

The airway inflammatory response in allergic asthma and its relationship to clinical disease

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Endobronchial biopsy and lavage studies have revealed the presence of mast cell, eosinophil, T-lymphocyte and epithelial cell activation in asthma, along with the structural changes of tissue eosinophil infiltration, loss of superficial columnar ciliated epithelial cells and enhanced collagen deposition in the lamina reticularis. As these cellular and structural changes underlie the clinical features of asthma, i.e., symptom expression, variable airflow obstruction and bronchial hyperresponsiveness, an understanding of their induction and regulation is essential to the understanding of the asthmatic process.

The acute airway response to allergen has been studied by the technique of local endobronchial allergen challenge with direct airway sampling in asthma. These studies identify allergen-mast cell interaction as the initial airway event, with mediator release inducing bronchoconstriction and enhancing vascular permeability. As preformed cytokines are present in mast cells, cytokine release from this cell population is likely to initiate the process of endothelial cell activation, with upregulation of cell adhesion molecules, and tissue cell recruitment. Subsequent cytokine elaboration from airway macrophages and T-lymphocytes will perpetuate this response while in chronic clinical disease T-lymphocytes, mast cells, matrix tissue, epithelial cells and eosinophils themselves are all likely to contribute to the cytokine pool within the airways and thus to the regulation of inflammatory cell migration and activation.

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Introduction

Over the last decade it has become increasingly apparent that airway inflammation is central to the development of asthma and underlies the physiological features of bronchial hyperresponsiveness and variable airflow obstruction which lead to the characteristic symptoms of chest tightness, wheeze, breathlessness and cough along with the typical nocturnal worsening (1). Evidence for the involvement of differing cell types in this airway inflammatory process has largely been derived from fiber-optic bronchoscopic studies which have enabled direct airway sampling by endobronchial lavage and biopsy. Such studies have been undertaken to investigate the acute airway response to allergen exposure in asthma as well as to assess the changes in cells and structure relevant to chronic clinical disease.

Acute Airway Inflammation

Early studies identified that following allergen aerosol inhalation an increase in activated eosino-

phils was apparent within the airways 4-6 hours following challenge (2, 3), at a time coincident with the late increase in bronchial responsiveness. To investigate the airway events leading to these cellular and physiological changes, local endobronchial allergen challenge has been used and the airways sampled at differing time points post challenge. Within 10 minutes of local allergen challenge in asthma, there is visual evidence of bronchoconstriction along with airway erythema suggestive of vasodilatation. Measurement of mediator levels in lavage identify acute elevations in histamine, cryptase and prostaglandin D₂ indicative of acute mast cell degranulation (4, 5, 6). In addition, elevations in leukotrienes have been reported following local endobronchial allergen challenge (5, 7) and biochemical and ultrastructural evidence of macrophage activation has been reported (2). Consistent with these mediator findings, partial inhibition of the immediate airway bronchoconstrictor response to allergen inhalation has been reported with H₁-antihistamines (8), leukotriene receptor antagonists (9) and antagonists of the TP thromboxane re-

ceptor through which prostaglandins exert their bronchoconstrictor actions (10). In addition to their action on airway smooth muscle, histamine, prostanoids and leukotrienes will interact to enhance plasma protein leakage through their action on the vascular endothelial cells (11, 12).

Visually it appears that airway edema contributes significantly to the airway narrowing that is evident 6 hours post local endobronchial allergen challenge in asthma. The airway is irregularly narrowed with a pale swollen appearance to the mucosa along with surface fluid exudation. It is probable that this airway wall thickening contributes to the increase in bronchial responsiveness at this time and that the inhibition of the late allergen bronchoconstrictor response and change in bronchial reactivity with leukotriene inhibition (9) may be related to inhibition of change in vascular permeability as much as to inhibition of airway smooth muscle constriction. Endobronchial biopsies 6 hours following acute local endobronchial allergen challenge identify leukocyte cell accumulation with an increase in leukocytes expressing the cell surface ligand LFA-1 in comparison with those findings from a saline challenge site (13). Immunostaining of the biopsies with monoclonal antibodies (MoAb) against neutrophil elastase, eosinophil cationic protein (EG2), T-lymphocytes (CD3) and mast cell tryptase (AA1) identify that increases in all these cell populations contribute to the leukocyte tissue accumulation (Fig. 1).

