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Implications of cytokine genes in allergic asthma

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Abstract Asthma is a complex disease involving numerous mediator molecules and effector cells, in combination with a range of environmental determining factors. Cytokines play a key role in the physiopathological mechanisms of asthma; the study of the structure, regulation and variations of the genes that encode for these molecules is therefore crucial. Cytokines have extremely diverse roles, and exert effects both as activators and inhibitors of the innate and adaptive immune response. Certain modifications in the expression or structure of these molecules, resulting from the presence of polymorphisms, may give rise to deregulation of the mentioned effects, and therefore to a predisposition to develop concrete asthma phenotypes.

Introduction

Asthma is characterised by inflammatory alterations of the airway, with the participation of numerous mediators that produce characteristic physiopathological changes. Cytokines play a key role in this context. These proteins are essential elements in the regulation of intercellular signalling, and are produced by different types of cells implicated in the host immune response. Cytokines control a range of physiological functions, such as cell differentiation and maturation, inflammation, local and systemic immune response, and tissue repair, among others. They comprise a genuine signalling system that could be compared to a neuronal network, with multiple interactions among the different molecules, pleiotropy and redundancy (Fig. 1). The present review describes different cytokines implicated in asthma and for which genetic studies have been made.

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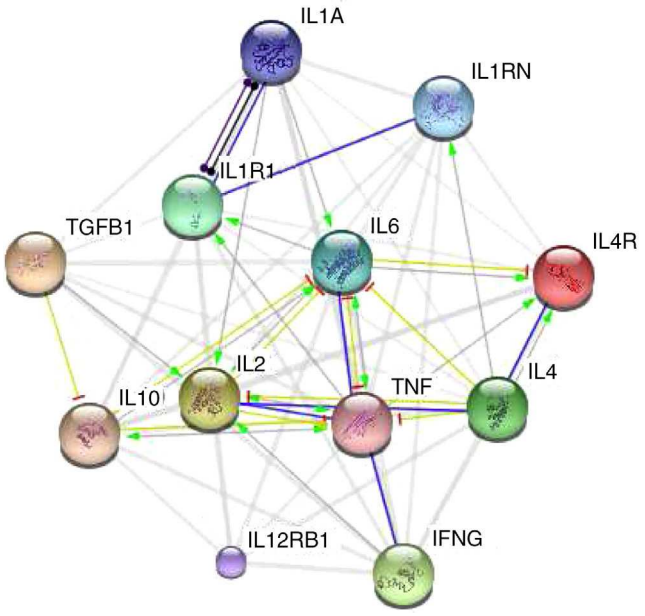

Figure 1 Schematic representation of the connections among the different cytokines implicated in asthma.

IL-1, IL-1RA and IL-1R1

Interleukin 1 (IL-1) is a pro-inflammatory cytokine with two forms, IL-1α and IL-1β, which have practically indistinguishable biological activities, but are encoded by different genes. These genes are located in chromosome 2q, forming a complex with other genes of the IL-1 family. Both genes, *IL1A* and *IL1B*, present six exons of similar structure, which suggests a genic duplication event.

IL-1α and IL-1β are synthesised as propeptides exhibiting 20% homology. They play a very important role in antigen presentation, contributing to T cell activation and proliferation, with an increase in the production of T lymphocyte-derived molecules such as interleukin 2 (IL-2) and the IL-2 receptors. They also increase the proliferation of B lymphocytes and immunoglobulin synthesis.

IL-1 stimulates leucocyte adherence to the endothelium, mediated by an increase in the expression of endothelial adhesion molecules (ICAM-1 and VCAM-1) and of selectin E. IL-1 induces vasodilatation, contributing to hypotension in septic shock. Many of the pro-inflammatory activities of IL-1 are related to its capacity to induce cyclooxygenase-2, with an increase in eicosanoid products, including prostaglandin E2 (PGE2) and leukotriene B4 (LTB4). Moreover, IL-1 induces the synthesis of tumour necrosis factor (TNF), interleukin 6 (IL-6) or granulocyte-macrophage colony stimulating factor (GM-CSF), with an increase in synthesis via a positive feedback mechanism. In the same way as TNF, IL-1 exerts cytotoxic effects upon cancer cells and virally infected cells. TNF and IL-1 share many biological activities – the main difference being that TNF does not exert a direct effect upon lymphocyte proliferation.

