Molecular mechanisms in allergy and clinical immunology

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Genetics of allergen-induced asthma

Angelika Daser, PhD,[a] Massoud Daheshia, PhD,[b] and George T. De Sanctis, PhD[c]

Berlin, Germany, and Boston, Mass

Antigen-induced airway hyperresponsiveness and airway inflammation are features of both human asthma and animal models of this disease. The genesis of these key asthma phenotypes represents the summation of a complex cascade of immune responses. It is hypothesized that multiple cell types are involved in the induction, propagation, and maintenance of these immune processes. Several molecules have been reported to be essential for cell-cell interactions, inflammatory cell recruitment, and effector functions leading to the overall expression of the asthmatic phenotype. This review summarizes the genetic evidence supporting a role for these molecules in antigen-driven airway hyperresponsiveness and inflammation. (J Allergy Clin Immunol 2001;108:167-74.)

Key words: *Airway hyperresponsiveness, antigen, asthma, genetics, inflammation, linkage*

The genetics of asthma, as investigated in animal models, has greatly increased our understanding of the genetic and environmental factors that contribute to the overall phenotypic expression of this complex disease. Asthma is characterized by 3 key phenotypes: intermittent airway obstruction, inflammatory cell infiltrates, and airway hyperresponsiveness (AHR). Numerous models of environmentally induced airway inflammation and AHR have been investigated in the inbred mouse. In comparison with outbred species, the inbred mouse represents a powerful tool for delineating the genetics of allergen-induced AHR. Because all animals in an inbred strain are genetically uniform, phenotypic variability is controlled solely by environmental influences. Furthermore, the short gestation period, large litter sizes, availability of numerous inbred mouse strains, well-defined genetic map, and ability to control the environment in which the animals are reared establish the inbred mouse as the ideal species for investigating both simple and complex genetic traits. A comprehensive review of the genetics of asthma, as studied in the inbred mouse, is presented in this review.

Abbreviations used

AE:	Airway eosinophilia
AHR:	Airway hyperresponsiveness
APC:	Antigen-presenting cell
BAL:	Bronchoalveolar lavage
NOS2:	Nitric oxide synthase 2
OVA:	Ovalbumin
PI:	Pulmonary inflammation
QTL:	Quantitative trait locus [loci]

This section briefly describes the cascade of events that occur during the induction of the T_H2 immune response and subsequently reviews the evidence that specific chromosomal genes are linked to these asthmalike phenotypes in murine models of allergic asthma. In particular, this section presents a review of all of the loci and potential candidate genes that have been reported to contribute to several of the many phenotypes observed in these models.

MURINE MODELS OF ALLERGEN-INDUCED PULMONARY INFLAMMATION AND AHR

Antigen-induced asthma is considered an inflammatory disorder induced by an uncontrolled cascade of immune responses. Different cell types—including antigen-presenting cells (APCs) and effector cells—and their secretory products are involved. T_H2 cells are the chief orchestrators, dendritic cells and alveolar macrophages being the APCs. Mast cells have been proposed as the triggering cells and the eosinophils as the main effector cells.

The main characteristic of antigen-induced asthma is pulmonary inflammation (PI), including airway eosinophilia (AE) coupled with high serum allergen-specific IgE titers and AHR. A schematic representation of the putative mechanisms is illustrated in Fig 1 (for reviews, see Corry and Kheradmand,[1] Holt et al,[2] Wills-Karp,[3] and Moser and Murphy[4]).

As in human beings, AHR in the mouse need not be accompanied by all three of these features (AE, PI, and IgE). Depending on the genetic background and immunization protocol adopted, AHR can be induced by diverse pathways. The IL-4/mast cells and IL-5/eosinophil pathways have been proposed as possible effector mechanisms.[5] A genetic predisposition has been strongly demon-

From [a]the Institute of Laboratory Medicine and Pathobiochemistry, Charite, Berlin, and [b]the Department of Rheumatology, Immunology and Allergy and [c]the Pulmonary and Critical Care Divisions, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston.

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Reprint requests: George T. De Sanctis, PhD, FCCP, Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital and Harvard Medical School, 75 Francis St, Boston, MA 02115.

