

Mechanisms of disease

Association of *IL12B* promoter polymorphism with severity of atopic and non-atopic asthma in children

Grant Morahan, Dexing Huang, Mark Wu, Barbara J Holt, Gregory P White, Garth E Kendall, Peter D Sly, Patrick G Holt

Summary

Background Severe asthma is a frequent cause of hospital admission, especially among children. The main environmental triggers of airway inflammation in asthma are viruses and aeroallergens. These agents elicit reciprocal immune responses, characterised by production of T helper 1 and T helper 2 cytokines, respectively. There is no genetic explanation for how hyper-responsiveness to these disparate environmental stimuli develops among individuals with asthma. Our aim was to assess relation between an *IL12B* promoter polymorphism and asthma.

Methods We did a cohort study in which we initially genotyped 411 6-year olds for the *IL12B* promoter polymorphism. We then assessed the relation between this polymorphism and asthma severity. A further 85 asthmatic children in an additional sample of 433 children from the same cohort were then assessed to confirm these findings. We also examined in-vitro interleukin-12 responses in a subgroup of individuals.

Findings Heterozygosity for the *IL12B* promoter polymorphism was observed in 76% (16) of atopic and non-atopic individuals with severe asthma in the initial sample. By comparison, heterozygotes comprised only 31% (17) of the moderate asthma group, and 48% (20) of individuals with mild asthma were heterozygous, as were unaffected controls. These findings were confirmed in the second sample (overall $p < 0.0001$). Our data suggest that *IL12B* promoter heterozygosity contributes to asthma severity rather than susceptibility per se. The severity-predisposing genotype was associated with reduced interleukin 12 p40 gene transcription and decreased interleukin 12 p70 secretion.

Interpretation Interleukin 12 plays a key part in antagonism of T helper 2 differentiation, and in induction of antiviral host defense. Genetically determined attenuation of interleukin-12 response capacity would, therefore, provide a plausible common immunological pathway to disease severity for the two major forms of asthma.

Lancet 2002; **360**: 455–59

See Commentary page 422

The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Victoria 3050, Australia (G Morahan PhD, D Huang MSc, M Wu BSc); and Telethon Institute for Child Health Research, and Centre for Child Health Research, University of Western Australia, PO Box 855, West Perth WA 6872, Western Australia (B J Holt BSc, G P White PhD, G E Kendall PhD, Prof P D Sly MD, Prof P G Holt FA)

Correspondence to: Prof Patrick G Holt
(e-mail: patrick@icmr.uwa.edu.au)

Introduction

Asthma is a complex disease associated with inflammation of the airways.¹ Several distinct asthma phenotypes exist, in particular atopic asthma driven by allergen-induced inflammation and non-atopic (intrinsic) asthma in which the key inducing stimuli are viruses.^{2–4} Results of studies of cellular mechanisms of atopic asthma over the past decade have implicated the local expression of T HELPER 2 (Th2)-mediated immunity in disease pathogenesis. This process is characterised by secretion of interleukin 4 and interleukin 5 by T lymphocytes; these cells, together with eosinophils, are present in the cellular infiltrate of airway tissues.^{1,4,5} Markers of Th2-associated inflammation have also been detected in airways of intrinsic asthmatics, despite the absence of evidence of any involvement of IgE in this form of the disease.^{4,6}

Findings of genetic studies show that asthma is a complex heritable disorder with many factors that contribute to susceptibility. Linkage has been consistently reported between asthma and atopy phenotypes, and genetic markers on chromosome 5q31–33,^{7,8} including the region containing *IL12B*.⁹ Several candidate genes in this region have been proposed to contribute to susceptibility to asthma or atopy, or both, but no genetic mechanism has been advanced that would account for progression from mild to severe forms of atopic and non-atopic asthma. The *IL12B* gene encodes the p40 subunit of INTERLEUKIN 12, a proinflammatory cytokine that is a major regulator of T HELPER 1 (Th1) and Th2 responses.¹⁰ We have previously identified polymorphisms in this gene¹¹ and have shown that these are in linkage disequilibrium with type 1 diabetes in Australian and British families.¹² *IL12B* is also a possible candidate for involvement in asthma susceptibility; interleukin 12 has an important immunoregulatory function, since it promotes Th1 differentiation and maintains the competence of interferon- γ transcriptional machinery within Th1 effector cells,^{10,13} and is thus likely to play a key part in infectious and allergic disease. Our aim was, therefore, to investigate associations between *IL12B* polymorphisms and development of asthma, in both atopic and non-atopic individuals.

