly unlikely that it can be cured through medication. In this article, some aspects involved in its pathogenesis that point to this conclusion will be discussed.

Asthma: cause and development

Apparently, in addition to genetic inheritance, changes in maternal diet, use of paracetamol during pregnancy, transmission of the maternal microbiota to the newborn during childbirth and prematurity are factors capable of introducing asthma into the body.[2] To cure asthma, it would be necessary to intervene in these factors. Defining a "preventive" diet, avoiding maternal exposure to risk factors are effective and feasible strategies. On the other hand, interfering with genetic inheritance, modulating the maternal microbiome or preventing prematurity are still not possible actions.

Immunological factors, age, sex and atopy influence the development of asthma.[3] There are differences in the age of onset of asthma symptoms according to sex. Probably due to a hormonal factor, asthma is more common among boys in early childhood, puberty and early adulthood. Symptom remission rates are also higher in males.[3-5] Asthma remission during adolescence is associated with a lower initial degree of bronchial hyperresponsiveness (BHR) and a greater gain in peripheral airway function when compared to asthma that starts after childhood.[5] Possibly, this variability between childhood-onset asthma (AII) and the late start (adult asthma - AIA) is associated with differences in the degree of environmental exposure.[6] Asthma that starts in older adults tends to be more severe than asthma that starts at younger ages.[7] Characteristically, asthma presents temporal heterogeneity. The same person presents different clinical forms throughout life, indicating variation in pathophysiological mechanisms over time. Possibly, a multifactorial arrangement is responsible for the clinical heterogeneity and temporal variation observed in asthma. Unraveling this arrangement and the elements involved would be a way to identify therapeutic targets to control asthma.

The causal trio of asthma and respiratory tract inflammation

"Complex systems" (area of physics and mathematics in which chaos theory became popular) deal with sets of numerous elements that interact with each other in different ways, making their behavior difficult to predict and simulate. The elements and processes involved in the pathogenesis of asthma constitute a "complex biological system". In it, genes/epigenetic mechanisms, dysbiosis in the intestinal/respiratory microbiota, and environmental/lifestyle factors interrelate in a coordinated and variable manner, involving multiple mechanisms and generating the full range of dysfunctions and anatomopathological changes observed in asthma. This variation is expressed by the different endotypes identified.

Asthma results from a coordinated and interdependent work between three factors: genetic, microbiomic and external. Through epigenetic mechanisms (DNA and miRNA methylation, mainly), environmental factors (environmental and climatic pollutants) and factors related to daily life/lifestyle (diet, physical activity, drug use, exposure to allergens and environmental pollutants, environment and others) cellular behavior in the respiratory tract is altered/modulated. At the same time, they cause dysbiosis in the intestinal and respiratory microbiota that lead to immune and inflammatory dysfunctions in the respiratory tract. Part of the processes involved still begins in the prenatal phase; the other part, from

the moment of birth. As a result of this orchestrated and organized work, distinct pathogenic processes are generated and lead to the variable clinical dysfunctions labeled as asthma.

The temporal and category variation between the inflammatory processes observed in asthma is one of the results of the complex multifactorial mechanisms involved in its pathogenesis.Inflammation is a central component of asthma, responsible for symptoms and for physiological and structural abnormalities. Several techniques have been and are being used to classify and size it in asthmatic airways, from stratified cell counts in lung material, assessment of cell response by quantitative cytometry and, more recently, omics technology. Cell type and its proportion in sputum/bronchial secretion began to be used to categorize asthma into eosinophilic, neutrophilic, mixed granulocytic and paucigranulocytic (non-elevated eosinophils and neutrophils) and to indicate the most appropriate drug approach. However, standardized cut-off points for these asthma categories have never been established, resulting in imprecision in the choice of therapeutic approach.