Endothelial-Leukocyte Cell Adherence/ Transendothelial Migration

The initial phase of cell recruitment into the tissue involves the local adherence of circulating leuko-

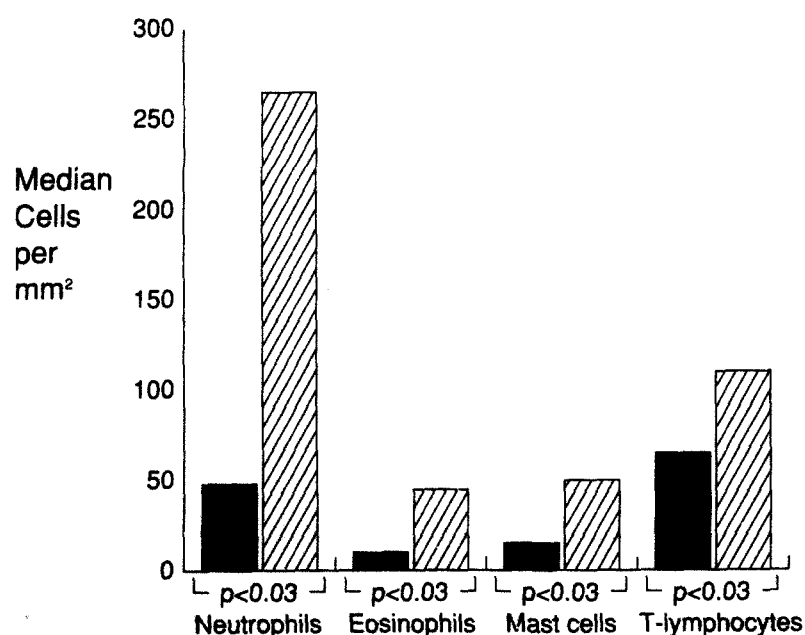


Fig. 1: Cellular change in endobronchial biopsies 6 hours following endobronchial allergen or saline challenge in 6 atopic asthmatic subjects (reference 13).

cytes to the vascular endothelium (14). In allergic reactions this is initiated by a loose adherence mechanism involving the selectin family of adhesion molecules. P-selectin, which is stored pre-formed in Weibel-Palade bodies, and E-selectin, which is generated in response to cytokines, are both expressed on endothelial surfaces while L-selectin is constitutively expressed on the cell surface of leukocytes. P-selectin is rapidly mobilized to the endothelial surface following exposure to mediators, such as histamine, and initiates a rolling margination through an interaction with a carbohydrate moiety Sialyl-Lewis^x on leukocytes (15). This adherence is enhanced by the binding of L-selectin to endothelial lectins and by the induced endothelial expression of E-selectin, which in vitro peaks at 2–4 hours following stimulation. Once the velocity of the margined cell is reduced, with the E-selectin-ligand interaction, the adherent cell undergoes changes including upregulation of intracellular calcium, polarization in shape, priming for enhanced activation, shedding of L-selectin and the expression of functional β 2 integrins, primarily CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac-1), which recognize the endothelial adhesion molecule ICAM-1 (16, 17). The expression of the immunoglobulin supergene adhesion molecule ICAM-1 and subsequently VCAM-1 on the vascular endothelium allow firm adherence and enable the cell to migrate into the tissue under a chemotactic stimulus.

The relevance of both the cell surface ligands and the endothelial adhesion molecules to this process has been suggested from studies incorporating blocking antibodies. Transendothelial migration of neutrophils in vitro is markedly inhibited by monoclonal antibodies against CD11a, CD11b and CD18 (18). In an animal model, antibodies against CD18, CD11b, ICAM-1 and E-selectin, but not MoAb against P-selectin, have all been shown to significantly attenuate LTB₄- and PAF-induced leukocyte endothelial adherence and transendothelial migration (19). Thus there is an interaction between lipid mediators and the specific cell adherence mechanisms involved in the process of tissue cell recruitment. In addition, although LTB₄ also reduces the leukocyte rolling velocity in post-capillary venules, this action of this eicosanoid appears independent of the expression of selectins, ICAM-1 or the β 2 integrins (19). The later temporal expression of vascular cell adhesion molecules (VCAM)-1, which in vitro peaks at 4–6 hours following cytokine stimulation of endothelial cells, has relevance to eosinophil but not neutrophil adherence. VCAM-1 recognizes the ligand, very late antigen (VLA)-4, which is not expressed on neutrophils but is evident on eosino-

phils, monocytes, T-lymphocytes and basophils. A VCAM-1-VLA-4 interaction thus permits a greater selectivity with respect to eosinophil airway recruitment.