Methods

Participants

Participants were part of an ongoing study on a large community-based birth cohort (n=2168).¹⁴ The cohort was recruited antenatally, and was not selected on any respiratory or atopic criteria. The ethnic mix of the cohort is broadly representative of the population living in Perth, Western Australia.

We assessed the atopic status of all children at age 6 years and ascertained whether or not they had asthma. Atopy was defined as production of a skin prick test wheal 2 mm or greater in diameter to at least one of a panel of the four most common inhalant allergens prevalent in Western Australia (house dust mite, cat, grass, and mould). We chose these allergens because they are known to detect 85% or more of atopic children in this geographical area. As an additional back-up, we assessed serum samples from all individuals with the Phadiatop atopy screening test (Pharmacia,

GLOSSARY**HARDY-WEINBERG EQUILIBRIUM**

A proportionality between the frequencies of heterozygotes and homozygotes for a genetic variant in the population determined by Mendel's laws of inheritance.

HETEROSIS

A condition in which a heterozygote is different from each of the homozygote classes, usually by displaying increased fitness; also referred to as hybrid vigour or overdominance.

INTERLEUKIN 12

The whole interleukin 12 molecule is 70 kDa in weight, and is made up of two chains, the larger (interleukin 12 p40) being 40 kDa in size. The *IL12B* gene encodes interleukin 12 p40.

T HELPER 1 (TH1) AND T HELPER 2 (TH2) CELLS

CD4+ T lymphocytes distinguished by patterns of cytokine production—Th1 produce interleukin 2 and interferon- γ , and Th2 produce interleukins 4, 5, and 13.

Uppsala, Sweden), which detects IgE antibodies against a broad panel of inhalant and dietary antigens. The results obtained with this procedure correlated strongly with those obtained via the skin prick test ($p < 0.0001$ by χ^2 analysis). Current asthma was defined as presence of all three of: episodes of wheeze, occurring within the past 12 months; an asthma diagnosis by a medical practitioner; and current use of asthma medications.¹⁵ Asthma severity was classified on the basis of each of: frequency of episodes of wheeze (mild=1–2, moderate=3–12, severe= ≥ 12 per year), frequency of nocturnal waking due to asthma (0, <1 , ≥ 1 per week), and severe asthma-limiting speech (0, 0, ≥ 1 per year) in the past 12 months. We used clinical criteria derived from consensus statements developed by the Australasian Paediatric Respiratory Group and Thoracic Society of Australia and New Zealand to ascertain severity of asthma.^{16,17}

DNA samples were available for 844 children enrolled, and selection of samples was simply on the basis of availability at time of study. In the overall cohort, 40% (867) of children were atopic and 18% (390) had current asthma,¹⁴ and in the sub-sample used here for genetic analysis 43% (363) were atopic and 24% (203) were currently asthmatic. We did the study in two stages, comprising an initial group of 411 children stratified by atopy, with a follow-up group (the 433 remaining children) being subsequently analysed to validate the findings obtained with the first group. In initial analyses, we compared all asthmatics (including atopic and non-atopic individuals) with the controls even though, in all, there were three disease classes: unaffected control, atopic asthmatic, and non-atopic asthmatic individuals. We also identified a fourth class of children, who had atopy but not asthma; these children did not show a significant difference in allele frequency by comparison with the control groups, and were not considered further in this study. The study was approved by the institutional human ethics committee, and parents provided written informed consent.