The IL-1 receptor antagonist (IL-1RA) is a protein of the IL-1 family that inhibits the activity of IL-1α and IL-1β, thereby modulating the immune responses mediated by these interleukins. The encoding gene is located in the same chromosome 2, adjacent to the genes that encode for IL-1α and IL-1β. It contains eight exons with a size of 22.9 kb and has four different isoforms, according to the processing undergone by the mRNA. The protein is secreted into the extracellular medium by monocytes, macrophages, neutrophils and fibroblasts in response to inflammatory stimuli. Certain polymorphisms of the *IL1RA* gene have been associated with the severity of asthma in both children and adults.[1]

The IL-1 type I receptor (IL-1RI) acts as a receptor for IL-1A, IL-1B and IL-1RA. It is involved in most of the immune and inflammatory responses mediated by these cytokines. The gene encoding for IL-1R is located on the long arm of chromosome 2 and has a size of 26 kb, comprising 12 exons that encode for up to 16 different mRNA molecules. The active IL-1R protein presents an extracellular region with three immunoglobulin-type domains that bind to IL-1, a transmembrane region, and a cytoplasmic domain homologous to toll-like receptors. The activation of IL-1R releases many pro-inflammatory cytokines such as TNFα, IL-2 and IL-1 (Fig. 1). Studies describing associations between allelic variants of this gene and different diseases are few in number. Some authors have attempted to relate the *IL1R* polymorphism rs2234650 to asthma, although no significant association has been found.

IL-2

The main function of IL-2 is to stimulate the growth and proliferation of T lymphocytes and other immune cells, and to induce the synthesis of other cytokines and pro-inflammatory molecules. It is encoded by a gene located in chromosome region 4q27. The gene has four exons that encode two known transcripts; no protein product has been detected in relation to one of them. IL-2 is a globular protein containing four alpha helixes. It is secreted into the extracellular medium, where it carries out its functions by binding to the corresponding receptor (IL-2R) – a heterotrimeric protein with a gamma region common to other interleukin receptors such as IL-4R or IL-7R. IL-2 expression occurs during the immune response, stimulating the growth, differentiation and survival of cytotoxic T lymphocytes. In addition, it acts as a differentiating factor in NK lymphocytes and stimulates the production of immunoglobulins. IL-2 also intervenes in T cell development in the thymus gland, playing a key role in the maturation of regulatory T lymphocytes.

Two polymorphisms have been described in the *IL2* gene. One of them is located in the promoter, in position −330 (rs2069762). It has been suggested that the presence of this polymorphism in homozygosis induces an increase in *IL-2* expression.[2] There is evidence of an association to the presence of asthma, allergy and atopic dermatitis.[3] The other described polymorphism is located in the coding region, in position +166 (rs2069763), and provokes a synonymous mutation.

IL-4

Along with interleukin 13 (IL-13), interleukin 4 (IL-4) is one of the main cytokines responsible for IgE response, and

moreover induces Th2 responses. It is encoded by a gene located in chromosome region 5q31.1, where other genes encoding for key cytokines in the development of asthma and atopy are also located – such as the genes that encode for IL-3, IL-5, IL-9, IL-12B, IL-13 or GM-CSF. The gene encoding IL-4 is about 9 kb in size and includes four exons and three introns. It can undergo alternative processing, giving rise to a protein lacking residues 46–61. The protein is mainly synthesised by CD4+ T lymphocytes, eosinophils and mast cells present in the airway of allergic individuals. It plays a crucial role in the development of Th2 cells. On one hand, IL-4 inhibits expression of the β2 subunit of the IL-12 receptor in T cells, thereby giving rise to inhibition of the production of Th1 cells, while on the other hand it induces the production and secretion of Th2 type cytokines and of NO, and the secretion of other pro-allergic cytokines. IL-4 is crucial in B cell isotype switching to IgE production. Once the change in isotype has taken place, IL-4 enhances IgE production and induces over-expression of IgE receptors in the mast cells of the airway. This in turn results in increased eosinophil recruitment. As has been commented above, *IL4* is one of the most widely studied genes related to asthma and atopic disease.[4] There is evidence that polymorphisms −33C>T (rs2070874) and −590 C>T (rs2243250) are associated to total IgE levels, asthma and other allergic phenotypes in different populations.[5] Polymorphism −1098 T>G (rs2243248) has been associated to chronic obstructive pulmonary disease, which has also been associated to a haplotype formed by the three polymorphisms of this gene.[6]

