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Systemic Lupus Erythematosus, Complement Deficiency, and Apoptosis

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I. Introduction

There are many links between the complement system and the autoimmune disease systemic lupus erythematosus (SLE). Soon after the identification of antinuclear antibodies, the major serological hallmark of the disease, it was discovered that complement proteins are deposited in the tissues of patients. An association was found between the degree of complement activation in blood samples from patients and the level of disease activity. Complement proteins were discovered to be co-located with antibodies in inflamed tissues, such as the glomeruli of patients with glomerulonephritis. These data suggested that the formation or deposition of immune complexes in tissues leading to complement and leukocyte activation could cause the pathogenesis of the tissue injury of SLE.

However, it was also discovered that the presence of antibodies and complement in tissues was not sufficient to cause inflammatory injury. Clinically normal tissues from patients with SLE (e.g., the skin) also contained deposited antibodies and complement proteins. Indeed, the presence of these proteins at the dermoepidermal junction in nonlesional skin was found to be a moderately specific diagnostic test for SLE, named the lupus band test.

As the spectrum of autoantibodies characterized in SLE increased, it was discovered that about one third of patients with the disease have autoantibodies to complement proteins, especially to C1q. A reduction was also discovered in the expression of a complement receptor on erythrocytes, CR1, which plays a role in binding immune complexes in the circulation.

Each of these links between complement and SLE, which we discuss in detail below, may be explained on the basis that the autoantibodies of SLE, on binding autoantigen, activate complement. Downstream events following complement activation could explain the development of autoantibodies to complement and to erythrocyte CR1 consumption.

However, the links between complement and SLE are much more complicated and curious. It was found that inherited complement deficiency is strongly associated with the development of SLE. Indeed, the very rare inherited homozygous deficiency of the first protein of the classical

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pathway of complement, C1q, is almost invariably associated with the development of severe disease. Thus, on the one hand, complement deficiency causes SLE. On the other, SLE causes complement consumption and tissue injury. Even more surprisingly, C1q deficiency causes SLE, yet SLE commonly causes autoantibodies to C1q to develop.

In this chapter, we attempt to resolve these paradoxes and, in doing so, discuss each of the associations between abnormalities of complement and SLE. First, we describe and tabulate the links between complement deficiency and SLE. Second, we describe some of the phenotypic abnormalities in mice with engineered mutations in complement genes. Third, we review the links between complement activation and inflammation in SLE. Fourth, we describe the autoantibody response to complement proteins and the significance of these autoantibodies. Finally, we develop the hypothesis that a major physiological activity of the complement system is to promote the clearance of autoantigens and mask them from the immune system. If this activity is deficient, in the presence of other disease susceptibility genes, autoantigens may drive a pathological autoantibody response leading to the development of SLE.

II. Description of the Associations between Complement and SLE in Humans

A. Genetic Deficiencies of Complement and SLE

1. Homozygous Classical Pathway Deficiency

Homozygous hereditary deficiency of each of the classical pathway components (C1q, C1r, C1s, C4, and C2) is associated with greatly increased susceptibility to SLE. There is a hierarchy of severity and susceptibility to the development of disease according to the position of the deficient complement protein in the activation sequence of the classical pathway of complement. The primary publications reporting these associations are cited in Tables I–V, which tabulate the reported cases of deficiencies of the classical pathway proteins and C3.

Thus, 39 of the 42 (93%) described individuals with homozygous C1q deficiency had SLE, which was frequently very severe. Next in the hierarchy comes C1r and C1s deficiency (usually combined) [SLE prevalence: 8 of 14 subjects (57%)], then C4 deficiency [SLE prevalence: 18 of 24 subjects (75%)]. There is then a significant step change in the strength of the association of SLE with deficiency of the next protein in the classical pathway, C2. Deficiency of C2 is the most common hereditary complement deficiency in western European white populations and is associated with the development of SLE in ~10% of cases. Finally, there is C3 deficiency, which, although strongly associated with the development of rashes and

glomerulonephritis, is typically not associated with the development of lupus autoantibodies.

These clinical observations strongly suggest that there is a physiological function of the classical pathway of complement activation that protects against the development of SLE. Furthermore, the hierarchy of susceptibility and severity of lupus, according to the missing classical pathway protein (C1q > C4 ⩾ C2), suggests that an activity of the early part of the classical pathway plays a key protective role against the disease. We now review the associations between inherited homozygous complement deficiency and SLE in detail. We consider each protein in turn and tabulate the published associations of hereditary complement deficiency and SLE, with the exception of C2 deficiency, for which the abundance of published cases would make a table summarizing all the case histories too cumbersome.

2. C1q Deficiency

Thirty-nine of the 42 recorded patients with homozygous C1q deficiency have developed a clinical syndrome similar to SLE (reviewed in Walport *et al.*, 1998). These cases are summarized in Table I. In the affected patients, rash occurred in 37, glomerulonephritis in 16, and cerebral disease in 8. Antinuclear antibodies were reported in 24 of the 35 patients tested, and antibodies to extractable nuclear antigens were present in 15 of 24 patients assessed. Notably, the incidence of anti–double-stranded DNA antibodies was low; only 5 of the 25 patients tested were positive. No clinical phenotype has been observed among any of the heterozygous C1q-deficient relatives of the homozygous deficient subjects.

Among C1q-deficient individuals, further analysis of their complement profile typically showed raised levels of C3 and C4 and elevated C1 inhibitor activity compared with serum samples from normal subjects. This demonstrates that in normal subjects there is significant physiological turnover of the classical pathway resulting from C1 activation. In the absence of C1 function, C4 and C3 levels, together with C1 inhibitor, the inhibitor of activated C1r and C1s, are elevated in concentration due to reduced turnover.

The molecular basis of C1q deficiency has been characterized in 12 families and is tabulated in Table I. Three genes, organized in tandem, encode C1q. In each family studied, a single mutation affecting one of these three genes has been found and mutations in all three genes have been characterized in different families. In every case, no functional C1q activity was detected. However, in some families, no C1q protein was detected, while in others, antigenic C1q which showed no functional activity could be detected.

TABLE I
HOMOZYGOUS C1q DEFICIENCY

Family	Age at Onset (yr), Gender, Race/ Ethnic Group	Clinical Features	Laboratory Tests	Notes	References
	Absent C1q				
1	37, M, Japanese	Discoid rash, erythema multiforme on soles and palms	ANA^+ 1/20, DNA^+, LE^-, ENA^+ (RNase resistant); IF lesional skin: negative for Ig and complement; 0.4 μg/mL C1q detected	FFP infusions: rash improved at 10 days, worse by 1 mo Brother died of SLE at age 10	Nishino *et al.* (1981)
2	5, F, Japanese	SLE, glomerulonephritis; died at age 6	ANA^-, DNA^-, latex positive, RNP^+		Komatsu *et al.* (1982), Orihara *et al.* (1987)
3	10, M, Turkish parents, consanguineous	Deformed fingernail and toenail with monilia, hyperkeratotic desquamative rash, fits, mouth monilia and aphthae, otitis media; died of septicemia at age 10	ANA^-, DNA^-, $latex^-$. ENA^-, anti–smooth muscle positive, anti-$HBsAg^+$; skin IF: positive for IgG and C3; necropsy: MCGN	Response to plasmapheresis and FFP in 10 days Genetic analysis of the family: C → T transition at position 186 of the A chain, Gln to stop codon, no DNA available from propositus	Berkel *et al.* (1977, 1979), Loos *et al.* (1980), Petry *et al.* (1997a)
4a	9, M, Spanish (Canary Islands)	Rash, hair loss, MPGN, Rothmund–Thompson syndrome (poikiloderma congenitale), bilateral posterior cataract	ANA^-, DNA^-, latex positive 1/640; renal IF: positive for IgG, IgM, C3, and C3PA		Mampaso *et al.* (1981)

4b	8, F, ?	Rothmund–Thompson syndrome, bilateral posterior cataract, rash, hematuria, MPGN	ANA$^+$ 1/80, DNA$^-$, latex positive 1/640; renal IF: positive for IgG, IgM, C3, and C3PA		
4c	5, M, ?	Rothmund–Thompson syndrome, bilateral posterior cataract, rash, hematuria, MPGN	ANA$^+$ 1/30, DNA$^-$, anti–smooth muscle positive 1/30, latex positive 1/80; renal IF: positive for IgA, IgG, IgM, C3, and C3PA		
5	29, M, Japanese	Discoid lupus	ANA$^-$, DNA$^-$, ENA$^-$		Nagaki *et al.* (1982), Orihara *et al.* (1987)
6	21, M, Japanese	Itchy, spreading rash on upper limbs and shoulder; leukopenia; discoid lupus	ANA$^-$, LE$^-$, latex negative		Uenaka *et al.* (1982)
7	1, F, Greek, white	Light-sensitive cutaneous vasculitis since 1 yr of age, mild alopecia, Raynaud's phenomenon, hyperkeratotic and atrophic skin, aphthous ulceration	ANA$^-$, LE$^-$; renal biopsy: mild mesangial proliferation; renal IF: capillary IgG, mesangial IgM and C3; skin IF: positive for IgG, IgM, C3, and C5		Minta *et al.* (1982)
8a	1, M, Yugoslav, white	Purulent otitis media, febrile then nonfebrile convulsions, rash on palms and soles, photosensitivity, "butterfly" rash, MPGN; died at age ?	ANA$^+$ 1/80 speckled, DNA 94% (<30%), anti-Sm$^+$, anti-RNP$^+$, anti-Ro$^+$, latex positive 1/40; IgG_2 deficiency	Transient improvement following plasma exchange and FFP Deletion of C at codon 43 in the C chain	Mikuska *et al.* (1983), Slingsby *et al.* (1996)

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TABLE I (*Continued*)

Family	Age at Onset (yr), Gender, Race/ Ethnic Group	Clinical Features	Laboratory Tests	Notes	References
8b	3, M, Yugoslav, white	"Butterfly" rash, cutaneous vasculitis on palms and toes, fever	ANA^+ 1/80 speckled; lupus band test: IgM^+		
9	13, F, Saudi Arabian	Grand mal seizures at ages 13 and 15; meningitis, polyarthritis, fever, extensive rash, alopecia, oral thrush, leukopenia, thrombocytopenia	ANA^+ 1/16, DNA^-, ENA^-; lupus band test: positive for IgG, IgM, and C3; skin biopsy: hyperkeratosis, basal vacuolation		Steinsson *et al.* (1983)
10	7, F, Turkish	Rash: crusted lesions on erythematous base; died of meningitis at age 9	ANA^+, DNA^-, ENA^-, LE^+, latex negative; skin IF: C3 at dermoepidermal junction		Berkel *et al.* (1981)
11a	4, F, Turkish	Malar rash, facial swelling, stomatitis, extensive macular eruption with erythema and desquamation; died of sepsis at age 6	ANA^+, DNA^-, ENA^-, LE^+, cryoglobulins negative; skin IF: IgG, IgM, and C3 in vessel walls	Abnormal immune response to T cell–dependent antigens	Berkel (1993), Berkel *et al.* (1981)
11b	6, F, Turkish	Facial and truncal edema, hematuria	ANA^-, antimitochondrial negative	C → T transition at position 186 of the A chain, Gln to stop codon	Petry *et al.* (1997a)

12a	?, M, Slovakian	Malar rash, arthritis, photosensitivity, necrotizing vasculitis, recurrent bacterial infections		C → T transition at position 6 of the A chain, Gln to stop codon	Petry *et al.* (1995), Toth *et al.* (1989)
12b	?, M, Slovakian	Malar rash, diffuse discoid lupus, arthritis, pericarditis, photosensitivity, vasculitis, alopecia, recurrent bacterial infections		C → T transition at position 6 of the A chain, Gln to stop codon	
12c	?, F, Slovakian	SLE-like syndrome, recurrent bacterial infections		C → T transition at position 6 of the A chain, Gln to stop codon	
13	9, F, white	Widespread rash, alopecia, photosensitivity, onychomycosis, fits, cerebral atrophy, basal ganglion calcification, cytomegalovirus retinitis; died at age 28	ANA^+ 1/2560, anti-RNP^+, anti-Sm^+, anti-Ro^+; latex positive 1/160, DNA^-; skin IF: positive for IgG, IgM, and C3 at the epidermal basement membrane	C → T transition at position 41 of the C chain, Arg to stop codon	Bowness *et al.* (1994), Slingsby *et al.* (1996)
14a	5, M, Turkish parents, unrelated	Erythematous desquamated skin lesions, aphthae, otitis media, bronchopneumonia; died of renal failure at age 9	ANA^-, DNA^-, ENA^-, latex negative, cryoglobulins negative, progressive renal failure and DNA^+; renal biopsy: "consistent with SLE"	No response to FFP therapy No DNA available	Berkel *et al.* (1997)
14b	?, F, Turkish	Asymptomatic at age 22		C → T transition of the A chain, Gln to stop codon	

(*continues*)

TABLE I (*Continued*)

Family	Age at Onset (yr), Gender, Race/ Ethnic Group	Clinical Features	Laboratory Tests	Notes	References
15a	3, F, Turkish	Arthralgia, photosensitive rash, oral monilia and aphthae, deformed finger and toe with monilia	ANA$^-$, DNA$^-$, latex positive 1/80, anti-Ro$^+$, ACA IgG$^+$ high titer but ACA IgM$^-$; renal biopsy: MPGN	No response to FFP or IVIG therapy Normal immune response to hepatitis B vaccine C → T transition of the A chain, Gln to stop codon	Topaloglu *et al.* (1996)
15b	15, F, Turkish	Photosensitive rash, 2 episodes of macroscopic hematuria, IgA nephropathy associated with MPGN	ANA$^-$, DNA$^-$, ENA$^-$, latex negative, ACA IgG weakly positive but ACA IgM$^-$, renal IF: mesangial IgA, IgM, C3, and C5b-9	Normal immune response to hepatitis B vaccine C → T transition of the A chain, Gln to stop codon	
16	16, F, Pakistani parents, consanguineous	Vasculitic rash, recurrent urinary tract infections, cerebral lupus	ANA$^-$, DNA$^-$, ENA$^-$, latex negative	Clinical improvement with weekly FFP infusions administered for 8 wk	Unpublished case
Dysfunctional C1q detected					
17a	4, M, Pakistani parents, consanguineous	Fever, MCGN, discoid facial lesions; died at age 8	ANA$^+$, DNA$^+$, latex positive; renal IF: positive for IgG, IgM, C3, and C5 but negative for C1q and C4	Point mutation resulting in premature stop codon in the B chain	McAdam *et al.* (1988), Reid and Thompson (1983), Thompson *et al.* (1980)