The search to identify the different inflammatory processes present in asthma and their detailed categorization brought the concept of endotyping. Endotype is defined as the mechanism that determines a particular form of asthma. Initially, with the assumption that CD4+ T lymphocytes were the main cell type in the coordination of inflammatory processes, the first endotypic categorization focused on Th1 and Th2 subgroups. Th2 cells, by generating large amounts of IL-4, IL-5 and IL-13, were the main inducers of eosinophilic airway inflammation. Subsequently, asthmatics began to be stratified into two categories, according to the presence or absence of eosinophilic inflammation and the response to corticosteroid therapy. Then, the polarization between two endotypic groups emerged: Th2-high (eosinophilic) and Th2-low (non-eosinophilic). In the first group, eosinophilia is predominant and treatment includes corticosteroid therapy and IL-4, IL-5 and IL-13 cytokine antagonists. In the second group, corticosteroids are not effective, the use of antibiotics may be useful and the cytokines responsible for neutrophil recruitment (TNF, IL-1, IL-6, IL-8, IL-23 and IL-17) may be targets of therapy. In the paucigranulocytic form, treatment with anti-inflammatory drugs does not seem to be helpful; the use of bronchodilators, bronchial thermoplasty or drugs targeting mast cells seems to be more effective.

Recently, another cell lineage has been incorporated into the pathogenesis of asthma, the innate lymphoid cells (ILCs). They are innate lymphocytes distinct from T and B cells. Group 2 ILCs (ILC2), which produce prototype type 2 cytokines, are important in the pathogenesis of asthma. They generate large amounts of IL-5 and IL-13 in the airways in response to alarmins and mediators released by epithelial cells activated by inflammatory stimuli. The discovery of the value of these cells in asthma brought a new endotypic terminology: high Th2 came to be called high T2, aggregating ILC2 to Th2. Both cells are primary regulators of type 2 immunity and express the transfer factor GATA3, which governs the production of type 2 cytokines. Subsequently, Inflammatory processes of the airways started to be classified as Type 2 (T2) and non-Type 2 (T1).[8] In T2, alarmins released by the bronchial epithelium (thymic stromal lymphopoietin - TSLP, IL-25 and IL-33) from inflammation caused by infectious or allergic stimuli initiate different cell signaling processes. While TSLP activates antigen-presenting cells (APC) leading to the activation of T and B cells, IL-33 and IL-25 activate ILC2. The expression of these markers is correlated with the severity of asthma.[9] When activated, ILC2 secretes ten times more potently than Th2 cells of IL-5 and IL-13, propagating/amplifying type 2 immune responses. There are indications that they are also associated with the process of remodeling and repair of lesions of the airways present in asthma.[10] IL-4, IL-5 and IL-13 are potent activators of eosinophils, a fundamental cell in T2.

The categorization of non-Type 2 (T1) asthma is not as clear as that of Type 2. In it, in addition to the number of eosinophils not being expressive, the role of Th1 and Th17 lymphocytes is relevant, as well as that of neutrophils and IL-1b, IL-6, IL-8, IL-17A, IFN-γ and TNF-α. In the T1 category, structural abnormalities in smooth muscle and in the neural network also participate in the pathogenesis. Neutrophilia is still a controversial point. There is no consensus on the cut-off point that classifies asthma as neutrophilic. Furthermore, neutrophilic inflammation is an inconsistent biomarker as it can be found in smokers or after inhalation of traffic pollutants or NO_2/ozone containing pollutants. Intense exercise and a cold environment can also induce a neutrophilic pattern, as well as bronchiectasis.[11]

Asthmatic airway inflammation is not a uniform or static process, it can be caused in different ways and

be composed of different mechanisms. The evolution of knowledge about the molecular and cellular processes involved makes it clear that asthma is a label that covers different respiratory disorders that have as common denominator episodes of dyspnea with variable intensity and frequency that, for the most part, arise in childhood. Often, the family history is positive and the cardinal symptoms are dyspnea, wheezing, cough and chest tightness. In some cases, allergic or environmental factors are associated with the onset of symptoms. In-depth examination reveals differences in the mechanisms that determine the dysfunctions (endotypes). Differences have a genetic and epigenetic basis, and result from cellular diversity, the wide variety of activated cytokines and the microbiomic dysbiosis involved. Epigenetic mechanisms translate external factors, such as allergens, environmental pollutants, diet, lifestyle, drug use, and others, into genomic modifications that alter cellular behavior in the respiratory tract.