IL-4 exerts its effect by binding to the corresponding receptor (IL-4R), located among others in the cell membrane of activated T lymphocytes. It consists of two subunits: the alpha chain (IL-4Rα) and the gamma chain. The receptors for IL-4 and IL-13 share the same alpha chain, which explains the similarity of their biological functions. The *IL4RA* gene is located in chromosome region 16p12, associated to asthma in certain populations. It is 50 kb in size and contains 12 exons. A number of polymorphisms have been described in the coding region of the *IL4RA* gene, many of which produce amino acid substitutions. A polymorphism located in position 1092 produces a change in amino acid 576 from glutamine to arginine (Q576R) (rs1801275). This change has been associated with an increased response to IL-4 in atopic patients, as well as to the presence of atopic asthma in Caucasians.[7]

IL-6

Interleukin 6 (IL-6) is an important acute phase response and chronic inflammation mediator, with established pro- and anti-inflammatory effects. The gene encoding IL-6 is located on the short arm of chromosome 7 (7p21), and is about 6 kb in size. It has five exons and four introns. IL-6 is a protein that exerts its functions in the form of a homodimer in which each subunit forms a globular domain comprising four alpha-helixes. It is mainly synthesised by macrophages, but is also produced by B and T lymphocytes, fibroblasts, endothelial cells, keratinocytes, hepatocytes, glial cells and other cells of the bone marrow. Its synthesis is stimulated by IL-1 and IL-2, TNF and IFN, but is inhibited by pro-Th2 cytokines such as IL-4 and IL-13 (Fig. 1). In adaptive immunity, IL-6 stimulates B lymphocyte growth and maturation. It also mediates in the activation, growth and differentiation of T lymphocytes. On the other hand, IL-6 possesses antiviral activity, since it shares with interferon (IFN) the capacity to induce expression of the type I major histocompatibility complex. In contraposition to these described pro-inflammatory effects, IL-6 also acts as a mediator in several anti-inflammatory actions: it is able to arrest the inflammatory cascade through inhibition of the synthesis of IL-1 and TNF, as well as its own synthesis. Furthermore, it stimulates the synthesis of IL-1 receptor antagonist (IL-1RA).

A number of studies have suggested that certain polymorphisms present in the promoter region of the *IL6* gene influence the level of expression of the cytokine. In particular, the polymorphism in position −174G>C (rs1800795) has been associated to the presence of childhood asthma.[8]

IL-10

Interleukin 10 (IL-10), also known as cytokine synthesis inhibiting factor (CSIF), is an anti-inflammatory cytokine produced mainly by monocytes. The *IL10* gene, located in chromosome 1q31–q32, is about 4.89 kb in size and has five exons. It encodes a 20.6 kDa homodimer with 178 amino acids, synthesised by a range of cell types (T and B lymphocytes and mast cells, but mainly monocytes). IL-10 plays a very important role in immune regulation and inflammation. It reduces the expression of other cytokines such as IL-1β, IL-6, IL-8, IL-12, TNF-α and interferon-gamma (IFN-γ), and inhibits the expression of type II histocompatibility molecules, as well as of other molecules implicated in the immune response in macrophage cells. These anti-inflammatory properties are in contrast to its effects upon B lymphocytes, where IL-10 acts as a cell proliferation and immunoglobulin secretion stimulating factor. It inhibits cytokines associated to cellular immunity and allergy, but stimulates the humoral immune response. Diminished IL-10 levels have been found in macrophages and monocytes in asthmatic patients. Studies of twins have shown this variability in the levels of IL-10 production to have a genetic basis.[9] Several polymorphisms have been described in the promoter region, including two microsatellites and three polymorphisms in positions −1082 (rs1800896), −819 (rs1800871) and −592 (rs1800872). Recent association studies have reported differences in the distribution of these haplotypes between controls and asthmatic patients.[10] Such polymorphisms are also associated to certain phenotypic characteristics of asthma, such as eosinophil counts and IgE levels.

