Clin Exp Immunol 1993; 92:284–287

Association of C4B deficiency (C4B*Q0) with erythema nodosum in leprosy

I. J. T. DE MESSIAS, J. SANTAMARIA, M. BRENDEN*, A. REIS* & G. MAUFF*
Laboratório de Immunopatologia, Departamento de Patologia Médica, Hospital de Clinicas, Curitiba-PR, Brazil, and
**Institut für Medizinische Mikrobiologie und Hygiene, University of Cologne, Cologne, Germany*

(Accepted for publication 25 January 1993)

SUMMARY

A considerable number of studies have postulated significant associations between susceptibility to the different clinical manifestations of leprosy and the MHC. In this investigation, the association between the MHC class III complement proteins C2, BF, C4A and C4B and leprosy in a patient population of Southern Brazil was studied. A total of 109 non-related leprosy patients was investigated; 73 presented with lepromatous leprosy (LL), 46 of them had the immunopathological reaction of erythema nodosum (ENL), the remaining 36 were tuberculoid, borderline and indeterminate leprosy (TIBL) patients. The control group included 172 healthy individuals matched with the patients according to their ethnic and geographical origin. C2, BF, C4A and C4B allotypes were determined by standard technologies including Western blots for C2 and C4 variant alleles with monoclonal and polyclonal antibodies. Non-expressed ('silent') C4 alleles in hemizygously deficient individuals were estimated semiquantitatively on the basis of the C4A and C4B isotype ratio and by the MASC ('minimal chi-square') method. The results showed a significantly elevated presence of the non-expressed C4B allele (C4B*Q0) in the LL and ENL patient groups in comparison with the controls. The most significant difference was observed in the ENL group when compared with the controls. In addition, all patients who were homozygously C4B-deficient had ENL, and most of them had the BF*F1 allele. The comparison between LL patients with and without ENL also showed a statistically significant difference in the presence of C4B*Q0, indicating that C4B deficiency itself is associated with ENL. The relative risk of LL patients with the C4B*Q0 allele suffering from ENL was 5·3 compared with LL patients without C4B*Q0. Since immune complexes (IC) are considered to be the pathogenic cause of ENL, our findings indicate that C4B deficiency may play an important role in the abnormal immune response against *Mycobacterium leprae* and in the lack of IC clearance, leading to ENL reactions. Individuals with this allele seem to be at a higher risk of developing pathological immune reactivity in lepromatous leprosy.

Keywords complement genetics major histocompatibility complex C2 BF C4 lepromatous leprosy erythema nodosum leprosum

INTRODUCTION

Leprosy presents a wide spectrum of clinical manifestations, ranging from polar tuberculoid (TT) cases with mild self-healing lesions to polar lepromatous leprosy (LL) showing chronic, progressive destructive disease. In between the polar forms, the clinically and histologically less characteristic forms of borderline lepromatous (BL), borderline (BB), borderline tuberculoid (BT) and indeterminate leprosy (IL) are to be found [1]. LL is the severe generalized form of the disease, frequently associated with the immunopathological reaction, erythema nodosum leprosum (ENL) in a large number of patients. Although the immunopathogenesis of ENL is not clearly known, it is considered to be an immune complex-mediated reaction.

Many studies have been carried out to demonstrate genetic influence on the susceptibility and clinical manifestations of leprosy. Besides information from classical twin studies [2], significant associations were found for specific MHC class I and class II antigens involving the LL and TT forms, as well as the ENL reactional episode [3–5]. Although a multi-centre study has abrogated clear MHC-association with the disease spectrum of leprosy, a significant association with HLA-DQ1 in the absence of lepromin skin reactivity was found [6].

The MHC includes a class III gene region which among others contains the structural genes for the complement components C2, Factor B (BF), and the two C4 isoproteins C4A and C4B. Their allotypes may be defined by electrophoretic separation of the expressed products and determination of haemoly-

Correspondence: Professor I. T. de Messias, Laboratorio de Immunopatologia, Dep. Patologia Medica, UFPR, Rua Padre Camargo 280, 80060 Curitiba-PR, Brazil.