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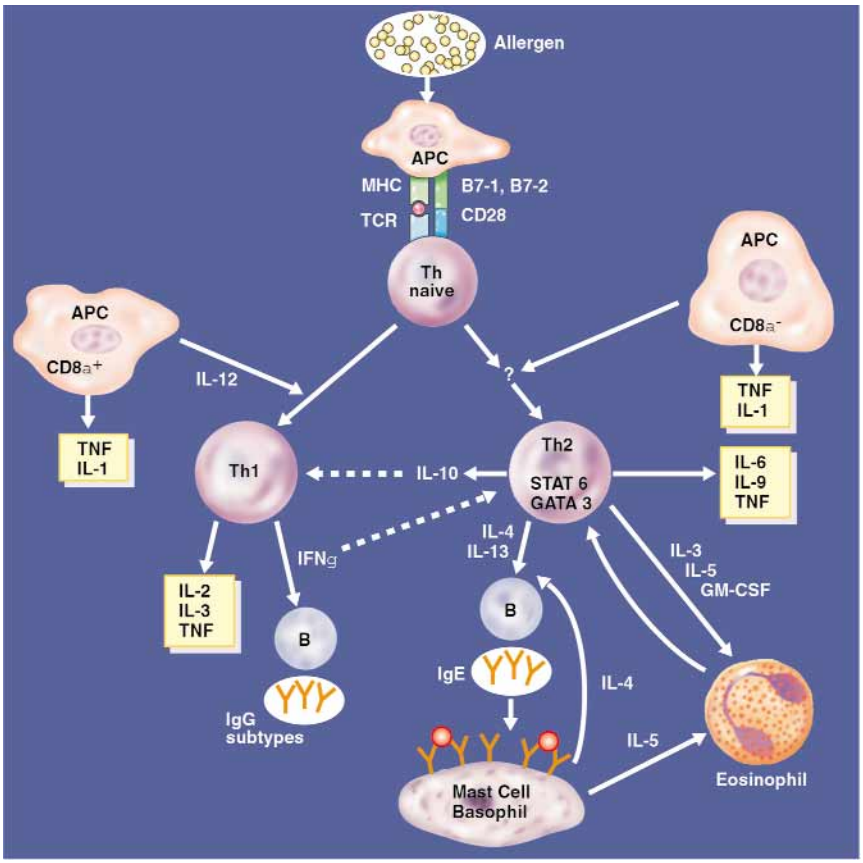


FIG 1. Antigens are taken up, processed, and presented by professional antigen-presenting cells (*APC*), such as dendritic cells, via MHC class I and class II molecules. T cells recognize the antigenic fragment in context of the MHC with their specific receptor (*TCR*). After this engagement and additional costimulatory molecule interactions such as the CD28/B7 pathway, activated T cells are generated. Depending on the cytokine milieu, the force of the T-cell/MHC interaction, and the nature of antigen-presenting cells, T_H0 cells develop into either T_H1 or T_H2 effector cells. It is postulated that the dendritic cells themselves could be polarized. The T_H1 cytokines, such as IL-12 and IFNγ, are responsible for cell-mediated immunity and immunoglobulin class–switching to IgG2a, whereas the T_H2 cytokines, such as IL-4 and IL-5, are responsible for humoral immune responses and class-switching to IgG1 and IgE. Through high-affinity IgE receptors, allergen-specific IgE binds to mast cells, and on allergen challenge there is cross-linking of IgE receptors, resulting in release of preformed bioactive molecules such as tryptase and leukotrienes. In addition, T_H2 cells can recruit and activate eosinophils at the site of allergic inflammation. The release of numerous mediators from various activated cell types leads to inflammation and AHR. (*Solid lines* indicate stimulatory pathways; *dotted lines* indicate inhibitory pathways.)

strated for allergic asthma in the mouse, as it has for intrinsic AHR, as described above. Two large genome screens have been carried out for ovalbumin (OVA)–induced AHR. The results of these studies have provided insight into the possible mechanisms responsible for the induction and maintenance of this important phenotype.

ASTHMALIKE PHENOTYPES ARE LINKED TO T_H1/T_H2-REGULATING AND -MODIFYING GENES

A genetic analysis of allergen-induced asthma was carried out in a cross of Biozzi and BALB/c mice.[6] In this study, Biozzi BP2 mice reacted to a single antigen application with high specific IgE production and development of AE, PI, and AHR.[7] The 2 phenotypes investigated in the F2 progeny were AHR and AE in histologic lung sections. Eosinophilia did not differ significantly in bronchoalveolar lavage (BAL) fluids from the parental strains. The 2 phenotypes segregated independently from each other in a linkage analysis with 180 microsatellite markers. Four chromosomal regions were linked to AHR, and 1 region was linked to AE. Table I lists the loci and potential candidate genes. As shown in this Table, numerous candidate genes were located around the chromosomal regions of interest. Many of these are involved in the induction and propagation of asthma.