IL-12

Interleukin 12 (IL-12) is a heterodimer composed of a 35 kDa subunit (p35 or α subunit) and a 40 kDa subunit (p40 or β subunit). This cytokine acts upon the T and NK lymphocytes, and has a broad range of biological effects. In the same way as in the case of IL-2α, the gene encoding IL-12β is located in region 5q31. It is 15.6 kb in size and has eight exons, of which the first and last are non-encoding. The IL-12β subunit (p40) is a 328-amino acid protein forming a heterodimer with p35 through disulphide bonds to conform a single structure (p70), which is the active IL-12 molecule. This cytokine

is expressed by activated macrophages and acts as an essential inducer of the Th1 response by increasing the levels of IFN-γ, and thus suppressing Th2 response. IL-12 is also important in cellular immune responses against many bacterial and parasitic infections, and its deficiency can lead to serious infections, as well as to recurrent pneumonia episodes. The *IL12B* gene is a candidate gene in asthma, due to its role in modulating the Th1/Th2 response, deviating the pathway towards the generation of Th1 cells and the production of IFN-γ, while at the same time inhibiting the release of Th2 response-inducing cytokines such as IL-4. Deficient IL-12 levels can cause the immune system to develop a type Th2 response – this representing a crucial pathway in the airway inflammation phenomena seen in asthma. Moreover, the *IL12B* gene is also a good candidate in view of the genomic region in which it is located, i.e. alongside other asthma-linked genes. The polymorphism in position 1888 3′UTR (rs3212227) has been associated to childhood asthma.[11]

IFN-γ

Interferon gamma (IFN-γ) or type II interferon is a cytokine that plays a critical role in the innate and adaptive immune response to viral or intracellular bacterial infections, as well as in tumour control. It is also a key molecule in the adaptive response. The gene encoding IFN-γ is 5 kb in size and is located on the long arm of chromosome 12 (12q14). The active form of the protein has 143 amino acids and forms a homodimer. IFN-γ is fundamentally secreted by Th1 lymphocytes, cytotoxic T cells, dendritic cells and NK cells. It is responsible for transcriptional modifications of up to 30 genes – thus giving rise to a great variety of physiological and cellular responses. These effects include increased antigen presentation in macrophages; increased lysosome activity likewise in macrophages; Th2 response inhibition; increased type I histocompatibility molecule expression in normal cells; NK cell activation; the activation of antigen-presenting cells; and the promotion of Th1 lymphocyte differentiation. Recently, Hussein et al. (2009) have studied polymorphism A>T present in position 874 (rs2430561) in atopic patients, and have found these individuals to exhibit an increased presence of the mutated homozygous genotype, in addition to lower serum IFN-γ levels.[12]

TGF-β

Cytokine TGF-β1 (transforming growth factor β) represents a peptide family that regulates cell growth. It is mainly produced by chondrocytes, osteocytes, fibroblasts, platelets, monocytes and T lymphocytes. TGF-β1 is encoded by a gene located on the short arm of chromosome 19 (19q13), and is 23 kb in size (seven exons). The cytokine is synthesised as an inactive precursor composed of 390 amino acids, and requires proteolytic processing in order to become activated. The active peptide consists of 112 amino acids and forms a 25 kDa homodimer in which both monomers are joined by disulphide bonds. TGF-β1 stimulates fibrosis, the formation of extracellular matrix, and cicatrisation. In relation to immune function, this cytokine inhibits B and T lymphocytes (helper and cytotoxic cells). It inhibits immunoglobulin secretion by B lymphocytes and cytotoxicity by mononuclear phagocytes and NK cells. The production of TGF-β1 in apoptotic cells gives rise to an immune suppressed environment that explains the absence of inflammation after cell death. However, TGF-β1 acts as a chemoattractant for macrophages and induces an isotype switch towards IgA in B cells.

