

Genetic Polymorphism and Th1/Th2 Orientation

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Key Words

ACP1 · Adenosine deaminase · Asthma · Crohn's disease · Haptoglobin · MN · Th1 · Th2

Abstract

Background: It is likely that besides developmental and environmental factors, genetic factors also play an important role in Th1/Th2 orientation and susceptibility to related disorders. Thus, for each genetic factor involved one would expect an opposite pattern of susceptibility towards Th1- and Th2-associated diseases. **Methods:** We report a comparative analysis of the pattern of association of four genetic polymorphisms with bronchial asthma (Th2 disease) and Crohn's disease (CD; Th1 disease). The study population included 291 Roman children with bronchial asthma and 72 adult Romans with CD, and haptoglobin, adenosine deaminase (ADA), acid phosphatase locus 1 (ACP1) and MN phenotypes were determined. **Results:** Compared with controls from the same population, the pattern of phenotype association observed in bronchial asthma is exactly opposite to that observed in CD. The analysis of pairwise gametic type distribution for ACP1, ADA and MN polymorphisms has shown that the pattern of differences between bronchial asthma and controls is opposite to

that observed between CD and controls. **Conclusions:** The pattern of differences between bronchial asthma versus CD is compatible with the hypothesis that some of the genetic systems considered contribute to Th1/Th2 orientation.

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Introduction

Besides developmental and environmental factors [1–6] it is likely that genetic factors also play an important role in Th1/Th2 orientation and susceptibility to related diseases. Thus, for each genetic factor involved one would expect an opposite pattern of susceptibility towards Th1- and Th2-associated diseases [7]. Indeed, such a pattern of association between Th1 and Th2 diseases has been found for haptoglobin (Hp) distribution [8].

Since we have previously shown an association between Hp, adenosine deaminase (ADA), acid phosphatase locus 1 (ACP1) and MN systems with allergic disorders [8–12], in the present study we have carried out a comparative analysis of these genetic systems in bronchial asthma (a Th2-associated disease) versus Crohn's disease (CD; a Th1-associated disease).

The Genetic Systems Considered

Haptoglobins

Hp, an α_2 -sialoglycoprotein well known for its hemoglobin-binding capacity, shows a genetic polymorphism with variations at the protein level. On starch gel electrophoresis, Hp displays three common phenotypes, Hp1, Hp2-1 and Hp2 ascribed to the presence of two alleles, Hp*1 and Hp*2 [13]. Compared with other genotypes, Hp*1/*1 produces more proteins and smaller polymers that diffuse more readily into the tissues [14, 15].

Several studies indicate that the genetic variability in Hp may have clinical relevance, influencing susceptibility to immunoallergic and infectious diseases. CD22 and the integrin CD11b/CD18 have been suggested as specific receptors for Hp in immune system cells [16, 17]. Recent observation indicates that Hp influences the Th1/Th2 balance [18].

Hp develops gradually during the first weeks of life [19] and exerts a protective action against bacterial infections [20]. Recent evidence from our laboratory suggests that in the neonatal period Hp1 develops earlier compared to other types. In subjects with Hp1 phenotype, this may help to maintain the immunological balance in favor of a Th2 response via a relative protection in the early stages of intrauterine life against infectious agents. The positive association between the Hp1 phenotype and asthma previously reported [8] supports this hypothesis. On the basis of these observations, we have considered two phenotypic classes for Hp: Hp1 and Hp2-1 plus Hp2 in the present analysis.

Adenosine Deaminase

ADA is a polymorphic enzyme controlled by a locus with two co-dominant alleles, ADA*1 and ADA*2. The corresponding three common ADA phenotypes have different enzymatic activity: ADA1 is 15% more active than ADA2-1 and 30% more active than ADA2 [21]. ADA occurs in a small molecular form (MW 33 kDa) called red blood cell (RBC) ADA and in a large molecular form (MW 200 kDa) called tissue-specific ADA. The various ADA tissue enzymes consist of one or more molecules of RBC ADA and one molecule of ADA-complexing protein [22].

ADA-complexing protein is identical with CD26 (a T-cell activator molecule). It has been suggested that ADA expression may be connected with the process of T-cell activation.

ADA polymorphism has been found to be associated with bronchial asthma in an Italian population [9], and,

recently, the association has been confirmed in a Chinese population [in preparation]. On the basis of these observations, we have considered two phenotypic classes: ADA1 and ADA2-1 plus ADA2.

Acid Phosphatase Locus 1

The ACP1 or cLMPTP (cytosolic low-molecular-weight protein tyrosine phosphatase) is an enzyme involved in T-cell receptor signal transduction. It specifically dephosphorylates the negative regulatory Tyr²⁹² of ZAP70, thereby counteracting inactivation of ZAP70. This indicates that ACP1 strengthens T-cell receptor signalling [23]. The association with serum IgE levels [24] and with age at onset of type 1 diabetes [25] recently reported suggest an involvement of this polymorphism in the Th1-Th2 balance.

