Advances in asthma pathophysiology: stepping forward from the Maurizio Vignola experience

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ABSTRACT   Maurizio Vignola was a superb and innovative researcher, who wrote seminal papers on the biology of airway epithelium in asthma. Inflammation and remodelling were the main topics of his research, mostly conducted in biopsy specimens from patients with asthma of variable severity, encompassing the entire spectrum of the disease from mild to severe asthma. His observations contributed to define the biology of asthma as we know it today, and opened the way to the personalised treatment of asthma. His group has successfully continued to investigate the biology and clinical aspects of bronchial asthma, with major interest in the clinical use of biomarkers to monitor disease activity, and in the development of new therapeutic perspectives. This review summarises the latest work on these topics proudly conducted by Maurizio's closest collaborators. The results indicate significant progress in our understanding of asthma in the last 10 years, in particular increased knowledge of the complex interaction between inflammatory and remodelling pathways, improved recognition of biological and clinical asthma phenotypes, and development of new treatment strategies, especially for patients with severe corticosteroid-resistant asthma.

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Introduction

The life of Maurizio Vignola (fig. 1) was too short, but was sufficient to leave durable signs in the development of a new paradigm for bronchial asthma. Since his first days as a research fellow, first in Montpellier, France, under the guidance of Jean Bousquet, then in Omaha, NE, USA, with Stephen Rennard, he showed innovative thinking and exceptional scientific maturity, a really uncommon finding at his young age. Lifelong friendship and fruitful collaboration were also established in those days with Pascal Chanez, who continued to collaborate with the research group in Palermo, Italy.

Maurizio was very interested in the biology of airway epithelium, and his studies on biopsy specimens from patients with asthma of variable severity allowed him to understand some aspects of the disease that had not been studied in depth. He used immunohistochemistry, a relatively "primitive" technique compared with the sophisticated methods used today, to study several markers of epithelial damage, inflammation, apoptosis, regeneration and remodelling [1–5], which allowed him to propose a new, more complex model of asthma, especially in its most severe form [6]. He addressed the pathogenesis of resistance to corticosteroid treatment [2, 7–9], and also studied the complex relationship between asthma
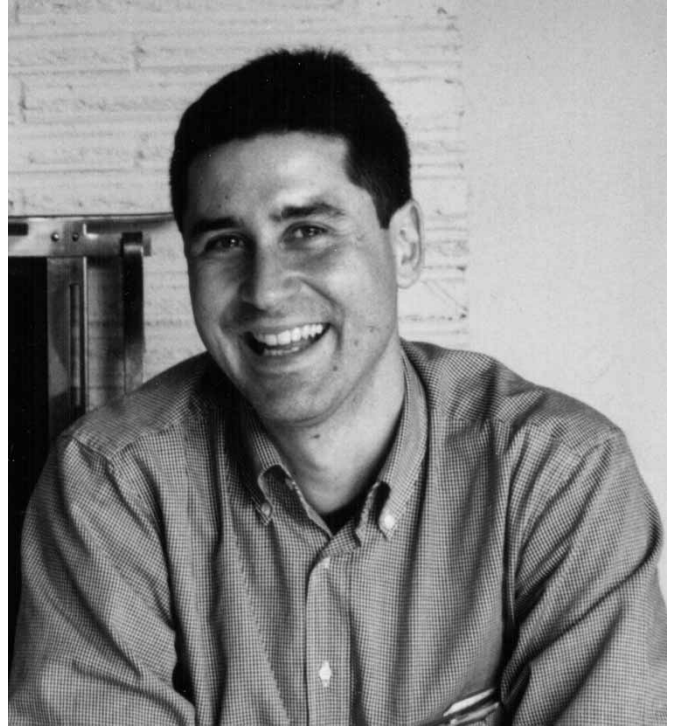
FIGURE 1 Maurizio Vignola (1964–2004).

and chronic obstructive pulmonary disease (COPD) when they coexist in the same patient [10]. His research soon extended to extracellular matrix, and he published seminal papers on the role of transforming growth factor (TGF)-β [11] and tissue metalloproteinases in asthma [12], setting a new paradigm of the disease that still stands today. He also realised that paediatric asthma could be used to study the earliest aspects of the disease, without the confounding effect of environmental exposures commonly found in adult patients [13]. Finally, he explored the dynamic nature of airway remodelling and the role of myofibroblasts in this process [14].