To investigate these processes in vivo, biopsies taken from allergen and saline challenged segmental airways in asthma (13) which had been processed into glycol methacrylate resin were immunostained with MoAb against the adhesion molecules ICAM-1 (6.5B6), VCAM-1 (1.4C3) and E-selectin (1.2B6) to assess changes in their expression in relationship to the tissue cell accumulation. Their expression was quantitated, as previously described (20), in relationship to the total number of vessels within an adjacent 2 μ m biopsy section. At 6 hours post challenge there was a significant increase in ICAM-1 and E-selectin but not in VCAM-1 (13). The increase in ICAM-1 expression on the vascular endothelium was paralleled by an increase in LFA-1 positive cells within the tissue. Consistent with the relevance of β 2 integrins to cell recruitment and the shedding of L-selectin in this process, a segmental allergen challenge study which assessed lavage cells 18 hours post challenge identified increased CD11b and diminished L-selectin expression on airway eosinophils, in comparison to their peripheral blood counterparts (21). This study also reported an accompanying increase in soluble E-selectin in bronchoalveolar lavage fluid after allergen but not saline challenge. By 24 hours post allergen challenge there is evidence in bronchial biopsies of enhanced endothelial expression of VCAM-1 (22).

The relevance of these adhesion molecule changes to acute allergic inflammation has been suggested in primate studies, in which administration of R6.5, a monoclonal antibody directed against ICAM-1, prevents allergen-induced airway eosinophilia and allergen-induced bronchial responsiveness (23). Within the same primate model, an anti-E-selectin MoAb has been shown to prevent the late phase bronchoconstrictor response following single-dose, allergen challenge as well as inhibiting the allergen-induced airway neutrophil recruitment (24). Although E-selectin recognizes a sialyl-fucosyl pentasaccharide moiety which is present in cell surface glycoproteins on eosinophils as well as neutrophils, the role of E-selectin in acute eosinophil recruitment is uncertain. While in vitro an E-selectin MoAb has been demonstrated to inhibit eosinophil adherence to stimulated endothelial monolayers (25), in vivo, within the primate model, allergen-induced eosinophil recruitment is unaffected by an E-selectin MoAb (26). As this primate model involves repeated allergen challenge, it is possible that this approach bypasses the regulation afforded by E-selectin, as in vitro leuko-

cyte transendothelial migration, while being E-selectin dependent at low levels of stimulation, is uninfluenced by E-selectin MoAb with high doses of cytokine or LTB₄ exposure, being CD11, CD18 dependent (27). Furthermore, within the animal model eosinophil airway recruitment is also likely to be dependent upon endothelial VCAM-1 expression. The VCAM-1-VLA-4 interaction allows selective eosinophil recruitment and retention within the airways in chronic allergic disease of the airways (28, 29).

Cytokine Regulation

The endothelial expression of the adhesion molecule ICAM-1, VCAM-1 and E-selectin are regulated locally by the generation and release of cytokines. Four cytokines appear to be of specific relevance: Interleukin (IL)-1, tumor necrosis factor TNF α , interferon gamma (IFN γ) and IL-4 (14, 30). Interleukin-1 and TNF α will induce the endothelial synthesis and expression of all these adhesion molecules while IFN γ will also enhance ICAM-1 expression and IL-4 will promote the action of TNF α on VCAM-1 expression. In human umbilical vein endothelial cell cultures (HUVECs), the cytokine induction of ICAM-1 expression starts at 2–4 hours following stimulation, reaches a plateau at 24 hours and remains expressed for up to 72 hours in the presence of continued stimulation (31). In contrast to ICAM-1, an allied cell adhesion molecule ICAM-2 is not cytokine inducible. The time course of upregulation of VCAM-1 in HUVECs is similar to that of ICAM-1, while E-selectin expression is evident by 30 minutes, peaks at 2–4 hours and declines to basal levels by 24 hours (31).