Adding complexity to the scenario, we have the dynamics of the processes involved. Asthma is not a "static" disease. On the contrary, it undergoes variations all the time. The different inflammatory processes involved can alternate or add up at different times. As a result, an asthmatic classified as eosinophilic at one time may be neutrophilic at another time and paucicellular at another time. Clinically, he may be asymptomatic or with occasional mild symptoms, and in others, have intense and continuous symptoms. Finally, he may have different "asthma" over time, or even total remission of symptoms.

As technological developments make it possible to clarify pathogenic mechanisms and their interactions, new therapeutic targets will be identified. This will certainly revolutionize the approach to asthma. The "palliative" treatment, which aims at symptomatic control and the prevention of symptoms/exacerbations, should not continue "uniform/rectilinear", as it is today; it will probably vary over time, molding itself to the pathogenic processes prevailing at each moment and variables in the same patient.

The role of each member of the causal trio (genes/epigenetics, microbiome and external factors) in the onset, progression and outcome of asthma in the human body is still unclear. Unraveling the mechanisms present in each one and their interrelationships is a fundamental step in defining the ideal treatment for asthmatics.

Genes/epigenetic mechanisms

Heredity is a hallmark of asthma. The phenotypic variety and underlying pathogenetic mechanisms demonstrate the complexity of the genetic processes involved. With the progress of genome-wide association studies (GWAS) technology, several genetic markers, single nucleotide polymorphisms (SNPs) and chromosomal regions have been associated with asthma susceptibility, age at onset and atopy.[12]

A recent study of the genetic variation between two distinct categories of asthma according to the age of onset of symptoms – childhood onset (AII) and adulthood (AIA) – observed partially different genetic architectures. Based on the findings, some assumptions can be made. In one, the different genes could lead to different molecular processes between the two forms. Therefore, the design of drugs designed specifically for each of the forms would be different. In another hypothesis, the same gene in particular would contribute to the pathophysiology of both forms, but its expression would be deregulated in AII by different risk alleles and, in AIA, by epigenetic modifications. SNPs explain only 5-10% of the variation in age at onset of symptoms. It may be that the wide range of differences observed in the alleles indicates that a specific association with certain alleles determines the age of onset. In this case, the internal difference in each group could be modulated by environmental factors (epigenetic factors). In AII, candidate environmental risk factors would be the timing, frequency and duration of respiratory infections, allergen exposure, domestic animals, maternal smoking and low-quality diet during pregnancy. In the EIA, occupational exposures, smoking and obesity.[13]

The study of epigenetics focuses on hereditary changes that affect gene expression without altering the DNA sequence.[14] The most common epigenetic mechanisms identified that play a regulatory role in immune responses and gene expression in asthma are DNA methylation, post-translational histone modifications and miRNA expression.[15] Changes in DNA methylation result in a differentiated genomic expression related to the production of cytokines and transcription factors associated with the phenotypic presentations of asthma. The environment and life habits (maternal smoking, atmospheric pollution, exposure to heavy metals, pesticides and microbes, certain foods and drugs)[16] are potent influencers of DNA methylation.[17] Briefly, it can be said that epigenetic mechanisms represent the bridge between external factors and genes,

leading to changes in genomic expression (cellular functioning).

In asthma, epigenetic changes can be induced in the prenatal phase, in early childhood and adolescence, and make the individual susceptible to asthmatic "triggers".[18] They play an important role in immune responses and in the regulation of various cellular functions, such as differentiation and balance between T cell classes, changes in the expression of inflammatory genes, in the cellular transformation of AII (Th2 asthma, predominantly eosinophilic-corticosteroid sensitive) to AIA (non-Th2 asthma, neutrophilic-paucigranulocytic – less sensitive corticosteroid), in remission/protection phenomena.