17b	1.5, F, Pakistani	Vasculitic rash on digits, face, and trunk; oral ulcers; pneumonia; died at age 7	ANA^+, DNA weakly positive, latex positive	Mucocutaneous lesions improved with thalidomide	
17c	0.5, F, Pakistani	Vasculitis with oral and facial lesions; died at age 2.5	ANA^+, DNA^- initially, no subsequent tests performed		R. Thompson (personal communication)
18a	?, M, Moroccan parents, consanguineous	Asymptomatic at age 42			Chapuis *et al.* (1982), Meyer *et al.* (1985), Petry *et al.* (1997b)
18b	3, M, Moroccan	Rash, widespread discoid lupus at age 15, thrombocytopenia (45×10^9/L), growth retardation	ANA^+, DNA weakly positive, anti-Ro^+; skin biopsy: lymphocytic infiltration of the upper epidermis with exocytosis; skin IF: positive for IgG and IgM but $C3^-$	Gly to Asp at position 15 of the B chain	
18c	16, F, Moroccan	Subacute cutaneous lupus erythematosus, arthralgia	ANA^+ 1/160, DNA^-, anti-Ro^+	Gly to Asp at position 15 of the B chain	
18d	23, M, Moroccan	Subacute cutaneous lupus erythematosus	ANA^+ 1/320, anti-Sm^+	Gly to Asp at position 15 of the B chain	
19a	7, F, Dutch, white	Glomerulonephritis at ages 7 and 20: fever, aphthae, "butterfly" rash, alopecia, myositis, grand mal seizure, somnolence; died of sepsis at age 20	ANA^+, DNA^-, anti-RNP^+, latex positive, LE^-; skin IF: positive for IgG and C3; renal IF: granular IgA, IgG, IgM, and C3		Hannema *et al.* (1984)
19b	4, F, Dutch, white	Nephritis at ages 4 and 23: discoid rash, arthralgia, alopecia, fever, "butterfly" rash, aphthae	ANA^+ 1/20,000, DNA^-, anti-RNP^+, latex positive, LE^-, leukopenia		

(*continues*)

TABLE I (*Continued*)

Family	Age at Onset (yr), Gender, Race/ Ethnic Group	Clinical Features	Laboratory Tests	Notes	References
19c	42, M, Dutch, white	Membranous glomerulonephritis	ANA$^+$ 1/80; renal IF: positive for IgA, IgG, and IgM but negative for C1q and C3		
20	6, F, German	Photosensitive rash, facial erythema, arthritis, Libman–Sacks endocarditis, fits and psychosis, peritonitis, MCGN, pneumonitis, generalized skin lesions; died at age 29	ANA$^+$ high titer, DNA$^+$, anti-Sm$^+$	150-kDa C1q molecule found comprising a single structural subunit containing two A chain–B chain dimers and a C–C chain dimer G to A at position 6 of the C chain, Gly to Arg	Kirschfink *et al.* (1993), Petry *et al.* (1995)
21a	19 mo, M, Indian parents, consanguineous	Discoid rash, facial erythema, parotitis	ANA$^+$, anti-Ro$^+$, ACA$^+$	G to A at position 6 of the C chain, Gly to Arg	Slingby *et al.* (1996)
21b	4, F, Indian	Discoid rash, photosensitivity	ANA$^+$ 1/80, ENA weakly positive		
22a	5, F, Saudi Arabian	Severe mucocutaneous lesions, alopecia, photosensitivity, renal disease, cerebral atrophy	ANA$^+$, anti-Ro$^+$, anti-La$^+$, anti-Sm$^+$	Sibling died at age 12, possibly of SLE G to A at position 6 of the C chain, Gly to Arg	Suwairi *et al.* (1997)

22b	14, M, Saudi Arabian	Discoid lupus, photosensitivity	ANA$^+$, anti-Ro$^+$	G to A at position 6 of the C chain	
22c	?, M, Saudi Arabian	Asymptomatic at age 5		G to A at position 6 of the C chain	
Low levels of C1q detected					
23	1.5, F, ?	Photosensitive rash, arthritis, "butterfly" rash, vasculitis on palms and soles, monilial stomatitis, staphylococcal meningitis, pneumococcal sepsis	ANA$^+$ speckled, DNA$^-$, lupus band test positive, ENA$^+$ 1/2 million (RNase resistant), latex positive 1/6400	C1q 44 μg/mL (normal range, 160 $\pm$ 50)	Wara *et al.* (1975)
24	10, F, Japanese parents, consanguineous	Rash, photosensitivity, aphthae, discoid lupus, syncope, peripheral numbness, basal ganglion and temporal lobe calcification	ANA$^+$ 1/160, latex positive 1/320, anti-RNP$^+$, anti-Sm$^+$, anti-Ro$^+$; skin IF: negative at the epidermal junction	C1q 5% of normal levels FFP transiently improved Sister died at age 7 with mucocutaneous candidiasis	Orihara *et al.* (1987)

A, Adenine; ACA, anticardiolipin antibodies; ANA, antinuclear antibody; Arg, arginine; Asp, aspartic acid; C, cytosine; ENA, extractable nuclear antigens; FFP, fresh-frozen plasma; G, guanine; Gln, glutamine; Gly, glycine; HBsAg, hepatitis B surface antigen; IF, immunofluorescence; Ig, immunoglobulin; IVIG, intravenous immunoglobulin; LE, lupus erythematosus; MCGN, mesangiocapillary glomerulonephritis; MPGN, mesangioproliferative glomerulonephritis; RNP, ribonucleoprotein; SLE, systemic lupus erythematosus; T, thymine. Modified from Walport *et al.* (1998).

3. C1s and C1r Deficiency

Hereditary deficiency of C1s and C1r is rarer than that of C1q deficiency and, of the 14 reported cases, 8 have developed a lupus-like illness and only 2 are healthy (Table II). One young Japanese boy suffered a virus-associated hemophagocytic syndrome and later developed a fatal unexplained febrile illness (Endo *et al.,* 1999). A further patient has suffered from severe recurrent pyogenic infections, another had chronic glomerulonephritis, and screening of a series of patients with disseminated gonococcal infection revealed a further case of C1r deficiency (Ellison *et al.,* 1987). In the majority of cases, deficiencies of both components coincided (Loos and Heinz, 1986), probably explained by the close proximity of the C1r and C1s genes on the short arm of chromosome 12 (Kusumoto *et al.,* 1988). In these cases, C1r levels were usually absent and C1s levels were ~50% of normal. Among these individuals, further analysis of their complement profile typically showed raised levels of C3 and C4 and elevated C1 esterase inhibitor activity, as seen in association with C1q deficiency.

Selective deficiency of C1s has been reported (Endo *et al.,* 1999; Suzuki *et al.,* 1992), and molecular analysis of one case has shown that homozygous C1s deficiency was the result of compound heterozygosity at the C1s loci. This individual possessed a 4–base pair deletion in exon 10 of the paternal C1s gene that resulted in a premature stop codon 90 base pairs downstream of the deletion. On the maternal gene, a single nucleotide substitution (guanine for thymine) in codon 608 of exon 12 was detected which results in the generation of a stop codon. Although both mutations would be predicted to result in truncated C1s proteins, no detectable C1s protein was found on Western blot analysis of the patient's serum (Endo *et al.,* 1999). Homozygosity for the 4–base pair deletion in exon 10 was demonstrated in the other reported case of selected C1s deficiency (Table II) (Inoue *et al.,* 1998).

4. C4 Deficiency

C4 is present in normal serum of humans as two isotypes, C4A and C4B, encoded by tandemly duplicated genes within the class III region of the major histocompatibility complex (MHC). Total C4 deficiency therefore requires the presence of mutations in both the C4A and C4B genes. Mutants (or null alleles) of the two isotypes of C4, associated with no expressed protein, are designated C4AQ*0 and C4BQ*0 (where Q*0 designates "quantity zero"). The individual frequency of C4AQ*0 and C4BQ*0 alleles among healthy populations of many different ethnic origins is high (see Section II,B,1). However, HLA haplotypes carrying null alleles at both the C4A and C4B loci are extremely rare. Individuals homozygous for C4AQ*0, C4BQ*0 haplotypes are totally deficient in C4.

TABLE II
HOMOZYGOUS C1r AND C1s DEFICIENCY

Family	Age (yr) at Onset, Gender, Race	Clinical Features	Laboratory Tests	Notes	References
1	60, F, ?	Hypertension, rash, temporal artery vasculitis, death from intracerebral hemorrhage 3 yr after illness onset	ANA$^+$ 1/1000, DNA$^+$, anti-Ro$^+$, skin biopsy: anti-μ^+ only C1s 37% (72–124, RID), C1r <12% (72–123, RID), CH50 <5% NHP, C1q 125% (72–123, RID) C4 646 mg/L (127–331), C3 1130 mg/L (451–861), C1 esterase inhibitor 601 mg/L (206–348) by nephelometry	CH50 partially corrected by addition of purified C1r Failure of C1r synthesis by patient's cultured monocytes to rise in response to γ-interferon despite normal rise in C1s Heterozygous for HLA-A1, B37, DR1/HLA-A2, B15	Chevailler *et al.* (1994)
2	2.5, M, Puerto Rican parents, unrelated	Recurrent otitis media, complicated pneumonia (empyema, pneumatocele), purulent staphylococcal lymphadenitis, staphylococcal liver abscess, pneumococcal bacteremia	C1r undetectable (Ouchterlony), C1s 50% normal levels (Ouchterlony), CH50 0% NHP, C1q detected (Ouchterlony) C4 85 mg/dL (16–44), C3 235 mg/dL (80–235), C1 esterase inhibitor activity not tested		Garty *et al.* (1987)
3a	1.5, F, Puerto Rican	Alopecia, facial rash, 3 episodes of meningitis, deforming polyarthropathy, febrile episode with seizures, hypertension	ANA$^-$, DNA$^-$, LE$^-$, latex positive 1/320, skin biopsy: "lupus erythematosus" C1r <0.01 OD units/mL (0.11, RID), C1s <10 mg/mL (24, RID), C1 hemolytic activity 1% NHP, C1q 130 mg/mL (158) C4 50.2 $\times$ 10^3 CH50 units/mL (20.5), C3 18.5 $\times$ 10^3 CH50 units/mL (13.5), C1 esterase inhibitor present	Improved with hydroxychloroquine, prednisolone, and methyldopa Heterozygous for HLA-A9, B5/HLA-A19, B12	Lee *et al.* (1978), Blum *et al.* (1976), Chase *et al.* (1976)
3b	12, M, Puerto Rican	Photosensitivity, severe discoid lupus, polyarthralgia, Raynaud's phenomenon, alopecia, cellulitis, pneumonia, proteinuria, hematuria	ANA$^+$ 1/80 speckled, DNA$^-$, LE$^-$, latex positive 1/640, immunofluorescence renal biopsy: positive for IgA, IgG, IgM, C3, C4, and C1q (mesangial)	Improved with hydroxychloroquine and prednisolone Heterozygous for HLA-A9, B5/HLA-A29, B12	

(*continues*)

TABLE II (*Continued*)

Family	Age (yr) at Onset, Gender, Race	Clinical Features	Laboratory Tests	Notes	References
			C1r <0.01 OD units/mL (0.11, RID), C1s <10 mg/mL (24, RID). C1 hemolytic activity 1% NHP, C1q 100 mg/mL (158) C4 50.2 × 10^3 CH50 units/mL (20.5), C3 16.9 × 10^3 CH50 units/mL (13.5), C1 esterase inhibitor present		
3c	31, M, Puerto Rican	Asymptomatic at time of report, history of tuberculous lymphadenitis	ANA weakly positive. latex positive 1/640 C1r <0.01 OD units/mL (0.11, RID), C1s 12 mg/mL (24, RID), C1 hemolytic activity 1% NHP, C1q 150 mg/mL (158) C4 31 × 10^3 CH50 units/mL (20.5), C3 22.5 × 10^3 CH50 units/mL (13.5), C1 esterase inhibitor present	HLA typing not performed	
3d	16, F, Puerto Rican	Asymptomatic	ANA^+ 1/20, latex positive 1/160 C1r <0.01 OD units/mL (0.11, RID), C1s <10 mg/mL (24, RID), C1 hemolytic activity 1% NHP, C1q 130 mg/mL (158) C4 40.8 × 10^3 CH50 units/mL (20.5), C3 16.6 × 10^3 CH50 units/mL (13.5), C1 esterase inhibitor present	Heterozygous for HLA-A2, B5/ HLA-A29, B12	
4	6, F, ?	Systemic lupus erythematosus	ANA^+ (initially negative), LE^-, CH50 0% NHP, absence of C1, elevated C1 esterase inhibitor level, CH50 restored after addition of purified C1s whereas unchanged following addition of purified C1r or C1q	Symptoms improved with corticosteroid therapy, but no change in complement component levels	Pondman *et al.* (1968)

5a	18, M, ?	Scaling, erythematous atrophic skin lesions at age 15, febrile episodes with polyarthritis, vasculitis, one brother died of "lupus illness" at age 12	C1r undetectable (Ouchterlony), C1s 11.7 μg protein/mL (30, RID), CH50 <12 units/mL (29–61), C1q 16 μg/mL (17–20) C4 hemolytic activity 401,800 units/mL (43,000–449,000), C3 hemolytic activity 4500 units/mL (1536–3664), C1 esterase inhibitor activity 180% NHP	Reduced C1 hemolytic activity restored following addition of purified C1r Serum bactericidal activity against *Escherichia coli in vitro* markedly impaired	Day *et al.* (1972), de Bracco *et al.* (1974)
5b	24, F, ?	Recurrent otitis media and upper respiratory tract infections since childhood, polyarthralgia, recurrent rash, one brother died of "lupus illness" at age 12	C1r undetectable (Ouchterlony), C1s 12.8 μg protein/mL (30, RID), CH50 <12 units/mL (29–61), C1q 20.8 μg/mL (17–20) C4 hemolytic activity 2,460,000 units/mL (43,000–449,000), C3 hemolytic activity 7800 units/mL (1536–3664), C1 esterase inhibitor activity 178% NHP	Reduced C1 hemolytic activity restored following addition of purified C1r Serum bactericidal activity against *E. coli in vitro* markedly impaired	Day *et al.* (1972) de Bracco *et al.* (1974), Moncada *et al.* (1972)
6	13, F, ?	Chronic glomerulonephritis	CH50 <1 unit/mL (45–58), C1 hemolytic activity 100 units/0.5 mL (21,000–30,000), C1s 16 μg/mL (20.4–45.2), C1q level normal C4 950 μg/mL (300–410), C3 1250 μg/mL (1100–1550), elevated C1 esterase inhibitor level	C1r not measured, but addition of purified C1r alone reconstituted total hemolytic and C1 hemolytic activity	Pickering *et al.* (1970)
7	14.5, F, Puerto Rican	Arthralgia, mesangioproliferative glomerulonephritis, history of rheumatic fever, varicella infection and postvaricella encephalitis	ANA^+ 1/320–640, DNA^-, anti-Sm^-, latex positive 1/640, immunofluorescence renal biopsy: positive for IgG and C3 CH50 <1% NHP, C1 hemolytic activity <0.5% NHP, C1r undetectable (RID), C1s 40% normal (RID), C1q 18.5% normal (RID)	HLA-Aw24, A10, Bw35, B18 haplotype C1 hemolytic activity partially restored after addition of purified C1r (<0.5% NHP to 39% NHP)	Rich *et al.* (1979)

(*continues*)

TABLE II (*Continued*)

Family	Age (yr) at Onset, Gender, Race	Clinical Features	Laboratory Tests	Notes	References
			C4 881 μg/mL (200–800), C3 860 μg/mL (800–1800), normal C1 esterase inhibitor level		
8	35, F, African American	Disseminated gonococcal infection, alcohol and intravenous drug abuse	Complete C1r deficiency		Ellison *et al.* (1987)
9	11, M, Japanese	Malar rash, glomerulonephritis with proteinuria and hematuria since age 11, previous pulmonary tuberculosis, previous nephrectomy for hydronephrosis, cardiomyopathy, renal replacement therapy commenced at age 26	ANA^+ 1/160, DNA^+, anti-Sm^-, latex positive, LE^-, anti-RNP^-, immunofluorescence renal biopsy: positive for lgG and C3 CH50 <1 unit/mL, C1 hemolytic activity 3 units/mL (87,000), C1s undetectable but C1r present (Ouchterlony), C1r 45% normal (RIE), C1q 16.7 mg/dL (8.8–15.3) (RID) C4 hemolytic activity 155,000 units/mL (66,000), C3 hemolytic activity 5100 units/mL (6000), C1 inhibitor hemolytic activity 23,710 units/mL (14,170)	Homozygous 4–base pair deletion on exon 10 of C1s gene (TTTG resulting in a premature stop codon), heterozygous deletion present in patient's mother	Suzuki *et al.* (1992), Inoue *et al.*(1998)
10	6, M, Japanese	Virus-associated hemophagocytic syndrome at age 4, pyrexia of undetermined origin at age 6, unconcious following convulsion during this illness and died 6 mo later without regaining consciousness	C1s undetectable (RIE), parents and one sibling possessed 50% normal C1s levels	C1s deficiency due to compound heterozygosity: 4–base pair deletion on paternal C1s gene (TTTG resulting in a premature stop codon), maternal nonsense mutation in codon 608 in exon 12; no truncated proteins detected on Western blot analysis of patient's serum	Endo *et al.* (1999)

Values in parentheses represent the reported normal range or normal mean. ANA, Antinuclear antibody; Ig, immunoglobulin; LE, lupus erythematosus; NHP, normal human pool; OD, optical density; RID, radial immunodiffusion; RIE, rocket immunoelectrophoresis, RNP, ribonucleoprotein.

