*Short communications***The complement component C4 in black Americans with Type 1 (insulin-dependent) diabetes mellitus**B. Budowle^{1, 4}, J. M. Roseman^{2, 4}, R. C. P. Go^{2, 4}, B. O. Barger^{1, 2, 3, 4} and R. T. Acton^{1, 2, 4}Departments of ¹Microbiology, ²Epidemiology and ³Surgery, and ⁴Diabetes Research and Training Center, University of Alabama in Birmingham, Birmingham, Alabama, USA

Summary. The complement component C4 variants C4A 4, C4B 4 and C4B Q0 were found to be significantly increased in 64 black patients with Type 1 (insulin-dependent) diabetes compared with 169 black control subjects, yielding relative risks of 3.3, 2.9 and 3.4, respectively. The increased frequencies of C4B 4 and C4B Q0 in black Type 1 diabetic patients are

similar to those found in Caucasoid Type 1 diabetic patients. The data suggest that Type 1 diabetes in black Americans may be partially due to admixture of genes from whites.

Key words: Complement component C4, Type 1 diabetes mellitus, black Americans.

Type 1 (insulin-dependent) diabetes mellitus has been shown to be associated with HLA, factor B (Bf) and complement component C4 in various white populations [7, 9, 11, 13, 20, 23]. With the exception of C4 similar findings have been reported for black Americans with Type 1 diabetes [5, 17, 19, 25].

The complement component C4 is determined by genes at two tightly-linked loci, C4A and C4B [1] which are closely linked to Bf and C2 [14] on chromosome 6 and probably lie between the HLA-B and -D/DR loci [4]. Studies of white subjects from the United States [1] have identified seven C4A variants and at least three C4B variants. Furthermore, data suggest that C4 is as polymorphic in the black population as in Caucasoids [6].

While recent studies have shown that C4B4 and C4B2 are significantly increased in Type 1 diabetes in whites [3, 18] and that C4BQ0 is increased in Type 1 diabetes in the Basques [8], at present no information is available on C4 phenotypes in black Type 1 diabetic patients. Studies by Reitnauer et al. [16] and Budowle et al. [5] suggest that the occurrence of Type 1 diabetes in black Americans may be partially due to admixture of genes from whites. Therefore, analysis of the C4 system is of interest, since it may offer additional support to the admixture hypothesis as well as identify an additional susceptibility marker for Type 1 diabetes in the black population. The purpose of this investigation was to determine whether or not there is an association of C4 with Type 1 diabetes in black patients from the southeastern United States.

Materials and methods

Plasma samples from 64 unrelated black Type 1 diabetic patients from the southeastern United States were phenotyped for C4 according to the method of Awdeh and Alper [1] and Awdeh et al. [2]. All patients were under age 40 years at onset of diabetes, insulin-dependent and ketosis-prone. The control sample, previously phenotyped for C4 [6], included unrelated normal black individuals from the same geographical region without a personal or family history of diabetes and with normal fasting blood glucose levels.

Table 1. Distribution of C4 phenotypes in 64 black Americans with Type 1 diabetes

Phenotypes		Number observed	Phenotypes		Number observed
C4A	C4B		C4A	C4B	
A U,Q0,	B 2,1	1	A 3,3,	B 3,1	1
A 5,2,	B 2,1	1	A 3,3,	B 2,1	1
A 4 ^a ,	B 2 ^a	2	A 3,3,	B 1,Q0	3
A 4 ^a ,	B 1 ^a	3	A 3,2,	B 3,Q0	1
A 4,4,	B 4,1	1	A 3,2,	B 1,1	1
A 4,4,	B 2,1	1	A 3,2,	B 1,Q0	1
A 4,Q0,	B 4,1	1	A 3,1,	B 1,1	1
A 4,Q0,	B 2,1	2	A 3,Q0,	B 4,2	1
A 4,Q0,	B 1,1	1	A 3,Q0,	B 4,1	1
A 3 ^a ,	B 4 ^a	1	A 3,Q0,	B 3,3	1
A 3 ^a ,	B 2 ^a	2	A 3,Q0,	B 3,1	2
A 3 ^a ,	B 1 ^a	13	A 3,Q0,	B 2,1	2
A 3 ^a ,	B Q0,Q0	1	A 3,Q0,	B 1,1	13
A 3,3,	B 4,1	4	A 2 ^a ,	B 1 ^a	1

^a either homozygous for that allele or heterozygous with a null allele. These possibilities cannot be differentiated with the present methodology and without family data. U refers to a new and yet unnamed phenotype

