CHAPTER IV

RESULT

1. C4 Phenotypes

The common C4 patterns produced from electrophoresis of neuraminidase-treated EDTA-plasma sample and immunoblotting with anti C4 are shown in Figure 10. Each C4 allotype consists of three bands, usually with one major (stronger anodal band) and two minor (weaker cathodal band) bands. Generally, The C4A alleles migrate more anodally than do C4B alleles but there is considerable overlap in the zone of migration of rare variant of the two loci. The pattern of C4 allotypes that carry a single null allele at either C4A or C4B locus, heterozygous C4 null allele, are shown in Figure 11. In this study, this could be identified from the relative densities of C4A and C4B bands.

Although neuraminidase treatment of plasma samples has improved resolution of C4 allotypes, one major problem in assigning pattern obtained on agarose gel electrophoresis is that each allotype gives three bands. When several allotypes are present in an individual (Figure 10) the multiple bands overlap and interpretation becomes difficult. In order to solve this problem the samples were treated with neuraminidase and carboxypeptidase B enzyme, yielding the simplified pattern of one single sharp, distinct band for each C4 allotypes (Figure 12). By this approach, identification of a hidden null allele is possible. As shown in Figure 13 , there is only one single C4A band in lane 3, the intensitity of which is less than that

of C4B allotype on the same lane and that of any other C4A bands in other lanes. The C4 allotypes in lane 3 was thus identified as C4A 4,QO B 2,1.

2. Detection of C4A and C4B Variants by a Functional Hemolytic Assay.

In the assignment of rare C4 variants distinguishing C4A from C4B products, it is necessary to perform hemolytic function assay. Bands of hemolysis was seen on the neuraminidase-treated sample showing almost exclusively C4B types.

3. Frequency of C4 Allotypes

3.1 Frequency of C4 Allotypes in Thai Populations.

The distribution of C4 allotypes in Thai normal subjects have been shown in Table 6. C4A3 and C4B1 are the most common C4A and C4B allotypes. The frequencies of phenotype and genotype being 0.7 and 0.452 for C4 A3 and 0.69 and 0.443 for C4 B1 respectively.

3.2 Frequency of C4 Null Allele in Thai Populations.

In 14 of the 100 normal subjects there were clear evidence of C4A*QO with C4A : B optical density ratios of 0.6 or less. Thus, the frequency of C4A*QO, the phenotype and genotype for C4A*QO are 14% , 0.14 and 0.0727 respectively (Table 6). There were one homozygous C4 A null allele (C4A*QO,QO) in normal subjects.

In 7 of the 100 normal subjects, C4B*QO could be assigned. The frequency of C4B*QO, the respective phenotype and genotype are 7%,

0.07 and 0.036 (Table 6). There were 2 homozygous C4B null allele (C4B*QO,QO) in normal subjects.

3.3 Frequency of C4 Allotypes in SLE Patients

As shown in Table 7, the common C4A and C4B allotypes in SLE patients are also C4A3 and C4B1 , the phenotype and genotype frequencies being 0.711 and 0.462 for C4A3; and 0.789 and 0.541 for C4B1 respectively.

3.4 Frequency of C4 Null Allele in SLE Patients

The frequencies of C4 null allele in SLE patients was shown in Table 7. Null alleles of C4A and C4B were found in 35.5% and 14.5% of patients with respectively gene frequency of 0.197 and 0.075. As shown in Table 8, there was a statistically significant increase of C4A null allele (C4A*QO) in SLE patients (35.5%) compared with controls (14%), ($p = 0.0015$, relative risk 3.38).

In contrast, there was a slight increase although no statistically significant in C4B*QO in SLE patients (14.5% versus 7% in controls ,$p = 0.17$).

No homozygous C4 A null allele has been found and, there was only one C4B null allele (C4B*QO,QO) in SLE patients.

3.5 Frequency of C4 Allotypes in RA Patients

C4 allotype frequencies in RA patients are shown in Table 9. C4A3 and C4B1 remain the common allotypes in RA patients with

phenotype and genotype frequencies of 0.735 and 0.485 for C4A3 and 0.853 and 0.616 for C4B1 respectively. As shown in Table 10. the frequency of C4B21 and C4B4 were increased in rheumatoid arthritis patients (8.8%, p = 0.17, R.R. = 3.13 and 2.9%, p = 0.44, R.R. = 3.00 respectively). The frequency of C4B2 was significantly decreased in rheumatoid arthritis (29.4% versus 53% , p = 0.029). Neither rare variants of C4B allotype such as C4B*29 , nor significant increase of C4A or C4B null alleles were observed.