Intestinal and respiratory microbiota

In 1989, Dr. David Strachan (United Kingdom) proposed a hypothesis to explain the increase in the prevalence of allergic diseases observed in previous years. According to him, *"These observations... could be explained if allergic diseases were prevented by infections in early childhood transmitted by unhygienic contacts with older siblings, or acquired in the prenatal period,... During the last century, the family size has decreased, indoor play has increased, and higher standards of personal hygiene have reduced the opportunity for cross-infection in young families. This may have resulted in greater generalized clinical expression of atopic diseases"*.[19] Called the "Hygiene Hypothesis", it was initially met with skepticism. Shortly thereafter, in the 1990s, the recognition that natural immunity against viral and bacterial infections induced a Th1 pattern of cytokine release, potentially suppressing the Th2 immune responses involved in IgE-mediated allergy, attracted the attention of allergists and immunologists.[20] With the advancement of studies on the subject, the Hygiene Hypothesis, which postulates that infections protect against atopy, is considered immunologically plausible and consistent with the epidemiological aspects of atopy. However, the inverse association between infection and atopy cannot be directly confirmed by epidemiological studies.[21] From then on, the participation of the microbiome in immune diseases began to be studied in depth. Under normal conditions, the interaction between the microbiome and the human organism confers mutual benefits (symbiosis). However, when the composition and diversity of the microbiome are altered (dysbiosis), these changes are translated into changes in immune responses and diseases, such as asthma, for example.

The bacterial portion of the microbiome alone contains about 3.3 million genes, 150 times more than the human genome.[22] This represents an epigenetic pressure on the human genome that makes evident the participation of the microbial ecosystem in the biological processes underlying health and diseases.[23] The intestinal microbial community is the most abundant, comprising more than a thousand bacterial species, apart from viral and fungal populations. The pulmonary microbiota (PM) is an ecosystem formed by a well-organized and metabolically active community, which includes microorganisms (viruses, bacteria and fungi, mainly), their genomes and environmental conditions. The microbiota (intestinal, oral, upper respiratory tract, genitourinary tract, skin and others) are in constant communication with each other in a bidirectional way through "axes", with each being able to influence the other. Communication between PM and the intestinal microbiota (IM) occurs through the "gut-lung axis".[24]

Growing evidence makes it clear that there is an interaction between PM and the host's immune system. Changes in MP diversity or abundance are associated with several chronic respiratory diseases, such as asthma, cystic fibrosis, bronchiectasis and chronic obstructive pulmonary disease (COPD). Bacteria, viruses and fungi from the microbiota of the upper and lower airways produce structural ligands and metabolites that interact with the host and change the progression of these diseases.[25] With the development of the omics sciences, it will be possible to begin to unravel all the molecular and genetic biology involved in the participation of the microbiome in asthma and to identify new therapeutic targets, certainly more specific.

Environmental factors and lifestyle

Environmental exposures are linked to the development and progression of diseases. In addition to exposure to allergens, air pollutants and climatic factors and microorganisms, everyday habits and lifestyle such as diet, exercise, medication, smoking, pets and infections are also risk factors for the development and exacerbations of allergic diseases and asthma.[26] The influence of all these factors is differentiated by the individual's genetic aspects, immunological aspects, time of exposure and microbiomic characteristics. Among the microbiological

factors, fungi play an important role. Some species, *Aspergillus*, *Candida*, *Alternaria*, *Clodosporium* and others are associated with asthma and its severity.[27]

If we want to cure asthma, we will have to be able to block the influences of these elements in the respiratory tract. This can only be possible after clarifying the complex interrelationships between genetic/epigenetic, environmental/lifestyle, microbiomic factors and the resulting dynamic biological processes. The identification of epigenetic markers is a fundamental step for the identification of asthmatic endotypes and for the definition of therapeutic and preventive approaches. The integration of the omic sciences and their instruments in studies to solve the still existing mysteries will be of great help in the identification of therapeutic agents, prediction of evolution/outcome and, eventually, preventive actions of their development. One of the goals of personalized medicine is to develop pharmacogenetic and pharmacoepigenetic approaches aiming to act on the factors responsible for the insertion/development of diseases. Interference can occur by preventing the inducers of the responsible alterations in each one of them or by restoring the original function of each element involved. In the first situation, we would be talking about preventive treatment, that is, preventing the development of asthma in the body. In the second, we would be talking about healing.