IL-10, a T_H2 cytokine, is a potent suppressor of T_H1-type responses. Thus the localization of the IL-10 receptor to this region on chromosome 9 represents a reasonable candidate gene. On chromosome 10, linkage was found in a region approximately 20 centi-morgans (cM) proximal to the major T_H1 gene, IFN-γ, whose absence or downregulation might be important in asthma. Its human

TABLE I. Evidence of genetic linkage to asthmalike phenotypes in a cross of Biozzi and BALB/c mice

Chromosome	Trait	Position (cM)	Syntenic loci (human)	Candidate gene products
9*	AHR	18	11q23	IL-10R
10	AHR	44	12q22-24	IFN-γ
11	AE	7	5q23-31	c-Rel, Tpm-1, IL-4 cluster
11	AHR	52	17q12-q22	NOS2, eotaxin, C-C chemokines
17*	AHR	10	6p21	MHC, TNF, Bhr3

cM, Centi-morgans.
*These 2 chromosomal regions reach suggestive levels of linkage (lod scores, 2.5 and 2.1, respectively).

syntenic region has been reproducibly linked to AHR and total IgE in studies in different ethnic backgrounds.[8-10] Moreover, it colocalizes with the microsatellite locus Cia8, a locus that regulates susceptibility to collagen-induced arthritis.[11] However, the distance of 20 cM between the AHR-linked region and IFN-γ is very great and might contain many other potential candidate genes that could contribute to this phenotype. The quantitative trait locus (QTL) for AE on the proximal part of chromosome 11 maps in the region of the autoimmunity locus Eae6a, which regulates susceptibility to experimental autoimmune encephalomyelitis,[12] and also to a locus that regulates the T_H2 phenotype of susceptibility to Leishmania major.[13] Thus far, only 1 good candidate gene has been placed in that region. Knockout studies have demonstrated a role for cRel, a transcription factor belonging to the NFκB family. Specifically, this transcription factor has been associated with AHR, PI, eosinophilia in BAL fluid, and total serum IgE[14] (see below). A cluster of genes, including IL-3, IL-4, IL-13, IL-5, interferon regulatory factor 1, and GM-CSF, maps at approximately 20 cM from the QTL for AE. The human syntenic region is associated with susceptibility to atopy with high natural IgE, specific IgE, and AHR.[10,15-17] This has not yet been confirmed in murine models of asthma. In addition, polymorphisms in the IL-4 and IL-13 promoter regions have been identified and postulated as the explanation for variations in susceptibility to atopic asthma.[18-20] This hypothesis has been reinforced by the finding that an amino acid exchange in the coding region of IL-13[21] is closely linked to asthma[22] (for a review, see Shirakawa et al[23]). This region contains another immunoregulatory gene, Tpm1 (T cell phenotype modifier 1),[24] which is associated with IL-12 responsiveness and T_H1/T_H2 balance. The gene differentially regulates the IL-12B receptor expression in BALB/c versus B10.D2 mice.[25]

The QTL for AHR on chromosome 11 maps to a region that includes nitric oxide synthase 2 (NOS2) and eotaxin as possible candidate genes. The syntenic region of human chromosome 17 has also been linked to asthma susceptibility,[10] though this has not been confirmed in more recent studies.[17,26] NOS2 is an immunoregulatory factor in innate immunity and, less directly, in adaptive immunity.[27] Although NOS2 has been suggested to play an important role in asthma, a recent study in a mouse model of allergic asthma suggests that the inducible NOS isoform NOS2 does not play a critical role in OVA-induced inflammation or AHR. Specifically, allergen-induced AHR did not differ between wild-type and NOS2 knockout mice in an OVA-sensitization and challenge.[28]

Eotaxin, a member of the family of chemokines, is found in the region of the QTL on chromosome 11 and is responsible for recruitment of eosinophils to tissues; it is therefore another candidate molecule for AHR.[29] Chemokines are expressed by many cell types, including T cells, monocytes, eosinophils, and basophils, in response to polyclonal and antigenic stimuli as well as to cytokines. They contribute to immunologic disorders and autoimmunity. The members of 1 subfamily, the conserved C-C chemokines, are especially involved in allergic diseases (for reviews, see Wills-Karp[3] and Rossi and Zlotnik[30]). The cluster at chromosome 11 segregates with asthmalike phenotypes and is also linked to experimental autoimmune encephalomyelitis. The syntenic region on chromosome 17p12-1711.2 has been associated with atopic dermatitis, and 1 member, RANTES, has shown to be functionally polymorphic in atopic versus nonatopic individuals.[31]