The first report of complete C4 deficiency was published in 1974 (Hauptmann *et al.,* 1974). Twenty-four cases have now been reported, among whom 18 suffered from lupus-like illness, often developing at an early age and associated with increased frequency of pyogenic infections (Table III). Only 2 of the 24 recorded cases of C4 deficiency were entirely healthy at the time of reporting (Table III). Antinuclear antibodies (ANAs) were positive in 15 of the 20 cases tested, although often present at low titer, while anti–double-stranded DNA antibodies were found in only 2 of the 11 tested. Anti-Ro antibodies were typically positive (7 of 10 tested), while anti-La antibodies, which frequently accompany anti-Ro antibodies, were not detected in any of these patients.

Molecular analysis has shown that 6 cases have a homozygous deletion containing the C4B and adjacent 21-hydroxylase A (CYP21A) genes, although the mechanism of C4A nonexpression was not elucidated (Fredrikson *et al.,* 1998; Fremeaux-Bacchi *et al.,* 1994; Lhotta *et al.,* 1996; Nordin Fredrikson *et al.,* 1991; Uring-Lambert *et al.,* 1989). Other work has shown that mutations resulting in C4A nonexpression may be due to either a 2–base pair insertion in exon 29 or a single base pair deletion in exon 20 of the C4A gene (Table III) (Fredrikson *et al.,* 1998; Lokki *et al.,* 1999). An identical 2–base pair insertion on exon 29 of the C4B gene also results in nonexpression, providing the first molecular evidence of a C4B pseudogene (Lokki *et al.,* 1999). A unique patient had C4 deficiency caused by uniparental isodisomy of an MHC haplotype containing null alleles of C4A and C4B (Welch *et al.,* 1990).

5. C2 Deficiency

Homozygous C2 deficiency is the most common inherited classical pathway complement deficiency, with an approximate prevalence in western European white populations of 1 : 20,000. In contrast to homozygous C1q deficiency, the majority of deficient individuals are probably healthy. SLE has been thought to occur in up to 33% of C2-deficient individuals (Agnello, 1978), although this figure is highly likely to be an overestimate due to ascertainment artifact. This is evident from the following argument.

Using data from population studies, the frequency of the C2Q*0 allele in healthy western European white populations is $\sim 6 \times 10^{-3}$ (Table IV) and therefore the predicted homozygote frequency is 4.8×10^{-5}, or 1 in 20,000. The population of the United Kingdom (UK) is presently estimated at 59 million. Using this figure, the number of homozygous C2-deficient individuals in the UK may be estimated to be ~2950. SLE in C2-deficient individuals is strongly biased toward female patients, in contrast to C1q and C4 deficiency (see Table VI). Therefore, approximately half of the homozygous C2-deficient subjects in the population form the major at risk

TABLE III
HOMOZYGOUS C4 DEFICIENCY

Family	Age (yr) at Onset, Gender, Race/ Ethnic Group	Clinical Features	Laboratory Tests	Notes	References
1	18, F, ?	Rash, alopecia, photosensitivity, hematuria, proteinuria at 2 mo gestation	ANA$^-$, DNA$^-$, anti-Ro$^-$, anti-La$^-$, cryoglobulins detected, LE test negative, lupus band test negative	Homozygous for HLA-A2, B40, Cw3 Homozygous deletion of C4B and 21-hydroxylase A genes	Hauptmann *et al.* (1974), Meyer *et al.* (1985), Uring-Lambert *et al.* (1989)
2	1.5, M, white	Rash, transient arthritis, fever, developed nephrotic syndrome; renal biopsy: mesangial sclerosis	ANA$^+$ 1/40, DNA binding raised, anti-Ro$^+$, anti-La$^-$; persistent lymphopenia and reduced neutrophil chemotaxis; immunofluorescence skin biopsy: negative; immunofluorescence renal biopsy: positive for IgG, IgM, IgA, and C3	Required chlorambucil therapy and prednisolone to control nephritis Reduced antibody response following immunization with bacteriophage ϕX174 and no IgM–IgG class switch Reduced lymphocyte response to mitogens and allogeneic cells Phagocytosis and bactericidal activity in the presence of C4-deficient serum reduced particularly in suboptimal conditions; latter reversed by addition of purified C4 Heterozygous for HLA-A2, B12, Dw2/HLA-A2, B15, Dw8	Awdeh *et al.* (1981a), Clark and Klebanoff (1978), Jackson *et al.* (1979), Meyer *et al.* (1985), Ochs *et al.* (1977), Schaller *et al.* (1977)
3a	17, M, white	Severe HSP at age 17 responsive to penicillin and corticosteroid, hypertension and nephrotic syndrome 6 yr later with renal failure, 2 yr after renal transplant recurrence of HSP in transplanted kidney	Immunofluorescence renal biopsy: positive for IgA, IgG, IgM, and C3; identical findings in transplanted kidney	Homozygous for HLA-A30, B18, DR7	Lhotta *et al.* (1990, 1993), Tappeiner *et al.* (1978)
3b	12, M, white	Asymptomatic	Not reported	Homozygous for HLA-A30, B18, DR7	Lhotta *et al.* (1990), Tappeiner *et al.* (1978)

4	24, F, ?	Discoid lupus, oral ulceration, leukopenia, malaise	ANA^+	Homozygous for HLA-A26, Bw49, DR2 No evidence of C4B gene deletion	Ballow *et al.* (1979), Goldstein *et al.* (1988)
5	19, F, ?	Photosensitive rash, arthralgia, dystrophic nail changes, proteinuria, thrombocytopenia	ANA^+, LE^+, DNA^+, lupus band test negative, anti-$Chido^-$, anti-$Rodgers^-$	2 sisters died of SLE at ages 14 and 17 Homozygous for HLA-A2, B40, Cw3, DR1	Minta *et al.* (1981), Urowitz *et al.* (1981)
6a	2, F, ?	Scarring atrophic skin lesions, reduced creatinine clearance at age 24; renal biopsy: mild focal and segmental mesangial expansion	ANA^-, DNA^-, anti-Ro^+, anti-La^-; immunofluorescence renal biopsy: positive for IgG, IgA, IgM, and C3	Rash improved with corticosteroids and chloroquine Homozygous for HLA-A24, B38, DR13	Lhotta *et al.* (1993), Meyer *et al.* (1985), Tappeiner *et al.* (1982)
6b	5, M, ?	Acute oliguric renal failure; renal biopsy: mild mesangial expansion, mild rash	ANA^+ 1/640; immunofluorescence renal biopsy: positive for IgA, IgM, and C3	Nephritis resolved with prednisolone Homozygous for HLA-A24, B38, DR13	Lhotta *et al.* (1993), Tappeiner *et al.* (1982)
6c	2.5, M, ?	Severe rash, fevers; died of septicemia and cerebral vasculitis at age 3; necropsy: normal renal histology on microscopy	ANA^+ 1/40	Homozygous for HLA-A24, B38, DR13	Lhotta *et al.* (1993), Tappeiner *et al.* (1982)
7	2, F, ?	Atypical rash, recurrent otitis media and purulent parotitis, polyarthritis, glomerulonephritis	$ANA^+ < 1/25$, DNA^-, RF^+, anti-$Chido^-$, anti-$Rodgers^-$	Heterozygous for HLA-A2, B40, Cw3, DR6/HLA-A30, B18, DR3 Homozygous deletion of C4B genes, C4A nonexpression due to a 2–base pair insertion in exon 29 in paternal gene (HLA-A2, B40, Cw3, DR6) and single nucleotide deletion in exon 20 in maternal gene (HLA-A30, B18, DR3)—both mutations generate premature stop codons	Fredrikson *et al.* (1998), Kjellman *et al.* (1982), Nordin Fredrikson *et al.* (1991)
8a	6, F, ?	Rash, proteinuria, hematuria, hypertension, mesangioproliferative glomerulonephritis	ANA^+ 1/320, anti-Ro^+, anti-La^-, immunofluorescence renal biopsy: positive for IgG, IgM, IgA, and C3	Nephritis improved with prednisolone and azathioprine Homozygous for HLA-A30, B18, DR7	Lhotta *et al.* (1993), Meyer *et al.* (1985), Tappeiner *et al.* (1982)

(*continues*)

TABLE III (*Continued*)

Family	Age (yr) at Onset, Gender, Race/ Ethnic Group	Clinical Features	Laboratory Tests	Notes	References
8b	5, M, ?	Minor rash, proteinuria, hematuria, hypertension, mesangioproliferative glomerulonephritis	ANA^+ 1/320; immunofluorescence renal biopsy: positive for IgG, IgM, IgA, and C3	Required pulse cyclophosphamide therapy in addition to prednisolone and azathioprine to control nephritis Homozygous for HLA-A30, B18, DR7	Lhotta *et al.* (1993), Tappeiner *et al.* (1982)
8c	5, F, ?	Nephrotic syndrome, mesangioproliferative glomerulonephritis	ANA^+ 1/40; immunofluorescence renal biopsy: positive for IgG and C3	Nephritis improved with prednisolone and azathioprine Homozygous for HLA-A30, B18, DR7	Lhotta *et al.* (1993)
9a	2, F, Moroccan parents, consanguineous	Recurrent severe respiratory tract infections, photosensitive rash	ANA^-, DNA^-, RF^+ 1/996, cryoglobulins negative, anti-Ro^-, anti-La^-, anti-Sm^-, anti-RNP^-, anti-$Chido^-$, anti-$Rodgers^-$; raised IgM 310 (82–33) mg/dL; reduced T_{helper} subset and increased $T_{suppressor/cytotoxic}$ subset; immunofluorescence skin biopsy: negative	IgG antibody response to tetanus, rubella, and EBV vaccination normal *In vitro* lymphocyte responses to concanavalin A, phytohemagglutinin, and allogeneic cells normal[g] C4-deficient neutrophil phagocytosis and bactericidal activity normal[g] Phagocytosis and bactericidal activity in the presence of C4-deficient serum normal in optimal but *not* suboptimal conditions; latter reversed by addition of purified C4[g] Homozygous for HLA-A11, Bw35, Cw4, DR1 Homozygous deletion of C4B and 21-hydroxylase A genes	Mascart-Lemone *et al.* (1983), Meyer *et al.* (1985), Uring-Lambert *et al.* (1989)
9b	7, F, Moroccan parents, consanguineous	Recurrent respiratory tract infections, pneumonia with pleurisy at age 18 mo, skin lesions similar to those in case 9a	Raised IgM 240 (82–333) mg/dL; normal lymphocyte subset numbers, increased B lymphocytes 25.2% (8–17%)	IgG antibody response to tetanus vaccination normal Homozygous for HLA-A11, Bw35, Cw4, DR1 (see also case 9a)	Mascart-Lemone *et al.* (1983)

10	6, F, German	Photosensitivity, rash, Raynaud's phenomenon	ANA$^+$, DNA$^-$, anti-Sm$^+$, anti-RNP$^+$	Homozygous for HLA-A30, B17, DR7	Klein *et al.* (1984)
11	44, F, ?	Positive test for syphilis at ages 17 and 23 treated with penicillin, developed photosensitive rash at age 41, polyarthritis, Coombs-positive hemolytic anemia, oral ulcers	ANA$^+$, biological false–positive test for syphilis, anti-Ro$^+$, anti-La$^+$; sister died of lupus nephritis, complotype unknown	Heterozygous for HLA-A11, B18, Cw5, DR2/HLA-A1, B7, Cw7, DR2 No evidence of C4B gene deletion	Goldstein *et al.* (1988), Reveille *et al.* (1985)
12	2, F, Algerian	Bacterial meningitis, rash, Raynaud's phenomenon, cicatricial atrophy, glomerulonephritis, later developed osteomyelitis of left femur, recurrent pulmonary infections, died of cardiopulmonary complications (Uring-Lambert *et al.*, 1989)	ANA$^+$ 1/1024 speckled, DNA$^-$, anti-Sm$^+$, anti-Ro$^+$, anti-La$^-$; immunofluorescence renal biopsy: positive for IgM and C1q mesangial deposits	Homozygous for HLA-A1, B17 No evidence of C4B gene deletion	Dumas *et al.* (1986), Meyer *et al.* (1985), Uring-Lambert *et al.* (1989)
13	9, F, ?, nonconsanguineous	Malar rash, vasculitic rash, photosensitivity	ANA$^+$, anti-DNA$^-$, anti-Ro$^+$, anti-Sm$^+$	Unilateral paternal isodisomy (identical paternal chromosomes without a maternal chromosome, in this case chromosome 6) Single paternal haplotype: HLA-A28, B40, Cw3, DR6, DRw52, DQw1 Homozygous deletion of C4B and 21-hydroxylase A genes	Welch *et al.* (1990)
14a	16, M, North African parents, consanguineous	Malar rash at age 6, hematuria, photosensitivity, polyarthralgia at age 14	ANA$^-$, DNA$^-$ anti-Ro$^+$, anti-La$^-$, anti-Sm$^-$; immunofluorescence renal biopsy: positive for IgG and C1q mesangial deposits	Homozygous for HLA-A2, B17, DR7 Homozygous deletion of C4B and 21-hydroxylase A genes	Fremeaux-Bacchi *et al.* (1994)
14b	12, M, North African parents, consanguineous	Asymptomatic	ANA$^-$, no antibodies to extractable nuclear antigens detected	Homozygous for HLA-A2, B17, DR7 Homozygous deletion of C4B and 21-hydroxylase A genes	

(*continues*)

TABLE III (*Continued*)

Family	Age (yr) at Onset, Gender, Race/ Ethnic Group	Clinical Features	Laboratory Tests	Notes	References
15[b]	10, M, ?	Recurrent fever, vomiting, hematuria, mesangioproliferative glomerulonephritis	ANA$^+$ 1/20, DNA$^-$, anti-Chido$^-$, anti-Rodgers$^-$; immunofluorescence renal biopsy: positive for IgG, IgM, (IgA), C3, and C1q	Attacks responded to alternate-day prednisolone therapy Homozygous for HLA-A24, B38, Cw7, DR13, DQ6 Homozygous deletion of both C4B and 21-hydroxylase A genes	Lhotta *et al.* (1996)
16a	30, F, Finnish	Photosensitivity, malar rash, polyarthritis, leukopenia, exacerbations during pregnancies	ANA$^+$ 1/320, RF weakly positive, anti-Sm$^+$ 1/1280; immunofluorescence skin biopsy: positive for IgM and C3	Heterozygous for HLA-A2, B39, Cw7, DR2 (DRB1°1501, DRB5°0101, DQB1°0602)/HLA-A2, B40, Cw3, DR2 (DRB1°1501, DRB5°0101, DQB1°0601) C4B deletion on maternal gene (HLA-A2, B40, Cw3, DR2), C4A gene on maternal haplotype, and C4A and C4B gene on paternal haplotype all contained identical 2–base pair insertion in exon 29 resulting in a premature stop codon in exon 30 No truncated C4 polypetide detected in culture	Lokki *et al.* (1999)
16b	?, M, Finnish	Photosensitivity only		Heterozygous for HLA-A2, B39, Cw7, DRB1°1501, DRB5°0101, DQB1°0602/HLA-A2, B40, Cw3, DRB1°1501, DRB5°0101, DQB1°0601	

ANA, Antinuclear antibody; EBV, Epstein–Barr virus; Ig, immunoglobulin; HSP, Henoch–Schönlein purpura; LE, lupus erythematosus; RF, rheumatoid factor; RNP, ribonucleoprotein.

