Rhinitis

Mechanisms and Management

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Chapter 3

The Immunopharmacology of Rhinitis

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Introduction

The National Morbidity surveys from the Royal College of General Practitioners Unit in Birmingham, UK, have identified a quadrupling of the number of consultations for seasonal allergic rhinitis between the years of 1955/56 and 1980/81. While a number of factors may account for this, a diagnostic change is unlikely, as the classical constellation of acute rhinitis symptoms on exposure to an allergen to which an individual is sensitised is easily recognised, with nasal itching, sneezing, rhinorrhoea and transient nasal blockage. These immediate symptoms are a consequence of mast cell degranulation and the local release of inflammatory mediators. Involvement of mast cells in the immediate response has been demonstrated both indirectly, with evidence of local mast cell mediator release, and directly by the identification of allergen-related mast cell degranulation on nasal biopsy. Prolonged allergen exposure to a relevant allergen, as in perennial allergic rhinitis, reproduces these symptoms with nasal blockage often becoming a more prominent feature. The role of the mast cell or the other metachromatic staining cell, the basophil, in the persistence of nasal symptoms and their relevance to the secretory eosinophilia identified in these patients has yet to be fully clarified.

The Mast cell

The mast cell was first described in 1877 by Paul Ehrlich, when investigating the histochemical staining characteristics of some synthetic basic aniline dyes. He identified the presence of a connective tissue cell whose granules altered the colour of the dye (metachromasia). As these cells were numerous in connective tissue whose nutrition was enhanced, he named them mastzellen (well nourished cells).

Granular contents

Electron microscopy of a quiescent mast cell identifies a cytoplasm full of defined secretory granules. It is the high content of proteoglycan within these granules that confers the metachromatic staining characteristics, a property shared only with basophils. In human mast cells this proteoglycan is a 60kD species of heparin whose highly sulphated glycosaminoglycan (GAG) groups combine with the predominant proteases, tryptase, chymase or carboxypeptidase to form the characteristic crystalline structure of the granules. Four structures of crystal have been described, scrolls, lattices, gratings and a serpentine-like structure, all of which have a common repeating periodicity of 7.5 pm or multiples thereof, suggesting a similar basic chemical structure. In addition to storing these potent proteolytic

enzymes, heparin, at the acid pH within the secretory granules, also stores the basic amine histamine through simple ionic binding. These three granular components, histamine, heparin and the neutral proteases comprise the major pre-formed mediators of human mast cells. In addition smaller quantities (on a weight basis) of exoglycosidases, chemotactic factors (eosinophil and neutrophil), oxidative enzymes and a kininogenase have been described in association with human mast cells.

Immunoglobulin E

The crucial link between the mast cell and the immediate allergic reaction is immunoglobulin E (IgE). Immunoglobulin E, following its isolation in 1966 from the sera of ragweed-sensitive subjects, was identified as being the agent responsible for the transmission of the immediate hypersensitivity response, an action previously attributed to an unidentified "reaginic antibody". Immunoglobulin E functions as a cell surface antibody, binding to cell surfaces. Two cell surface receptors are described, a high affinity F_{CI} receptor and a low affinity F_{CII} receptor. The high affinity F_{CI} receptors are present on human mast cells and basophils, with these cells possessing approximately 300.000 and 40.000-100.000 such receptors respectively. The more recently described F_{CII} receptors are present in low numbers on macrophages, eosinophils, platelets and lymphocytes. In the atopic state, in which there is a propensity for the excessive production of IgE to environmental allergens, IgE binds to IgE Fc receptors on mast cell surfaces. These sensitised cells are thus primed to react to specific environmental allergens. Exposure to the relevant allergen leads to cross linking of the surface IgE molecules which brings the high affinity IgE-F_{CI} receptors into apposition and initiates a series of membrane and cytoplasmic events culminating in the release of the granule-associated, pre-formed mediators and the *de novo* synthesis of newly generated mediators. Enhanced IgE production also occurs in conditions associated with diminished T-lymphocyte suppressor cell activity, as in the primary immunodeficiency syndromes, such as the Wiskott-Aldrich syndrome and ataxia-telangiectasia, and in acquired immunodeficiency states such as transient hypo-gammaglobulinaemia in children and some forms of lymphoma (eg, Hodgkin's). These conditions may also be associated with symptoms characteristic of the atopic state.