Table 2. C4A and C4B phenotypes in patients and control subjects

C4 phenotypes	Patients (n=64)		Control subjects (n=169) ^a		Relative ^b risk	p (uncorrected)
	Number	(%)	Number	(%)		
C4A U ^c	1	1.6	0	0.0	undefined	NS
6	0	0.0	1	0.6	undefined	NS
5	1	1.6	8	4.7	0.3	NS
4	11	17.2	10	5.9	3.3	0.007
3	50	78.1	147	87.0	0.5	NS
2	5	7.8	23	13.6	0.5	NS
1	1	1.6	3	1.8	0.9	NS
Q0	25	39.1	57	33.7	1.3	NS
C4B 4	9	14.1	9	5.3	2.9	0.026
3	5	7.8	14	8.3	0.9	NS
2	13	20.3	42	24.9	0.8	NS
1	55	85.9	152	89.9	0.7	NS
Q0	6	9.4	5	3.0	3.4	0.039

^a Data from Budowle et al. [6]; ^b relative risks were estimated according to Woolf [24]; ^c U refers to a new and yet unnamed phenotype

The relative risks were estimated using Woolf's odds ratio method [24]. The comparisons of C4 phenotypes between black patients and control subjects were made by use of the χ^2 statistic, with Yates' correction where appropriate. A probability level of 0.05 or less was considered significant.

Results

Twenty-eight different C4 phenotypes were observed in the sample of black Type 1 diabetic patients (Table 1). The percentage phenotypic frequencies of the C4A and C4B antigens in patients and controls are shown in Table 2. The C4A 4 frequency is significantly increased in the patients (17.2% versus 5.9%; $p=0.007$) for a relative risk of 3.3. C4B 4 is also significantly increased (14.1% versus 5.3%; $p=0.026$) in this patient population for a relative risk of 2.9. In addition, the significant increase of C4B Q0 (9.4% versus 3.0%; $p=0.039$) results in a relative risk of 3.4.

Discussion

This is the first report of an association of C4 with Type 1 diabetes in black Americans. The genetic associations of C4B 4 with Type 1 diabetes found in whites [3, 18] and C4B Q0 in the Basques [8] also occur in blacks. Furthermore, if confirmed the C4A 4 phenotype may be a unique association with Type 1 diabetes in blacks, although here the association is not significant after correction for the number of comparisons made. Interestingly, of the six patients positive for C4B Q0, five carried the Bf FI allele [5] which supports linkage of C4B Q0 and Bf FI as described in the Basque population [8]. This possibility cannot be confirmed at present because of sample size.

Since C4 is intimately involved with the immune response and host defence mechanisms [10, 15, 18, 23] it has been suggested that it may be causally related to

Type 1 diabetes. Supporting this is the occurrence of the non-functional variant C4A6 [22], and the high frequency of C4 null phenotypes in the population [1, 6]. It is possible that some C4 products may be more or less functional than others and might, therefore, be directly involved in the aetiology of Type 1 diabetes.

Obviously, all patients do not carry C4A 4, C4B 4 and/or C4B Q0, while a portion of healthy controls do possess these risk factors. However, our understanding of the genetics of Type 1 diabetes suggests that the aetiology of this disease is the result of at least two genes. Thus, C4 may be part of an array of closely linked genes within the major histocompatibility complex which are functionally interrelated and may operate as a single gene. Included in this array may be the genes for Bf, C2 and HLA as well as others yet unidentified, leading to the possibility that a combination of altered gene products may be necessary for the development of Type 1 diabetes. However, there is as yet no evidence that C4A 4, C4B 4 and C4B Q0 are directly involved in the aetiology of Type 1 diabetes.

Studies suggest that there is a decreased incidence of Type 1 diabetes in the black American population compared with white Americans [12]. In addition, Type 1 diabetes has been shown to be rare in black Africans [21]. Reitnauer et al. [16] observed that there was a greater degree of racial admixture in the same black patient sample used in this study than seen in healthy black controls. This report adds further support to the racial admixture hypothesis since we found that the genetic association of C4B 4 with Type 1 diabetes is similar in both races, as already reported for HLA [17, 19, 25] and Bf [5].