[a] Also demonstrated for case 9b.

[b] Related to family 6.

TABLE IV
C2Q*0 Alleles and Systemic Lupus Erythematosus

Reference	Race	No. of		C2Q*0 Deficiency % (No.)				C2Q* Allele Frequency			
				Homozygous		Heterozygous		HLA-A25, B18, DR2 Haplotype Analysis		Detection of the 28–Base Pair Genomic Deletion by PCR Analysis	
		Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
Glass *et al.* (1976)	White	137	509	0.7 (1)	0	5.1 (7)	1.2 (6)	0.0328[a]	0.0058[a]	—	—
Christiansen *et al.* (1983)	White	43	176	0	0	0	1.7 (3)	0	0.0088	—	—
Fielder *et al.* (1983)	White	29	42	0	0	3.4 (1)	0	0.0208	0	—	—
Hartung *et al.* (1989)	White	248	2163	0.4 (1)	—[b]	2.0 (5)	—[b]	0.0141	—[b]	—	—
Christiansen *et al.* (1991)	White	62	76	0	0	1.6 (1)	1.3 (1)	0.0080	0.0065	—	—
Truedsson *et al.* (1993)	White	86	100	0	0	5.8 (5)[c]	1 (1)[c]	—	—	0.0291	0.0050
Sullivan *et al.* (1994)	White	122	427	1.6 (2)	0	1.6 (2)	1.4 (6)	—	—	0.0246[d]	0.0070[d]
	African American	127	194	—	—	—	—	—	—	0	0
Unpublished data	White	219	406	0	0	3.6 (8)	1.7 (7)	—	—	0.0183	0.0086

PCR, Polymerase chain reaction.

[a] Of the C2-deficient individuals HLA typed, all possessed either HLA-A25 or HLA-B18 or both.

[b] The haplotype frequency was not stated in the control group, but the individual antigen frequencies (%) in patients and controls were: HLA-A 25—6.5% versus 4.7%, HLA-B 18—11.3% versus 10.7%, HLA-DR2—40.6% versus 29.1% ($p = 0.006$, relative risk = 1.7).

[c] $p = 0.0999$ using Fisher's exact test.

[d] $p < 0.05$.

group (i.e., 1475). If the incidence of SLE among C2-deficient individuals was as high as 33%, there would be ~500 C2-deficient lupus patients in the UK. The prevalence of SLE in the UK is ~1 in 3000, giving a national total of 19,600 cases. If 33% of C2-deficient individuals developed lupus, then such cases would represent 2.5% of the lupus population.

However, there are data showing that the frequency of homozygous C2-deficient individuals among patients with SLE is, at most, 1% (Table IV). Therefore, of the 19,600 UK lupus patients, a maximum of 196 might be C2 deficient. This gives a maximum prevalence of SLE among the UK C2-deficient population of ~13%. It is clear from these figures that the incidence of SLE among C2-deficient women is not 33%, but much more likely to be ~10%.

Consistent with the fact that the majority of homozygous C2-deficient individuals are well was the finding that the first 8 reported individuals with homozygous C2 deficiency (from 4 families) were all healthy (Agnello, 1978; Cooper *et al.,* 1968; Klemperer *et al.,* 1966, 1967) and, indeed, 2 of them were immunologists! However, following these initial reports, many cases were subsequently recorded of patients with C2 deficiency and SLE (Agnello, 1978). At present, at least 100 such cases have been reported.

The severity of SLE associated with homozygous C2 deficiency is comparable to "idiopathic" SLE, but certain phenotypic differences exist. Renal and cerebral involvement appears less common, while arthralgia is more frequent (Agnello, 1978; Ruddy, 1986). Cutaneous involvement, typically widespread erythematous annular lesions, is common. Serological differences include the rarity of antibodies to double-stranded DNA and ANAs, while the frequency of anti-Ro antibodies appears to be high compared with idiopathic lupus (Agnello, 1978; Provost *et al.,* 1983). For example, in a study of 9 homozygous C2-deficient female lupus patients, 7 were anti-Ro antibody positive, while only 5 possessed low-titer ANAs and only 3 were anti-DNA antibody positive (Provost *et al.,* 1983). Skin immunofluorescence studies in individuals with homozygous C2 deficiency and SLE typically do not show the presence of either complement or immunoglobulin at the dermoepidermal junction (Agnello, 1978).

There are also reports of associations of C2 deficiency with recurrent infections, although these are less frequent than the reports of the association of SLE and C2 deficiency (Borzy *et al.,* 1984; Hyatt *et al.,* 1981; Leggiadro *et al.,* 1983; Newman *et al.,* 1978; Sampson *et al.,* 1982; Thong *et al.,* 1980). The reason for this association, in some cases, may be partly explained by coexistent abnormalities of the alternative pathway function in C2-deficient individuals. Two C2-deficient children with recurrent septi-

cemia were shown to have 50% normal factor B levels and reduced alternative pathway hemolytic activity, the latter normalized upon addition of purified factor B (Newman *et al.,* 1978).

Deficiency of C2 may be either due to a failure to synthesize the protein (termed type I deficiency) or due to a selective defect in its secretion (type II deficiency). Type I C2 deficiency is by far the most common cause of C2 deficiency and, in at least 90% of cases, is associated with the extended haplotype: HLA-25, B18, DR2, C2Q*0, C4A4, C4B2, BfS (Agnello, 1978; Hauptmann *et al.,* 1982). The molecular basis for type I deficiency, associated with this haplotype, is due to a 28–base pair genomic deletion which causes skipping of exon 6 during RNA splicing, resulting in generation of a premature termination codon (Johnson *et al.,* 1992). Molecular analysis of a $3\frac{1}{2}$-year-old white boy with complete C2 deficiency has elucidated a further cause of type I C2 deficiency. On one allele, the 28–base pair genomic deletion was detected, while on the other, a novel mutation consisting of a single base pair deletion in exon 2 that caused a frameshift mutation and premature stop codon was present (Wang *et al.,* 1998). This deletion was present on the haplotype HLA-A3, B35, DR4, C2Q*0, C4A32, C4BQ*0, BfS. Type II C2 deficiency is due to missense mutations at highly conserved residues in the C2Q*0 allele (Wetsel *et al.,* 1996). A cytosine-to-thymine substitution in exon 5 ($C^{566}T$, $Ser^{189}Phe$) was associated with the haplotype HLA-A11, B35, DR1, C2Q*0, C4AQ*0, C4B1, BfS. The second is a guanine-to-adenine substitution ($G^{1930}A$, $Gly^{444}Arg$) associated with the haplotype HLA-A2, B5, DR41, C2Q*0, C4A3, C4B1, BfS. A third mutation associated with type II C2 deficiency has been described ($G^{392}A$, $Cys^{111}Tyr$) on another haplotype: HLA-A28, B58, DR12. The precise mechanisms by which these mutations result in the failure to secrete the C2 protein are not known.

6. C3 Deficiency

Homozygous C3 deficiency is strongly associated with recurrent and severe bacterial infections, particularly those caused by encapsulated organisms such as *Neisseria meningitidis, Streptococcus pneumoniae,* and *Haemophilus influenzae.* These infections illustrate the important role of C3 as a bacterial opsonin. Major infections in patients with C3 deficiency are most prominent in childhood and are less of a clinical problem in adults. This presumably reflects the lesser importance of complement in host defense to pyogenic bacteria as antibody responses mature in response to repeated infectious challenges. Among C3-deficient subjects, there is also an increased susceptibility to "immune complex"–mediated disease, particularly glomerulonephritis.

Twenty-three individuals from 16 families have been reported to date (Table V). Seventeen of these suffered from severe and recurrent pyogenic infections. Only 2 were apparently healthy, while 1 individual developed mesangiocapillary glomerulonephritis (MCGN) but did not have a history of recurrent infection. In contrast to homozygous classical pathway component deficiency, complete absence of C3 has been associated with an SLE-like illness in only 3 individuals, all of whom were ANA negative (Imai *et al.*, 1991; Sano *et al.*, 1981). MCGN has been reported in 4 of the 23 cases, while 3 individuals had clinical evidence of nephritis (e.g., proteinuria and hematuria) and 1 individual developed immunoglobulin A (IgA) nephropathy. Many family studies have shown that heterozygotes possess 50% normal C3 levels. With the exception of 1 heterozygous sibling who developed MCGN (Pussell *et al.*, 1980), heterozygote individuals are healthy (Botto and Walport, 1993). An additional family has been described containing 3 female siblings who expressed a dysfunctional C3 protein, among whom 1 suffered from SLE (Nilsson *et al.*, 1992).

The first molecular analysis of human homozygous C3 deficiency demonstrated the presence of a GT-to-AT mutation at the 5′donor splice site in intron 18 (Botto *et al.*, 1990). This splice site mutation caused a 61–base pair deletion in exon 18 that resulted in a frameshift mutation and premature stop codon in exon 18. Further characterized mutations have included the presence of a 5′ donor splice site mutation in intron 10 (Huang and Lin, 1994), the presence of an 800–base pair deletion which included exons 22 and 23 (Botto *et al.*, 1992), and a point mutation that affected a factor I cleavage site (Watanabe *et al.*, 1993). An individual with C3 deficiency but normal C3 cDNA has also been reported (Katz *et al.*, 1994; Peleg *et al.*, 1992; Singer *et al.*, 1994). In this case, a maternally inherited point mutation in exon 13 resulted in a single amino acid substitution in the β-chain and caused impaired C3 secretion (Singer *et al.*, 1994).

7. Terminal Pathway Component Deficiency

There is a small number of patients with SLE and homozygous deficiency of the membrane attack complex proteins. These include isolated case reports of SLE in individuals with deficiencies of C5 (Ross and Densen, 1984), C6 (Tedesco *et al.*, 1981; Trapp *et al.*, 1987), C7 (Segurado *et al.*, 1992; Zeitz *et al.*, 1981), C8 (Jasin, 1977), and C9 (Kawai *et al.*, 1989). These associations are more likely to be explained by ascertainment artifact than by a causal link between deficiency of the membrane attack complex protein and the development of SLE. The reasons for this are set out in Section II,A,9.

8. Mannose-Binding Lectin Deficiency

The third pathway for activation of the complement system is the mannose-binding lectin (MBL) pathway, which is homologous to the classi-

TABLE V
HOMOZYGOUS C3 DEFICIENCY

Family	Age Onset of Infection, Gender, Race/Ethnic Group	Clinical Features: Infections	Clinical Features: Other Complications	Notes/Laboratory Tests	References
1	15 yr, infancy, F, South African, white, parents consanguineous	Pneumonia ×4; meningitis: *Neisseria meningitidis;* otitis media	Erythema gyratum perstans, Sweet's syndrome	800–base pair deletion involving exons 22 and 23 No blood neutrophilia during infection	Alper *et al.* (1972, 1976), Botto *et al.* (1992), Weiss and Schulz (1989)
2	4 yr, during first year, F, American but? race (adopted)	Otitis media: *Haemophilus influenzae* b; UTI; *Escherichia coli;* septicemia: *Streptococcus pneumoniae*	MCGN	Normal leukocytosis to infection Normal Rebuck window	Ballow *et al.* (1975), Berger *et al.* (1983)
3	4 yr, 1 yr, F, South African, white, parents unrelated	Meningitis: *S. pneumoniae* ×3; lobar pneumonia: *S. pneumoniae*	Died at age 7.5; necropsy: purulent meningitis with polymorphs present in subarachnoid space	Peripheral lymphoid tissues: barely discernible germinal centers, low IgG levels (3.8–6 g/L)	Grace *et al.* (1976)
4	3 yr, N/A, M, white, parents consanguineous	No infections	Maculopapular rash, fever, arthralgia in wrist	Illness resolved following whole blood transfusion	Osofsky *et al.* (1977)
5	5 yr, 5 mo, F, American but? race	Pneumonia, septic arthritis, otitis media, febrile convulsions		Blunted leukocytosis to infection	Davis *et al.* (1977)
6a	13 yr, ?, F, Lebanese	Frequent earache, sore throat	Proteinuria, microhematuria		Pussell *et al.* (1980)
6b	7 yr, 6.5 yr, F, Lebanese	Peritonitis: *S. Pneumoniae*	Proteinuria, microhematuria	Left renal artery stenosis	
6c	5 yr, 3 yr, M, Lebanese	Peritonitis	Proteinuria	Heterozygous sibling with MCGN	
7a	19 yr, early childhood, F, Japanese parents, consanguineous	Bronchitis	SLE-like illness at age 16: erythematous rash, fever, arthralgia, photosensitivity	ANA^-, LE^-	Sano *et al.* (1981)
7b	14 yr, N/A, F, Japanese parents, consanguineous	No infections	SLE-like illness at age 10: photosensitive facial rash, arthralgia	ANA^-, LE^-	

(*continues*)

TABLE V (*Continued*)