Protocol

We extracted DNA from Buffy-coat lymphocytes, and identified *IL12B* polymorphisms as previously described.^{11,12} The *IL12B* promoter polymorphism arose as a result of a microinsertion event at a point 3 kb upstream of the transcriptional start site. We amplified DNA samples with two primers: 5' TCAGACACATTAACCTTGCA 3' and 5' ACCTGCCAATGACCACATTA 3', one of which was radiolabelled with ³²pATP. We did PCR reactions with 50 ng DNA, 25 ng each primer, 200 nmol/L deoxyribonucleoside triphosphate, 2 mmol magnesium chloride, and 0.3 U Taq polymerase (Gibco BRL; Life technologies, Gaithersburg, MD, USA). DNA was denatured at 95°C for 3 min, then PCR was done for 35 cycles of 95°C 20 s, 55°C 20 s, 72°C 30 s, with a final 2 min extension. We detected alleles after separation on polyacrylamide gels. We designated the 4 bp larger—ie, insertion—allele as allele number 1, and the smaller product allele number 2. Significance of genotypic differences between groups was assessed by Fisher's exact test.

We obtained peripheral blood mononuclear cells (PBMC) from all children in the study and stored them under liquid nitrogen until use. We selected PBMC by genotype and tested them for production of interleukin 12. The samples were cultured in aliquots of 10⁶ cells in round-bottomed tissue-culture tubes in 1.0 mL RPMI 1640 medium (Gibco; Grand Island, NY, USA) supplemented with 10% fetal calf serum, and test cultures were stimulated with 10 ng/mL interferon- γ for 3 h before addition of 10 ng/mL lipopolysaccharide (LPS). This method provides maximum stimulation for production of interleukin 12. We harvested the cultures at 24 h, and collected cells and supernatants. We measured interleukin 12 p70 concentrations in culture supernatants, using an in-house time-resolved fluorescence immunoassay with antibodies and standards from Pharmingen (Torrey Pines, San Diego, CA, USA). There were no differences in production of interleukin 12, according to disease status of the donor (not shown).

To quantify *IL12B* gene expression, we extracted RNA from stimulated PBMC and reverse-transcribed for Taqman analysis with the ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA), using predeveloped reagents (Applied Biosystems, Scoresby, Australia) for *IL12B* target (interleukin 12 p40 mRNA) and the endogenous control 18S ribosomal RNA. We expressed transcription concentrations as Taqman units relative to internal 18S rRNA.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report, or in the decision to submit the paper.

Results

Table 1 shows the genotypes of the 411 children initially assessed for presence of *IL12B* polymorphisms. Although

	Asthma severity				Controls		
	Mild (n=42)	Moderate (n=55)	Severe (n=21)	Total (n=118)	Non-atopic (n=124)	Atopic (n=169)	Total (n=293)
Genotype of <i>IL12B</i> promoter							
1:1	10 (24%)	20 (36%)	2 (10%)	32 (27%)	26 (21%)	43 (25%)	69 (24%)
1:2	20 (48%)	17 (31%)	16 (76%)	53 (45%)	67 (54%)	81 (48%)	148 (51%)
2:2	12 (29%)	18 (33%)	3 (14%)	33 (28%)	31 (25%)	45 (27%)	76 (26%)

Data are number (%).

Table 1: *IL12B* promoter genotype and asthma severity

there was no significant relation between individual *IL12B* polymorphisms and asthma susceptibility per se—ie, considering the asthmatic individuals as a group—there was a differential distribution of *IL12B* promoter alleles within the asthma group ranked by severity of disease (table 1). We noted no differences between the groups with respect to expression of the *IL12B* 3'UTR polymorphism previously described (data not shown). The expected ratio of 1 to 2 to 1 of *IL12B* promoter genotypes was noted in both the control groups—ie, atopic and non-atopic individuals without asthma—and in the group with mild asthma. By contrast, comparison of the most severely affected individuals with all others—ie, those with mild or moderate asthma—showed a significant difference between groups, with a clear trend toward heterozygosity in the most severely affected group (table 2; analysis 1, $p=0.0015$).