Space constraints do not allow a thorough discussion of all his scientific papers. Instead, this review focuses on the results obtained in the last few years by his group, who kept working on asthma, first by continuing research on the biology of asthmatic epithelium and on the interactions between upper and lower airway inflammation, and secondly by testing the results obtained through experimental studies in asthmatic patients.

Airway inflammation and remodelling

Asthma is an inflammatory disorder of the airways, characterised by infiltration of mast cells, eosinophils and T-helper cell type 2 (Th2) CD4$^+$ T-lymphocytes in the airway wall [15]. Eosinophils within the airways of asthmatics: 1) are recruited *via* a chemical gradient due to the release of chemotactic agents (interleukin (IL)-5 and eotaxin), 2) show a prolonged survival, and 3) induce epithelial damage and remodelling *via* the release of eosinophil toxic products (eosinophil peroxidase and the eosinophil cationic protein).

Similarly, the accumulation of T-lymphocytes within the airways of asthmatics may be due to both increased recruitment and decreased cell apoptosis [16]. The increased recruitment of lymphocytes may be promoted by the release of lymphokines including thymic stromal lymphopoietin (TSLP) [17]. TSLP is expressed by structural and immune cells at the site of allergen entry into the airways and has been identified as a master switch for allergic inflammation, since it regulates and drives Th2 responses [18]. Th2 lymphocytes induce and regulatory T-cells (Treg) (characterised by Foxp3 expression) suppress several features of allergic inflammation in asthmatics [15].

The Th2 response, and in particular increased expression of IL-13 within the lungs of asthmatic subjects, triggers an aberrant injury-repair mechanism leading to remodelling of the airway wall. Some of the most prominent effects of IL-13 include increases in goblet cell differentiation, activation of fibroblasts and increases in bronchial responsiveness. Moreover, IL-13 induces bronchial epithelial cells to secrete periostin, which in turn enhances profibrotic TGF-β, a tissue growth factor whose dysregulation is associated with airway remodelling and might affect disease chronicity and severity [19].

Airway epithelium is one of the principal actors in the pathological events associated with both bronchial inflammation and remodelling in asthma [20]. Airway epithelium represents more than a protective barrier against external insults, since it actively secretes pro-inflammatory mediators, *i.e.* leukotrienes and cytokines such as IL-8 and TSLP, which in turn amplify the initial inflammation due to external insults by recruiting and activating neutrophils in the early phase, and eosinophils and lymphocytes during the late response. Bronchial epithelial cells are activated by cysteinyl leukotrienes including leukotriene (LT)D$_4$, *via* activation of extracellular signal-regulated kinase (ERK) 1/2 and of the signal transducer and activator of transcription (STAT)-1, increasing their adhesiveness to eosinophils [21].

Studies on bronchial biopsies demonstrated that in asthmatics, especially if severe, the airway epithelium highly expresses pro-inflammatory transcription factors including nuclear factor (NF)-κB [2] and cAMP response element binding protein (CREB) [22]. Both transcription factors induce acetylation of chromatin and transcription of pro-inflammatory genes, leading to persistent inflammation and severe disease.

The increased expression of phosphorylated (p)CREB in the airway epithelium is positively correlated with leukocyte infiltration within the bronchi (CD45RB$^+$ cells), strongly supporting the concept that activation of the airway epithelium is crucial in the recruitment and activation of leukocytes within the airways, as confirmed by the elevated expression of pCREB also in these cells [22]. Furthermore, increased pCREB expression was also observed in peripheral blood mononuclear cells (PBMCs) from untreated and severe asthmatics, suggesting that some of the molecular events crucially involved in the pathogenesis of asthma may be evaluated in systemic compartments, thus avoiding bronchial biopsies to monitor asthma inflammation.