Linkage to this region on chromosome 11 has been found with other experimental diseases: immediate cutaneous hypersensitivity (Daser, unpublished data; see also Daser et al[32]), autoimmune diabetes (Idd4),[33] autoimmune orchitis (Orch3),[34] and autoimmune encephalomyelitis (Eae7).[35] For Eae7, three polymorphic candidate genes have been identified within the C-C chemokine subfamily: Scya1 (TCA-3, CCR8 ligand), Scya2 (monocyte chemoattractant protein [MCP] 1), and Scya12 (MCP-5).[12]

It has been suggested that a common set of susceptibility loci regulate susceptibility to immunologic diseases in general and that disease-specific loci determine the target organ of each particular disease.[34,36] This region of chromosome 11 thus constitutes a common susceptibility locus.

Finally, there is suggestive linkage of AHR with a microsatellite-defined locus in the proximal part of chromosome 17. This is the location of H-2, the mouse MHC, together with non-MHC loci such as TNF-α. In human beings, MHC class II alleles and (independently) polymorphic TNF alleles are associated with asthma (for a review, see Cookson and Moffatt[37]). From this genome-wide screen, it appears that (1) AHR is a polygenic trait linked to chromosomal regions with important candidate genes for atopic immunodeviation, (2) syntenic regions cosegregate with atopic phenotypes in human beings, and (3) the contributing loci are either atopy-specific loci or immunoregulatory loci shared with T_H1-driven phenotypes.

TABLE II. Genome scan of ovalbumin-induced AHR conducted in the A/J and C3H/HeJ genetic background

Chromosome	Locus	Position (cM)	Synteny (human)	Candidate gene products
2	Abhr1	5	10p11-13	GATA-3
			2q12-q14	IL-1R antagonist
			9q22-q34	IL-15R_
2	Abhr2	20	9q33-q34	C5, lipocalin
			2q14-q24	Prostaglandin
				Synthase 1
7*	D7Mit21 to D7Mit56	1,5	19q13	IL-11
				GABA receptors
				Bhr4 (15 cM)

cM, Centi-morgans.
*This chromosomal region reaches suggestive linkage (lod score 1.9).

Complement factors C5 and C3a are candidate genes for AHR

In a second genome scan of OVA-induced AHR conducted in A/J × C3H backcross mice,[38] the rank order of allergen-induced AHR was found to be similar to that of native (intrinsic) AHR as reported in 2 previous studies.[39,40] Specifically, the A/J mice expressed the high-responder phenotype and the C3H/HeJ the low-responder phenotype. The phenotype segregated with significant linkage to 2 adjacent loci on chromosome 2 and a single locus on chromosome 7 with suggestive linkage. Table II lists the loci and potential candidate genes.

There are several intriguing candidates around the QTL on chromosome 2. In particular, the transcription factor GATA-3 (see below), IL-1 receptor (IL-1R) antagonist, IL-15Rα chain (Table III), and complement factor 5 are located within this region. It is important to note that all of these are different from the loci and candidate genes that were identified in the previous scan through use of Biozzi × BALB mice. This suggests that (1) strain-specific genes determine the expression of the asthmalike phenotypes and (2) different pathways might be involved in this process. Whether the previously reported QTL Bhr4, from the native AHR phenotype in a cross of A/J and C3H mice (see above), is the same locus as the one on chromosome 7 in the allergen-induced AHR in the same strain progeny has not been determined, but there is a suggestion that the intrinsic allele is a predisposing allele for the lung manifestation of atopy.

