Immunoglobulin allotypes in Crohn's disease in the Netherlands

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SUMMARY An association between Crohn's disease and immunoglobulin allotypes has been reported. Confirmation of this association in other populations would be of particular importance for the study of hereditary factors in Crohn's disease. In the present study we have typed 155 unrelated Dutch patients with Crohn's disease and 450 blood donors for the Gm, Am, and Km markers. No significant differences were found in Gm phenotypes and haplotypes between patients and controls. It therefore seems unlikely that the immunoglobulin allotypes play an important role in the susceptibility of individuals to Crohn's disease.

The aetiology of Crohn's disease is unknown, but individual susceptibility seems to be influenced by genetic and environmental factors.

The high familial incidence, especially in first degree relatives, the rarity of nuclear families with both parents affected, and the high concordance of the disease in monozygotic twins as compared with dizygotic twins, strongly suggest an important role of genetic factors in the causation of the disease.¹ Until now, however, such a factor has not been established unequivocally and consistently. An association between Crohn's disease and immunoglobulin allotypes was described recently by Kagnoff *et al*,² who found that for caucasoids with the Gm phenotype (a,x,f;b,g) the relative risk of developing Crohn's disease was 3.17. The influence of the immunoglobulin allotypes on the incidence of Crohn's disease in a particular population can be estimated with the population attributable risk which is often referred as the aetiologic fraction,³ which takes into account the relative risk and the relative frequency of the factor under study. Further analysis of the data reported by Kagnoff *et al*² gave a population attributable risk of 26%, which means that 26% of the incidence of Crohn's disease can be attributed to the existence of individuals with the described Gm phenotype (a,x,f;b,g) in the general population under study.

The presence of this phenotype could therefore contribute considerably to the susceptibility for Crohn's disease. Confirmation of this association in other populations would be of particular importance for the study of hereditary factors in Crohn's disease. This led us to type 155 Dutch patients with Crohn's disease for the G1m, G2m, G3m, A2m, and Km allotypes, which are polymorphic determinants of immunoglobulins located on the heavy chains of IgG1, IgG2, IgG3, and IgA2, and on the kappa light chains, respectively. The phenotype and haplotype frequencies were compared with those of healthy individuals.

Methods

PATIENTS

One hundred and fifty five unrelated patients suffering from Crohn's disease were bled between July 1981 and July 1982. Most of them were outpatients; the others were investigated during an admission to the ward of the department of gastroenterology. The diagnosis of Crohn's disease was established on the basis of clinical, radiological, and histological criteria.⁴

The mean age at the time of the study was 39 years (range 16-79 years). There were 69 men and 86 women. The site of the disease at time of first referral to the Leiden University Hospital was in the distal part of the small intestine in 38.1% of patients, in both the ileum and the colon in 30.3%, and in the colon alone in 29.7%; the other patients had the

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Received for publication 3 October 1986.

disease at that time in the distal part of the rectum and perianal lesions or only the latter, but during the follow up the disease developed in a way that fitted to Crohn's disease.

CONTROLS

Phenotype and haplotype frequencies were compared with those of 450 Dutch blood donors.

TYPING

Typing was carried out in the Central Laboratory of the Netherlands Red Cross Transfusion Service in Amsterdam for the following markers: G1m(z,a,x,f), G2m(n), G3m(g,b0,b1,b3,b5,s,t,c3,c5), A2m(1,2), and Km(1,3). The notation used for allotypic markers on immunoglobulins follows the recommendations of the World Health Organisation.⁵ Typing was carried out with the conventional agglutination inhibition technique.⁶ The reagents used are described elsewhere.⁷ All samples were tested in two dilutions, 1:20 and 1:60, in microtitre plates. When disturbing antibodies were present, the samples were heated for 10 min at 65°C and retested in three dilutions.⁷

STATISTICAL ANALYSIS

The χ^2 for 2xk and 2x2 contingency tables was calculated according to the principles of Pearson.⁸ The χ^2 value for the 2x2 tables was corrected for continuity according to Yates.⁹

Haplotype frequencies were estimated with the maximum likelihood method, using the computer program MAXLIK.¹⁰

Comparison of haplotype frequencies between patients and controls was done by estimating the standardised normal deviate (Z) from the quotient of the difference in haplotype frequencies of cases and controls and the standard error of the pooled haplotype frequency¹¹ corrected for continuity.⁹

Results

The absolute and relative frequencies of Gm phenotypes are shown in Table 1. The distribution of phenotypes did not differ between patients and controls ($\chi^2=9.48$; df=8; $0.25 < p < 0.5$). The frequencies of haplotypes in patients with Crohn's disease are almost the same as those found in the Dutch controls, as can be seen in Table 2.