As a further support to the assessment of systemic inflammation to manage asthmatics, it has been shown that IκB kinase (IKK), a complex tightly regulating NF-κB activation, and in particular IKKβ activity, was higher in PBMCs in asthmatic patients than in controls [23]. IKKβ activation seems to be specific for asthma, since increased IKKα activity was found in patients with COPD and healthy smokers compared with asthmatic patients or controls [23]. The different profile in the upstream regulation of the IKK-driven NF-κB system in asthmatic and in COPD patients may explain, at least in part, the different pro-inflammatory and pharmacological responses in asthmatic *versus* COPD patients.

Persistence of chronic inflammation may alter the homeostasis of lung tissue, leading to airway remodelling. Tissue remodelling includes epithelial alterations (epithelial shedding), increased matrix deposition, matrix degradation and accumulation of plasma proteins. Genetic influences, fetal exposures and early life events, and long-term uncontrolled inflammation may all contribute to airway remodelling. Structural changes may alter the protective functions of the airways by affecting mucociliary clearance and increasing the risk of infections and/or exacerbations. Furthermore, activated fibroblasts and smooth muscle cells may release pro-inflammatory mediators, leading to amplification of subsequent tissue damage [24].

The adipokine leptin is a potential new player in bronchial epithelial homeostasis, since it counteracts some molecular events crucially involved in airway remodelling [25]. Leptin decreases the spontaneous release of TGF-β and promotes tissue repair by increasing epithelial cell proliferation. Conversely, TGF-β decreases leptin receptor expression in the airway epithelium. The bronchial epithelium of asthmatics undergoes shedding, and shows decreased expression of leptin and its receptor, increased thickness of the reticular basement membrane and increased TGF-β expression compared with the values found in healthy volunteers [26].

Biomarkers

Allergic diseases, including allergic rhinitis and asthma, are chronic inflammatory disorders with a prevailing Th2 immune response. Inhalation of allergens leads to bronchial hyperreactivity and recruitment of eosinophils, mast cells and lymphocytes in the upper and lower airways, triggering the inflammatory cascade and generating local and systemic inflammatory responses. Uncontrolled inflammation in the upper and lower airways and in the systemic circulation may compromise the control of allergic rhinitis and asthma, with subsequent disease progression [27]. Therefore, from a clinical point of view, it is important to detect and monitor the occurrence of inflammation and remodelling in the upper and lower airways and in the systemic circulation of patients with asthma and allergic rhinitis. This goal may be achieved using noninvasive methods such as induced sputum, exhaled breath condensate (EBC), nasal wash and collection of blood samples. This approach might also be useful to characterise different inflammatory phenotypes and monitor them in order to provide "personalised" treatment to each individual patient.

Exhaled nitric oxide fraction (*F*eNO) measurements and collection of EBC are noninvasive techniques for the assessment of nasal and oral airway inflammation in atopic subjects with and without asthma [28], providing useful clinical information even in the absence of symptoms. In children with atopic dermatitis, allergic rhinitis and asthma, *F*eNO, and pH and IL-5 levels in the EBC from upper and lower airways were

studied to identify children with atopic disorders who might develop bronchial asthma, and to predict the development of exacerbations in asthmatic children [29].

A large number of molecules measured in EBC, including LTB_4, prostaglandin E_2 and 8-isoprostane, are considered useful markers of airway inflammation and oxidative stress in the airways. In children with persistent rhinitis and asthma of variable severity, such markers and FeNO were measurable in both upper and lower airways, but showed a large variability. Correlations between these markers and their persistence despite treatment might help to identify a relationship between biomarkers in the two airway compartments that might be useful to phenotype the "united allergic disease of the airways" [30].

