INTRACELLULAR EVENTS IN ALLERGIC BRONCHIAL ASTHMA

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Current evidence suggests that in allergic asthma, mast cells, and possibly other mediator cells, are triggered by specific immunological stimuli to produce mediators which have both direct and indirect actions on bronchial tissue (1). The direct acting mediators such as histamine lead to a rapid spasmogenic response of the airways. This acute bronchoconstriction may be followed by a late and sustained asthmatic response which probably depends on the recruitment of inflammatory cells (eosinophils, neutrophils, mononuclear leukocytes) by mast cell-derived chemotactic factors and subsequent activation of these cells (1,2). The late asthmatic response is generally associated with an increase of the bronchial hyperreactivity which may lead to a vicious circle of exacerbation and the progressive nature of allergic bronchial asthma (3).

Many of the pathogenetic processes involved in allergic asthma, including mediator release from mast cells, contraction of airway smooth muscle, and infiltration and activation of inflammatory cells, are Ca^{2+} -dependent processes and are regulated by the concentration of free calcium ions in the cytoplasm of the cells ($(\text{Ca}^{2+})_c$) (4,5). The $(\text{Ca}^{2+})_c$ is under important control of two opposing receptor-mediated signal pathways:

1. a stimulatory pathway, employing Ca^{2+} -mobilizing receptors such as IgE-receptors on mast cells, histamine, prostaglandin, leukotriene, or muscarinic receptors on bronchial smooth muscle, and chemotactic (e.g. LTB_4) receptors on inflammatory leukocytes;
2. an inhibitory pathway, employing adenylate cyclase activating receptors (mainly β_2 -adrenergic receptors on all cell types) to produce the second messenger cAMP which ultimately leads to a decrease of free intracellular Ca^{2+} ions.

Antigen-, mediator-, or hormone-induced activation of Ca^{2+} mobilizing receptors is generally accompanied by two events:

- a. passive influx of extracellular Ca^{2+} through transmembrane Ca^{2+} channels down to a steep, 10,000-fold concentration gradient. The transduction mechanism of Ca^{2+} gating is presently unknown, but may, at least in mast cells, involve methylation of plasma membrane-bound phosphatidylethanolamine to phosphatidylcholine by stimulation of methyltransferases (6);
- b. stimulation of the phosphatidylinositol (PI) turnover (4,5,7). The initial event in PI-turnover is a guanine nucleotide-dependent activation of the enzyme phospholipase C with subsequent breakdown of the plasma membrane-bound phospholipid, phosphatidylinositol 4,5-bisphosphate. This leads to generation of two intracellular messengers: inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG). IP_3 releases intracellularly stored calcium from endoplasmic reticulum, thereby also raising $(\text{Ca}^{2+})_c$. DG activates the Ca^{2+} - and phospholipid-dependent protein kinase C (pkC).

The enhanced $(\text{Ca}^{2+})_c$ and activated pkC display a concerted action in the further stimulation of the cells by phosphorylation of various cellular proteins. Free calcium ions bind to the Ca^{2+} binding protein, calmodulin, which subsequently activates the enzyme myosin light chain kinase (MLCK). This results in

phosphorylation of myosin light chains which ultimately permits the interaction between myosin and actin filaments. Actin-myosin interaction causes contraction of smooth muscle and is probably involved in the degranulation of mast cells and inflammatory leukocytes. DG-induced activation of pkC is another pathway of cellular protein phosphorylation which contributes to the same physiological responses (7).

In mast cells and inflammatory leukocytes, Ca^{2+} mobilization has also shown to activate the formation and release of membrane-derived mediators such as prostaglandins, thromboxane, leukotrienes, and platelet activating factor (8-10).

Contraction of smooth muscle and activation of mast cells and leukocytes are inhibited by receptor-mediated stimulation of the plasma membrane-bound enzyme adenylate cyclase. The stimulation of adenylate cyclase involves a guanine nucleotide-dependent regulatory coupling protein (N-protein), which transduces the receptor stimulus to the catalytic unit of adenylate cyclase. This catalytic unit converts ATP to 3',5'-cyclic adenosine monophosphate (cAMP), which acts as an intracellular messenger to activate a third pathway of cellular protein phosphorylation: the cAMP-dependent protein kinase (pkA) pathway. In airways smooth muscle, pkA activation leads to augmented sequestration of Ca^{2+} by the endoplasmic reticulum and enhanced Ca^{2+} -efflux from the cell by activation of Ca-Mg ATPase and Na-K ATPase, respectively (11). In addition, pkA activation exerts an inhibitory effect on MLCK and PI-turnover (11). Similar mechanisms are also likely to occur in mast cells and leukocytes (8).