To further characterize the relevance of candidate genes encoded in the 3 QTL regions, pulmonary expression profiles were investigated with oligonucleotide microarrays.[41] In lung tissue of A/J and C3H mice, 739 of the 2718 genes are differentially expressed, and 21 of these 739 genes are differentially expressed in the high- and low-responder backcross offspring. The only gene that was both different in pulmonary gene expression and linked to a chromosomal region in the genome scan was *Abhr2*. This QTL colocalizes with complement C5 on chromosome 2. Interestingly, the A/J mice carry a 2–base pair deletion in the first exon of C5,[42] whereas the hyporesponsive C3H/HeJ strain expresses the wild-type gene.[41]

An examination of the expression array data revealed that the amount of C5 mRNA correlated with AHR; the low-responders had high C5 expression and the high-responders had low C5 expression. The F1 progeny exhibited intermediate C5 mRNA levels. In a high percentage of animals, the 2 C5 alleles segregated accordingly: backcross high-responders were homozygous for the A/J allele and low-responders were heterozygous for the A/J and C3H allele. That this pattern of inheritance was not uniformly observed in all hyporesponsive and hyperresponsive mice indicates that there are additional genes involved in the expression of antigen-induced AHR.

Segregation analysis of the alleles from the high- and low-responders suggests that expression of the wild-type allele is protective, inasmuch as the AHR low-responder C3H/HeJ possesses the highly expressed wild-type allele. Conversely, the AHR high-responder A/J possesses the low-expressed allele with the 2–base pair deletion. This deletion leads to a translation stop within the first exon and therefore to the absence of any C5 protein. It is not known whether the different amounts of C5 mRNA in high- and low-responder mice are due to differential transcription or to differential mRNA stability (or to some other secondary effect).

It is tempting to speculate that the level of C5a might be crucial in the genetics of allergen-induced AHR. Anaphylatoxin C5a is a biologically active fragment of C5 that has both proinflammatory activity and anti-inflammatory activity. It has been detected in BAL fluids from atopic patients with asthma,[43] and direct application of C5a to the airways led to AHR[44] in a rat model. On the other hand, a protective role has been reported for brain inflammation in C5a receptor–deficient mice.[45] Its protective role in AHR and brain inflammation strengthens this hypothesis. One possible mechanism for this protective effect is modulation of IL-12 production. This is supported by the following observations: (1) reduced IL-12 production of in vitro stimulated macrophages from A/J mice in comparison with C3H-derived macrophages and (2) suppression by C5aR blockade of IL-12 production by activated human monocytes. A cautionary note: treatment with C5a itself can also suppress IL-12 production.[46,47] The authors therefore proposed that C5aR signaling up to a certain level makes monocytes and macrophages become competent for IL-12 pro-

TABLE III. Proteins involved in antigen-induced AHR and airway inflammation by gene-targeting strategies

Targeted gene	Phenotype	Effect	Reference
Il4–/–	PI	Decrease	Brusselle et al[73]
	BAL eosinophilia	Decrease	
Il4r–/–	BAL eosinophilia	Absence	Cohn et al[74]
	Mucus	Absence	
IL-13 blockade	AHR	Decrease	Grunig et al[75]
	AE	Decrease	Wills-Karp et al[76]
	Mucus	Decrease	
Il13–/–	T_H2 cytokines	Decrease	McKenzie et al[77]
	Total IgE	Decrease	
Il5–/–	AHR	Absence	Foster et al[52]
	BAL eosinophilia	Absence	
	Specific IgE	As Il5+/+ mice	
Il5r–/–	AHR	Protection	Tanaka et al[78]
	BAL eosinophilia	Decrease	
	AE	Decrease	
	IL-5	As wild-type	
	Specific IgE	As wild-type	
B7-1–/–	AHR	Absence	Mark et al[58]
B7-2–/–	AE	Absence	
B7-1,2–/–	PI	Absence	
	Total IgE	Decrease	
	T_H2 cytokines	Absence	
	T_H1 cytokines	Increase	
Stat6–/–	AHR	Protection	Kuperman et al[79]
	Mucus	Protection	Akimoto et al[80]
	Specific IgE	Negative	
	BAL eosinophilia	Negative	
	PI	Decrease	
Gata3–/–	AE	Decrease	Zhang et al[66]
	Mucus	Decrease	
	Specific IgE	Decrease	
Bcl6–/–	AI	Spontaneous	Dent et al[63]
	Total IgE	Increase	Ye et al[64]
Rel–/–	AHR	Protection	Donovan et al[14]
	AI	Absence	
	BAL	Absence	
	Total IgE	Negative	
Cd81–/–	AHR	Protection	Deng et al[60]
	AI	Decrease	
	T_H2 cytokines	Decrease	
	Specific IgE	As Cd81+/+	
DP–/–	PI	Decrease	Matsuoka et al[68]
	T_H2 cytokines	Decrease	