In asthma, symptoms are often not specific and do not closely reflect the presence or severity of airway inflammation [31]. GAGLIARDO et al. [32] showed that several biomarkers in induced sputum, including IL-8, matrix metalloproteinase (MMP)-9, tissue inhibitor of metalloproteinase (TIMP)-1 and number of eosinophils, as well as the levels of FeNO, were higher in children with intermittent asthma and moderate asthma compared with healthy subjects, suggesting that the inflammatory process is associated with biological and clinical heterogeneity. TGF-β1 in induced sputum supernatants was also higher in children with intermittent or moderate asthma compared with controls, and was associated with activation of the intracellular TGF-β/Smad signalling pathway in induced sputum cells. Therefore, a dynamic mechanism results from the interaction between TGF-β1 signalling activity and inflammatory cells through the commitment of bronchial epithelium, indicating a putative immunoregulatory role of TGF-β1 in the progression of airway inflammation in paediatric asthma. As a consequence, treatment strategies should be aimed at controlling inflammation and preventing airway structural changes in young asthmatic patients [33].

Recent studies have identified an IL-23-dependent T-cell population, a Th lineage distinct from Th1 and Th2 called Th17, which plays an important role in inflammation and tissue injury [34]. Th17^{+} cells primarily produce IL-17A, and include CD4^{+} and CD8^{+} T-cells showing either the Th1 or Th2 cytokine profile [35]. IL-17^{+} cells are overexpressed in the lung during acute neutrophilic inflammation and asthma [36]. In mouse models of airway inflammation, such as those driven by house dust mite allergen, ovalbumin [37, 38] or ozone [39], IL-17A controlled bronchial hyperresponsiveness and airway remodelling but did not affect airway eosinophilia and Th2 cell recruitment to the airways [40, 41]. These studies suggest that some of the pathogenic effects of IL-17 are mediated directly on bronchial smooth muscle cells and local fibroblast progenitors, rather than indicating a major effect on airway inflammation. At variance with these experimental results, ALBANO et al. [42], using noninvasive methods (induced sputum, nasal wash and plasma samples), showed that increased levels of IL-17A are involved in the systemic and upper and lower airway inflammation in children with allergic asthma and rhinitis. In particular, higher levels of IL-17A were documented in nasal wash and induced sputum supernatants of allergic children. In vitro, IL-17A increased the production of IL-8 in nasal and bronchial epithelial cell lines. Moreover, increased levels of intracellular IL-17A and RAR-related orphan receptor (ROR)Ct, associated with lower levels of Foxp3 in T-lymphocytes, together with higher plasma levels of IL-17A, suggest a Th17/Treg imbalance in moderate forms of allergic asthma and rhinitis. Therefore, Th17 immunity appears to be involved in allergic asthma and rhinitis in children, reinforcing the concept of the "united airway disease" in allergic diseases [42]. Despite the progress in our understanding of the pathophysiological functions of Th17 cells in inflammatory diseases of the airways, the regulation of Th17 responses remains relatively poorly understood and controversial. Research on the regulation of Th17 responses in airway disease is quite limited, and the underlying mechanisms have not been thoroughly studied. However, altogether the available studies indicate that the Th17 responses may be differently regulated under in vitro or ex vivo conditions, in animal or human models, in different cell types, or according to variable patterns of endogenous molecules, drugs, steroids or bacterial products in the setting of inflammatory disease [43].

Nebulisers are still frequently used in clinical practice to overcome problems with the technique of inhalers [44], and nebulisation is particularly useful to deliver bronchodilators in children, the elderly and severe or steroid-dependent asthmatics [45]. PROFITA et al. [46] conducted a randomised, double-blind, placebo-controlled study to determine whether nebulised beclomethasone dipropionate (nBDP) may positively affect inflammation and improve the clinical and functional status in children with both allergic asthma and allergic rhinitis with exclusive exacerbation during the pollen season. nBDP (400 g twice a day for 4 weeks) was administered through a facial mask, looking at specific markers of inflammation from the nose and bronchi during the season of allergen exposure. Treatment with nBDP reduced the levels of oral FeNO and oral EBC IL-5, while it did not affect the levels of oral pH. In the nasal compartment, nBDP reduced the levels of EBC IL-5 and increased EBC pH, while it did not affect FeNO. Therefore, noninvasive techniques are very useful to study and monitor the effects of treatment, especially in paediatric asthma [46].