Among several other mechanisms, a systematically reduced functioning of the β_2 -receptor adenylate cyclase system has been proposed to be involved in the pathogenesis of airway hyperreactivity (12,13). Using peripheral lymphocytes as a model, we found evidence that a decreased beta-adrenergic responsiveness may indeed contribute to enhanced cellular reactivity in the airways of patients with allergic asthma, however, only after immunological triggering of the allergic response (13-16). The origin of the reduced responsiveness is localized in the N-protein rather than in the receptor itself, thereby causing a generalized dysfunctioning of the adenylate cyclase system under this condition (15,16). Recent investigations at our laboratory have indicated that the observed alteration in adenylate cyclase activity may be caused by activation of pkC (17), thus possibly linking the stimulatory pathway of the allergic response to the development of allergen-induced bronchial hyperreactivity.

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PRODUCTION OF, AND INTERACTIONS BETWEEN, IMMUNOGLOBULINS

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Immunoglobulins are the product of plasma cells. Plasma cells are derived from lymphocytes by a complex differentiation pathway that involves both antigen-independent and antigen-dependent stages. All the early differentiation steps are antigen-independent; this is understandable because the cells do not yet have the capability to recognize the antigen until a number of differentiation steps have taken place. Some of these differentiation steps require DNA rearrangements, notably the joining of a V- (= variable region) gene to a D- (= diversity region) gene and subsequently the joining of the VD gene to a J (= joining region) gene and thus to the C- (= constant region) gene. This DNA rearrangement generates a gene for a heavy chain of an IgM molecule. After another set of DNA rearrangements on a different chromosome an immunoglobulin light chain gene is generated. After this event the cell starts to synthesize surface-bound immunoglobulin, predominantly of the IgM type. This surface-bound immunoglobulin enables the cell to recognize a fitting antigen, so from now on the cell is immuno-competent. Interaction with such an antigen under suitable conditions results in cell proliferation and differentiation. During the cell divisions a further DNA rearrangement may take place: the isotype switch, or class-switch. In this DNA rearrangement the VDJ-gene on the heavy-chain chromosome is linked to another C-region gene, e.g. the IgG₁ or the IgE-C region gene.

The differentiation leads to plasma cells committed to the production of one single type of immunoglobulin and to the production of secondary (memory) B cells which may respond upon a subsequent antigenic stimulation.

Factors governing the isotype regulation are largely unknown. Some regulation is evident in the isotype switch event; further isotype specific regulation occurs at the level of the isotype-committed B-cell.

If we take the regulation of the IgE production as an example of isotype-specific regulation, it is clear that certain parasitic infections stimulate the production of IgE B-cells, presumably by stimulating isotype-switching to IgE; a further stimulus for the enhanced production of IgE are IgE-binding factors, produced by certain activated T-cells, which acts by stimulating IgE-bearing B-cells to differentiate into IgE-producing plasma cells.

In the response to environmental non-microbial glycoprotein antigens, three immunoglobulin isotypes are prominent: IgG₁, IgG₄ and IgE. IgE is prominent only in allergic individuals; IgG₄ is prominent only after prolonged antigen exposure. The role of antibodies of the IgG₄ isotype is still controversial; presumably IgG₄ antibodies protect against the potential harmful effects of IgE- and IgG₁ antibodies by preventing mast cell degranulation and complement activation.

GENETIC DISEASE: PATIENTS; MOLECULES AND PREVENTION H. Galjaard

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Congenital disorders become the major cause of infant mortality and morbidity in an increasing number of countries. The possibilities of primary prevention are very limited and the main emphasis has therefore been on newborn screening and early treatment, and on the early identification of couples at risk, genetic counseling and prenatal diagnosis followed by selective abortion. Enzyme replacement therapy has been attempted in patients with various types of lysosomal storage diseases and "gene therapy" is foreseen in patients with genetic disorders of the haemopoietic system. It seems, however, that in this area more basic research is needed about the exact nature of the molecular defect involved, the routing of gene products within the body, the efficiency of their uptake by various types of mutant cells and the corrective effect on cell metabolism of exogenous (enzyme) protein. Some of these aspects will be illustrated by experimental work on the genetic, molecular and cellular background of a number of lysosomal storage diseases.

At present the most effective approach towards prevention of handicaps is the early diagnosis of index patients with a chromosomal aberration, a genetic disease or other types of congenital anomalies followed by genetic counseling of relatives at increased risk. Various follow-up studies indicate that a high proportion of couples who are informed about an increased risk of affected offspring are deterred from a (next) pregnancy. For those who still want to reproduce, there is the option of chorion analysis, amniotic fluid (cell) analysis or ultrasonography in each pregnancy. The scope of prenatal diagnosis will be further widened by the possibilities of demonstrating gene mutations or polymorphisms closely linked to disease using recombinant DNA technology. Also for genetic diseases with a relatively high incidence, carrier screening programmes will enable couples to prevent the birth of a first affected baby in their family. In the future, the technology will also allow the prediction of diseases or of increased susceptibility to environmental factors. Such developments require timely attention to psychological, social and educational aspects. Such factors also play a major role in the participation of presently available technology as will be illustrated by