To confirm this finding, we randomly selected a second group of 433 children from the cohort and analysed their *IL12B* genotypes. The second group included 85 asthmatic children, including 18 severely affected individuals. Again, there was an excess of *IL12B* promoter heterozygotes in the severely asthmatic children (table 2). The overall p value for combined data was less than 0.0001. When we considered all study groups, only the moderate and severe asthma cases showed significant departure from HARDY-WEINBERG EQUILIBRIUM ($p<0.0001$ and $p=0.0003$, respectively). Among all asthmatic children, the relative risk for *IL12B* promoter heterozygotes developing severe asthma was 4.6 (95% CI 2.1–11.1). We conclude that *IL12B* promoter heterozygosity was strongly associated with a predisposition to severe asthmatic responses.

Furthermore, the distribution of genotypes in the group with moderate asthma was also distorted (table 2). It is noteworthy that, in addition to the strong trend towards heterozygosity in the most severely affected group, there was a tendency to a ratio of 1 to 1 to 1 genotypes in patients with moderate asthma. This ratio was probably due to the association of heterozygotes with more severe asthma, since combining the moderate and severe groups returns the expected ratio of 1 to 2 to 1 seen in the general population. This explanation accounts for the apparent lack of association between *IL12B* genotypes and susceptibility for asthma per se. The implication of this result is that the *IL12B* genotypes are not associated with a raised risk of children becoming asthmatic, but in those who progressed beyond mild asthma, heterozygosity at the *IL12B* promoter was associated with significant risk for further progression to the most severe form of disease.

	Asthma severity		p
	Mild or moderate	Severe	
Genotype <i>IL12B</i> promoter			
Analysis 1			
Homozygous	60	5	0.0015
Heterozygous	37	16	
Analysis 2			
Homozygous	35	2	0.0013
Heterozygous	32	16	
Analysis 3			
Homozygous	54	7	<0.0001
Heterozygous	30	32	

Analysis 1—the most severely affected individuals from table 1 compared with all other patients with asthma with Fisher's exact test. Analysis 2—data from second, independent, group of 433 children. p value calculated with Fisher's exact test. Analysis 3—comparison of all 39 patients with severe asthma with individuals with moderate asthma from each of the two groups.

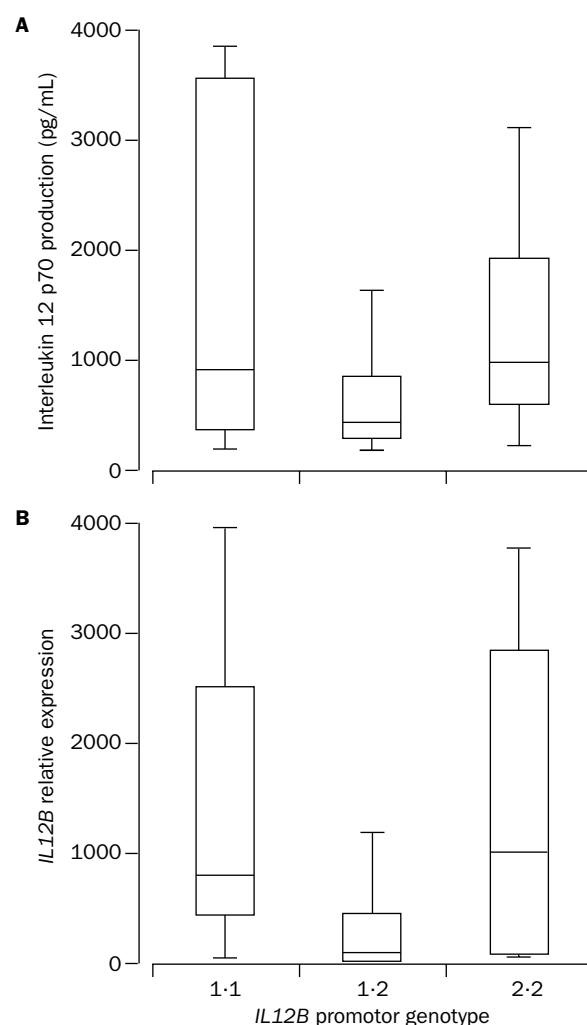
Table 2: Effect of *IL12B* promoter heterozygosity on asthma severity

	<i>IL12B</i> promoter genotype	Asthma status	
		Non-atopic	Atopic
Mild	Heterozygous	19	20
	Homozygous	15	26
Moderate	Heterozygous	13	17
	Homozygous	21	36
Severe	Heterozygous	12	20
	Homozygous	2	5

Table 3: *IL12B* genotype and asthma severity by atopic status

(An alternative view is that *IL12B* promoter homozygosity maintained asthma at moderate levels.)