Recent studies have also examined the role of vitamin D in allergic diseases. Vitamin D is a potent immune system modulator involved in the development of allergy-associated Th2 cells [47]. BONANNO et al. [48]

investigated the relationship between different plasma levels of 25-hydroxyvitamin D (25(OH)D) in children with allergic disease of the airways. Children with concomitant asthma and rhinitis who showed deficient 25(OH)D had higher levels of blood eosinophils, a higher number of exacerbations in the last year, a longer disease duration and higher total allergen IgE score and total atopy index compared with children with normal 25(OH)D levels. Therefore, low levels of 25(OH)D in plasma samples could be useful to identify children with allergic rhinitis prone to develop asthma [48].

Stepping forward from the Maurizio Vignola experience, the components of his group are still involved in studies on noninvasive biomarkers of inflammation and remodelling in allergic disease. Together, these studies have contributed to defining the phenotype of disease and to identifying the benefits of a pharmacological approach in the control of the innate and adaptive immunity, and of the related symptoms, in patients with allergic disease of the airways.

Asthma phenotypes and disease control: a new perspective

In the attempt to better characterise and treat bronchial asthma, several studies have been performed over the last decade to define asthma phenotypes. A variety of parameters have been identified for this definition: functional parameters, frequency of exacerbations, age of patients, presence of allergy, geographical localisation [49] and biomarkers. In a recent pan-European study, the stability of phenotypes in adults with controlled and severe asthma has been analysed in the BIOAIR cohort (longitudinal assessment of clinical course and BIOmarkers in severe chronic AIRway disease study), to compare phenotypes defined either by biomarkers (the "Hastie approach") or by physiological variables (the "Moore approach") over 1 year of observation [50]. There are still conflicting data on the repeatability of inflammatory phenotypes defined by sputum analysis. FLEMING et al. [51] found that sputum results were unstable in children with asthma, whereas studies in adults suggested reasonable stability and repeatability of sputum measurements [52, 53]. This BIOAIR study concluded that the inflammatory phenotypes determined by induced sputum cellularity were less stable than those defined by physiological variables, confirming that asthma is a variable inflammatory disease and, therefore, phenotyping to understand mechanisms of the disease needs to be long-term and based on repeated measures, and should ideally integrate several different outcomes [54].

On the basis of these considerations, it is necessary to define what we mean by "asthma control". The most used definition refers to the stability of clinical and functional parameters. A patient with normal spirometry and no symptoms is generally defined as a "controlled" patient. However, some recent papers have shown that the inflammatory profile of an asthmatic patient should also be taken into account in the definition of asthma control. In this regard, within populations of patients with allergic rhinitis, intermittent asthma (treated only with short-acting β_2-agonists (SABA) on demand) or moderate asthma (treated with inhaled corticosteroids (ICS) and long-acting β_2-agonists plus SABA on demand), and also within a population of patients with atopic dermatitis, some subjects show ongoing bronchial inflammation, i.e. low pH and high IL-5 concentrations in the EBC, as well as increased FeNO levels [29]. Therefore, some specific "phenotypes" of these patient populations show evidence of bronchial inflammation. Although these patients were not treated with ICS because they did not have bronchial asthma (i.e. atopic dermatitis or rhinitis) or had intermittent asthma, they were probably representing a specific phenotype of patients who may develop symptoms on an accelerated "allergic march". Moreover, within the group of patients with moderate asthma, who were treated with ICS, some of them displayed bronchial inflammation according to the above-mentioned biomarkers, suggesting that these patients might have been candidate to "step up" in the classification of their disease [29]. Accordingly, within a population of patients with intermittent asthma, some subjects displayed increased expression of pCREB and its receptor expression [22], and low expression of leptin and its receptor [26] in bronchial epithelium, suggesting that in these patients bronchial inflammation occurs and can be demonstrated at the tissue level. These latter studies also demonstrate that bronchial tissue inflammation occurs in some patients with moderate asthma treated with ICS, suggesting that "specific phenotypes" of not fully controlled inflammation do occur in patients.