PI, Pulmonary inflammation; *BAL,* bronchoalveolar lavage; *AHR,* airway hyperresponsiveness; *AE,* airway eosinophilia; *AI,* airway inflammation.

duction and that above that level it has an antagonistic effect. In addition, one should bear in mind that C5b, the other fragment of C5, could also have a modulating effect. These possibilities could be tested in C5aR knockout mice[48] in antigen-induced AHR. Final proof will bring the phenotype of A/J mice restored with the C5 wild-type gene, which is currently underway.[41] In human beings, the C5 gene containing region on chromosome 9q has been linked to asthma in 2 genome screens[17,26] as well as to the C5aR-encoding region—thus demonstrating the high value of animal models for providing additional information to the human system.

Another complement factor contributes to nonspecific AHR: anaphylatoxin C3a binds to its specific C3a-receptor (C3aR) and evokes, as does C5a, leukocyte activation, smooth muscle contraction, and vascular permeability. Moreover, it activates and attracts human eosinophils and mast cells.[49,50] In another model of OVA-induced AHR, homozygous deletion of C3aR abrogates AHR in BALB/c mice.[51] In this study, anaphylatoxin was seen to participate as usual in an inflammatory reaction. But the underlying mechanisms remain unclear, as AE, IgE levels, and the T_H2 cytokine pattern remain unaltered and the nonspecific methacholine response is even increased. As has been shown for C5a, C3a is present in BAL fluids of allergen-challenged asthmatic individuals. The other major features of atopic asthma—AE, IgE, and T_H2 cytokines—are identical in C3aR–/– and wild-type

mice. Accordingly, the authors suggest a division of asthma into 3 phases: an initiation phase dependent on IgE and mast cells, a T_H2- and eosinophil-dependent propagation phase, and a final effector phase, in which the inflammation leads to nonspecific smooth muscle reactivity.

Taken together, 2 of the players in the innate immune system—C3a and C5a—appear to participate in the expression of allergen-induced AHR but to do so in opposite directions, C3a as a susceptibility factor and C5a as a protective one.

Candidate gene analysis by homologous deletion

In addition to the previous knockout data presented, other candidate genes have emerged in murine models of allergic asthma (Table III). These are presented in the following section. Individual candidate genes can be investigated in animal models by genetic modifications (one of these being gene disruption by homologous recombination and the other being overexpression) and by treatment with blocking monoclonal antibodies. As genomics leads to proteomics, it is expected that a large range of specific drug inhibitors will emerge.

Cytokines and their receptors. Several pathways might lead to allergen-induced AHR. Two that have been detected by comparing inbred strains of mice are the IL-5/eosinophil-dependent pathway and the mast cell/IgE-dependent pathway.[5] Appropriately sensitized mice develop AE, AHR, and specific IgE. On a B6 background, Il5–/– mice treated in this manner remained IgE-positive but became eosinophil- and AHR-negative.[52] Thus IL-5 is here required to develop AHR; IgE and mast cells are not sufficient. On the other hand, in BALB/c mice, depletion of IL-5 abrogated AE but not AHR; conversely, AHR could be abrogated through depletion of CD4 T cells or through use of antibodies directed against IL-4, thus inhibiting IgE production and mast cell activation.

The complexity of allergen-induced asthmalike phenotypes is illustrated by genetic manipulation. Knockout of genes encoding IL-4, IL-5, IL-13, IL-4Ra, and Stat6, as well as various double knockouts, indicates that each of these proteins is important but not absolutely required for the ultimate expression of asthmalike phenotypes. This indicates that different signaling pathways can lead to the expression of a defined phenotype and that deletion of a single protein could be overcome by the action of alternative molecules.[53] This is underlined by the observation that the mode of inheritance of complex diseases is polygenic—ie, no single allele is either necessary or sufficient for the expression of the phenotype, and several genes cause the disease, in combinations that vary.[34]