Mast Cell Activation/Secretion

Following immunological activation, there is an increase in intracellular calcium, solubilisation of the mast cell granules, movement of the granules by cytoskeletal proteins into apposition with the cell membrane, fusion of the perigranular and cytoplasmic membranes and liberation of the granule contents into the extracellular environment. This process involves the activation of the phosphatidyl inositol (PI)-diacyl glycerol (DAG) pathway and the production of inositol triphosphate. Phosphatidyl inositol is a membrane phospholipid from which DAG is cleaved following cell activation, either by phospholipase C or after sequential methylation of PI by a phosphodiesterase. Subsequent metabolism of DAG by the calcium-dependent enzyme diglyceride lipase leads not only to the production of L-monoacyl glycerol (MAG) but also arachidonic acid. The products of this pathway regulate intracellular events. Both DAG and MAG are potent membrane fusagens. In addition DAG activates the calcium-dependent enzyme protein kinase C which is responsible for the conversion of myosin to its active phosphorylated form, and hence induces contraction of smooth muscle (in this instance the contraction of the cytoskeletal thin filaments), leading to the movement of the granules

towards the cell plasma membrane. Inositol triphosphate, formed during generation of DAG, can mobilise calcium from intracellular stores in the presence of ATP, making it available for calcium dependent enzymes. In addition to these intracellular changes, cell activation is accompanied by a transient increase in cellular levels of cyclic AMP which precedes mediator secretion.

Newly generated mediators

The cleavage of arachidonic acid from the phospholipid membrane, following mast cell activation, is likely to involve primarily the phospholipase C/diglyceride lipase pathway as described, rather than the steroid-sensitive phospholipase A_2 pathway pertinent to some other cell types. The mobilised arachidonic acid is then metabolised by either cyclooxygenase enzymes to prostaglandins (PGs) and thromboxanes (TXs) or by lipooxygenase enzymes to hydroxyeicosatetranoeic acids (HETEs) and leukotrienes (LTs). Immunological stimulation of purified mast cells produces predominantly PGD_2 as the major prostanoid, with only small quantities of TXB_2, PGF_{2a}, PGE_2 and 6-keto-PGF_{1a} being produced. In addition to generating prostanoids, activation of human mast cells is associated with generation of 5HETE, LTB_4, LTC_4 and to a lesser extent LTD_4. There is little evidence for human mast cells as being a major source of platelet activating factor (PAF).

Mediator relevance *in vivo*

An understanding of the mediators and their secretory processes is only of relevance if this can be directly related to the disease under consideration. With respect to allergic rhinitis, it is possible to explain many of the features of this disease on the basis of mast cell mediator release. Histamine nasal insufflation produces itching, sneezing, rhinorrhoea and transient nasal blockage. These effects are predominantly mediated via H_1-receptors with only a small contribution to nasal blockage being related to vascular H_2-receptors. The hypersecretion is bilateral following unilateral nasal challenge, while the blockage is only on the challenged side. The bilateral hypersecretion is related to H_1-stimulation of sensory nerve endings with reflex parasympathetic activity to contralateral nasal glands.

In clinical practice, H_1-antihistamines reduce seasonally related sneezing and rhinorrhoea but have little effect on nasal blockage, suggesting that non-histamine mediators are of greater importance in the everyday genesis of this symptom. Possible contributors to nasal blockage are LTC_4, LTD_4 and PGD_2. Both LTC_4 and LTD_4 produce a dose-dependent sustained nasal blockage and an increase in nasal secretions with local application, in the absence of itching or sneezing. PGD_2 infusion in man is reported to be associated with "nasal stuffiness" consistent with the known vasodilator properties of this prostanoid. The interaction between histamine, PGD_2, PGI_2, PGE_2, LTC_4, LTD_4 which vasodilate, and LTC_4, LTD_4 and histamine, all of which enhance vascular permeability will lead to local fluid extravasation and tissue oedema, further compromising nasal airflow. These effects on nasal blockage could be compounded further by the chemoattractant properties of eosinophil chemotactic factor (ECF) and the more potent LTB_4, attracting eosinophils into tissue and mucosal sites. Eosinophils also possess IgE-receptors, of lower affinity than mast cells, and if immunologically activated would elaborate the inflammatory response, being able to synthesise the sulphidopeptide leukotrienes, to degranulate mast cells and to produce ciliary motility abnormalities impairing clearance of nasal secretions.