Partially or not controlled disease, as indicated by positive biomarkers of inflammation in asthma, may have important consequences on treatment. The longitudinal study conducted in paediatric patients with asthma discussed earlier [32] confirmed that a specific phenotype of controlled disease on the basis of symptoms and spirometry, with increased bronchial inflammation on the basis of inflammatory biomarkers, is associated with poorly controlled disease and frequent exacerbations.

As already mentioned, the involvement of the upper airways strongly influences the control of bronchial asthma both from a functional and an inflammatory point of view. In a pan-European study conducted by the Global Allergy and Asthma European Network (GA²LEN), chronic rhinosinusitis was positively associated with adult-onset asthma. This association has been interpreted as chronic rhinosinusitis causing

asthma, because of the release of inflammatory mediators and cytokines from the diseased sinus tissue into the circulation and recruitment of eosinophils into both upper and lower airways from the bone marrow and circulation [55]. These observations confirm that patients with asthma and chronic rhinosinusitis represent a phenotype where upper airway inflammation has to be controlled to achieve a complete control of asthma. Chronic rhinosinusitis and asthma are linked by a common inflammatory pathway, in which eosinophils and airway epithelium play an important role [56, 57]. Many asthmatic patients, whatever their severity, have chronic rhinosinusitis demonstrated on computed tomography scans. Patients with severe asthma, uncontrolled asthma or both have more severe computed tomography scan abnormalities than other patients. In many patients with severe asthma, surgical treatment of chronic rhinosinusitis or nasal polyposis might improve asthma symptoms [57]. Accordingly, a recent study from our group used an asthma-specific gene array to assess the inflammatory profile of nasal biopsies from patients with chronic rhinosinusitis and nasal polyps [58]. Among the cytokines whose expression was altered, IL-19, a cytokine contributing to the increased activation of Th2 lymphocytes, was increased in patients with chronic rhinosinusitis with nasal polyps [58]. Moreover, IL-19 protein was found to be highly expressed in the metaplastic nasal epithelium when compared with normal or hyperplastic epithelium [58]. IL-19 could therefore contribute to the increased proliferation of nasal epithelial cells and to remodelling processes in these patients.

Phenotyping patients with bronchial asthma represents an important strategy to achieve control of this complex disease. Future therapeutic strategies will certainly take into account the characterisation of these "phenotypes" made on the basis of the predominant pathogenetic pathway(s) of inflammatory processes.

Mechanisms in glucocorticoid control of inflammation

Glucocorticosteroids are widely used to treat various inflammatory and immune diseases. The most common use of glucocorticosteroids today is in the treatment of asthma and other allergic diseases, and inhaled glucocorticosteroids have now become established as a first-line treatment in adults and children who have persistent asthma. Suppression of inflammation by corticosteroids positively affects asthma symptoms and airway inflammation in most patients. The major action of glucocorticosteroids is to switch off activated inflammatory genes that encode for cytokines, chemokines, adhesion molecule, inflammatory enzymes and receptors [59].

Several studies have examined the efficacy of glucocorticosteroid-based therapies in airway inflammation, cell activation and immunological responses. PACE et al. [60] showed that treatment with budesonide was effective in controlling asthma inflammation by reducing both T-lymphocyte activation and survival and inducible T-cell co-stimulator (ICOS) in $CD4^+/CD25^-$ cells, and by increasing survival and activation of $CD4^+/CD25^+Foxp3^+$ cells (Treg) both in vitro and in vivo. Furthermore, fluticasone propionate was shown to control peripheral blood T-cell activation and induce T-cell apoptosis through activation of caspases 8 and 3 in mild-persistent asthmatics [61]. VIGNOLA et al. [62] showed that in mild-persistent asthmatics, low-dose fluticasone propionate was effective in improving symptoms due, at least in part, to the resolution of eosinophilic bronchial inflammation and downregulation of remodelling markers, such as MMP-9 and TIMP-1 levels in induced sputum samples. Other in vitro studies confirmed these findings: flunisolide treatment of sputum cells isolated from asthmatic subjects with mild-to-moderate asthma inhibited the release of MMP-9, TIMP-1, TGF-β and fibronectin and increased eosinophil apoptosis [63]. Exposure of induced sputum cells from mild to moderate asthmatic subjects to beclomethasone dipropionate reduced granulocyte-macrophage colony-stimulating factor, RANTES and IL-8 levels and induced transcription of the β_2 receptor [64]. Furthermore fluticasone furoate is able to upregulate the leptin/leptin receptor pathway also in nasal epithelial cells, thus increasing epithelial homeostasis upon allergen exposure [65].