Table 1. MHC class III alleles in leprosy patients compared with healthy controls

		Patients				
		All LL/TIBL		LL		
Alleles	Controls ($n=172$)	($n=109$)	Total ($n=73$)	With ENL ($n=46$)	No ENL ($n=27$)	TIBL ($n=36$)
C4B*1	161	96 (NS)	62 (NS)	36 (3.9×10^{-3}*)	26 (NS)	34 (NS)
C4B*Q0	20	32 (4.4×10^{-5}*)	26 (2.7×10^{-5}*)	22 (3.6×10^{-7}*)	4 (NS)	6 (NS)
BF*F1	1	4 (NS)	3 (NS)	3 (0·03)	0 (NS)	1 (NS)

All other C4B, factor B and all C4A and C2 alleles showed no significant differences.

Level of significance in Fisher's exact test (in parentheses) against the control group if corrected for the number of observed alleles: * C4B, $P=0.0045$; † BF, $P=0.0122$.

LL, Lepromatous leprosy; TIBL, all other non-lepromatous patients; ENL, erythema nodosum leprosum; NS, not significant.

tic isotype function and relative protein level. In addition, deficiencies of C2 and much more so of the C4 isoproteins occur which may be traced back to gene deletions at the DNA level [7] or non-expression of genes. In contrast to the expressed class III proteins, a limited genetic variability has been observed at the DNA level [8].

Numerous studies have shown significant associations between class III alleles and diseases such as juvenile onset diabetes mellitus, multiple sclerosis, subacute sclerosing panencephalitis, rheumatoid arthritis, and others. Besides the association between C2 and C4 deficiency alleles and immune complex-mediated diseases, such as systemic lupus erythematosus (SLE) [9,10], a more direct involvement of infections with non-expressed C4 alleles, also designated C4*Q0, has recently been postulated from findings in patients with systemic viral, bacterial, and fungal diseases [11–14]. In leprosy, significant associations of the C4A6 and the BF F1 and S07 alleles were observed in Thai and South African Negro patients, respectively, presenting the LL form of the disease [15,16].

In this investigation we studied the association between the MHC class III complement components and leprosy in a patient population of Southern Brazil. We were especially interested in investigating a possible association of non-expressed C4 alleles with ENL.

PATIENTS AND METHODS

Patients

A total of 109 non-related leprosy patients, age range 18–79 years, from the Hospital de Dermatologia Sanitaria, Piraquara, and Centro de Saude Metropolitano de Curitiba, Brazil, were studied. Seventy-three patients presented the LL form, 46 of them had ENL; 12 patients were classified with the TT/BT form, eight with BL, and 16 with the IL form of the disease. By ethnic background, 31·1% were of Caucasoid, 47·7% Mullatos, and 21·1% of Negroid origin. Because of the small number of patients in the TT, IL and BL groups, they were combined as 'TIBL-group'.

The disease classification was done on the basis of clinical and histopathological findings according to Ridley & Jopling [1]. The Mitsuda (lepromin A) skin reactivity and 'bacillary index' were recorded in all patients.

Controls

One hundred and seventy-two non-related healthy Brazilian individuals were used as the control group. These had been matched with the patients according to their geographic and ethnic origin. Thus, the control individuals had the following ethnic background: 35·5% Caucasoids, 48·8% Mullatos, and 15·7% Negroids.

Sera

Venous blood (10 ml) was collected from each subject. The blood was allowed to clot at room temperature and after centrifugation at 4°C the sera were divided into aliquots and stored at −70°C. One aliquot of each sample was transported within a maximum of 6 months by one of us on dry ice to the Institute of Medical Microbiology and Hygiene at the University of Cologne, Germany, where the C2 and C4 typing was performed.

Complement protein allotyping

BF typing was carried out using high voltage agarose gel electrophoresis, followed by immunofixation with anti-human BF antibody (Atlantic Antibodies, Scarborough, ME) [17]. C2 types were determined by isoelectric focusing followed by haemolytic overlay [18] and on Western blots [19].