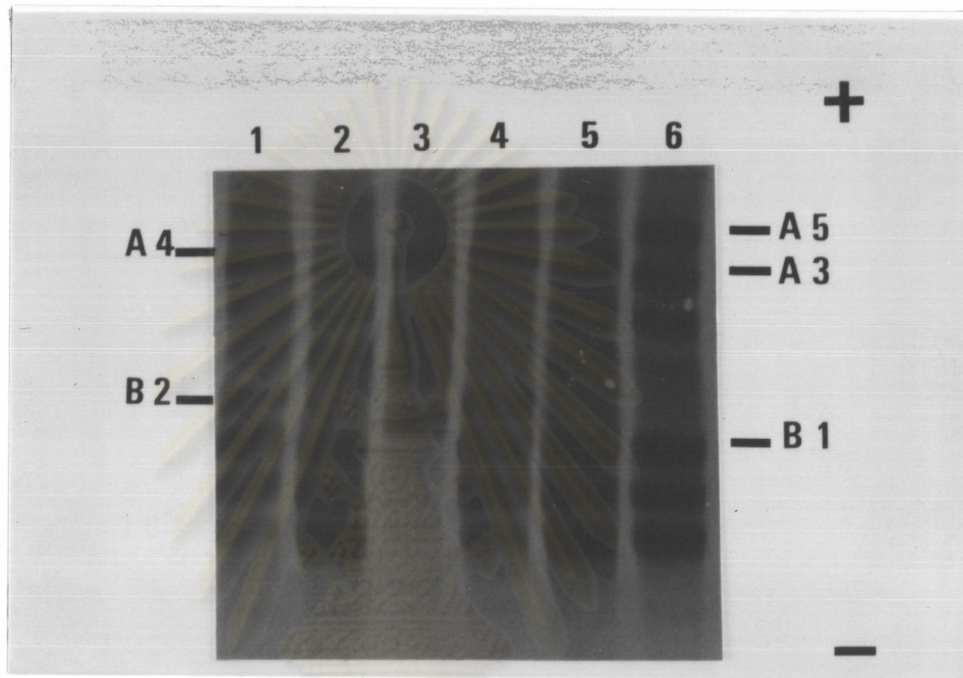

Figure 10 C4 eletrophoretic patterns of neuraminidase-treated EDTA plasma from reference plasma selected to illustrated the diversity of variants. The C4 allotype determined from these patterns are : lane 1 C4 A4,3 B2,1 ; lane 2 A3,3 B1,1 ; lane 3 A3,3 B5,5 ; lane 4 C4 A4,3 B2,1 ; lane 5 A3,3 B1,1 ; lane 6 A5,3 B1,1.

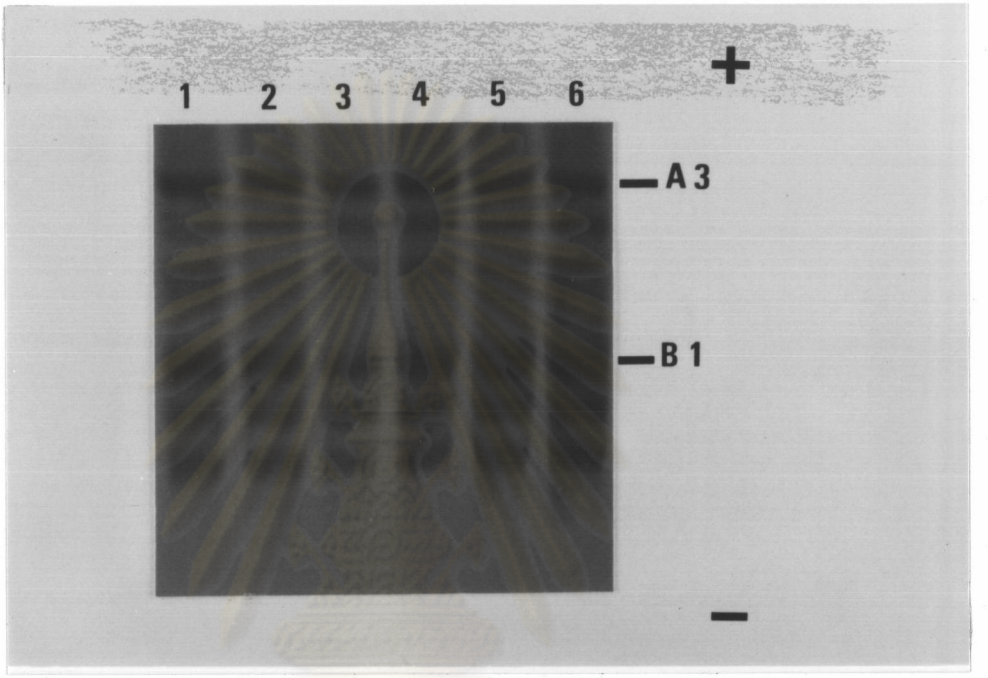

Figure 11 C4 electrophoretic patterns of neuraminidase-treated EDTA plasma (reference), that carry C4 null allele at either C4A or C4B locus. The C4 allotype determined from these patterns are ; lane 1,2,3 = C4A 3,QO B1,QO (reference) ; lane 4,5,6 = C4A 3,QO B1,1 (Reference)