Family	Age Onset of Infection, Gender, Race/Ethnic Group	Clinical Features: Infections	Clinical Features: Other Complications	Notes/Laboratory Tests	References
8	10 yr, 8 mo, F, Aborigine of the Atayal tribe in Taiwan, parents unrelated	Pneumonia, septic arthritis; otitis media: *H. influenzae*	Rash during infection, arthralgia Elder sister died of pneumonia and meningitis at age 6 mo	Normal leukocytosis to infection GT–TT mutation at the 5′ donor splice site of intervening sequence 10 Elder sister died of pneumonia and meningitis at age 6 mo	Hsieh *et al.* (1981), Huang and Lin (1994)
9a	26 yr, 7 mo, F, Dutch	Meningitis: *N. meningitidis;* meningitis: *S. pneumoniae;* sepsis: *Staphylococcus aureus*			Roord *et al.* (1983)
9b	19 yr, 21 mo, F, Dutch	Meningitis: *S. pneumoniae;* otitis media	Transient maculopapular rash		
9c	16 yr, 8 mo, F, Dutch	Osteomyelitis, otitis media	Transient maculopapular rash, MCGN type I	Administration of FFP of no benefit	Roord *et al.* (1983, 1989)
10	7 yr, 5 mo, M, Laotian parents, unrelated	Lobar pneumonia; meningitis: *S. pneumoniae* ×2	MCGN C3 ~4 μg/ml (0.3% normal)	Acute administration of FFP not associated with renal deterioration Normal size but greatly reduced C3 mRNA (1% normal)—defect unknown	Borzy and Houghton (1985), Borzy *et al.* (1988), Singer *et al.* (1996)
11	12 yr, N/A, F, Kuwaiti:	No infections	Microhematuria, nephrotic syndrome, renal failure, MCGN type I		Cozma *et al.* (1987)

12	6 yr, 3 mo, M, Brazilian parents, consanguineous	Meningitis: *N. meningitidis* ×3; bronchopneumonia ×4, otitis media, osteomyelitis ×2		Normal leukocytosis to infection	Grumach *et al.* (1988)
13	10 yr, ?, M, English parents, consanguineous	Otitis media; URTI: *Streptococcus pyogenes*	Transient erythema multiforme at time of infection	GT–AT mutation at the 5′ donor splice site of intervening sequence 18	Botto *et al.* (1990)
14a	23 yr, 4 yr, M, Japanese parents, consanguineous	Meningitis	IgA nephropathy		Imai *et al.* (1991)
14b[a]	14 yr, N/A, F, Japanese parents, consanguineous	No infections	Lupus-like illness	ANA^-	
15a	19 yr, childhood, M, New Zealand	Meningitis: *N. meningitidis;* periorbital cellulitis, pneumonia, 2 episodes of impetigo, recurrent cold sores in childhood		ANA^+ C3 mRNA normal size and quantity Normal-sized proC3 molecule but aberrant trypsin cleavage profile Impaired C3 synthesis due to nucleotide substitution in exon 13 resulting in single amino acid change in β chain of C3 Paternal C3 gene defect uncharacterized	Katz *et al.* (1994), Peleg *et al.* (1992), Singer *et al.* (1994)
15b	7 yr, N/A, F, New Zealand	Asthma, rhinitis			
16	4 yr, ?, F, ?	Meningitis ×4, recurrent otitis	2 male siblings died with infections at age 6 mo	C3 0.8% normal 2 male siblings died with infections at age 6 mo	Sanal *et al.* (1992)

ANA, Antinuclear antibody; FFP, fresh-frozen plasma; Ig, immunoglobulin; LE, lupus erythematosus; MCGN, mesangiocapillary glomerulonephritis; N/A, not applicable; SLE, systemic lupus erythematosus; URTI, upper respiratory tract infection; UTI, urinary tract infection. Modified from Botto and Walport (1993).

[a] First-cousin of case 14a.

cal pathway. MBL is homologous to C1q but binds to terminal mannose groups on the surface of many pathogens. Following ligand binding, MBL activates two serine esterases, MASP-1 and MASP-2, which are homologous to C1r and C1s, and these in turn cleave C4 and C3 (Lu *et al.,* 1990; Matsushita and Fujita, 1992). MBL is structurally and functionally analogous to complement C1q and this led to the hypothesis that individuals with deficiency of MBL might be predisposed to the development of lupus.

The MBL gene comprises four exons and is located on chromosome 1. Five polymorphisms that result in reduced serum MBL have been identified (Lipscombe *et al.,* 1995). Three point mutations have been described in exon 1 that result in reduced MBL serum levels: Asp^{54}, Glu^{57}, and Cys^{52} mutations (Lipscombe *et al.,* 1995). Two linked promoter polymorphisms, at nucleotide positions −550 (H/L variants) and −221 (X/Y variants), have also been described (Madsen *et al.,* 1995). These polymorphisms occur on three haplotypes: HY, LY, and LX, which are associated with high, intermediate, and low levels of serum MBL, respectively.

A point mutation (guanine to adenine) at nucleotide 230 of exon 1 that results in the substitution of aspartic acid for glycine in codon 54 (Asp^{54} mutation) is associated with severe, recurrent infections in children and adults (Summerfield *et al.,* 1995, 1997), and the mutant MBL protein is unable to activate complement (Super *et al.,* 1992). In a study of 102 white lupus patients, both the frequency of this allele (41% versus 30%) and the number of homozygous individuals (10% versus 7%) were increased in patients compared to 136 healthy controls, although this did not reach statistical significance (Davies *et al.,* 1995a). However, in a small study of 50 Spanish lupus patients, the frequency of the Asp^{54} allele was significantly increased in the patient group (52% versus 31%, $p = 0.03$) (Davies *et al.,* 1997). In both of these studies, the combination of a C4 null allele and a dysfunctional MBL allele was more strongly associated with SLE than either allele alone (Davies *et al.,* 1995a, 1997). An association between low levels of serum MBL and SLE has been reported in Chinese lupus patients, among whom an increased frequency of the Asp^{54} mutation was found (0.33 versus 0.23) (Lau *et al.,* 1996).

In a study of 92 African American lupus patients, the frequencies of both the Asp^{54} mutation (0.163 versus 0.087, $p = 0.0225$) and the Glu^{57} mutation (0.125 versus 0.047, $p = 0.0067$) were significantly increased compared with 86 healthy controls (Sullivan *et al.,* 1996). Furthermore, the frequency of the promoter polymorphisms associated with high levels of MBL were significantly decreased among the lupus patients (HY haplotype frequency: 0.078 versus 0.164, $p = 0.0115$). Although the increase in the frequency of the LX haplotype among lupus patients did not reach statistical significance, the number of LX/LX homozygotes was higher among the

patient group (11% versus 2.6%, $p = 0.0324$). Moreover, an association between the LX haplotype and SLE has been demonstrated in a study of 112 Chinese lupus patients (LX haplotype frequency: 0.259 versus 0.154, $p = 0.019$) (Ip *et al.,* 1998).

In summary, the data show that both structural and promoter polymorphisms associated with low serum MBL are increased among patients with SLE from different ethnic backgrounds. These results suggest that MBL may play a similar role to C1q in conferring protection against the development of SLE, though with a much weaker protective effect.

9. Complement Deficiency, SLE, and Ascertainment Artifacts

Ascertainment artifact is a common trap in studies of associations in medicine. Thus, as described above, the first two individuals identified with C2 deficiency were both immunologists. Similarly, the first two humans with IgA deficiency were both immunologists working in Dr. Henry Kunkel's laboratory in New York. It is implausible that C2 deficiency is a disease susceptibility gene for becoming an immunologist or that IgA deficiency was a cause for working in Henry Kunkel's laboratory! There are analogous reasons for worrying that the association of complement deficiency with SLE is a similar though less extreme type of artifact induced by the selective assay of complement levels among patients with the disease.

However, there are compelling data that the link between deficiency of classical pathway complement proteins and SLE is causal rather than artifactual. First, two large population surveys in Switzerland (of 4000 consecutive recruits into the army) and Japan (of 145,640 consecutive blood donors) identified no individuals with homozygous deficiency of any classical pathway protein or of C3 (Hassig *et al.,* 1964; Inai *et al.,* 1989). Second, surveys of the inbred populations local to some of the C1q-deficient patients in Turkey have failed to reveal any asymptomatic C1q-deficient individuals (Berkel *et al.,* 2000).

Third, among families in which a C1q- or C4-deficient proband was identified, the great majority of the sibships who were also found to be homozygous complement deficient also had SLE or later developed disease (Table VI). The concordance of SLE between siblings with C1q, C1r/C1s, and C4 deficiency is 90%, 67%, and 80%, respectively. This very high concordance provides additional evidence against the association of complement deficiency with SLE being due to ascertainment artifact. It is interesting to contrast these concordance data with the best published study of twin concordance of the expression of SLE, which showed concordance of disease of 2% among dizygotic twins and 24% among monozygotic twins (Deapen *et al.,* 1992).

TABLE VI
HOMOZYGOUS COMPLEMENT DEFICIENCY AND SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

Homozygous Complement Deficiency	C1q	C1r/C1s	C4	C2[a]	C3
Incidence of SLE					
Total no. of reported cases	42	14	24	77	23
Individuals with SLE					
No.	39	8	18	24	3
%	93	57	75	32[b]	13
Sex ratio					
All cases					
F : M (no.)	23 : 19	8 : 6	14 : 10	43 : 30[c]	16 : 7
F : M (ratio)	1.2 : 1	1.3 : 1	1.4 : 1	1.4 : 1	2.3 : 1
Individuals with SLE					
F : M (no.)	22 : 17	5 : 3	12 : 6	21 : 3	3 : 0
F : M (ratio)	1.3 : 1	1.7 : 1	2 : 1	7 : 1	—
Sibling concordance data for SLE					
No. of families with SLE and >1 deficient sibling	11	2	4	8	1
Total no. of siblings from these families	29	6	10	17	2
Sibling concordance for SLE (%)	90	67	80	58	N/A

N/A, Not applicable.
[a] From Ross and Densen (1984).
[b] See also text.
[c] Gender not reported for four cases.

Fourth, several conditions in which there is chronic acquired deficiency of complement, for example, caused by deficiency of C1 inhibitor (hereditary angioedema) or autoantibodies to the C3 convertase enzyme (C3 nephritic factor), show a markedly raised prevalence of SLE. We review this association in the next section. Finally, mice in which a gene-targeted mutation of C1q has been engineered developed spontaneous lupus-like disease (reviewed in Section III).

However, it remains likely that there is still some ascertainment artifact that may tend to overestimate the strength of the association between complement deficiency and the development of SLE. This is most obvious in the case of C2 deficiency (discussed above), in which simple mendelian calculations using the Hardy–Weinberg equation show that 1 : 20,000 of western European white populations have homozygous C2 deficiency. Only a minority of these individuals can have symptomatic SLE, or lupus clinics would be swamped with C2-deficient patients!

It is likely that ascertainment artifact is the explanation for the very rare reported cases of patients with SLE and inherited deficiency of a membrane

attack complex protein (reviewed in Section II,A,7). A survey of Japanese blood donors identified 138 subjects of 145,640 with a homozygous deficiency of a membrane attack complex protein, mainly of C9; none of these subjects had SLE (Inai *et al.,* 1989). In this same population, no subjects were identified with a classical pathway protein deficiency (Fukumori *et al.,* 1989). By contrast, 5 Japanese patients with SLE and C1q deficiency have been reported, but only 1 with SLE and C9 deficiency (Kawai *et al.,* 1989).

10. Acquired Complement Deficiency and SLE

Patients with heterozygous deficiency of C1 inhibitor suffer from the disease hereditary angioedema. The angioedema in this disease is caused by a failure of the reduced levels of C1 inhibitor to regulate the activity of kallikrein, C1r, and C1s, leading to the production of kinins which increase vascular permeability. This failure to regulate C1r and C1s is also associated with increased turnover of C4 and C2. Patients with this disease have chronically severely reduced levels of these complement proteins, even in the absence of attacks of angioedema. It is of great interest that there are now a number of reports of patients with hereditary angioedema developing SLE (Table VII). This association reinforces the data that complement deficiency is a cause of SLE and illustrates that acquired, as well as inherited, deficiency of classical pathway proteins may be a cause of disease.

There are several autoantibodies to complement proteins that interfere with the physiological regulation of complement activation *in vivo,* and each of these has been associated with the development of SLE. These antibodies are C3 nephritic factor, anti-C1 inhibitor autoantibodies, and anti-C1q antibodies. In each of these cases, there is a "chicken and egg" dispute, since it could be argued that development of the anticomplement autoantibody is itself part of the SLE process. However, in the case of C3 nephritic factor, which stabilizes the C3bBb C3 convertase enzyme of the alternative pathway, 8 cases of SLE have been described (Table VIII) (Cronin *et al.,* 1995; Font *et al.,* 1990; Jasin, 1979; Sheeran *et al.,* 1995; Walport *et al.,* 1994). In each of these, the onset of SLE occurred many years after the development of the partial lipodystrophy or dense-deposit MCGN (the main clinical phenotypes associated with the presence of C3 nephritic factor), supporting the idea that the C3 nephritic factor was the "egg" rather than the "chicken".

B. Complement Null Alleles

Because of the strong association between hereditary homozygous classical pathway complement component deficiency and SLE, it was hypothe-

TABLE VII
Hereditary C1 Inhibitor Deficiency and Systemic Lupus Erythematosus (SLE) or Discoid Lupus Erythematosus (DLE)

Case No.	Age (yr), Gender, Race/Ethnic Group	Age (yr) at Diagnosis		Clinical Features		Laboratory Tests/Notes	References
		C1 Inhibitor Deficiency	SLE/DLE	C1 Inhibitor Deficiency	SLE/DLE		
1a[a]	18, M, ?	17	8	Angioedema, recurrent abdominal pain and vomiting	Discoid rash, alopecia, photosensitivity, splenomegaly	ANA^+, $ssDNA^+$, $dsDNA^-$, C1q 0.166 mg/mL (0.134–0.245), C4 0.054 mg/mL (0.258–0.780), C1, inhibitor level 0.037 mg/mL (0.094–0.318)	Kohler *et al.* (1974)
1b[a]	18, M, ?	8	5	Facial and scrotal angioedema, abdominal pains	Severe discoid rash, alopecia, photosensitivity, splenomegaly	ANA^+, $ssDNA^+$, $dsDNA^-$, C1q 0.212 mg/mL (0.134–0.245), C4 0.108 mg/mL (0.258–0.780), C1 inhibitor level 0.043 mg/mL (0.094–0.318)	
1c[b]	?, F, ?	16	10	Angioedema affecting the face and extremities, recurrent abdominal pain and vomiting	Malar rash, polyarthralgia, fever, grand mal seizure at age 25, died 4 yr later from cardiac failure; necropsy: atherosclerosis and inflammatory arteritis	Not available (normal levels of C1 inhibitor present in father and maternal grandparents)	
2	?, F, ?	23	21	Angioedema	Malar rash, photosensitivity	ANA^+, DNA^+, C4 1–2.3 mg/dL (30–70) (mother and brother have hereditary angioedema but not SLE)	Donaldson *et al.* (1977)
3	?, F, ?	?	?	Angioedema	Photosensitivity, discoid rash	ANA^+, DNA^+, C4 5.8 mg/dL (30–70), functional but not antigenic C1 inhibitor deficiency (son has hereditary angioedema but not SLE)	
4	?, F, ?	30	48	Angioedema	Photosensitivity, discoid rash	ANA^+, C4 5.5 mg/dL (30–70), (3 relatives have hereditary angioedema but not SLE)	
5	?, F, ?	6	14	Angioedema	Proliferative glomerulonephritis	ANA^+, DNA^+, (identical twin has C1 inhibitor deficiency but no symptoms of either SLE or hereditary angioedema)	Rosenfeld *et al.* (1974), Donaldson *et al.* (1977)