No difference ($\chi^2=0.53$; $p>0.05$) was detected between the frequency of the Km(1) allotypic marker in patients (16.1%) and controls (13.3%). For the patients, a relative frequency of 3.2% was found for the A2m(2) allotypic marker and the frequency of this marker in the Dutch population was found to be 4.9%, which is not significantly different ($\chi^2=0.41$; $p>0.05$).

Discussion

To our knowledge only four case control studies have been done to investigate the distribution of immunoglobulin allotypes in Crohn's disease. Three studies including our own have been done independently in Dutch patients suffering from Crohn's disease. Regrettably, the data of the previous study, done in Amsterdam,¹² have not been published in detail, but the report stated that no deviation from control frequencies was observed and in a study done in Groningen¹³ the same conclusion was reached. These three independent studies done in The Netherlands failed to detect any Gm phenotype or haplotype associated with Crohn's disease. This is in contrast with the findings of Kagnoff *et al*² in 68 North American caucasoids of North European ancestry suffering from Crohn's disease, which showed a strong association with Gm phenotype (a,x,f;g,b) and haplotype Gm^{a,x;g}. The antigens G1m(z) and

Table 1 Gm phenotypes in patients with Crohn's disease and blood donors

Gm phenotype G1m;G2m;G3m	Crohn's disease patients (n=155)		Controls (n=450)	
	n	%	n	%
f; n;b	62	40.0	207	46.0
zaf; n;gb	23	14.8	71	15.8
zaxf; n;gb	18	11.6	34	7.6
zaf;...;gb	13	8.4	35	7.8
f;...;b	11	7.1	29	6.4
zax;...;g	7	4.5	16	3.6
zaxf;...;gb	6	3.9	26	5.8
za;...;g	4	2.6	18	4.0
Others	11	7.1	14	3.1

Table 2 Frequency of Gm haplotypes in patients with Crohn's disease and blood donors

Gm haplotype G1m;G2m;G3m	Crohn's disease patients (n=155)		Controls (n=450)	
	HF*	SE	HF	SE
f; n;b	0.4429	0.0331	0.4628	0.0195
f;...;b	0.2465	0.0299	0.2574	0.0177
zaf;...;g	0.1648	0.0219	0.1747	0.0129
zax;...;g	0.1096	0.0185	0.0895	0.0098
Others	0.0361	0.0107	0.0157	0.0042
ΣHF_i	0.9999		1.0001	
χ^2_{df}	1.655 (NS)		3.97 (NS)	

*Haplotype frequency, that is, number of haplotypes divided by twice the number of individuals; $\dagger\chi^2$ value for departure from Hardy-Weinberg equilibrium with 4 degrees of freedom.

G2m(n) were not included in the North American study.

Comparison of the phenotype distribution between patients in North America and Leiden revealed a significant difference in the phenotypic distribution ($\chi^2=12.24$; $df=4$; $p<0.025$). This difference can be attributed to the significantly higher frequency of the Gm phenotype (a,x,f;b,g) in the North American Crohn's patients (38.2 v 16.7%; χ^2 Yates corrected=10.76; $p=0.0014$).

Distortion of the Gm phenotypic distribution can also be explained by selection bias. Selection bias in Kagnoff *et al*'s study could have been introduced by, for example, consanguinity or the inclusion of patients recently given a blood transfusion. It is unclear from this report whether all of their patients were unrelated, and the exclusion criteria of the National Cooperative Crohn's Disease Study do not include blood relationship.¹⁴ This seems to be unlikely, however.

The presence of phenotype Gm (a,x,f;b,g) could be caused by recent blood transfusions, but in that case we would have expected a higher frequency of the Gm(n) and the Km(1) markers in the patients too, and this was not found.

In summary, at present we do not have an adequate explanation for the different results obtained in the USA and in The Netherlands. We have concluded that as immunoglobulin allotypes have not been found consistently associated with Crohn's disease in different populations, this genetic polymorphism seems to be of little value for an understanding of the genetic predisposition of Crohn's disease.

The authors wish to thank Dr W S Volkers of the Department of Human Genetics of Leiden University for providing the computer program MAXLIK, Mr P H van Eede and Mr A M van Leeuwen for excellent technical assistance, and Miss Karlien Kruijer for typing the manuscript.

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