The Mast cell *In Vivo*

Indirect approach

Lavage of the nasal cavity following intranasal allergen instillation in sensitised subjects has been used to detect the local release mediators. During the immediate nasal response to allergen instillation there is an increase in histamine, PGD_2, LTC_4, LTD_4, kinins and TAME esterase. While a number of these mediators could have other cell sources, their appearance in conjunction with PGD_2 would support a mast cell origin.

A number of patients also develop a late nasal obstructive response following allergen nasal challenge. Measurement of mediator release during the late response identifies elevations in histamine, kinins, LTC_4 and TAME esterase. There is no apparent elevation in PGD_2. Short-term corticosteroid therapy (<48 hours) inhibits both the obstruction and mediator release occurring during the late nasal response to allergen challenge but has no effect on the immediate response. As glucocorticoids inhibit basophil but not mast cell degranulation *in vitro* and PGD_2 is mast cell and not basophil derived, this has led to the hypothesis that the basophil and not the mast cell is important in the late nasal obstructive response. By analogy with the relationship between the late phase airway response to allergen and clinical asthma, it has been suggested that the basophil (which also possesses high affinity, high density IgE receptors) is of greater relevance to perennial allergic rhinitis than the mast cell.

Direct approach

Direct evidence for mast cell degranulation following allergen nasal challenge has been investigated in nasal biopsy specimens. During the immediate nasal response substantial mast cell degranulation has been identified. During the late nasal obstructive response an increase in secretory eosinophils, neutrophils and basophils but not mast cells is described.

Direct evidence for mast cell involvement has also been investigated in the clinical context. During natural seasonal exposure in grass pollen or birch pollen sensitive subjects there is an increase in metachromatic staining cells in the nasal mucosa. Sequential biopsies show a migration of mast cells from the lamina propria to the mucosal surface and a total increase in mast cell numbers. These newer mucosal metachromatic cells do not exhibit the classical staining properties of mast cells and may have differing morphological characteristics. These have been referred to as basophil or "basophiloid-like" cells. Similar metachromatic cells have been described in nasal smears, epithelial scrapings and mucosal sections in allergic nasal conditions, their number being in direct relationship with the clinical disease activity. This differentiation between basophils and mast cells may be a reflection of cellular heterogeneity, in response to local stimuli rather than representing separate cell populations of distinctly different lineage. It is now realised that mast cells are not a homogeneous population.

Mast Cell Heterogeneity

The concept of mast cell heterogeneity was first described from rodent cells. In these animals there are two major mast cell populations based on cell size, number of secretory granules, histamine content, secretory characteristics and histochemical fixation and staining

properties. Of these the connective tissue mast cell is larger and contains more histamine than the mucosal mast cell. It is the latter which has been shown to proliferate during parasitic infestation or following allergen sensitisation under the regulation of cytokines, including interleukin-3, secreted from activated T-lymphocytes.

It is therefore tempting to extrapolate these findings to the nose and liken the "basophiloid-like" cells, which increase on mucosal surfaces in response to allergen exposure, to the rodent mucosal mast cell, the standard staining technique being based on the connective tissue mast cell. In support of this is the identification of a proliferation of formaldehyde-sensitive mast cells throughout the pollen season in patients with allergic rhinitis and the inhibition of their appearance by regular corticosteroid therapy. The availability of monoclonal and polyclonal antibodies to the mast cell proteases tryptase and chymase should now permit a more definitive appraisal of nasal mast cell heterogeneity. It has been shown in human lung and intestinal tissue that the proportion of tryptase and chymase within cells and the dependence of these cells upon T-derived maturation factors varies depending upon their location in relationship to the mucosal surface.