ACP1 is a polymorphic enzyme showing a strong quantitative variation in total enzymatic activity. In the Caucasian population, six genotypes have been observed (designated A, BA, B, CA, CB and C attributed to the presence of three co-dominant alleles ACP*A, ACP*B and ACP*C at the autosomal locus). Spencer et al. [26] found the following activities ($\mu\text{mol } p\text{-nitrophenol produced in 30 min per g of Hb at } 37^\circ\text{C}$): ACP1 A = 122.4; ACP1 BA = 153.9; ACP1 B = 188.3; ACP1 CA = 183.8 and ACP1CB = 212.3. The ACP1C phenotype is very rare.

On the basis of previous observations showing association with enzymatic activity [10], ACP1 genotypes have been divided into two classes: A plus BA (low activity) and B plus CA plus CB (medium-high activity).

MN Blood Groups

MN is a polymorphic system controlled by two closely linked genes on chromosome 4q28–q31 encoding MN and Ss antigens. There are three phenotypes: M, MN and N. The two major glycoproteins of the human red cell membrane (glycophorins A and B) carry the antigen specificity of the system. Glycophorin A (MN sialoglycoprotein) is the most abundant in the red cell membrane (about one million copies per cell). Less is known about glycophorin B (Ss sialoglycoprotein) that is similar in structure to glycophorin A [27–29]. Glycophorin A is a the major ligand of *Plasmodium falciparum*. It is also an important ligand for some bacteria and for viruses (orthomyxovirus, reovirus and picornavirus) that recognize the N-acetylneuraminic acid which is abundant on glycophorins [29].

We have reported an association between MN blood groups and asthma onset in children [12]. Based on our previous findings, we have considered two phenotypic classes: M and MN plus N.

Materials and Methods

A total of 291 Roman children (202 males and 89 females) with bronchial asthma were included in the study. Their age ranged from 1 to 12 years. The criteria for inclusion were a history of two or more episodes of wheezing in the preceding 6 months irrespective of the possible etiology/pathogenesis of the attacks. The children were treated at the outpatient Pediatric Pulmonary Clinic of the University of Rome or were admitted to the wards of the same clinic for acute respiratory episodes. ADA phenotype was determined in all children, while the other markers were studied in randomly selected patients of the study cohort.

A total of 72 Roman patients with CD (37 males and 35 females, aged 27–58 years) were studied in the St. Camillo Hospital. The diagnosis of CD was performed correlating clinical symptoms with endoscopic findings, mucosal biopsy studies and radiological evaluation of the small and large intestine. ADA phenotype was determined in all patients while other markers were studied in patients randomly selected from the CD patients.

The investigation was approved by our Institutional Review Board, and all subjects (or their parents for children) gave verbal informed consent to be included (or to include their children) in this investigation.

Various samples of normal healthy Romans studied either by us or by colleagues of our Department have been included as controls [30–32]. These controls consist of adults studied in the Transfusion Center and/or consecutive newborn infants from the Maternity Department of the University of Rome. All individuals were White Caucasians.

For Hp, the control sample is composed of 1,956 adults of both sexes. The control sample for ACP1 includes 417 adults of both sexes and 609 consecutive newborns. No significant difference was observed between newborns and adults. The control sample for ADA comprises 572 consecutive newborn infants and 845 adults of both sexes. No significant difference was observed between newborns and adults. The control sample for MN consists of 548 adults of both sexes.

Hp phenotype has been determined by starch gel electrophoresis on hemoglobin-supplemented sera according to Smithies [13]. ACP1 and ADA phenotypes have been determined by starch gel electrophoresis on red blood cell hemolysates according to Harris and Hopkinson [33]. MN phenotype has been determined according to standard serological procedures [34].

Two polymorphic system [phosphoglucosyltransferase locus 1 (PGM₁) and adenylate kinase locus 1 (Ak₁)], for which we had no 'a priori' evidence indicating a possible involvement in Th1/Th2 orientation, were also studied in asthmatic children, a control group of consecutive healthy newborns and in CD patients. PGM₁ and Ak₁ phenotypes were determined on red blood cell hemolysates according to Harris and Hopkinson [33]. PGM₁ is a key enzyme in glucose metabolism that catalyzes the reaction glucose-1-phosphate ↔ glucose-6-phosphate. Ak catalyzes the reaction ATP + AMP ↔ 2ADP and has an important role in the synthesis of nucleotides.

Gametic type and haplotype frequencies are maximum likelihood estimates (program Mendel Department of Biostatistics, University of Michigan, Ann Harbor, Mich., USA). The χ^2 test of independence has been performed according to SPSS programs (SPSS/PC+, version 5.0; SPSS, Chicago, 1992).

Most of the present study has been carried out during the last 20 years.

Results

Table 1 shows the distribution of Hp1, of low-activity ACP1 (A plus AB), ADA1 and of M plus MN phenotypes in bronchial asthma, in normal controls and in CD patients. Compared with controls, the pattern of association observed in bronchial asthma is exactly the opposite of that observed in CD.

Table 2 provides the data on PGM₁ and Ak₁. No significant difference has been observed among CD patients, controls and asthma patients.