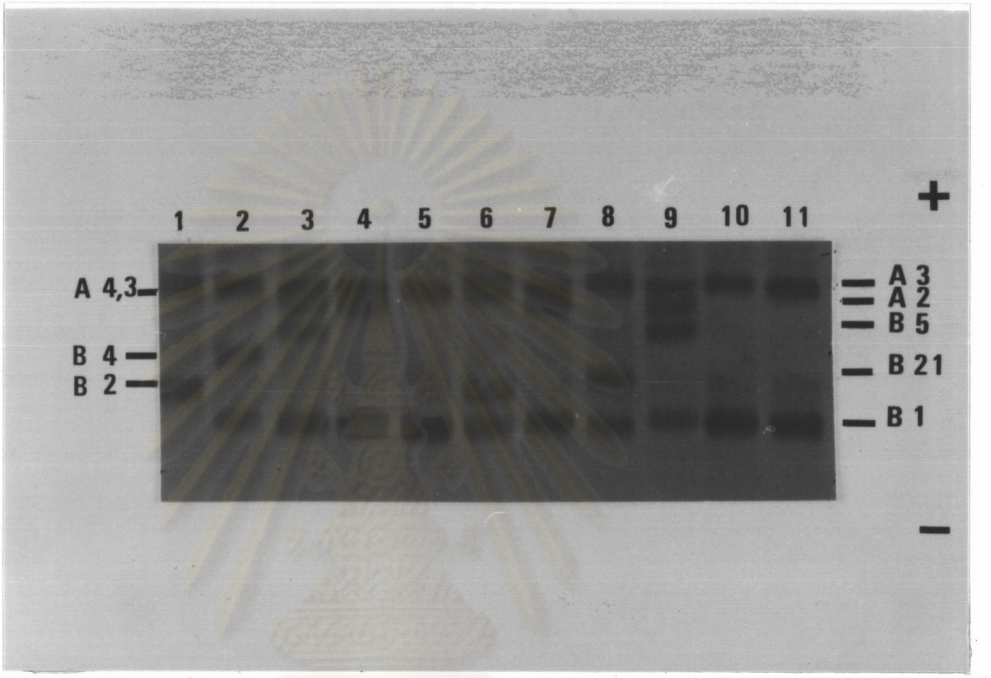

Figure 12 C4 electorphoretic patterns of neuraminidase and carboxypeptidase-treated plasma. This pattern is a single, sharp distinct band for each C4 allotypes. The position of each C4 variant has been shown.

1. A 4,3 B 2,2
2. A 3,3 B 4,1
3. A 3,2 B 5,1
4. A 3,2 B 1,1
5. A 3,3 B 1,1 (Reference)
6. A 4,3 B 2,1
7. A 3,2 B 1,1
8. A 4,- B 21,1
9. A 3,2 B 5,1
10. A 3,Q0 B 1,1
11. A 3,3 B 1,1 (Reference)