Involvement of Other Cell Types

It is apparent from the foregoing discussion that cells other than mast cells participate in the pathogenesis of rhinitis. Their exact involvement and their relationship to the mast cell has yet to be fully unravelled. The role of the basophil has already been discussed, in relationship to the late nasal response and to clinical disease. Considerable interest also centres on the eosinophil, a cell known to possess IgE receptors and to be activated on immunological challenge. This cell is identified in nasal smears from patients with non-infective rhinitis, not all of whom, apparently, have an allergic basis for their disease. Its granules contain eosinophil peroxidase (EPO) and major basic protein (MBP) which are both capable of degranulating mast cells. MBP has in addition a potential for disrupting cilial and epithelial function, an effect apparent on ciliated human nasal epithelial cells *in vitro* but not apparent in the nose in seasonal allergic rhinitis. The end organ effect of eosinophil derived newly generated mediators is uncertain, as both LTD_4 and PAF are produced, each having opposing actions. LTD_4 causes nasal blockage while PAF reduces nasal resistance by decreasing mucosal blood flow and constricting venous capacitance vessels. Other cells identified within nasal tissue following allergen exposure are monocytes and lymphocytes. Both these cell types possess low affinity IgE receptors and are likely to be involved in immune responses. Indeed it is possible that interleukin-5 produced by lymphocytes is one of the major chemotactic stimuli for eosinophil accumulation. The interrelationship between these different cell lines is thus complex, a situation not clarified by the realisation that non-immunological mechanisms may also lead to mast cell activation with mediator release.

Non-Immunological Mast Cell Secretagogues

Non-immunological stimuli inducing mast cell degranulation, in addition to eosinophil derived produces, include the complement components C3a and C5a, bacterial cell wall lectins, the neuropeptide substance P, the presence of a hyperosmolar environment and histamine releasing factors (HRF). The mechanism of mediator release with these agents is distinct from IgE orchestrated release. It is relevant to note, however, that interleukin-3 (IL-3) and granulocyte colony stimulating factor (G-CSF), both HRF in their own right, potentiate

IgE-mediated histamine release when present in concentrations too low to promote secretion themselves. Substance P-containing nerves are present in the nasal mucosa in a perivascular, periglandular and epithelial distribution. Nasal insufflation with substance P in man causes nasal blockage, facial flushing and tachycardia, identifying a systemic as well as a local effect. This could be due to a direct effect of substance P on vessels but may alternatively be related to substance P-induced histamine release, which occurs in the absence of the generation of PGD_2 or LTC_4.

Therapeutic Implications

Although an allergic basis for rhinitis in atopic individuals is undisputed, avoidance of relevant allergens such as grass pollen or birch pollen in seasonal allergic disease is not practical without major modifications to lifestyle. A reduction in house dust exposure, however, through standard mite control procedures coupled with the use of acaricidals can significantly reduce house dust mite colonies with symptomatic benefit. Simple mite control measures in mite sensitive rhinitis (as detailed in Chapter 8) should therefore be standard before considering therapeutic intervention. Similarly a search for other relevant allergens, such as household pets, may be pertinent in some individuals.

Allergy testing

The relevance of an environmental allergen to rhinitis is often apparent from the history of either symptoms on specific exposure or with a characteristic seasonal periodicity. This can be confirmed by testing for the presence of IgE either bound to cutaneous mast cells (skin tests), or free in the circulation (RAST). Due to their simplicity, rapidity of performance, low cost and high sensitivity, skin tests remain a corner stone of allergy testing. They can be done either by intradermal administration of allergen or by epidermal prick or scratch testing. Of these, sin-prick testing (SPT) is most commonly employed as it is easy to perform, is virtually free from adverse effects, has a clearly discernible negative and positive response, is repeatable (especially with the recently introduced Morrow-Brown, stallerpointe or allergy-prick lancets which standardise the depth of penetration) and is acceptable in children. Intradermal testing (IDR) is disadvantageous in comparison with SPT as it (1) has a higher false positive response, (2) is painful and (3) may be associated on occasions with systemic reactions. If testing extracts of low allergenicity, however, IDR testing may offer advantages over standard SPT as it is a more sensitive method. Scratch testing has now been largely discontinued as it introduces a variable quantity of allergen.