6	43, F, white	6	43	Angioedema, including laryngeal attacks	Polyarthralgia, proliferative glomerulonephritis	ANA$^+$, DNA$^+$, C1 inhibitor >5% normal	Young *et al.* (1980)
7	38, F, Ojibwa Indian and German	?	38	Recurrent angioedema	Fever, polyarthralgia, generalized rash, proteinuria, pulmonary fibrosis	ANA$^+$, DNA$^-$, anti-RNP$^+$, anti-Sm$^+$, C4 <7 mg/dL (12–72), C1 inhibitor level 6.2 mg/dL (14.8–26.1) (several sisters, a nephew, and 1 child have hereditary angioedema but not SLE)	Massa and Connolly (1982)
8	48, F, Japanese	20	48	Angioedema affecting the face and extremities	Fever, facial rash, polyarthralgia, photosensitivity	ANA$^-$, C4 3 mg/dL, C1 inhibitor levels 10% normal (4 family members have hereditary angioedema but not SLE)	Shiraishi *et al.* (1982)
9	24, F, white	22	24	Recurrent abdominal pain	Facial rash, photosensitivity	ANA$^-$, DNA$^-$, C1 inhibitor level undetectable (mother and 4 siblings have hereditary angioedema but not SLE)	Youinou *et al.* (1983)
10	17, M, French	—	—	Angioedema	Proliferative lupus glomerulonephritis	ANA$^-$, DNA$^-$	Hory *et al.* (1981), Hory *et al.* (1983)
11	33, F, ?	—	17	No symptoms attributable to hereditary angioedema	Polyarthralgia, rash, pericarditis, membranoproliferative glomerulonephritis	ANA$^+$, DNA$^+$, C4 2 mg/dL (12–60), C1 inhibitor level 1.2 mg/dL (21–41) (mother, brother, and aunt have hereditary angioedema but not SLE)	Suzuki *et al.* (1986)
12	24, F, white	15	—	Recurrent abdominal pains	Facial rash, photosensitivity (sister, who had C1 inhibitor deficiency, developed photosensitive facial rash during danazol therapy)	ANA$^-$, DNA$^-$, anti-Ro$^+$ (mother and 2 brothers have hereditary angioedema but not SLE)	Guillet *et al.* (1988)
13	24, F, white	14	14	Angioedema of the extremities, abdominal pain	Nonscarring rash in sun-exposed areas	ANA$^-$, anti-Ro$^+$, C4 6 mg/dL (20–50), C1 inhibitor level 4.8 mg/dL (15–35) (father and sister have hereditary angioedema but not SLE)	Gudat and Bork (1989)
14	33, F, Japanese	4	20	Angioedema affecting the face and extremities, abdominal pain	Photosensitivity, malar rash	ANA$^+$, reduced C1q and C4 levels, reduced C1 inhibitor levels (mother and sister have hereditary angioedema but not SLE)	Horiuchi *et al.* (1989)

(*continues*)

TABLE VII (*Continued*)

Case No.	Age (yr), Gender, Race/Ethnic Group	Age (yr) at Diagnosis		Clinical Features		Laboratory Tests/Notes	References
		C1 Inhibitor Deficiency	SLE/DLE	C1 Inhibitor Deficiency	SLE/DLE		
15	38, F, ?	16	22	Angioedema affecting the face, larynx, and extremities; abdominal pain	Scarring facial rash; skin biopsy: DLE	ANA^-, DNA^-, C4 2 mg/dL (12–60), C1 inhibitor level 0.02 g/L (0.18–0.26) (son had asymptomatic hereditary angioedema)	Duhra *et al.* (1990)
16	26, F, ?	3	19	Angioedema affecting the extremities, abdominal pain	DLE in sun-exposed areas, alopecia, polyarthralgia	ANA^+, anti-Ro^+, C4 0.06 g/L (0.14–0.42), C1 inhibitor level 0.04 g/L (0.18–0.54)	Cox *et al.* (1991)
17	9, F, ?	?	1	Intermittent facial swelling	Photosensitivity	ANA weakly positive, anti-Ro^+, anti-La^+, DNA^-, C4 undetectable, functional but not antigenic C1 inhibitor deficiency, reduced levels of free protein S (mother and sister have hereditary angioedema but not SLE)	Perkins *et al.* (1994)
18	44, F, white	20	?	Subcutaneous edema, vomiting, abdominal pain	Photosensitivity, later hypertension, proteinuria, hematuria, cerebral vasculitis	ANA^+ (ANA^- at age 20, when clinical symptoms of hereditary angioedema developed), DNA^-, anti-Ro^+, C4 undetectable, C1 inhibitor antigenically and functionally undetectable (mother and brother have hereditary angioedema but not SLE)	Donaldson *et al.* (1996)

?, —, Information not reported; ANA, antinuclear antibody; ds, double-stranded; RNP, ribonucleoprotein; ss, single-stranded.
[a] Identical twins.
[b] Mother of cases 1a and 1b.

TABLE VIII
C3 Nephritic Factor and Systemic Lupus Erythematosus (SLE)

Case No.	Age (yr), Gender, Race	Age (yr) at Onset		Clinical Features		Laboratory Tests	Reference
		C3 Nephritic Factor	SLE	C3 Nephritic Factor	SLE		
1	34, F, ?	5	17	Partial lipodystrophy	Polyarthritis, serositis, photosensitivity	ANA^+, LE^+	Jasin (1979)
2	35, F, ?	7	31	Partial lipodystrophy, MPGN type II	Polyarthritis, serositis, thrombocytopenia	ANA^+, DNA^+,	Font *et al.* (1990)
3	35, F, ?	19	21	Partial lipodystrophy	Discoid rash, photosensitivity	ANA^+, anti-Ro^+, IgG anticardiolipin positive	Walport *et al.* (1994)
4	20, M, ?	9	16	MPGN type II	Malar rash, discoid rash	ANA^+, anti-Ro^+	Walport *et al.* (1994)
5	38, F, ?	7	23	Partial lipodystrophy	Polyarthritis, photosensitivity, discoid rash	ANA^+, lymphopenia	Walport *et al.* (1994)
6	37, F, ?	7	35	Partial lipodystrophy	Polyarthritis, photosensitivity	ANA^+, anti-Ro^+, lymphopenia, neutropenia	Walport *et al.* (1994)
7	13, F, Afro-Caribbean	13	25	MPGN type III	Meningococcal septicemia polyarthralgia, Raynaud's phenomenon, recurrent angioedema	ANA^+ 1/6400, DNA^+, anti-Ro^+	Sheeran *et al.* (1995)
8	44, F, white	Childhood	43	Partial lipodystrophy	Fatigue, photosensitivity, polyarthralgia, Raynaud's phenomenon, hepatosplenomegaly	ANA^+ 1/2560, DNA^-, anti-Ro^-	Cronin *et al.* (1995)

ANA, Antinuclear antibody; IgG, immunoglobulin G; LE, lupus erythematosus. MPGN, membranoproliferative glomerulonephritis. Modified from Walport *et al.* (1994).

sized that partial deficiencies of C4 or C2 may increase disease susceptibility. This has been an extremely difficult hypothesis to test, for two reasons. The first of these is the difficulty of accurately ascertaining individual C4 null alleles and the second is due to the phenomenon of linkage disequilibrium within the MHC region.

There are three reasons that it is difficult to ascertain C4 null alleles with precision. The first is because there are two isotypes of the protein, which require resolution either by electrophoretic separation (Awdeh and Alper, 1980) [which is easier after removal of C-terminal basic amino acids with carboxypeptidase B (Sim and Cross, 1986)] or by using monoclonal antibodies (Chrispeels *et al.*, 1989; O'Neill, 1984). The second, more fundamental difficulty is that the levels of expressed C4A and C4B show overlap in the presence of one or two functional alleles (Hammond *et al.*, 1992; Moulds *et al.*, 1993; Wilson *et al.*, 1989). The third is that patients with SLE often have severely reduced C4 levels in plasma because of complement activation *in vivo.* Several strategies have been devised to get around these problems, but none are perfect. The ratio of C4A to C4B expression has been used to deal with the problem of variable total C4 turnover in serum. Family studies provide increased confidence, because low C4A or C4B levels can be shown to be heritable. The molecular basis for the common C4A and C4B null alleles has been identified which allows accurate genotyping for some, though not all, null alleles (Schneider *et al.*, 1986). However, the majority of published studies have been based on protein phenotyping.

The phenomenon of linkage disequilibrium in the MHC raises a more fundamental difficulty. There is no doubt at all that there is one or more disease susceptibility genes for SLE located within the MHC—but after more than 20 years of research in this area, it still remains uncertain what is the relevant gene or genes. The best approach to trying to identify the relevant gene within the MHC that causes disease susceptibility is to study populations of different ethnic origins, in which the combinations of alleles at the many genes within the MHC are different. However, at present, a level of agnosticism is necessary about which are the relevant loci in the MHC that are associated with disease susceptibility to SLE in the majority of patients. It is universally agreed that total deficiency of either C2 or C4 causes a powerful predisposition to the development of SLE, but only a tiny minority of cases can be explained on this basis. In the majority of patients with SLE, there are three main groups of candidate genes within the MHC. The first are the genes that encode MHC class II proteins and components of the antigen-processing machinery, which control the repertoire of peptide presentation to T cell receptors (Beck and Trowsdale, 1999). The second are the mutated genes, which are responsible for C4A

and C4B null alleles (which we review in the next section). The third are the allelic variants at the loci for the cytokines tumor necrosis factor (TNF-α) TNF- and lymphotoxin. Very many other genes have been discovered within the MHC, and some of these may turn out to be important in determining susceptibility to SLE.

1. C4 Null Alleles

Null alleles at either the C4A or C4B locus are very common, although, as discussed above, haplotypes carrying null alleles at both loci are very rare, accounting for the extreme rarity of total C4 deficiency. A single C4 null allele may be seen in up to 30% of healthy white subjects, with ~4% having homozygous C4A deficiency and 1% exhibiting homozygous C4B deficiency. Low levels of C4 are present on normal erythrocytes. It was found that polymorphic variation of C4A is responsible for the blood group named Rodgers, and likewise, variation of C4B is responsible for the Chido blood group (O'Neill *et al.,* 1979). This means that the erythrocytes from individuals with C4A deficiency are negative for the Rodgers blood group and those from subjects with C4B deficiency are negative for the Chido blood group.

There is a strong association between C4AQ*0 null alleles and SLE among patients of western European white origins (Table IX). Moreover, in these patients the association shows a gene "dose-dependent" effect. For example, one study of white patients demonstrated a relative risk in heterozygotes of 3.23, rising in homozygotes to 16.86 (Howard *et al.,* 1986). However, in the majority of these studies, the association with C4AQ*0 occurs in conjunction with an increased incidence of the 8.1 ancestral haplotype: HLA-A1, C7, B8, C4AQ*0, C4B1, DR3, DQ2. This particular MHC haplotype is of special interest to immunologists because it is associated with other diseases of immune dysfunction, including type 1 diabetes, autoimmune thyroid disease, myasthenia gravis, dermatitis herpetiformis, and celiac disease (Price *et al.,* 1999). The 8.1 ancestral haplotype is also associated with IgA deficiency, reduced responsiveness to vaccination by hepatitis B surface antigen (Alper *et al.,* 1989), and accelerated progression of human immunodeficiency virus (HIV) (Cameron *et al.,* 1990). It is therefore a matter of some frustration that, after many years of studies of MHC associations with disease, it is still uncertain which gene or genes within this, the most common haplotype in western European white populations, are responsible for these important associations.

In this context, it is important to note that an association between C4AQ*0 alleles and SLE was also demonstrated in the majority of studies of patients from different ethnic groups in which an association with HLA-DR3 was not found (Table IX). For example, in African American patients,

TABLE IX

C4AQ*0 Allele Frequencies in Different Populations of Patients with Systemic Lupus Erythematosus

Reference	Race Ethnic Group	Study Number		Homozygous C4AO*0 Deficiency			C4AQ*0 Allele Frequency			DR3 Association with SLE?
		Patients	Controls	Patients % (No.)	Controls % (No.)	p Value, Relative Risk	Patients	Controls	p Value, Relative Risk	
Fielder *et al.* (1983)	White	29	42	13.8 (4)	0	—	0.38	0.08	$p < 0.01$	Yes
Christiansen *et al.* (1983)	White[a]	41	176	12 (5)	0	—	0.32[b]	0.20[b]	—	Not tested
Reveille *et al.* (1985)	White	15	64[c]	13.3 (2)	6 (4)	—	0.366	0.281	NS	Yes and DR2
Howard *et al.* (1986)	White	63	63	11.1 (7)	0	$p < 0.006$, 16.86	0.254	0.095	$p = 0.003$, 3.23	No, but DR2 increased
Dunckley *et al.* (1987)	White	63	197	7.9 (5)	1.5 (3)	$p < 0.05$	0.317	0.169	$p < 0.01$	Not tested
Batchelor *et al.* (1987)	White, DR3 negative	30	60	0	0	—	0.083	0.05	—	All DR3 negative
Gougerot *et al.* (1987)	White	20	108	—	—	—	0.054	0.106	NS	Yes
Kemp *et al.* (1987)	White	88	236	10.2 (9)	1.7 (4)	$p < 0.002$	0.159	0.008	—	Yes
							0.153[d]	—	—	
Hartung *et al.* (1989)	White	196	204	—	—	—	19.8[d]	12[e]	$p < 0.001$, 1.0	Yes and DR2
So *et al.* (1990)	White	54	62	3.7 (2)	0	—	0.277[d]	0.121[d]	$p < 0.005$	Yes and DR2
Schur *et al.* (1990)	White	101	1731	—	—	—	0.188	0.176	NS	No
	English/Irish	27[e]	144[e]	7	5	—	0.41	0.22	$p = 0.03$	Yes
	Other Europeans	62[e]	310[e]	3	1	—	0.11	0.12	NS	No
Sturfelt *et al.* (1990)	Southern Sweden	80	330	16 (13)	2.4 (8)	$p < 0.001$	0.1625	0.024	$p < 0.001$	Not tested
Kumar *et al.* (1991)	White	32	—	6 (2)	—	—	0.391	—	—	Yes
	Mexican	11	9[e]	9.1 (1)	11.1 (1)	—	0.318	0.389[e]	—	All DR3 negative
Christiansen *et al.* (1991)	Australian	62	133	12.9 (8)	0	—	0.298	0.15	—	No
	Aborigine	9	—	0	—	—	0.11	0.29[g]	—	No
Reveille *et al.* (1991)	White	48	—	6 (3)	—	—	0.302[d]	—	—	Yes
Hartung *et al.* (1992)	White	396	204	—	—	—	29[e]	12[e]	$p < 10^{-6}$	Yes
		310[e]	155[e]	—	—	—	0.30[d]	0.181[d]	$p < 10^{-6}$	Yes
		310[e]	155[e]	—	—	—	0.055[h]	0.052[h]	NS	No
Goldstein and Sengar (1993)	French Canadians	43	44	0	0	—	0.12[d]	0.06[d]	NS	No, but DQ6 increased
	Non-French Canadians	43	36	7 (3)	0	—	0.31[d]	0.10[d]	$p = 0.001$, 4.3	Yes
Cornillet *et al.* (1993)	French	74	130	2.7 (2)	0	—	0.149	0.031	—	Not tested
De Juan *et al.* (1993)	Spanish	58	69	—	—	—	27[e]	34[e]	NS	Yes
		53	52	—	—	—	17[d, e]	15[d, e]	NS	Yes