Costimulatory pathways. For the activation and proliferation of T cells, 2 signals are required: the antigen-specific signal through the trimolecular MHC/peptide/TCR complex (Fig 1) and the costimulatory signal provided by the B7/CD28/CTLA4 system. CD28 and CTLA4 are expressed on T cells and are counteracted by B7-1 and B7-2 on APCs. That B7-1 and B7-2 are important regulators for both T_H1 and T_H2 responses has been shown in various mouse models of immunologic diseases (for a review, see Green[54]). Moreover, B7-1 and B7-2 blockade in vivo abrogates the development of pulmonary disease in mice.[55-57] Both B7-1 and B7-2 participate in the expression of AHR: B7-1, B7-2, and B7-1,2 double-knockout mice develop similar phenotypes despite the differences in signaling strength and kinetics between B7-2 and B7-1.[58] The B-cell antigen receptor is modulated by a coreceptor complex consisting of the CD-19, TAPA-1, and CR2 cell-surface molecules. CD81 (also known as TAPA-1) belongs to the transmembrane 4 superfamily, whose function is incompletely understood. By means of a monoclonal antibody, it has been implicated in the motility, adhesion, proliferation, and differentiation of hematopoietic cells.[59] CD81–/– mice have an impaired humoral immune response and are T_H2-deficient. Moreover, they show decreases in all asthma-associated phenotypes except allergen-specific IgE.[60] Coligation of CR2/CD19/TAPA-1 with the B-cell receptor results in phosphorylation of the B-cell receptor and enhanced activation. The absence of AHR in the presence of allergen-specific IgE could be due to direct inhibition of mast cell degranulation by the CD81 pathway.[61]

Transcription factors. The proto-oncogene BCL-6 is a transcription factor participating in T_H1/T_H2 differentiation. Its gene product functions as a transcriptional repressor by binding to DNA motifs recognized by the STAT family. One such motif is in the promoter of the immunoglobulin heavy chain to which both BCL-6 and STAT6 can bind.[62] Bcl6–/– mice develop high levels of IgE and IgG1 accompanied by tissue inflammation of various organs, including the lungs, with infiltration of T_H2 cells, eosinophils, and monocytes.[63,64] Interestingly, the elevated IgE levels and organ inflammations seem to result from different pathways, inasmuch as Bcl6–/– Stat6–/– mice still suffer from organ inflammations but lack detectable IgE antibodies.[65] C-Rel, a member of the NF-κB/Rel transcription factor family, is involved in immune and inflammatory responses. C-Rel–deficient mice have reduced MCP-1 expression, and allergen-sensitized cRel–/– mice do not exhibit AHR, PI, BAL eosinophilia, or increased total serum IgE.[14] The transcription factor GATA-3 is expressed in T_H2 cells but not T_H1 cells. That expression of GATA-3 can influence the amounts of the T_H2 cytokines IL-4, IL-13, and IL-5 has been demonstrated in gene-targeted Gata3–/– mice.[66] As a consequence, AE, mucus production, and allergen-specific IgE are drastically reduced in these mice.

Chemokines. Toney et al[67] reported an increase in the expression of the 3 chemokines MCP-1, MCP-3, and MRP-1 in Bcl6–/– mice. The promoter of MCP-1, the most prominently elevated chemokine, contains 3 BCL-6 binding sites, and transcription of a reporter construct is decreased by BCL-6 in a dose-dependent manner.[67] These findings demonstrate that transcriptional repressors are integrated into the regulation of lymphocyte activity. Whether the elevated IgE levels and lung inflammation also lead to allergen-induced AHR has not been investigated.

Prostaglandins. Another mediator of asthmalike phenotypes is prostaglandin D2 (PGD2), as has been demonstrated in PGD2 receptor–deficient (DP–/–) mice.[68] DP–/– mice do not develop PI, have a significant reduction in lymphocyte number and T_H2 cytokines, and do not express eosinophilia and AHR, in contrast to their C57BL/6 littermates. This parallels the observation in human beings that PGD2 is released in large quantities during asthmatic attacks.[69-71]

Granule proteins. Major basic protein (MBP) is released by activated eosinophils and has been implicated into the IL-5/eosinophil–dependent pathway of AHR. However, in a recent study by Denzler et al,[72] there were no differences in AHR, AE, and PI between the Mbp–/– mice and their wild-type littermates.[72]

Summary of linkage and candidate gene studies

It is evident from the results of the linkage studies of allergen-induced airway inflammation and AHR that numerous genes contribute to the "asthmalike" phenotypes. Although the precise genetic mechanisms are unclear, a number of promising candidate genes have emerged. Some of these are of biological relevance in asthma and could yield new therapeutic approaches once they are fully validated. What remains to be established is whether there are functional polymorphisms in these candidate genes that determine the severity of the disease phenotypes. These polymorphisms might result in several scenarios, including a reduction in gene expression and an absence or alteration in the protein of interest.

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