The need for an alternative method other than SPT is due to the limitations of this method when testing patients with dermatographism or with extensive atopic dermatitis. The responses are unreliable in these conditions and the responses are also reduced in patients receiving H_1-antihistamines. In these circumstances measurement of specific circulating IgE by the radioallergosorbent test (RAST) offers an alternative diagnostic method. RAST correlates closely with SPT. It is an immunometric "sandwich"-type immunoassay in which excess amounts of allergen are attached to a solid phase. After the patient's serum is incubated with a solid phase allergen, the amount of allergen-specific IgE in the serum is quantified by incubation with radiolabelled anti-IgE. More recently, the radioactive labels used in earlier RAST assays have been replaced with enzyme labels that generate colour or fluorescence. A

number of systems now offer a panel of different allergens that can be tested from one serum sample. These systems are, however, expensive compared with skin-prick testing.

Pharmacotherapy

With respect to pharmacotherapy, H_1-antihistamines and corticosteroids still remain the main therapeutic agents for allergic rhinitis. Histamine, whether mast cell or basophil derived, whether immunologically released or released as a consequence of mast cell activation by neuropeptides, eosinophil products, cell derived histamine releasing factors or local osmotic changes will have the same symptomatic effects, producing itching, sneezing and rhinorrhoea. Treatment with H_1-antihistamines reduces all these symptoms in both seasonal and perennial allergic rhinitis. Symptoms are not, however, abolished and there is little effect on nasal blockage with this form of therapy. Despite this, H_1-antihistamines remain the most appropriate therapy for patients with transient and intermittent symptoms, in whom the immediate mast cell response is of prime importance. Additional or alternative treatment may be required in patients with more persistent symptoms.

As mediators other than histamine are important in allergic rhinitis and it is not possible to antagonise all their end organ effects, treatment has been directed at inhibiting cell activation to prevent mediator release. In respect to mast cells, both beta-agonists and cromoglycate-like drugs possess this action. Beta-agonists, by raising intracellular cyclic AMP, inhibit immunologically stimulated mast cell mediator release, and are several thousand times more potent in this respect than cromoglycate, according to one *in vitro* study employing human lung mast cells. Although intranasal administration of fenoterol, a $beta_2$-selective agonist, reduces the immediate laboratory response to allergen challenge, it has proved disappointing in clinical practice. This may be due to a vasodilator effect limiting its efficacy by increasing nasal resistance, or to pharmacological heterogeneity of the mucosal metachromatic cell. While beta-agonists are effective on mast cells they have little effect on immunologically-mediated basophil degranulation.

Cromoglycate-like drugs are thought to inhibit mast cell degranulation through actions on either protein kinase C or through phosphorylation of a 78kD protein, both effects being associated with an interruption of the intracellular activation process. Like beta-agonists, these drugs overall have proved disappointing, tending to be more effective in limiting sneezing and runny nose than nasal blockage. The relevance of the discovery that cromoglycate has a differential effect on newly generated mediator release as compared to preformed mediator release from stimulated mast cells is difficult to put into this perspective. One report demonstrates an 85% reduction in PGD_2 release but only a 25% reduction in histamine release following immunological mast cell stimulation in the presence of cromoglycate. A further possible limiting factor with cromoglycate therapy is the lack of effect of this drug on basophil degranulation. The newer cromoglycate-like drug, nedocromil, is more potent than cromoglycate in inhibiting immunological mast cell degranulation and has additional effects on eosinophil and neutrophil activation. Preliminary results with this agent suggest beneficial effects in the treatment of seasonal allergic rhinitis, although again with less of an effect on nasal blockage than on sneezing and rhinorrhoea.