The affected children included both atopic and non-atopic individuals, allowing us the opportunity to assess whether *IL12B* alleles were associated with either of these major asthma subtypes. Comparison of *IL12B* genotypes for all disease severity states indicated no significant



Production of interleukin 12 p70 protein and relative production of interleukin 12 p40 mRNA by LPS-stimulated PBMC

A: Interleukin 12 p70—pooled results from three separate experiments are shown for 19 cell cultures of PBMC from *IL12B* promoter heterozygotes, 14 promoter allele 1 homozygotes, and 17 promoter allele 2 heterozygotes. B: Results derived from nine *IL12B* heterozygotes and five samples each of the 1.1 and 2.2 genotypes. The top, bottom, and middle lines of each box correspond to the 75th percentile, 25th percentile, and median, respectively. Bars extend to the top 90th percentile and to the 10th percentile of each group.

difference between non-atopic and atopic children with asthma (table 3) and, furthermore, we noted no significant association between *IL12B* promoter heterozygosity and any of the indices of atopy (data not shown). We did note significant differences between severe asthmatics and individuals with mild or moderate asthma, irrespective of whether they had non-atopic ($p=0.007$) or atopic ($p=0.0001$) asthma. Thus, *IL12B* promoter genotypes were associated with asthma severity irrespective of disease cause, but not with atopy per se.

An obvious question raised by our results is whether the promoter polymorphism is associated with variations in gene expression. We and others have previously shown that the *IL12B* 3'UTR polymorphism affected gene expression,^{12,18} but have not tested directly the promoter polymorphism. Accordingly, we cultured PBMCs from a subset of children under optimum conditions for stimulation of production of interleukin 12, which was measured as both interleukin 12 p70 in culture supernatants and *IL12B* transcript concentrations in extracts from cell pellets. The figure shows data comparing mean interleukin 12 p70 production in cultures from 19 *IL12B* promoter heterozygotes and from 31 *IL12B* promoter homozygotes (comprising 14 *IL12B* promoter 1.1 and 17 *IL12B* promoter 2.2 individuals). There was no difference between the 1.1 and 2.2 homozygotes, but individuals with each of these genotypes produced significantly more interleukin 12 p70 than did heterozygotes (figure, A; $p=0.01$). Similarly, we noted differences at the RNA level by Taqman analysis: heterozygotes produced significantly less *IL12B* transcripts than did homozygotes (figure, B; $p=0.008$). The within-group variability evident in the figure is attributable to the effects of other polymorphisms, including the 3'UTR polymorphism, which we previously showed had an effect on *IL12B* expression.¹² It should be noted that the reduced *IL12B* expression was also observed in cultures derived from individuals without asthma (mean homozygotes [$n=22$] 1358 [SEM 266]; heterozygotes [$n=9$] 685 [164]; $p=0.02$).

Discussion

Our results show that heterozygosity for the *IL12B* promoter polymorphism is associated with an increased severity of both atopic and non-atopic asthma in childhood. Furthermore, our findings indicate a biologically relevant effect of heterozygosity on reduced expression of the *IL12B* gene and reduced production of the biologically active interleukin 12 p70 protein. We know of no other example of heterozygote deficiency at the level of gene expression when each of the homozygotes have the same amounts of expression. It is possible that an *IL12B* variant mediates these effects. However, we believe this possibility unlikely, since such a hypothetical polymorphism would need to be in linkage equilibrium with each of the promoter alleles and would thus appear in promoter homozygotes, which are under-represented among the severely affected individuals. The *IL12B* gene has limited variability,¹¹ and we have noted no other polymorphism that could account for the findings presented here. Furthermore, among other indications of linkage of asthma to chromosome 5q, Noguchi and colleagues¹⁹ reported linkage to the region near *IL12B*, yet noted no significant transmission of *IL12B* polymorphisms, including the 3'UTR allele. Their results were similar to ours (data not shown). These other polymorphisms do not seem to be in strong linkage disequilibrium with the *IL12B* promoter polymorphism,

emphasising the importance of the promoter genotype itself, rather than linked polymorphisms, in mediating asthma severity.