For C4A and C4B typing, EDTA-added sera were used. Allotypes were determined by high-voltage agarose-gel electrophoresis with neuraminidase and carboxipeptidase B-treated sera, developed by immunofixation with anti-human C4 (Atlantic Antibodies) and in parallel by haemolytic overlay. Some alleles were defined on Western blots using MoAbs against the C4A and C4B isoproteins [20,21]. The estimation of non-expressed C4A and C4B alleles in hemizygous individuals was done by semiquantitative evaluation of the electrophoretic plates and calculation of the minimal chi-square (MASC) method according to the Hardy–Weinberg equilibrium [22,23].

RESULTS

The following C2, BF, C4A and C4B alleles were seen in the patient groups and in the controls: C2*C, B03, and B variants; BF*F, S, F1, SO7; C4A*6, 51, 5, 4, 3, 2, the duplicated C4A allele (3, 2), and the non-expressed C4A*Q0; C4B*5, 4, 3, 2, 12,

Table 2. MHC class III alleles in leprosy patients with erythema nodosum leprosum (ENL) and in other patient groups

	LL		
Alleles	With ENL ($n=46$)	No ENL ($n=27$)	TIBL ($n=36$)
C4B*1	36	26 (0·0456*)	34 (0·058*)
C4B*Q0	22	4 (0·0053*)	6 (0·058*)

All other C4B alleles, and all C4A, factor B and C2 alleles showed no significant differences.

* Level of significance in Fisher's exact test (in parentheses) against ENL patients if corrected for the number of observed alleles: $P=0·0042$.

LL, Lepromatous leprosy; TIBL, all other non-lepromatous patients.

11, 1, 91, 92, 95, the duplicated C4B allele (3, 1), and the non-expressed C4B*Q0. For both investigated groups Hardy-Weinberg equilibrium was seen in the distribution of the alleles for C2 and BF, and for C4A and B for the raw phenotype data and after applying the MASC method. There was no significant deviation between the patient and the control groups for the expressed C4A and C4B alleles. DNA for restriction fragment length polymorphism (RFLP) analysis was not available. However, for the recognition of non-expressed C4 alleles an estimate from relative electrophoretic protein patterns of carboxypeptidase B-treated sera has now been shown to be of comparable reliability [24].

Most strikingly, the results for C4B showed a significantly elevated presence of the non-expressed C4B allele, C4B*Q0, in the LL and ENL patient groups in comparison with the controls ($P<0·00003$ in Fisher's exact test, Table 1). The most significant difference was observed in the ENL group ($P<0·000\,0004$) when compared with the controls. The comparison between LL patients with and without ENL also showed a statistically significant difference for the presence of C4B*Q0 ($P<0·006$, Table 2), indicating that the non-expressed C4B silent allele itself is associated with ENL. Twenty-two of 46 ENL patients possessed the C4B*Q0 allele against four of 27 LL patients without ENL. Most remarkable was the presence of all the C4B homozygously deficient individuals in the ENL group, the majority of these possessing the BF*F1 allele. A decrease of the C4B*1 allele was observed in the ENL group, although this was secondary to the C4B*Q0 increase. C4A*3 was less frequent in the ENL patients, but with no statistical significance. Comparable results for C4B*Q0 were seen in patients with negative lepromin skin reactivity ($P<0·000\,01$). The relative risk of LL patients with the C4B*Q0 allele suffering from ENL was 5·3 compared with LL patients without C4B*Q0. In addition, an increased frequency of BF F*1 was observed in the ENL patients. There was no difference in the distribution of C2 alleles.

DISCUSSION

The mechanisms of natural resistance to *Mycobacterium leprae* are still not well understood. It depends, however, on the ability of macrophages to inhibit the multiplication of the mycobacteria [25]. The presence of positive cellular reactivity against *Myco. leprae* antigens (Mitsuda test) in the skin of most healthy contacts of leprosy patients in endemic areas suggests the existence of a natural resistance mechanism against the disease in individuals having an efficient immune response [25]. The type of clinical manifestation that an individual develops depends on the capacity of his own immune response and this was shown to be under genetic control of the MHC [27].