Corticosteroids remain the most effective treatment for allergic rhinitis, reducing all symptoms including nasal blockage. They are known to have several modes of action of

possible relevance: they influence eosinophil function, reducing both cell activation, through inhibiting production of lymphokines and monokines involved in the activation process, and total circulating eosinophil numbers. The eosinopenia following systemic corticosteroid administration may be related both to the cell margination on the intravascular endothelial surface limiting their chemotaxis, and to suppression of bone marrow production. Intranasal corticosteroid therapy inhibits the eosinophil accumulation in the nasal secretions in the late nasal response and in clinical practice reduces nasal eosinophil accumulation. Corticosteroids are also known to stimulate the production of an intracellular protein, lipomodulin, which inhibits phospholipase A_2, an enzyme involved in arachidonic acid cleavage. Support for the phospholipase C/diglyceride lipase pathway for arachidonic acid cleavage in mast cell activation, is the failure of corticosteroids to have any influence on mast cell activation *in vitro.* Consistent with this is the failure of short-term corticosteroids to inhibit the immediate nasal response to allergen challenge. Corticosteroids do, however, inhibit the release of histamine, kinins, and TAME-esterase, and the accumulation of eosinophils within the nasal mucosa during the late nasal response to allergen challenge and are effective inhibitors of immunologically related basophil degranulation. Of further interest is the discovery that long therapy inhibits the early response to allergen challenge within the nose. A clue to the mechanism of this action, and highlighting the importance of the mode of therapy, is the finding that inhaled corticosteroids inhibit the seasonally related migration of mast cells into the nasal mucosa, thereby limiting any allergen/antibody interaction relevant to the immediate response. This effect may be related to inhibition of lymphokine production by T-lymphocytes.

Other potential modes of therapy currently under clinical evaluation are the dipeptide, N-acetyl-aspartyl-glutamic acid (magnesium sulphate) (NAAGA) and the pentapeptide (HEPP), a synthetic peptide derived from the Fc region of human IgE. NAAGA is a naturally occurring brain dipeptide with a high affinity for glutamate receptors. It has been shown experimentally to (1) prevent anaphylactic and non-anaphylactic mast cell degranulation, (2) to inhibit both the cytolytic effect of the activated complement and the generation of anaphylatoxins, and (3) to attenuate allergen-induced nasal blockage in the laboratory. HEPP has been more extensively investigated, inhibiting both experimentally-induced and naturally occurring sneezing, rhinorrhoea and nasal blockage in allergic rhinitis when administered either subcutaneously or intranasally. The mechanism of action of HEPP is still under evaluation as it does not act by competing for native IgE on Fc receptors on mast cells and basophils as originally proposed.

Immunotherapy

Immunotherapy involves administering increasing concentrations of allergen extracts, to which a patient is sensitive, in an attempt to ameliorate the associated symptomatology through modulation of the immune response. This is usually administered subcutaneously, but oral and nasal routes of administration have been explored. Its introduction in 1911 was based on the assumption that it would work as for immunisation, through the stimulation of protective antibodies, before the relevance of IgE to symptom generation was appreciated. Despite its widespread use from this date, it was not until 1954 that a placebo-controlled study identified efficacy in the treatment of allergic rhinitis. The mode of action of immunotherapy was initially considered to be due to the production of a "blocking antibody" of the IgG subclass, but this is now not considered a major mechanism of action in inhalant

allergy. More recently immunotherapy has been found to reduce the mediator secretion during the immediate nasal response to allergen challenge *in vivo*, this effect correlating with individual efficacy of treatment. *In vitro* studies have identified a decreased sensitivity of basophils to allergen stimulation after immunotherapy, an effect possibly due to cytokines as during pollen immunotherapy mononuclear cells synthesise less histamine releasing factor. Immunotherapy (like corticosteroids) has also been reported to reduce the number of metachromatic staining cells (mast cells and basophils) and eosinophils in the nasal mucosa in allergic rhinitis, possibly through regulation of T-lymphocyte activity.

Conclusion

Despite the increase in information concerning the immunology of rhinitis, the majority of this has centered on the immediate response and the mast cell. The relevance of this cell to the persistence of symptoms and its interaction with other cell types, now realised to be of relevance to symptom generation, has yet to be clarified. Further research directed towards an understanding of factors regulating local cell accumulation, cell maturation and cell activation within the nose is required to clarify these points. In addition, an understanding of mast cell maturation during migration and the influence on this process of pharmacological and immunotherapeutic agents is required to define therapeutic approaches and to determine if the circulating basophil serves as a better model for therapeutic effects within the nose than the present *in vitro* animal or human mast cell systems. In the current state of knowledge topical corticosteroid therapy which inhibits both mast cell and eosinophil accumulation and eosinophil and basophil activation within the nose is the most consistently effective treatment.