References

1. Awdeh ZL, Alper CA (1980) Inherited structural polymorphism of the fourth component of complement. *Proc Natl Acad Sci* 77: 3576-3580

2. Awdeh ZL, Raum D, Alper CA (1979) Genetic polymorphism of the fourth component of human complement. *Nature* 282: 205–207
3. Bertrams J, Hintzen U, Schlicht V, Schoeps S (1982) C4: another marker for Type 1 diabetes. *Lancet* 1: 41
4. Bruun-Petersen G, Lamm U, Sorensen JJ, Buskjaer L, Mortensen JP (1981) Family studies of complement C4 and HLA in man. *Human Genetics* 58: 260–263
5. Budowle B, Reitnauer PJ, Barger B, Go R, Roseman JM, Acton RT (1982) Properdin factor B in black Type 1 (insulin-dependent) diabetic patients. *Diabetologia* 22: 483–485
6. Budowle B, Roseman JM, Go R, Louv W, Barger B, Acton RT (1983) Phenotypes of the fourth complement component (C4) in black Americans from the southeastern United States. *J Immunogenetics* 10: 199–204
7. Cudworth AG, Usher N, Woodrow JC (1977) Factor B phenotypes in 'juvenile onset' diabetes. *Diabetologia* 13: 388 (Abstract)
8. deMouzon A, Ohayon E, Ducos J, Hauptmann G (1979) Bf and C4 markers for insulin-dependent diabetes in Basques. *Lancet* 2: 1364
9. Dornan J, Allan P, Noel EP, Laren B, Farid N (1980) Properdin factor B (Bf) allele BfF1 specifies an HLA-B18 diabetogenic haplotype. *Diabetes* 29: 423–427
10. Ferrone S, Pelligrino MA, Cooper NR (1976) Expression of C4 on human lymphoid cells and possible involvement in immune recognition phenomena. *Science* 193: 53–58
11. Kirk RL, Theophilus J, Whitehouse S, Court J, Zimmet P (1979) Genetic susceptibility to diabetes mellitus. The distribution of properdin factor B (Bf) and glyoxalase (GLO) phenotypes. *Diabetes* 28: 949–951
12. LaPorte R, Fishbein H, Drash AL, Kuller H, Schneider BB, Orchard JJ, Wagener DK (1981) The Pittsburgh insulin-dependent diabetes mellitus registry. The incidence of insulin-dependent diabetes in Allegheny County, Pennsylvania (1965–1976). *Diabetes* 30: 279–284
13. Nerup J, Platz P, Ortvad-Anderson O, Christy M, Lynsroe J, Paulsen J, Ryder L, Thomsen M, Staub-Nielsen L, Svejgaard A (1979) HLA antigens and diabetes mellitus. *Lancet* 2: 864–866
14. O'Neill GJ, Yang S, Dupont B (1978) Two HLA linked loci controlling the fourth component of human complement. *Proc Natl Acad Sci* 75: 5165–5169
15. Porter RR, Reid K (1979) Activation of the complement system by antibody-antigen complexes: the classical pathway. *Adv Prot Chem* 33: 1–76
16. Reitnauer PJ, Go R, Acton RT, Murphy CC, Budowle B, Barger B, Roseman JM (1982) Evidence for genetic admixture as a determinant in the occurrence of insulin-dependent diabetes mellitus in US blacks. *Diabetes* 31: 532–537
17. Reitnauer PJ, Roseman JM, Barger B, Murphy C, Kirk K, Acton RT (1981) HLA associations with insulin-dependent diabetes mellitus in a sample of the American black population. *Tissue Antigens* 17: 286–293
18. Rittner C, Bertrams J (1981) On the significance of C2, C4 and factor B polymorphisms in disease. *Human Genetics* 56: 235–247
19. Rodey GE, White N, Frazer TE, Duquesnoy RJ, Santingo JV (1979) HLA-DR specificities among black Americans with juvenile-onset diabetes. *N Engl J Med* 301: 810–812
20. Sachs JA, Cudworth AG, Jaraúemada D, Gorsuch A, Festenstein H (1980) Type 1 diabetes and the HLA-D locus. *Diabetologia* 18: 41–43
21. Seftel H, Schultz E (1981) Diabetes mellitus in the urbanized Johannesburg, Africa. *S Afr Med J* 35: 66–90
22. Teisberg P, Olaisen B, Nordhagen R, Thorsby E, Gedde-Dahl T (1980) A haemolitically non-active C4 gene product. *Immunobiology* 158: 91–95
23. Walsh L, Ehrlich R, Falk J, Cox D, Simpson N (1980) Bf and HLA in Type 1 diabetes. *Amer J Hum Genet* 32(b): 135A
24. Woolf B (1955) On estimating the relation between blood groups and disease. *Ann Hum Genet* 19: 251–253
25. Zeidler A, Loon J, Frasier SD, Kumar D, Penny R, Terasaki P (1980) HLA-DRw antigens in Mexican-American and black American diabetic patients. *Diabetes* 29: 247–250

Received: 26 January 1983
and in revised form: 29 September 1983

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