Evidence for MHC involvement in leprosy was drawn from studies on various patient populations in the world, which showed significant correlation between some HLA class I and class II antigens and the clinical classification of the disease [3,4,27]. Family studies have also been reported on the susceptibility to *Myco. leprae* and the control of clinical expression of the disease by MHC-linked genes [5,27].

With regard to the MHC class III region, complement proteins as C3 activators play an important role in the modulation of the immune response. It is also known that besides the large number of non-expressed C4 alleles, MHC class III allotypes show differences in their functional activities or their plasma levels [10,28]. Whereas it has long been observed that homozygous deficiency of a single complement component might result in a defect of physiological complement action [10,29], hemizygous C2 or C4 deficiencies are known to be associated with immune complex diseases such as SLE or SLE-like pathological reactions [9,30]. Immune complexes (IC) are considered to be the pathogenic cause of erythema nodosum. They are also found in most patients' sera with ENL, and the reactions are associated with systemic involvements similar to those seen in other immune complex diseases. Thus, a deficiency in immune complex solubilization in the sera of these patients was considered to be responsible for ENL [31].

We present here evidence which permits the assumption of a positive association between ENL and the non-expressed C4B allele (C4B*Q0). It increasingly emerges that functional impairment of the C4A isoprotein, which shows higher affinity to amide groups on membranes than C4B [32], might be associated with IC diseases involving viral infectious agents as antigens. Deficiency of the C4B isoprotein with higher binding capacity for thiol groups has been found in association with IC diseases involving bacterial or fungal antigens [11–14]. Considering the critical role of complement in the solubilization and clearance of IC, we conclude that the C4B*Q0 allele may be one important cause of the abnormal immune response against the *Myco. leprae* and of the lack of IC clearance, leading to ENL reactions. Individuals with these alleles seem to be at higher risk of developing pathological immune reactivity in lepromatous leprosy.

ACKNOWLEDGMENTS

Part of this work was supported by a grant of the Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), Brazil.

REFERENCES

1 Ridley DS, Jopling WH. Classification of leprosy according to immunity: a five-group system. Int J Leprosy 1966; **34**:255–73.

2 Chakravarrti MR, Vogel FA. A twin study on leprosy. In: Becker PE, Lenz W, Vogel F, Wendt GC, eds. Topics in human genetics, Vol. 1. Stuttgart: Georg Thieme Verlag, 1973:1–123.

3 Agrewala IN, Ghei SK, Sudhakar KS *et al.* HLA antigens and erythema nodosum leprosum (ENL). Tissue Antigens 1988; **33**: 486–7.