It is biologically plausible that reduced production of the interleukin 12 p70 protein is associated with more severe disease in asthmatic individuals. Reduced production of interleukin 12 could result in attenuation of Th1 function, leading to more intense or more long-term allergen-specific Th2 responses. Additionally, the efficiency of Th1 responses against respiratory viruses could be compromised by feedback inhibition from antagonistic Th2 cytokines. In this respect, researchers have reported that progression to severe wheeze after respiratory viral infection in children is associated with attenuated Th1 function as measured by interleukin 12 or interferon- γ production.²⁰⁻²⁵ Furthermore, respiratory infections arise with increased frequency, and tend to be more severe, in children who subsequently develop asthma than in those who do not.^{26,27} That the Th2 component of host-antiviral responses in these individuals might be asthmaticogenic per se is also feasible. It is noteworthy that virus infections, in particular respiratory syncytial virus infection, represent the most frequent cause of severe wheeze in preschool children,^{2,3,28} and that antiviral responses in these individuals exhibit prominent Th2 features, including eosinophilia^{29,30} and IgE antibody against viral proteins.³¹ Additionally, respiratory viral infections represent a major cause of severe asthma exacerbations in atopic asthmatics of all ages,^{5,32} presumably by superimposing a further layer of inflammation-induced airway pathology over that initiated by chronic Th2-mediated responses to aeroallergens.

Attenuation of interleukin 12 p70 production in these individuals could predispose toward development of hyper-responsiveness of the airways. In murine models of asthma, administration of exogenous interleukin 12 protects against expression of airway hyper-responsiveness,^{33,34} and depletion of interleukin 12 increases susceptibility to development of airway hyper-responsiveness.³⁵ The precise mechanism by which interleukin 12 protects against hyper-responsiveness has not been established. However, interleukin 13 does play a central part in development of airway hyper-responsiveness in mice with asthma, and interleukin-13 responses are counterbalanced by interleukin 12 in these models.³⁶

In conclusion, our results suggest a potential role for *IL12B* gene polymorphisms in asthma pathogenesis in human beings. Importantly, our data provide a plausible final common pathway for progression to disease severity for the two different forms of asthma, which have traditionally been viewed as related but mechanistically distinct pathological entities. Three important issues that arise from these findings need to be addressed in future research. Our most pronounced finding is that individuals who are heterozygous for the *IL12B* promoter polymorphism have the greatest risk for progression to severe asthma. Although there is no known molecular mechanism to explain this effect, this differential heterozygous control of gene expression might contribute to other cases of HETEROSIS—ie, hybrid vigour—and to balancing of selection of alleles at the population level. Hence, this finding should be confirmed in other populations. Second, the effects of the *IL12B* promoter polymorphism described here were associated with progression to severe asthma but not with mild or moderate disease, so the key genes that determine asthma susceptibility per se still remain to be identified. It is pertinent in this respect to note that polymorphisms in the

gene that encodes the β 2-andrenergic receptor have been associated with moderate but not mild or severe asthma,³⁷ providing a precedent for a single gene variation promoting progression along the pathway towards asthma severity, as well as an explanation for the absence of association in the mildly affected children in our study. Finally, we focused exclusively on 6-year-old children. In this context, it is noteworthy that the interleukin-12 response capacity in early childhood is much lower than in adults,^{38,39} suggesting that the relative importance of the *IL12B* polymorphisms in asthma pathogenesis could vary with age. We will examine this hypothesis in subsequent studies.

Contributors

G Morahan, P G Holt, and P D Sly designed the study, analysed and interpreted data, and wrote the report; D Huang and M Wu genotyped individuals; B J Holt and G P White prepared DNA samples and did in-vitro studies on interleukin-12 responses; G E Kendall did 6-year follow-up on the study cohort and associated analyses of clinical phenotypes.