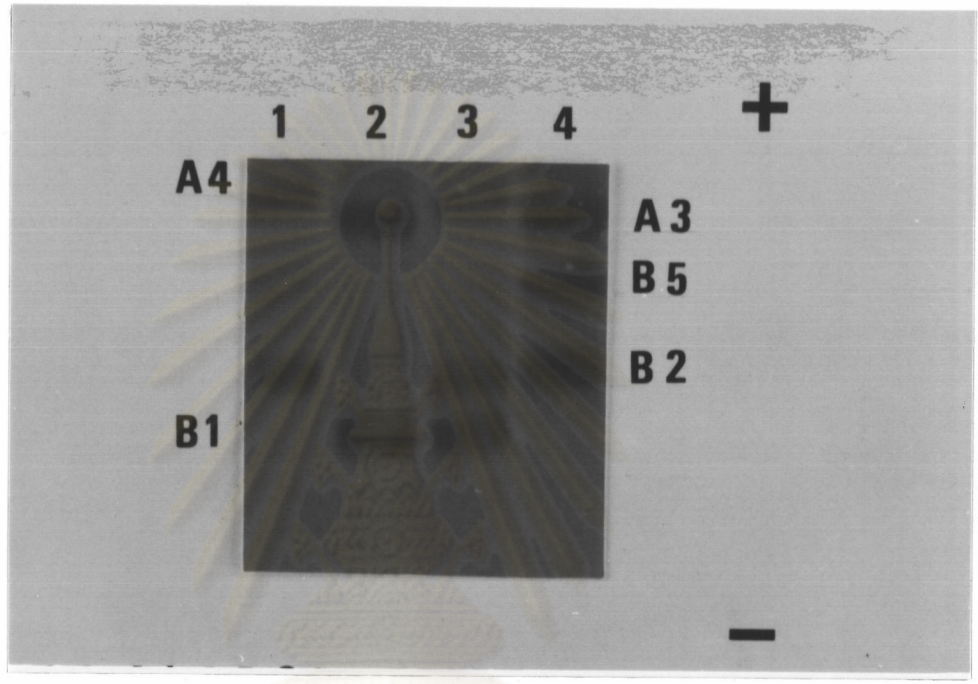

Figure 13 C4 electrophoretic pattern of neuraminidase-carboxypeptidase treated EDTA plasma from individuals selected to illustrate the different intensity of C4A and C4B of heterozygous null allele. The C4 allotype determined from these pattern are : lane 1 C4 A 4,4 B 2,2 ; lane 2 : C4 A 3,3 B 1,1 ; lane 3 : C4 A4,QO B2,1 ; lane 4 : C4 A 3,3 B 5,2

Table 6. C4 Phenotype and Genotype Frequencies of 100 Thai Normal Subjects.

Allotype	Subjects (n)	Phenotype frequencies	Genotype frequencies
C4 A 1	0	0.00	0.000
2	10	0.10	0.051
3	70	0.70	0.452
4	40	0.40	0.225
5	0	0.00	0.000
6	5	0.05	0.254
QO	14	0.14	0.073
C4 B 1	69	0.69	0.443
2	53	0.53	0.315
21	3	0.03	0.015
3	1	0.01	0.005
4	1	0.01	0.005
5	12	0.12	0.062
QO	7	0.07	0.036

Table 7. C4 Phenotype and Genotype Frequencies of 76 SLE Patients.

Allotype	Subjects (n)	Phenotype frequencies	Genotype frequencies
C4 A 1	0	0.000	0.000
2	4	0.053	0.027
3	54	0.711	0.462
4	26	0.342	0.189
5	1	0.013	0.006
6	1	0.013	0.006
QO	27	0.355	0.197
C4 B 1	60	0.789	0.541
2	37	0.486	0.284
21	4	0.053	0.027
3	0	0.000	0.000
4	0	0.000	0.000
5	8	0.105	0.054
QO	11	0.145	0.075

Table 8. Frequency of C4 allotypes in SLE and Controls.

Allotype	% Patients (n=76)	% Controls (n=100)	X^2	P-value	R.R.
C4 A 1	0	0	-	-	-
2	5.3	10	0.76	0.384	0.50
3	71.1	70	0.00	0.987	1.05
4	34.2	40	0.40	0.529	0.78
5	1.3	0	-	0.431^F	-
6	1.3	5	-	0.182^F	0.25
QO*	35.5	14	10.03	0.0015	3.38
C4 B 1	78.9	69	1.70	0.191	1.68
2	48.6	53	0.17	0.678	0.84
21	5.3	3	0.14	0.710	1.80
3	0	1	-	-	-
4	0	1	-	-	-
5	10.5	12	0.00	0.947	0.86
QO	14.5	7	1.88	0.170	2.25

* = Statistical significance

F = Fisher exact test

Table 9. C4 Phenotype and Genotype Frequencies of 34 RA Patients.

Allotype	Subjects (n)	Phenotype frequencies	Genotype frequencies
C4 A 1	0	0.000	0.000
2	5	0.147	0.076
3	25	0.735	0.485
4	10	0.294	0.159
5	0	0.000	0.000
6	1	0.029	0.015
QO	6	0.176	0.092
C4 B 1	29	0.853	0.616
2	10	0.294	0.159
21	3	0.088	0.045
3	0	0.000	0.000
4	1	0.029	0.015
5	4	0.118	0.061
QO	4	0.118	0.061

Table 10. Frequency of C4 Allotypes in RA Patients and Control.

Allotype	% Patients (n=34)	% Controls (n=100)	X^2	P-value	R.R.
C4 A 1	0	0	-	-	-
2	5	10	0.19	0.662	1.55
3	73.5	70	0.03	0.862	1.19
4	29.4	40	0.81	0.369	0.63
5	0	0	-	-	-
6	2.9	5	-	0.522^F	0.58
QO	17.6	14	0.06	0.812	1.32
C4 B 1	85.3	69	2.65	0.103	2.61
2	29.4	53	5.20	0.029^S	0.37
21*	8.8	3	-	0.17^F	3.13
3	0	1	-	-	-
4*	2.9	1	-	0.44^F	3.00
5	11.8	12	0.07	0.79	0.98
QO	11.8	7	0.26	0.61	1.77

F = Fisher exact test

S = Significant

* = increase in RA patients