4 Blavy G, Thiam D, Ndoye B *et al.* HLA and leprosy in Dakar: distribution of histocompatibility antigens in leprous patients and their relationship to ENL reactions. Acta Leprol Geneva 1986; **100**:93–99.
5 Van Eden W, de Vries RRP, Mehra NK *et al.* HLA segregation of tuberculoid leprosy: confirmation of the DR2 marker. J Infect Dis 1980; **141**:693–701.
6 De Vries RRP, Serjeantson SW, Layrisse Z. Leprosy. In: Albert ED, Bauer MP, Mayer WR, eds. Histocompatibility testing. Berlin: Springer-Verlag 1984:362–7.
7 Carroll MC, Campbell RD, Bentley DR *et al.* A molecular map of human major histocompatibility complex class III region linking complement genes C4, C2 and factor B. Nature 1984; **307**:237–41.
8 Schneider PM, Carroll MC, Alper CA *et al.* Polymorphism of the human complement C4 and 21 H steroid hydroxylase genes. J Clin Invest 1986; **78**:650–7.
9 Agnello V. Lupus diseases associated with hereditary and acquired deficiencies of complement. Spring Sem Immunopathol 1986; **9**: 161–78.
10 Alper CA, Rosen FS. Inherited deficiencies of complement proteins in man. Spring Sem Immunopathol 1984; **7**:251–61.
11 Schifferli JA, Paccaud JP. Two isotypes of human C4, C4A and C4B, have different structure and function. Complement Inflamm 1989; **6**:19–26.
12 Rowe PC, McLean RH, Wood RA. Association of homozygous C4B deficiency with bacterial meningitis. J Infect Dis 1989; **160**:448–51.
13 Bishof NA, Welch TR, Beischel LS. C4B deficiency: a risk factor for bacteremia with encapsulated organisms. J Infect Dis 1990; **162**:248–50.
14 De Messias IJT, Reis A, Brenden M *et al.* Association of major histocompatibility complex class III complement components C2, BF, and C4 with Brazilian paracoccidioidomycosis. Complement Inflamm 1991; **8**:288–93.
15 Greiner J, Weber FJ, Mauff G *et al.* Genetic polymorphisms of properdin factor B (Bf), the second component (C2), and the fourth component (C4) of complement in leprosy patients and healthy controls from Thailand. Immunobiol 1980; **158**:134–8.
16 Mauff G, Hitzeroth HW, Kleeberg HH *et al.* Complement haplotypes ("complotypes") of BF, C4, and C2 are associated with lepromatous leprosy in Negroid patients. Immunobiol 1983; **164**:274.
17 Geserick G, Abbal M, Mauff G *et al.* Factor B (BF) nomenclature statement. Complement Inflamm 1990; **7**:255–60.
18 Alper CA. Inherited structural polymorphism in human C2: evidence for genetic linkage between C2 and BF. J Exp Med 1976; **144**:1111–4.
19 Uring-Lambert B, Gas S, Goetz J *et al.* Detection of the genetic polymorphism of human C2 (native protein and C2a fragment) by immunoblotting after polyacrylamide gel isoelectric focusing. Complement 1985; **2**:185–92.
20 Doxiadis G, Grosse-Wilde H. C4 allotyping by prolonged gel electrophoresis and immunoblotting using monoclonal and polyclonal antibodies. Complement Inflamm 1990; **7**:269–76.
21 Mauff G, Alper CA, Dawkins R *et al.* C4 nomenclature statement (1990). Complement Inflamm 1990; **7**:261–8.
22 Mauff G. Application of the MHC-Class III complement markers to population genetics. In: Ohayon E, Cambon-Thomson A, eds. Colloque INSERM 142, Paris 1986: 143–66.
23 Clerget-Darpoux F, Babron MC, Prum B *et al.* A new method to test genetic models in HLA associated diseases: the MASC method. Ann Hum Genet 1988; **52**:247–59.
24 Plum G, Siebel E, Bendick C *et al.* Host genetic factors in HIV related disease: a comparative study of major histocompatibility complex alleles in AIDS Kaposi's sarcoma patients. In: Schauzu M, ed. Progress in AIDS research in the Federal Republic of Germany. München: Medizin Verlag 1990:93–98.
25 Sansonetti P, Lagrange PH. The immunology of leprosy: speculations on the leprosy spectrum. Rev Infec Dis 1981; **3**:422–69.
26 Smith DG. The genetic hypothesis for susceptibility to lepromatous leprosy. Hum Genet 1979; **50**:163–77.
27 De Vries RRP, Ottenhoff THM, van Schooten WCA. Human leukocyte antigens (HLA) and mycobacterial disease. Spring Sem Immunopathol 1988; **10**:305–18.
28 Isemann DE, Young IR. The molecular basis for the difference in immune hemolysis activity of the Chido and Rodgers isotypes of human complement component C4. J Immunol 1984; **132**:3019–27.
29 Lachmann PJ. Inherited complement deficiencies. Phil Trans R Soc Lond B 1984; **306**:419–30.
30 Hauptmann G, Schifferli J. Inherited deficiency of the fourth component of human complement. Immunodef Rev 1988; **1**:3–22.
31 Chakrabarty AK, Kashyap A, Sehgal VN *et al.* Solubilization of preformed immune complexes in sera of patients with type 1 and type 2 lepra reactions. Int J Lepra Mycobact Dis 1988; **56**:559–65.
32 Rittner C, Notghi A, Gutfreund K *et al.* The significance of C4A deficiency and HLA-DR allotypes for HIV-1 seroconversion. In: Schauzu M, ed. Progress in AIDS research in the Federal Republic of Germany. München: Medizin Verlag 1990:87–92.