Further members of the IL-4 cytokine gene family, which are encoded for on chromosome 5, are relevant to allergic inflammation (32). These comprise IL-3, IL-5, IL-13 and granulocyte macrophage colony stimulating factor (GM-CSF), in addition to IL-4. Interleukin-13 has many overlapping functions with IL-4, promoting IgE synthesis as well as β -cell growth and differentiation (33). Interleukins 3 and 5, along with GM-CSF, exert a range of actions relevant to tissue eosinophil accumulation.

They promote the development of eosinophils in the bone marrow from progenitor cells (34, 35), prime eosinophils for increased functional activity (36, 37), have weak chemotactic properties at high concentrations while priming the eosinophils to chemotact to other stimuli, such as LTB₄ and IL-8, at lower concentrations (38, 39), and also prolong eosinophil survival (40). For IL-5, this action on survival has been shown to be due to a delay in

apoptosis (41). Other cytokines of relevance to tissue eosinophil recruitment are TNF α , which enhances eosinophil VLA-4 and members of the chemokine family, such as IL-8, RANTES, and lymphocyte chemotactic factor (LCF), which all have the potential to promote eosinophil chemotaxis (42–44).

Investigation of the expression of these cytokines in the acute allergic reaction has identified an increase in cells expressing mRNA for IL-4, IL-5 and GM-CSF in lavage 24 hours following allergen inhalation challenge (45). Within biopsies of the airways at the same time point post allergen challenge, there is a significant increase in cells expressing mRNA for IL-5 and GM-CSF along with a trend, which did not reach statistical significance, for an increase in gene expression for IL-4 and IL-2 (46). Assessment of released cytokines has identified that IL-5 is the major contributor to the chemotactic activity within lavage fluid post allergen challenge (47).

Chronic Airway Inflammation

Post-mortem studies and more recently fiber-optic bronchoscopy studies, with airway sampling by lavage and biopsy, have revealed the mucosal inflammatory changes in naturally occurring clinical asthma (1). These studies have identified abnormalities of mast cells, eosinophils, T-lymphocytes, macrophages, epithelial cells and myofibroblasts in relationship to disease expression.

Mast cells: Although comprising a small percentage of cells recovered by lavage (normally <0.5%) several studies have identified an increase in lavage mast cell recovery in asthma (48, 49). Consistent with this, the majority of studies also reveal an increase in epithelial mast cells within mucosal biopsies (50, 51). These cells are in an activated state, with increased levels of histamine, tryptase and prostaglandin (PG) D₂ reported in bronchoalveolar lavage (BAL) samples (52–54), in asthma.

In support of these lavage findings, mast cell ultrastructural changes indicative of degranulation are evident on transmission electronmicroscopic examination of endobronchial biopsies (55–56). Several workers have demonstrated correlations between the numbers and/or percentages of mast cells recovered by lavage, or indices of their activation, with either the magnitude of airflow obstruction or the level of bronchial responsiveness (49, 54, 57, 58).

More recently it has become evident that human airway mast cells generate and store preformed cytokines such as IL-3, IL-4, IL-5, IL-6, GM-CSF and TNF α (59, 61). There is increased mast cell

expression of TNF α and secretory IL-4 in asthma (59).

Eosinophils: A fairly universal finding in asthma is airway eosinophil recruitment. This has been reported from analysis of sputum (62, 63), lavage (49, 58) and biopsy (55, 56) samples. The eosinophils are activated, as evidenced by morphological changes on transmission electronmicroscopy (55, 56) and by the identification of increased concentrations of the eosinophil products, eosinophil cationic protein (ECP) and major basic protein (MBP), in recovered lavage in asthma in comparison to those levels in lavage fluid from normal airways (58, 64). Again, as for mast cells and their products, there are reports correlating eosinophil numbers and/or their activation status to the level of resting airflow obstruction and bronchial responsiveness (58, 64).

Eosinophils within the airways in asthma have been shown to contain IL-5 (59).