Understanding molecular mechanisms whereby corticosteroids suppress inflammation explains how glucocorticosteroids are able to inhibit inflammatory pathways, and also provides insights into the complex molecular mechanisms of steroid unresponsiveness. glucocorticosteroids activate and suppress many pro- and anti-inflammatory genes, such as NF-κB and correlated kinase proteins [59]. Several distinct molecular mechanisms contributing to decreased anti-inflammatory effects of glucocorticosteroids have been identified. Excessive activation of NF-κB can cause reduced or absent responsiveness to glucocorticoids in asthma, since NF-κB binds the glucocorticoid receptor, preventing its interaction with the glucocorticoid response element and other transcription factors. PBMCs from uncontrolled severe adult asthmatics showed higher levels of NF-κB p65 protein expression, IκBα phosphorylation and IKKβ kinase activity than normal individuals, despite long-term inhaled and oral glucocorticosteroid treatment [9, 23]. A similar state of overexpression and activation of NF-κB p65 subunit protein in PBMCs and induced sputum cells has been shown in children with moderate to severe asthma [13, 32].

Activated glucocorticoid receptor interacts with co-repressor molecules to attenuate NF-κB-associated co-activator activity, thus reducing histone acetylation and chromatin remodelling. Chromatin modification

plays a critical role in the regulation of inflammatory genes and there is an extensive literature concerning histone acetylation and mechanism of action of glucocorticosteroids. CHIAPPARA et al. [22] showed that the increased expression of pCREB can be modulated by glucocorticoids in responsive asthmatic patients.

Stepping forward from the Maurizio Vignola experience, our research group has further contributed to the definition of the molecular mechanisms in glucocorticoid control of inflammation. The new insights into corticosteroid action may lead to new pharmacological approaches to treat inflammatory lung diseases, and in particular to counteraction of reduced steroid responsiveness in complex inflammatory diseases, such as asthma.

New therapies in corticosteroid-resistant asthma

Since multiple asthma phenotypes with distinct pathogenetic profiles have been identified, a personalised strategy in treatment directed towards disease-specific features is expected to improve outcomes in these specific patients [66].

Allergic asthma is characterised by increased levels of IgE. Omalizumab, a humanised anti-IgE monoclonal antibody specifically binding free IgE, has been introduced in asthma treatment. Omalizumab interrupts the allergic cascade by preventing the binding of IgE with its high-affinity FcεRI receptors on target cells (mast cells, basophils, antigen-presenting cells, etc.) [67]. Its clinical efficacy has been well documented in a number of clinical trials in patients with moderate-to-severe and severe allergic asthma and with increased levels of IgE. Omalizumab reduced exacerbations, asthma symptoms and use of ICS and rescue medication, and improved quality of life relative to placebo [68]. Results from recent pooled data from randomised clinical trials and from a large prospective cohort study provided reassurance about the long-term safety of omalizumab. Long-term treatment with omalizumab was able to reduce inflammation [69] and improved clinical control in severe asthmatics [70]. In severe uncontrolled asthma, omalizumab increased Foxp3 expression and decreased the pCREB/CREB ratio and IL-8 expression. These findings correlated with the forced expiratory volume in 1 s (FEV1) values and symptom scores [70].