Reveille *et al.* (1995a)	Mexican	55	112	—	—	—	0.14	0.07	$p < 0.005$	Yes
Reveille *et al.* (1995b)	Greek	36	33	0	0	—	0.11	0.07	NS	No
Davies *et al.* (1995)	White	82	59	—	—	—	63[e]	41[e]	$p = 0.008$	Yes
Skarsvag (1995)	Scandinavian	51	121	13.7 (7)	1.6 (2)	$p = 0.0028$, 9.7	0.294[d]	0.141[d]	$p = 0.0172$, 2.3	Yes
Reveille *et al.* (1998)	White	69	186	—	—	—	30[e]	20[e]	NS	Yes
	Hispanic	68	119	—	—	—	19[e]	13[e]	NS	Yes and DR8
Naves *et al.* (1998)	Spanish	84	96	—	—	—	0.143	0.156	NS	No
Steinsson *et al.* (1998)	Icelandic	64	194	—	—	—	0.266	0.126	$p = 0.002$	Yes (DRB1*03)
Sullivan *et al.* (1999)	White	104	140	—	—	—	0.034[i]	0.004[i]	$p = 0.012$	No[i]
Howard *et al.* (1986)	African American	35	35	2.9 (1)	0	—	0.200	0.071	$p = 0.046$, 3.25	No
Wilson *et al.* (1988)	African American	59	59	1.7 (1)	0	—	0.177	0.079	$p = 0.02$	Not tested
Olsen *et al.* (1989)	African American	79	68	5.1 (4)	0	—	0.12[d]	0.04[d]	$p = 0.05$, 4	No
Reveille *et al.* (1998)	African American	88	73	—	—	—	20[e]	20[e]	NS	No, but DR2 increased
Sullivan *et al.* (1999)	African American	84	82	—	—	—	0.018[i]	0[i]	NS	No[i]
Hong *et al.* (1994)	Korean	60	72	0	0	—	0.208	0.125 Increased	$p < 0.05$, 2.1	No, but DR2 and DR9
Dunckley *et al.* (1987)	Chinese	75	76	2.7 (2)	0	NS	0.304	0.188	$p < 0.05$	Not tested
Hawkins *et al.* (1987)	Southern Chinese	72	61	—	—	—	0.307	0.155	$p < 0.05$	No, but DR2 increased
Zhao *et al.* (1989)	Chinese	58	89	8.6 (5)	0	—	0.397	0.169	$p < 0.001$	Not tested
Dunckley *et al.* (1987)	Japanese	51	50	11.8 (6)	0	$p < 0.05$	0.347	0.121	$p < 0.01$	Not tested
Yukiyama *et al.* (1988)	Japanese	53	166	0	0	—	0.339	0.207	$p < 0.004$	Not tested
Yamada *et al.* (1990)	Japanese	59	166	0	0	—	0.220	0.066	$p < 0.005$	Not tested
	Japanese	59	159	0	0	—	0[d]	0.003[d]	—	Not tested

NS, Not significant.
[a]Two female Burmese and three Australian Aborigines were included in this patient group.
[b]Minimal estimated C4A null allele frequency.
[c]Healthy relatives.
[d]Frequency of the deleted C4A gene.
[e]Population frequency (%).
[f]Number of haplotypes.
[g]C4A gene frequency in healthy Darwin Aborigines was taken from Ranford *et al.* (1987).
[h]Frequency of the silent, nondeleted C4A gene.
[i]Frequency of C4AQ*0 due to a 2–base pair insertion in exon 29 which is associated with HLA-B60 and -DR6.

the presence of a C4AQ*0 allele conferred a relative risk of 4.5 despite no association with HLA-DR3 (Olsen *et al.,* 1989). Furthermore, in Japanese and other Asian populations in which HLA-DR3 is extremely rare, the association with C4AQ*0 persisted (Dunckley *et al.,* 1987; Yamada *et al.,* 1990). However, a number of studies in white (De Juan *et al.,* 1993; Goldstein and Sengar, 1993; Reveille *et al.,* 1995b, 1998) and African American (Reveille *et al.,* 1998) populations have not found significant associations between the presence of SLE and C4AQ*0 alleles.

Reported associations between C4BQ*0 alleles and SLE are much weaker than for C4AQ*0 alleles (Table X). Many studies have found no increase in C4BQ*0 gene frequency in white populations and in other ethnic groups. It is therefore necessary to explain why partial deficiency of C4A and not C4B might predispose an individual to SLE. There are functional differences between C4A and C4B that could account for this. The C4A isotype shows preferential binding to amino groups, forming amide bonds, and binds particularly to proteins, for example, in immune complexes (Schifferli *et al.,* 1986). C4B shows preferential binding to hydroxyl groups, forming ester bonds, and binds predominantly to carbohydrates. C4B binds more effectively to the surface of erythrocytes than C4A, and for this reason is more active in hemolysis than C4A.

Complement activation by immune complexes interferes with lattice formation, thereby maintaining complexes in solution (Heidelberger, 1941). Hence, deficiency of C4A may cause less effective processing of immune complexes with deposition in tissues and resultant damage. The role of complement in the processing of immune complexes is discussed in detail in VI,C. There is also increasing evidence that the complement pathway plays a role in the clearance of apoptotic cells (discussed in Section VI). It is not known whether the C4A and C4B isotypes show any difference between their abilities to bind to apoptotic cells.

The molecular basis of a number of the more common C4AQ*0 and C4BQ*0 alleles has been characterized. The C4AQ*0 allele on the extended haplotype HLA-A1, B8, DR3 is most commonly caused by a large deletion involving C4A and the adjacent 21-hydroxylase A pseudogene locus (CYP21A) (Carroll *et al.,* 1985; Goldstein *et al.,* 1988; Kumar *et al.,* 1991; So *et al.,* 1990). The deleted C4AQ*0 allele is easy to ascertain using molecular techniques and—as would be expected from the association between this deletion and the HLA-A1, B8, DR3 haplotype—a significant association between this deletion and SLE has been reported in white lupus patients (Hartung *et al.,* 1992; So *et al.,* 1990). However, the deleted C4AQ*0 allele was also significantly increased in a study of African American lupus patients (24% versus 7.4%, $p = 0.05$, relative risk = 4), although all patients with the deletion were either HLA-DR2$^+$ or HLA-DR3$^+$ (Olsen

et al., 1989). Among individuals who possess C4AQ*0 alleles originating from haplotypes other than HLA-A1, B8, DR3, this gene deletion is rare (Goldstein *et al.,* 1988; Hong *et al.,* 1994; Kumar *et al.,* 1991; Yamada *et al.,* 1990). For example in Japanese patients, despite a significant increase in C4AQ*0 allele frequency (44.1% versus 13.3%, $p < 0.005$), no patient had a deletion of the C4A gene (Yamada *et al.,* 1990).

Deficiency of C4A may also be a consequence of nonexpression of the C4A gene. A C4A pseudogene can result from a 2–base pair insertion in exon 29 of the C4A gene, which results in the generation of a premature stop codon, most commonly in association with HLA-B60, DR6 (Barba *et al.,* 1993) but also described on the HLA-A2, B40, Cw3, DR6 haplotype (Nordin Fredrikson *et al.,* 1991). This non–DR3-associated mutation has also been found in the C4B gene on the haplotype HLA-A2, B39, Cw7, DR2 (Lokki *et al.,* 1999). A recent study of 188 lupus patients and 222 healthy controls demonstrated that the 2–base pair C4A gene mutation was significantly increased compared to controls (gene frequency: 0.027 versus 0.002, $p = 0.004$) (Sullivan *et al.,* 1999). When the patients were analyzed by ethnicity, the gene frequency remained significantly elevated in the 104 white lupus patients (gene frequency: 0.034 versus 0.004, $p = 0.012$). In the 84 African American lupus patients studied, the gene frequency was lower (0.018) compared to the white patient group, while none of the 82 African American control population possessed this mutation. This supports the hypothesis that C4A null alleles may be the relevant disease susceptibility for lupus. However, the molecular basis for the other C4AQ*0 alleles is not yet known and this prevents the molecular epidemiology work that will be essential in establishing whether C4AQ*0 alleles are important independent disease susceptibility genes in all populations.

2. C2 Null Alleles

C2 deficiency is the most common inherited deficiency of the classical pathway of complement. The case that homozygous C2 deficiency is a powerful predisposing factor for SLE has been considered above. Is there any evidence that heterozygous C2 deficiency may also be a disease susceptibility gene for the development of lupus?

As is the case for C4, it is extremely difficult to detect C2 null alleles by measuring protein levels in blood samples from patients with active SLE because of the complement activation *in vivo* associated with disease activity. However, the C2 null allele (C2Q*0) occurs, in the large majority of cases, in association with the haplotype HLA-A25, B18, Cw-, DR2, C4A*4, C4B*2, C2Q*0, Bf*S (Agnello, 1978; Awdeh *et al.,* 1981b; Hauptmann *et al.,* 1982). Identification of this haplotype, especially of the unusual

TABLE X

C4BQ*0 Allele Frequencies in Different Populations of Patients with Systemic Lupus Erythematosus

Reference	Race/Ethnic Group	No. of		Homozygous C4BQ*0 Deficiency			Frequency of C4BQ*0 Allele			DR3 Association with SLE?
		Patients	Controls	Patients % (No.)	Controls % (No.)	p Value, Relative Risk	Patients	Controls	p Value, Relative Risk	
Fielder *et al.* (1983)	White	29	42	0	0	—	0.086	0.14	—	Yes
Christiansen *et al.* (1983)	White[a]	41	176	5 (2)	4 (7)	—	0.15[b]	0.10[b]	—	Not tested
Howard *et al.* (1986)	White	63	63	—	—	—	0.087	0.111	NS	No but DR2 increased
Dunckley *et al.* (1987)	White	63	197	3.2 (2)	4 (8)	NS	0.231	0.195	NS	Not tested
Batchelor *et al.* (1987)	White, DR3 negative	30	60	0	0	—	0.25	0.266	NS	All DR3 negative
Gougerot *et al.* (1987)	White	19	108	—	—	—	0.368	0.134	$p < 0.001$	Yes
Hartung *et al.* (1989)	White	196	204	—	—	—	*c*	*c*	NS	Yes and DR2
So *et al.* (1990)	White	54	62	—	—	—	0.065[d]	0.024[d]	NS	Yes and DR2
Schur *et al.* (1990)	White	101	1731	—	—	—	0.069	0.147	$p < 0.04$	No
	English/Irish	27[e]	144[e]	0	1	—	—	—	—	Yes
	Other Europeans	62[e]	310[e]	0	2	—	—	—	—	No
Christiansen *et al.* (1991)	Australian	62	133	3.2 (2)	—	—	0.12	0.17	NS	No
	Aborigine	9		22.2 (2)	—	—	0.33	0.22[f]	—	No
Hartung *et al.* (1992)	White	396	204	—	—	—	10[g]	14[g]	NS	Yes
De Juan *et al.* (1993)	White	58	69	—	—	—	41[g]	29[g]	NS	Yes
Reveille *et al.* (1995a)	Mexican	55	112	—	—	—	0.09	0.05	NS	Yes
Reveille *et al.* (1995b)	Greek	36	33	—	—	—	0.055	0.106	NS	No

Reveille *et al.* (1998)	White	69	186	—	—	—	23[g]	18[g]	NS	Yes
	Hispanic	8	19	—	—	—	21[g]	8[g]	$p = 0.03$	Yes and DR8
Naves *et al.* (1998)	Spanish	84	96	—	—	—	0.286	0.063	$p = 0.6 \times 10^{-4}$	No
Steinsson *et al.* (1998)	Icelandic	64	194	—	—	—	*c*	*c*	NS	Yes (DRB1*03)
Howard *et al.* (1986)	African American	35	35	—	—	—	0.171	0.071	NS	No
Wilson *et al.* (1988)	African American	59	59	5.1 (3)	5.1 (3)	NS	0.156	0.127	NS	Not tested
Reveille *et al.* (1998)	African American	88	73	—	—	—	19[g]	19[g]	NS	No but DR2 increased
Hong *et al.* (1994)	Korean	60	72	—	—	—	0	0.0208	NS	No but DR2 and DR9 increased
Dunckley *et al.* (1987)	Chinese	75	76	1.3 (1)	1.3 (1)	NS	0.143	0.126	NS	Not tested
Hawkins *et al.* (1987)	Southern Chinese	72	61	—	—	—	0.168	0.147	NS	No, but DR2 increased
Zhao *et al.* (1989)	Chinese	58	89	1.7 (1)	2.2 (2)	—	0.216	0.73	$p < 0.001$	Not tested
Dunckley *et al.* (1987)	Japanese	51	50	0	2 (1)	NS	0.142	0.193	NS	Not tested
Yukiyama *et al.* (1988)	Japanese	53	166	0	2.4 (4)	—	0.321	0.298	NS	Not tested

NS, Not significant.

[a]Two female Burmese and three Australian Aborigines were included in this patient group.

[b]Minimal estimated C4B null allele frequency.

[c]Numerical data were not reported, but the authors stated that the C4BQ*0 gene frequency did not differ between patients and controls.

[d]Frequency of the deleted C4B gene.

[e]Number of haplotypes.

[f]C4B gene frequency in healthy Darwin Aborigines was taken from Ranford *et al.* (1987).

[g]Population frequency (%).

C4 allotypes in combination with the Bf*S allotype of factor B, is a reasonable surrogate for the detection of the C2 null allele.

Results of the first study to test the hypothesis that C2 deficiency might be a disease susceptibility gene for autoimmune disease showed that there was a significant association between SLE and heterozygous C2 deficiency (5.9% versus 1.2%, $p = 0.0009$) (Glass *et al.,* 1976). This result has not been confirmed by subsequent studies. The common haplotype containing the C2 null allele was not raised in a study of 248 central European lupus patients (Hartung *et al.,* 1989). Similarly, none of the HLA-B18$^+$ lupus patients in another white population possessed the complotype C4A*4, C4B*2, Bf*S (Christiansen *et al.,* 1983), suggesting that a single null C2 allele does not confer increased susceptibility to disease.

As discussed earlier, the molecular basis of C2 deficiency is most commonly due to a 28–base pair genomic deletion, associated with the HLA-A25, B18 haplotype (type I C2 deficiency) (Johnson *et al.,* 1992). This has enabled precise identification of null alleles, and several groups have now measured the prevalence of the deleted C2 null allele using molecular techniques. Among 86 Swedish lupus patients and 100 controls, no homozygous C2-deficient individuals were found and the frequency of C2 null alleles did not differ significantly between the groups (5.8% versus 1%, not significant using Fisher's exact test) (Truedsson *et al.,* 1993). A further study of 122 white lupus patients and 427 North American white controls did not demonstrate a significant increase in C2 heterozygotes in the patient group compared to ethnically matched controls (1.6% versus 1.4%, respectively) (Sullivan *et al.,* 1994). Furthermore, the 28–base pair deletion was not detected at all in 127 African American lupus patients or in 194 African American healthy controls (Sullivan *et al.,* 1994).