T-Lymphocytes

Although a recent electronmicroscopic study of bronchial biopsies reported an increase in lymphocytes in both the epithelium and lamina propria in asthma (51), studies assessing T-lymphocytes (CD3) and their subpopulations (CD4 and CD8) have not found any disease-related increase in either bronchial biopsies or BAL (49, 65, 66) fluid. Airway T-lymphocytes are, however, in an activated state in asthma, with increased expression of the cell surface markers CD25, HLA-DR and VLA-1 on T-cells recovered by lavage, (66, 67) and in biopsies there are increased numbers of CD⁺ cells in atopic asthma (65), non-atopic asthma (68) and fatal asthma (69). In addition, morphological changes in airway T-lymphocytes have been described in asthma which have been interpreted as being indicative of activation (70).

An important recent advance has been the recognition that CD4⁺ T-lymphocytes can be divided into sub-populations according to their profile of cytokine production. In long-term murine T-cell clones, those classified as TH₂ produce IL-4, IL-5, IL-6 and IL-10, while TH₁ clones produce IFN γ and IL-2 (71). In addition IL-3, GM-CSF and TNF α are produced by both types (72). In BAL fluid from asthmatics there is an increase in cells expressing mRNA for IL-2, IL-3, IL-4, IL-5 and GM-CSF (73). Immunomagnetic separation techniques have defined the IL-4 and IL-5 generating cells as CD2⁺ lymphocyte, indicative of a potential expansion of the TH₂ CD4 T-lymphocyte subpopulation in clinical asthma (73). In bronchial biopsies there is an increase in IL-5 mRNA positive cells in asthma, consistent with the lavage

findings, although the cell provenance is undefined (74).

Monocytes and Macrophages

The majority of early bronchoscopy studies failed to demonstrate any difference between asthmatics and normal subjects in the number of macrophage cells in BAL fluid or biopsies. However, two recent studies, one employing transmission electron-microscopy (64) and the other detailed immunohistochemistry (75) have reported an increase in macrophage numbers in the epithelium and lamina propria in asthma. The immunohistochemical analysis also identified that the cells had phenotype features characteristic of peripheral blood monocytes, suggestive of their recent recruitment into the airways from the circulation.

Monocyte adhesion *in vitro* depends on interactions involving E-selectin and VCAM-1, consistent with the concept that they may be recruited in association with other leucocytes utilizing these molecules.

Ex vivo, macrophages from asthmatic airways exhibit a greater leukotriene generation than macrophages obtained from normal airways (77, 78) consistent with this, several workers have demonstrated that airway macrophages are more activated in asthmatic patients than healthy controls (79). There are, however, discrepancies as to the cytokine profile of activated airway macrophages in asthma. Potentially these cells can generate IL-1, IL-6, GM-CSF and TNF α , and enhanced basal release of IL-1, GM-CSF and (80, 81) TNF α has been observed in asthma (80). This has not been a consistent finding, however, and stimulated airway macrophages from asthmatics have been reported to release an IL-1 inhibitory factor (82). The basis for these contrasting findings awaits further evaluation.

Epithelial Cells

A feature noted in some of the early post mortem studies of asthma was partial or complete disruption of the bronchial epithelium (83). Most bronchoscopic studies have also reported similar findings (56, 70, 84), although there is debate about the extent to which the findings may be artefactual (85) as well as conflicting reports (86). It is likely, however, that epithelial fragility is indeed a feature of asthma with a tendency for the superficial cells to slough off with a resultant increase in epithelial cells in lavage fluid (56) and sputum specimens from asthmatics (49). The increased recovery of epithelial cells in bronchoalveolar lavage fluid cor-

relates inversely with the level of bronchial reactivity (56).

The traditional view has been that epithelial shedding is secondary to cytotoxicity from eosinophil products such as ECP and MBP (87). Alternative causes have to be considered, however, as many of the shed epithelial cells are viable and a plane of cleavage appears to exist between the basal and suprabasal cells (88), resulting in intact clumps of columnar ciliated cells in the airway lumen. One explanation for this observation is proteolytic cleavage, perhaps by eosinophil derived proteolytic enzymes, as the protease inhibitor α_2 macroglobulin inhibits eosinophil mediated epithelial damage *in vitro* (89).