Current and future therapeutic approaches

The pharmacological groups used in the treatment of asthmatic patients aim to reduce inflammation (glucocorticosteroids – GCS), reverse peribronchial smooth muscle contraction (bronchodilators – BD), antagonize key cytokines in pathogenesis, inflammatory mediators or block IgE. All of them act on the effects resulting from the interrelated actions of the trio genetics-microbiome-external factors. All drug approaches aim to antagonize definitively established abnormal cellular behaviors. For this reason, none of them allows talking about healing, only about control. They are, in fact, palliative treatments. Bronchodilators relax peribronchial smooth muscle, relieving dyspnea. Corticosteroids reduce inflammation partially, as they do not act on all inflammatory processes present. Biologicals antagonize/block specific cytokines.

The first biologic used in asthma was Omalizumab, which prevents the binding of IgE to its receptor on mast cells, basophils and dendritic cells, preventing the subsequent release of inflammatory mediators by these cells. Subsequently, other biologics – Mepolizumab, Reslizumab, Benralizumab and Dupilumab – were included in the therapeutic arsenal against asthma. The first two bind to the IL-5 ligand preventing its binding to its receptor. Benralizumab also binds to the IL-5 receptor, causing apoptosis of eosinophils and basophils. Finally, Dupilumab binds to the IL-4 α-receptor, blocking IL-4 and IL-13 signaling.[28]

Biologics are being indicated for severe asthmatics who need to take three or more courses of oral corticosteroids per year despite adequate adherence to prescribed treatment. As they have not yet been compared drug to drug, it is not possible to affirm the superiority of any of them.[29-31] In general, all reduce the exacerbation rate by around 50% and the effects are greater when the absolute number of eosinophils is higher. As the predominant biological role of IL-5 is linked to maturation, survival and recruitment of eosinophils to the airways, better effects of anti IL-5 are to be expected when symptoms/dysfunctions are driven by intraluminal eosinophils. However, as IL-4 and IL-13 (acting on a single IL-4R receptor) have a broader action, acting on eosinophil recruitment, goblet cell hyperplasia/mucus secretion, smooth muscle contraction and HRB, beneficial effects would be expected in a larger population of asthmatics, and not only in those with airway eosinophilia.[32]

Investigations seeking to identify new biologicals continue. Further on, others that target IL-25 and 33, TSLP and an alarmins will be included in the therapeutic arsenal.[28] Certainly, the development of predictive or monitoring biomarkers will help to select the most appropriate biologic for each patient. In any case, their therapeutic value is partial and they are adjuvant drugs for the control of asthma.

Conclusion

In medical ethics, the principle of non-maleficence has always been related to the maxim *Primum non nocere*, which can be interpreted "above all (or above all) not to cause harm". According to some authors, despite being essentially associated with the thought of Hippocrates expressed around the year 430 BC - "above all, do not cause harm" - the phrase does not appear in any Hippocratic text. What is established in paragraph 12 of the first book of his work, *Epidemic*, is that the doctor "practices two things in dealing with diseases; help and do not harm the patient".[33] To

imagine curing asthma, we have to change levels. We will have to move to a quantitative rather than qualitative, mechanistic rather than organic, indefinite rather than finite, challenging scenario.

Based on current knowledge about its pathogenic mechanisms, we can assume that the effective approach to asthma should include agents that modulate the genome and correct the microbial dysbiosis involved. If so, we would be talking about an "epigenetic and microbiomic" therapeutic approach that will include agents acting on DNA methylation, histone modifications, miRNA and dysbiosis in the microbiome.[34] In this case, in which the therapeutic approach will basically act on the human genome, the ethical principles mentioned above cannot be forgotten.

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