Conflict of interest statement

The Walter and Eliza Hall Institute of Medical Research has filed a patent, covering usefulness of *IL12B* polymorphisms in testing for susceptibility to immune-related disorders.

Acknowledgments

We thank Devinda Suriyaarachchi and Jenny Tizard for skilled technical assistance, and Profs Terry Speed and Nick de Klerk for advice on statistical analysis. This work was supported by the National Health and Medical Research Council of Australia, the RAINE Foundation, and by a Special Program Grant from the National Health and Medical Research Council (NHMRC) and the Juvenile Diabetes Research Foundation.

References

- Holgate S. The inflammation-repair cycle in asthma: the pivotal role of the airway epithelium. *Clin Exp Allergy* 1998; **28** (suppl 5): 97–103.
- Martinez FD, Wright AL, Taussig LM, et al. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995; **332**: 133–38.
- von Mutius E. Paediatric origins of adult lung disease. *Thorax* 2001; **56**: 153–57.
- Humbert M, Menz G, Ying S, et al. The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: more similarities than differences. *Immunol Today* 1999; **20**: 528–33.
- Busse WW, Banks-Schlegel S, Wenzel SE. Pathophysiology of severe asthma. *J Allergy Clin Immunol* 2000; **106**: 1033–42.
- Burke CM, Poulter LW. Relevance of atopy in the pathogenesis of bronchial asthma. *Allergy Immunol (Life Sci Adv)* 1995; **14**: 75–84.
- Anderson GG, Cookson WOCM. Recent advances in the genetics of allergy and asthma. *Mol Med Today* 1999; **5**: 264–73.
- Rosenwasser LJ, Klemm DJ, Dresback JK, et al. Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. *Clin Exp Allergy* 1995; **25**: 74–78.
- Yokouchi Y, Nukaga Y, Shibasaki M, et al. Significant evidence for linkage of mite-sensitive childhood asthma to chromosome 5q31-q33 near the interleukin 12 B locus by a genome-wide search in Japanese families. *Genomics* 2000; **66**: 152–60.
- Trinchieri G, Scott P. The role of interleukin 12 in the immune response, disease and therapy. *Immunol Today* 1994; **15**: 460–63.
- Huang D, Cancilla MR, Morahan G. Complete primary structure, chromosomal localisation, and definition of polymorphisms of the gene encoding the human interleukin-12 p40 subunit. *Genes Immunity* 2000; **1**: 515–20.
- Morahan G, Huang D, Ymer SI, et al. Linkage disequilibrium of a Type 1 diabetes susceptibility locus with a regulatory *IL12B* allele. *Nat Genet* 2001; **27**: 218–21.
- Yap G, Pesin M, Sher A. IL-12 is required for the maintenance of IFN- γ production in T cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii*. *J Immunol* 2000; **165**: 628–31.
- Oddy WH, Holt PG, Sly PD, et al. Association between breast feeding and asthma in 6 year old children: findings from a prospective birth cohort study. *BMJ* 1999; **319**: 815–19.
- Haby MM, Peat JK, Marks GB, Woolcock AJ, Leeder SR. Asthma in preschool children: prevalence and risk factors. *Thorax* 2001; **56**: 589–95.
- Isles AF, Robertson CF. Treatment of asthma in children and adolescents: the need for a different approach. *Med J Aust* 1993; **158**: 761–63.
- Henry RL, Robertson CF, Asher I, et al. Management of acute asthma. *J Paediatr Child Health* 1993; **29**: 101–03.
- Davoodi-Semiromi A, Yang JJ, She JX. IL-12p40 is associated with type 1 diabetes in caucasian-American families. *Diabetes* 2002; **51**: 2334–36.
- Noguchi E, Yokouchi Y, Shibasaki M, et al. Identification of missense mutation in the *IL12B* gene: lack of association between *IL12B* polymorphisms and asthma and allergic rhinitis in the Japanese population. *Genes Immun* 2001; **2**: 401–03.