A further consideration in the airway epithelial disruption is the role of nitric oxide (NO). Elevated levels of nitric oxide have been reported in exhaled air in asthma (90, 91), and increased expression of the inducible form of the enzyme nitric oxide synthase (iNOS) which generates NO from L-arginine, is described within the epithelium of asthmatics (92). The proinflammatory cytokines IL-1, TNF α and IFN- γ upregulate iNOS *in vitro*, and NO generation by this enzyme is less well regulated by the constitutive form of the enzyme. At low concentrations, NO, which has potential bronchodilator and vasodilator actions, will physiologically antagonize the actions of the 21 amino acid peptide endothelin, which is also generated by the airway epithelium and has increased expression in asthma (93). At high concentrations, NO may have detrimental effects on the epithelium. Nitric oxide will interact with superoxide generated by inflammatory cells to produce peroxynitrite which will inhibit enzymatic activity and impair cell function and viability.

It is now also appreciated that airway epithelial cells cultured *in vitro* generate and release cytokines, in particular IL-6, IL-8 and GM-CSF (94, 95). Immunostaining of endobronchial biopsies reveals expression of IL-8, GM-CSF and the chemokine MCP-1 with increased expression of the latter two in asthma (96–98). Interleukin 8 and GM-CSF will contribute to eosinophil airway recruitment and MCP-1 to airway macrophage accumulation.

Myofibroblasts: Myofibroblasts are a selective group of collagen synthesizing cells with contractile capabilities. They are found within the airways beneath the basement membrane. The numbers of these cells are increased in asthma, and a positive correlation exists with the thickness of the collagen layer within the lamina reticularis (99). This collagen is beneath the true basement membrane and is composed predominantly of types III and V collagen (100). Consistent with the myofibroblast pro-

liferation in asthma, the sub-basement membrane collagen deposition is increased in asthmatic airways in comparison with non-asthmatic controls (99).

Chronic Mucosal Inflammation and Clinical Disease

In allergic asthma the interaction between inhaled allergen and cell surface bound IgE induces cell activation. High affinity IgE receptors are found in high density both on mast cells and on Langerhan's cells, which may represent the primary antigen presenting cell within the airways. Lower affinity IgE receptors may become upregulated on other cell populations, such as macrophages, T-lymphocytes, platelets and eosinophils in response to cytokine stimulation, in particular IL-4 and IL-13. The appreciation that mast cells contain cytokines, including IL-4, suggests that this cell population may initiate the cellular response to allergen exposure and that the response is elaborated and perpetuated by cytokine generation from many airway cell populations, including T-lymphocytes, eosinophils themselves, epithelial cells and possibly macrophages. The initiating potential for mast cell IL-4 is also suggested by the identification that T-lymphocytes need a pulse of IL-4 to proliferate and generate a TH₂-like cytokine profile (101).

Within the airways, bronchodilator tone will reflect a balance between epithelially derived endothelin and NO as well as mediator release from activated cells. In this respect mast cells and eosinophils are the primary effector cells, both generating leukotrienes (LTs) which will contribute to airway smooth muscle constriction, mucus hypersecretion and plasma protein extravasation. With inhalation studies LTC₄ and LTD₄ are 150–1000 more potent than histamine in inducing bronchoconstriction (102–104). In addition, leukotrine release may contribute to bronchial hyperresponsiveness as in normal individuals LTD₄ inhalation can induce prolonged hyperresponsiveness to methacholine (105), and LTE₄ has a similar effect in asthmatics although not non-asthmatic subjects (106). This influence on airway reactivity may be mediated via an action on cell infiltration and activation as LTE₄ inhalation has been shown to induce an airway eosinophil infiltration (107).

The potential for eosinophil products to damage the airway epithelium may explain, in part, the genesis of bronchial responsiveness while geometric factors due to airway wall thickening will also contribute (108). In the short term, mucosal edema will contribute to airway wall thickening while enhanced collagen deposition and smooth muscle hyperplasia/hypertrophy will contribute to

the chronicity. The differing components of bronchial responsiveness, acute inflammatory and chronic structural responsiveness, offers an explanation for the progressive improvement in bronchial responsiveness with anti-inflammatory therapy in contrast to the rapid and plateaued bronchodilator response. As bronchial responsiveness contributes to airflow variability and symptom generation in asthma, treatment of the underlying inflammatory components of asthma rather than physiological antagonism of the airway smooth muscle response has been the focus of current international guidelines for asthma management (109).

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