Table 3 demonstrates the pairwise gametic type distribution for ACP1, ADA and MN polymorphisms. Hp has been excluded from this analysis since Hp determination was performed only in a small number of CD patients. Therefore, there are three possible pairwise gametic types.

The pattern of differences between bronchial asthma and controls appears opposite to that observed between

Table 1. Distribution (%) of Hp, ADA, ACP1 and MN phenotypes in controls and in patients with bronchial asthma and CD

Groups	Hp1 phenotype (low molecular weight)	ACP1A and AB phenotypes (low ACP1 activity)	ADA1 phenotype (high activity)	N and MN phenotypes (carriers of L ^N allele)
Bronchial asthma	18.9 (95)	50.0 (146)	88.7 (291)	72.7 (162)
Normal controls	11.1 (1,956)	40.5 (1,026)	83.3 (1,417)	69.0 (548)
CD	0.0 (28)	32.4 (71)	79.2 (72)	40.7 (42)
χ^2 test of independence	p < 0.01	p < 0.05	p < 0.05	p < 0.001

The numbers of subjects studied are shown in parentheses.

Table 2. Distribution (% , except for total n) of PGM1 and Ak1 phenotypes in controls and in patients with bronchial asthma and CD

Groups	PGM1				Ak1			
	1	2-1	2	total n	1	2-1	2	total n
Bronchial asthma	47.0	47.7	5.3	151	95.0	5.0	0.0	160
Controls	49.6	42.9	7.5	375	92.0	7.7	0.3	350
CD	50.0	44.0	6.0	50	98.0	2.0	0.0	49
χ^2 test of independence	p = 0.826				p = 0.18			

Table 3. Pairwise gametic type distribution in patients with CD and bronchial asthma and in controls

		ACPI(*A/*nonA) ADA				
		gametic types				total
		*A/*1	*A/*2	non*A/*1	non*A/*2	
a	CD frequency	0.166	0.034	0.713	0.088	140
	SE	0.034	0.019	0.040	0.027	
b	Controls frequency	0.215	0.057	0.684	0.044	158
	SE	0.034	0.021	0.039	0.019	
c	Asthma frequency	0.272	0.015	0.668	0.046	248
	SE	0.029	0.011	0.031	0.015	
Comparisons		χ^2	d.f.	p		
a vs. b vs. c		12.989	6	0.043		
a vs. c		8.650	3	0.044		
		MN ADA				
		gametic types				total
		*L ^M /*1	*L ^M /*2	*L ^N /*1	*L ^N /*2	
a	CD frequency	0.640	0.078	0.232	0.050	78
	SE	0.056	0.033	0.050	0.020	
b	Controls frequency	0.531	0.046	0.380	0.042	464
	SE	0.024	0.011	0.023	0.011	
c	Asthma frequency	0.519	0.006	0.428	0.047	322
	SE	0.028	0.006	0.028	0.012	
Comparisons		χ^2	d.f.	p		
a vs. b vs. c		21.811	6	0.001		
a vs. c		23.760	3	0.000		
		MN ACPI(*A/*nonA)				
		gametic types				total
		*L ^M /*A	*L ^M /non*A	*L ^N /*A	*L ^N /non*A	
a	CD frequency	0.156	0.563	0.050	0.232	78
	SE	0.042	0.057	0.027	0.049	
b	Controls frequency	0.186	0.464	0.068	0.283	148
	SE	0.038	0.046	0.028	0.042	
c	Asthma frequency	0.138	0.374	0.139	0.348	238
	SE	0.030	0.037	0.030	0.037	
Comparisons		χ^2	d.f.	p		
a vs. b vs. c		16.398	6	0.012		
a vs. c		11.530	3	0.012		

Differences in the distribution of gametic types are evaluated by the χ^2 test of independence.

CD patients and controls. All differences are statistically significant. The most significant difference is observed for the pair MN-ADA.

Discussion

The presence of a consistent pattern of association between the systems investigated and the class of Th disease shown is compatible with the hypothesis of an involvement in Th1/Th2 orientation and encourages further investigations. A correlation between the phenotype of the systems studied and immunological parameters that are a direct expression of Th1/Th2 orientation would represent the proof of causal involvement. In this context, it is interesting that no significant association has been found for two polymorphisms (PGM₁ and Ak₁) for which we had no 'a priori' evidence for a possible involvement in Th1/Th2 orientation.

For ADA, ACP1 and Hp, biochemical and observational data suggest a direct involvement in Th1-Th2 orientation. Concerning MN polymorphisms, an indirect involvement appears more likely through an increased susceptibility to bacterial and/or viral infections that have an important role in the clinical manifestation of asthma and CD.

Our data indicate that the effects of Hp and MN are stronger than those of ACP1 and ADA. These differences might depend on specific effects related to the organ involved in the class of disease.

Assuming an involvement in Th1/Th2 balance, additive and epistatic interactions among the systems investigated could be operating. The analyses in table 3 suggest the presence of such interactions. The mechanism may involve local interaction between receptors and other structural components of the cell membrane such as MN or interactions at the biochemical level, e.g. those previously observed between ADA and ACP1 [35].

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