Another asthma phenotype is characterised by increased numbers of neutrophils in the airways of a subgroup of patients with severe asthma, characterised by inadequate symptom control and high rate of inflammation despite high doses of steroids. Since IL-8, a neutrophil chemotactic agent, exerts pro-inflammatory responses in leukocytes by activating the G-protein coupled receptor CXCR2, a selective CXCR2 receptor antagonist (SCH 527123) was tested in a randomised clinical study in patients with severe asthma and increased number of neutrophils in sputum. A mean reduction of 36.3% in sputum neutrophil percentage was shown in the treated arm, compared with a 6.7% increase in the placebo arm. In addition, asthmatics treated with SCH 527123 experienced fewer mild exacerbations without additional adverse events compared with the placebo group [71]. Conversely, a randomised clinical study on the use of golimumab (50, 100 or 200 mg), a human monoclonal antibody against tumour necrosis factor (TNF)-α, did not demonstrate a favourable risk–benefit profile in patients with severe persistent asthma [72]. Golimumab-treated asthmatics experienced major adverse events, including serious infections, without any advantage in terms of asthma control or improved FEV1. The potential use of anti-TNF monoclonal antibodies in asthmatics remains undefined, since this study showed inaccurate selection of severe asthmatics, i.e. patients were not selected based on elevated levels of TNF before treatment.

To further underline the need for accurate phenotyping of asthmatic patients, a recent clinical trial using the anti-IL-13 lebrikizumab demonstrated that patients with high pre-treatment levels of serum periostin had greater improvement in lung function with lebrikizumab than did patients with low periostin levels [73].

New advanced biotechnologies may provide further perspectives in the therapy of severe asthma by re-directing the immune and inflammatory responses in these patients. In this regard, synthetic hypoallergenic fragments of Parietaria judaica upregulated innate immunity receptors and increased interferon-γ, and appeared capable of re-orienting the immune system towards a more physiological balance between Th1 and Th2 responses [74]. In summary, personalised therapy aimed at targeting disease-specific features will allow better control in asthmatics, but further studies are still needed to achieve this important goal.

Finally, nonpharmacological therapeutic approaches could also be useful in asthma treatment. Physical exercise has been shown, in animal models of allergic asthma, to significantly reduce airway inflammation [75]. In healthy humans, studies on induced sputum cells at rest and after a marathon [76] or half-marathon [77] race documented that intense exercise caused mild epithelial injury and influx of neutrophils into the airways, possibly due to hyperosmolarity of the airway lining fluid secondary to the hyperpnoea of exercise. However, no significant activation of inflammatory cells occurred [76, 77], in line with the anti-inflammatory effects of exercise documented in animal models. However, such inflammation could negatively affect airway muscle tone. Subsequent studies in healthy runners showed that trained

subjects were less responsive to single-dose methacholine inhaled in the absence of deep breaths [78], suggesting a positive effect of repeated airway smooth muscle stretching during exercise hyperpnoea. Moreover, healthy sedentary subjects showed decreased bronchial responsiveness after 12 weeks of training [79], and the same effect occurred in sedentary subjects with mild asthma undergoing the same training protocol [80]. Similar results of decreased bronchial responsiveness have been recently reported after application of continuous positive airway pressure (CPAP) during sleep for a week in asthmatic patients free from sleep disordered breathing [81]. Population studies also documented an inverse relationship between physical activity and bronchial responsiveness [82]. Therefore, exercise training might be beneficial in asthmatics, and mechanical stretch of the airways is a possible mechanism of the beneficial effects of exercise or CPAP. However, more data are needed before these results can be applied clinically.

Conclusion

Maurizio Vignola enthusiastically set up a solid research group that in the last 10 years has carried forward the large amount of work that he had so successfully started. Research activity has continued on both the experimental and the clinical aspects of asthma, with special regard to the biology of inflammation and remodelling of the airways, the relationship between upper and lower airway inflammation, and the complex mechanisms responsible for steroid-resistant asthma. On the clinical side, the group is part of European networks and actively contributes to the studies on the definition of asthma phenotypes based on biomarkers. Finally, studies on new treatment strategies will help to achieve the personalised treatment of asthma that Maurizio Vignola had foreseen as "the future" for asthma patients.

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