In allergic inflammation, eosinophils are the main source of TGF-β1, and expression of the latter is associated to bronchial epithelial remodelling characteristic of asthma. The levels of this cytokine are increased in the bronchoalveolar lavage of asthmatic patients – this increment being more pronounced after exposure to respiratory allergens. Studies have been made of the association between different polymorphisms present in the *TGFβ1* gene and asthma. One of the most extensively studied polymorphisms is located in position −590. The presence of the mutated allele has been associated to an increased predisposition to asthma related to a possible increase in the transcription levels, in both children and adults. Single nucleotide polymorphism (SNP) −590 C>T has also been associated to rhinosinusitis, increased IgE levels and bronchial hyperresponsiveness.[13] Another polymorphism that has also been associated to increased expression of the cytokine and is located in the coding region, is 869T>C, which induces a change from proline to leucine in the tenth amino acid. This polymorphism has been associated to increased asthma susceptibility in atopic patients.[14] SNP 915G>C (rs1800471), which causes a change from arginine to proline in position 25, has been associated to bronchial hyperresponsiveness and a predisposition to asthma.[13] Recently, the polymorphism in position 915 has been associated to irreversible air flow obstruction in asthmatic males.

TNF-α

Tumour necrosis factor alpha (TNF-α) is a pleiotropic cytokine mainly produced by macrophages and T lymphocytes. It was originally identified in mouse serum in studies of tumour haemorrhagic necrosis produced by endotoxin action. The *TNFA* gene is located on the short arm of chromosome 6, in region 6p21.3, where the major histocompatibility complex is also found. The gene is about 3 kb in size and has four exons. The last exon encodes approximately 89% of the soluble protein (active form). The TNF-α protein is composed of two folded, anti-parallel layers conforming a typical molecular structure also seen in viral capsid proteins. TNF-α exerts its influence as a pro-inflammatory cytokine and its action upon the T cells through signalling pathways activated by *TNF-α* binding to its receptor. In this context, TNF-R1 is expressed by most tissues, and can be activated by both the membrane-bound form and the trimeric soluble forms of TNF-α. In contrast, TNF-R2 is only found in immune system cells and responds to the membrane-bound forms of the TNF-α homotrimer. Many polymorphisms have been described in the *TNFA* gene. Polymorphism (−308) has been related to TNF-α production levels, and some studies suggest that the presence of allele A is associated to asthma and influences the expression of *TNFA*.[15]

To summarise, a description has been made of a range of genic associations of different polymorphisms in the genes

Table 1 Studies of genic associations to asthma and atopic disease for different polymorphisms of genes encoding for cytokines.

Gene	Location	SNP	Association	Reference
IL2	4q27	−330 (rs2069762)	Asthma, allergy and atopic dermatitis	Christensen et al., 2006
IL4	5q31.1	−33C>T (rs2070874)	Total IgE levels, asthma and allergic phenotypes	Michael et al., 2003
		−590 C>T (rs2243250)	Total IgE levels, asthma and allergic phenotypes	Michael et al., 2003
IL4RA	16p12	Q576R (rs1801275)	Atopic asthma	Beghé et al., 2003
IL6	7p21	−174G>C (rs1800795)	Childhood asthma	Settin et al., 2008
		−1082 (rs1800896)	Asthma	Movahedi et al., 2008
IL10	1q31-q32	−819 (rs1800871)	Asthma	Movahedi et al., 2008
		−592 (rs1800872)	Asthma	Movahedi et al., 2008
IL12	5q31	1888 3′UTR (rs3212227)	Childhood asthma	Tomomitsu et al., 2005
INFG	12q14	874 A>T (rs2430561)	Atopy	Hussein et al., 2009
		−590 C>T (rs1800469)	Asthma, rhinosinusitis,	Judith et al., 2006
TGFB	19q13		IgE levels and bronchial hyperresponsiveness	Sharma et al., 2009
		869T>C	Bronchial hyperresponsiveness, asthma	Judith et al., 2006
		915G>C (rs1800471)	Bronchial hyperresponsiveness, asthma	Sharma et al., 2009
TNFA	6q21.3	−308G>A (rs1800629)	Asthma	Padrón-Morales et al., 2013

that encode for cytokines (Table 1). Certain modifications in the expression or structure of these molecules generated by the presence of such polymorphisms may give rise to changes in the mechanisms of action of the cytokines – a situation which in turn could influence patient predisposition to allergic disease.

Conflict of interest

The authors have no conflict of interest to declare.

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