- Aberle JH, Aberle SW, Dworzak MN, et al. Reduced interferon-gamma expression in peripheral blood mononuclear cells of infants with severe respiratory syncytial virus disease. *Am J Respir Crit Care Med* 1999; **160**: 1263–68.
- Renzi PM, Turgeon JP, Marcotte JE, et al. Reduced interferon-gamma production in infants with bronchiolitis and asthma. *Am J Respir Crit Care Med* 1999; **159**: 1417–22.
- Blanco-Quirós A, González H, Arranz E, Lapeña S. Decreased interleukin-12 levels in umbilical cord blood in children who developed acute bronchiolitis. *Pediatr Pulmonol* 1999; **28**: 175–80.
- Leech SC, Price JF, Holmes BJ, Kemeny BJ. Nonatopic wheezy children have reduced interferon-gamma. *Allergy* 2000; **55**: 74–78.
- Rowe J, Macaubas C, Monger T, et al. Heterogeneity in diphtheria-tetanus-acellular pertussis vaccine-specific cellular immunity during infancy: relationship to variations in the kinetics of postnatal maturation of systemic Th1 function. *J Infect Dis* 2001; **184**: 80–88.
- Holt PG, Sly PD. Interactions between respiratory tract infections and atopy in aetiology of asthma. *Europ Respir J* 2002; **19**: 1–8.
- Forastiere F, Agabiti N, Corbo GM, et al. Socioeconomic status, number of siblings, and respiratory infections in early life as determinants of atopy in children. *Epidemiology* 1997; **8**: 566–70.
- von Mutius E, Illi S, Hirsch T, Leupold W, Keil U, Weiland SK. Frequency of infections and risk of asthma, atopy and airway hyper-responsiveness in children. *Eur Respir J* 1999; **14**: 4–11.
- Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999; **354**: 541–45.
- Ehlenfeldt DR, Cameron K, Welliver RC. Eosinophilia at the time of respiratory syncytial virus bronchiolitis predicts childhood reactive airway disease. *Paediatrics* 2000; **105**: 79–83.
- Gaga M, Lambrou P, Papageorgiou N, et al. Eosinophils are a feature of upper and lower airway pathology in non-atopic asthma, irrespective of the presence of rhinitis. *Clin Exp Allergy* 2000; **30**: 663–69.
- Welliver RC, Wong DT, Sun M, Middleton E, Vaughan RS, Ogra PL. The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. *N Engl J Med* 1981; **305**: 841–46.
- Johnston SL, Pattemore PK, Sanderson G, et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. *Am J Respir Crit Care Med* 1996; **154**: 654–60.
- Gavett SH, O'Hearn DJ, Li X, Huang S-K, Finkelman FD, Wills-Karp M. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J Exp Med* 1995; **182**: 1527–36.
- Hofstra CL, Van Ark I, Hofman G, Kool M, Nijkamp FP, Van Oosterhout AJ. Prevention of Th2-like cell responses by coadministration of IL-12 and IL-18 is associated with inhibition of antigen-induced airway hyper-responsiveness, eosinophilia, and serum IgE levels. *J Immunol* 1998; **161**: 5054–60.
- Keane-Meyers A, Wysocka M, Trinchieri G, Wills-Karp M. Resistance to antigen-induced airway hyper-responsiveness requires endogenous production of IL-12. *J Immunol* 1998; **161**: 919–26.
- Wills-Karp M. IL-12/IL-13 axis in allergic asthma. *J Allergy Clin Immunol* 2001; **107**: 9–18.
- Weir TD, Mallek N, Sandford AJ, et al. Beta2-Andrenergic receptor halotypes in mild, moderate and fatal/near fatal asthma. *Am J Respir Crit Care Med* 1998; **158**: 787–91.
- Joyner JL, Augustine NH, Taylor KA, La Pine TR, Hill HR. Effects of group B streptococci on cord and adult mononuclear cell interleukin-12 and interferon-gamma mRNA accumulation and protein secretion. *J Infect Dis* 2000; **182**: 974–77.
- Lee SM, Suen Y, Chang L, et al. Decreased interleukin-12 (IL-12) from activated cord versus adult peripheral blood mononuclear cells and upregulation of interferon-gamma, natural killer, and lymphokine-activated killer activity by IL-12 in cord blood mononuclear cells. *Blood* 1996; **88**: 945–54.