The MHC haplotype carrying the C2Q*0 allele is sufficiently common that it must carry a selective advantage to compensate for the disadvantage of the raised incidence of lupus among the homozygotes. While it is quite likely that such an advantage lies in other genes in the haplotype, it is also plausible that the C2 deficiency itself has a compensating advantage. Schorey *et al.* (1997) have reported that *Mycobacterium tuberculosis* makes a C4-like molecule that can scavenge C2a to generate an active C3 convertase, which in turn causes C3 deposition on the mycobacteria and facilitates their entry into macrophages through complement receptors. Absence of C2 would subvert this mechanism and could therefore result in increased resistance to tuberculosis (Lachmann, 1998).

In conclusion, the majority of studies (Table IV) have failed to demonstrate either a significant increase in heterozygous C2 deficiency or an increase in its associated haplotype. Hence, partial C2 deficiency does not appear to be a disease susceptibility factor for the development of SLE.

C. Complement Receptor Type 1 (CR1, CD35, C3b/C4b Receptor)

CR1 (the "immune adherence" receptor, C3b/C4b receptor, or CD35) is predominantly a cell surface protein with a transmembrane domain. It is distributed on B cells, neutrophils, monocytes, macrophages, erythrocytes, follicular dendritic cells, and glomerular epithelial cells. Its principal ligands are C3b, iC3b, and C4b. There are also data that suggest that CR1 may play a role as a C1q receptor (Klickstein *et al.,* 1997; Tas *et al.,* 1999).

CR1 has several important physiological functions. First, it has a crucial role in the protection of host tissues from damage by autologous complement activation. CR1 present on autologous cell membranes promotes the dissociation of C3b from Bb and the dissociation of C2a from C4b, resulting in the "decay acceleration" of the alternative and classical pathway C3 convertases, respectively. CR1 also acts as a cofactor to the serine esterase factor I, which cleaves C3b to iC3b. Factor H, a plasma protein, acts as an alternative cofactor to CR1 for this cleavage. However, only CR1 acts as a cofactor to factor I in the subsequent cleavage of iC3b to C3dg with the release of C3c.

Second, CR1 acts as an opsonic receptor on neutrophils and macrophages, enhancing phagocytic uptake of C3b-, C4b-, or iC3b-coated material. Third, in humans, CR1 present on erythrocytes enhances the physiological transport of opsonized bacteria and complement-fixing immune complexes from the bloodstream to the fixed mononuclear phagocytic system of the liver and spleen. Fourth, ligation of CR1 and CR2 (CD21, Epstein–Barr virus receptor) on B cells lowers the threshold for B cell activation in conjunction with the binding of the membrane-bound antigen receptor (antibody) by antigen. These last two functions explain the important accessory role of complement in the optimal induction of antibody responses.

1. Inherited Numerical Polymorphism of CR1

In 1981, Miyakawa and colleagues demonstrated that erythrocytes from the majority of patients with SLE failed to adhere to aggregated human Ig in the presence of complement. This finding was independent of disease activity and was also found in some of the relatives of the patients. It was suggested that reduced expression of CR1 might be an inherited disease susceptibility factor for the development of SLE. These findings were confirmed and amplified using antibodies in radioligand binding assays to quantitate CR1 numbers on erythrocytes (Iida *et al.,* 1982). Among healthy individuals, it was possible to detect three numerical phenotypes consisting of high, intermediate, and low erythrocyte CR1 expression (Wilson *et al.,* 1982). Analysis of pedigrees suggested that the defect in expression on the erythrocytes of SLE patients was inherited.

After the identification and sequencing of CR1 cDNA (Wong *et al.,* 1985, 1986), a CR1 genomic polymorphism that correlated with erythrocyte CR1 numbers was identified (Wilson *et al.,* 1986). Hybridization of *Hin*dIII-digested human genomic DNA with a CR1 cDNA probe showed that individuals with high numbers of CR1 sites per erythrocyte possessed a single 7.4-kb restriction fragment, whereas individuals with low numbers per erythrocyte had a single 6.9-kb restriction fragment (Wilson *et al.,* 1986). Individuals possessing both restriction fragments had intermediate erythrocyte CR1 numbers. This polymorphism of CR1 has been found in all populations studied so far and appears to be ancient in human phylogeny. It has been speculated that variation in CR1 expression may play a role in host defense against infectious and parasitic disease (Xiang *et al.,* 1999). This hypothesis is considered further in Section II,C,3.

The identification of an inherited numerical polymorphism of CR1, together with the discovery that CR1 numbers were reduced on erythrocytes from patients with SLE, led to the hypothesis that the reduced erythrocyte CR1 expression in SLE was inherited. The *Hin*dIII 6.9-kb restriction fragment length polymorphism (RFLP) could be a marker of a disease susceptibility allele for the disease (Wilson *et al.,* 1982). A reduction in the number of patients homozygous for the 7.4-kb "high" allele was reported (Wilson *et al.,* 1987) compared with normal subjects.

All subsequent studies have confirmed the reduced expression of CR1 on erythrocytes from patients with SLE (Holme *et al.,* 1986; Inada *et al.,* 1982; Minota *et al.,* 1984; Walport *et al.,* 1985). However, the balance of the evidence now supports the hypothesis that there is an acquired loss of CR1 from the erythrocytes of patients with SLE. The evidence for this is considered in the next section.

2. The Reduction in Erythrocyte CR1 Numbers Associated with SLE Is Acquired, Not Inherited

Although among consanguineous relatives a reduction in erythrocyte CR1 levels compared to healthy controls was reported (Wilson *et al.,* 1982), a subsequent study did not detect a significant difference (Walport *et al.,* 1985). The identification of the RFLP correlating with numerical expression of CR1 meant that robust molecular epidemiological studies could be performed. Several groups found no difference in the allele frequency of this polymorphism among SLE patients compared with control populations (Cohen *et al.,* 1989; Moldenhauer *et al.,* 1987; Satoh *et al.,* 1991; Tebib *et al.,* 1989). In three of these studies, patients with SLE, despite being homozygous for the 7.4-kb allele associated with high expression of CR1, had low erythrocyte CR1 numbers (Mitchell *et al.,* 1989; Moldenhauer *et al.,* 1987; Satoh *et al.,* 1991).

In addition, several studies have provided evidence for acquired loss of CR1 from the erythrocytes of patients with SLE. The reduction in erythrocyte CR1 numbers was correlated with measures of disease activity (Corvetta *et al.,* 1991; Holme *et al.,* 1986; Ross *et al.,* 1985). Additional correlations were reported between low erythrocyte CR1 numbers and increased circulating immune complexes (Iida *et al.,* 1982; Inada *et al.,* 1982), increased erythrocyte C3dg expression (Ross *et al.,* 1985), and reduced C4 levels (Iida *et al.,* 1982). Erythrocyte CR1 numbers increased significantly during SLE disease remission (Holme *et al.,* 1986; Iida *et al.,* 1982). The correlation between reduced erythrocyte CR1 and increased erythrocyte-bound C3dg demonstrated during active lupus also reversed during remission (Ross *et al.,* 1985).

Direct *in vivo* evidence of acquired reduction in erythrocyte numbers was obtained by studying patients with SLE receiving blood transfusions (Walport *et al.,* 1987). In patients with active disease, as much as 60% of erythrocyte CR1 was lost during the first 5 days following transfusion. Normal expression of CR1 was found on reticulocytes from patients with SLE, showing that loss of CR1 occurs from these cells during their time in the circulation (Lach-Trifilieff *et al.,* 1999).

Finally, a reduction in erythrocyte CR1 numbers has been found to be associated not only with SLE but also with other diseases characterized by the presence of immune complexes and systemic complement activation. These diseases include rheumatoid arthritis (Corvetta *et al.,* 1991; Iida *et al.,* 1982), autoimmune hemolytic anemia (Ross *et al.,* 1985), primary Sjögren's syndrome (Ross *et al.,* 1985; Thomsen *et al.,* 1986), juvenile rheumatoid arthritis (Thomsen *et al.,* 1987), paroxysmal nocturnal hemoglobinuria (Pangburn *et al.,* 1983; Ross *et al.,* 1985), hepatitis C infection (Kanto *et al.,* 1996), acquired immunodeficiency syndrome (AIDS) (Tausk *et al.,* 1986), and lepromatous leprosy (Tausk *et al.,* 1985).

It is not known how CR1 is lost from erythrocytes in SLE. As noted above, reduced CR1 numbers are found in diseases associated with antibody and complement deposition on erythrocytes, that is, autoimmune hemolytic anemias and diseases associated with the presence of immune complexes. Indeed, in blood samples from patients with SLE, an inverse correlation was found between mean numbers of bound C3dg fragments and CR1 numbers per erythrocyte (Ross *et al.,* 1985). Erythrocytes bearing C3 fragments and immune complexes interact with mononuclear phagocytic cells in the liver and spleen (see Section VI,C). During these interactions, any erythrocyte-bound immune complexes are stripped from the cell and erythrocyte-bound iC3b is catabolized to C3dg. CR1 is exceptionally sensitive to proteolytic degradation (Ripoche and Sim, 1986). It is possible that CR1 is cleaved from erythrocytes by the action of proteolytic enzymes

from macrophages during their interactions with erythrocytes bearing complement or immune complexes.

3. Inherited Structural Polymorphisms of CR1

In addition to the inherited numerical polymorphism, CR1 has a complicated structural polymorphism. There are four codominantly inherited allotypes showing large size variation (Dykman *et al.,* 1983, 1984, 1985). These have molecular weights of 220 (CR1-A, or F allotype), 250 (CR1-B or S allotype), 190 (CR1-C, or F^1 allotype) (Dykman *et al.,* 1984), and 280 (CR1-D) (Dykman *et al.,* 1985). The size differences reflect changes in the number of long homologous repeats (LHRs) in the extracellular domain of CR1, which in turn are accompanied by changes in the number of C3b and C4b binding sites (Klickstein *et al.,* 1988; Wong *et al.,* 1989). The most common allele, CR1-A, consists of four LHRs, termed A, B, C, and D. It possesses a single C4b binding site in the N-terminal two SCRs (short consensus repeats) of LHR-A, while the N-terminal two SCRs of LHR-B and LHR-C each contain a C3b binding site (Klickstein *et al.,* 1988). The largest allotype, CR1-B, has five LHRs and is predicted to have a third C3b binding site (Wong *et al.,* 1989). The smallest allotype, CR1-C, has three LHRs with only a single C4b and C3b binding site (Wong and Farrell, 1991). These allotypic structural variants are thought to have arisen from variable duplication of the gene sequences encoding the LHR (Wong *et al.,* 1986).

In addition, it has been discovered that a number of blood group antisera—Knops, McCoy, Swain-Langley, and York—recognize specificities on CR1 (Moulds *et al.,* 1991). Of particular interest, a malaria-encoded erythrocyte surface molecule, PfEMP1, has been shown to mediate rosetting of erythrocytes, by adhesion to CR1 molecules on other erythrocytes (Rowe *et al.,* 1997). A common African structural variant of CR1, Sl(a−), shows reduced binding to PfEMP1 and may have a protective role against severe malarial infection.

Several studies have analyzed the prevalence of these structural variants of CR1 among patients with SLE (Cornillet *et al.,* 1992; Dykman *et al.,* 1984; Moldenhauer *et al.,* 1988; Moulds *et al.,* 1996; Van Dyne *et al.,* 1987). The frequency of the A, B, and C alleles has not differed between lupus patients and ethnically matched healthy controls in most studies (Dykman *et al.,* 1984; Moldenhauer *et al.,* 1987; Moulds *et al.,* 1996). One study of 63 French lupus patients demonstrated an increase in the frequency of the CR1-B (S allotype) allele when compared with ethnically matched healthy controls (51% versus 26%, $p = < 0.001$) (Cornillet *et al.,* 1992).

III. Animal Models of Complement Deficiency

C5 deficiency was discovered as a spontaneous mutant in laboratory mice many years ago (Nilsson and Muller-Eberhard, 1967), and many laboratory strains now in common use lack expression of C5 protein (Rosenberg and Tachibana, 1986). C8-deficient mice have also been described (Tanaka *et al.,* 1991). However, there have been no natural examples of any other complement deficiency occurring in mice.

The advent of gene-targeting technology has allowed the development of mouse strains deficient in many different complement proteins. From the investigation of these mice, significant advances have been made in the study of the role of complement in the pathogenesis of SLE, which we now describe. We focus on the data from the mouse, which is an excellent model species for the development of SLE. We also briefly describe the findings from other species in which spontaneous complement-deficient mutants have arisen.

A. C1q-Deficient Mice

C1q-deficient ($C1qa^{-/-}$) mice were generated by insertional mutagenesis of the first exon of the C1qA chain gene, *C1qa,* resulting in mice with no *C1qa* transcripts detectable on Northern blots and no circulating C1q protein detectable by Western blot or enzyme-linked immunosorbent assay (ELISA) (Botto *et al.,* 1998). As expected, these mice lacked classical pathway-mediated lytic activity and the ability to opsonize immune complexes with C3, but retained a functional alternative pathway. To date, C1q deficiency is the only complement deficiency in mice which has been associated with the spontaneous development of an SLE-like autoimmune disease.

A large cohort consisting of 226 $C1qa^{-/-}$ and 108 wild-type control mice, on a hybrid 129 × C57BL/6 genetic background, were monitored for the development of autoimmune disease. By 8 months of age, more than half of the C1q-deficient animals had detectable levels of ANAs and 25% had histological evidence of proliferative glomerulonephritis (Botto *et al.,* 1998). The glomerulonephritis was associated with the presence of electron-dense subendothelial and subepithelial immune deposits and the glomeruli stained positively for IgG and C3, suggesting activation of complement via the alternative pathway. The presence of a large number of apoptotic bodies was noted in the glomeruli of the nephritic animals. C1q-deficient mice with no histological evidence of glomerulonephritis also exhibited a significantly increased number of glomerular apoptotic bodies compared with the control animals. The expression of SLE-like disease was critically

dependent on the genetic background of the knockout animals. Only *C1qa*$^{-/-}$ mice of the hybrid 129 × C57BL/6 genetic background developed an SLE-like disease. It is relevant that mice of this genetic background are already predisposed to the development of autoimmunity (Botto *et al.,* 1998; Obata *et al.,* 1979), and the C1q deficiency augmented the development of autoimmunity in these mice.

B. Factor B/C2-Deficient Mice

Generation of mice deficient in complement components factor B and C2 (*H2-Bf/C2*$^{-/-}$) by gene targeting resulted in a loss of both classical and alternative pathway-mediated complement activity and antibody-mediated C3 opsonic activity in the serum of deficient animals (Taylor *et al.,* 1998). However, in contrast to C1q-deficient mice, these animals did not develop spontaneous autoimmunity. This suggested that the protective role of complement from autoimmunity did not require C3 activation and that the early components of the classical pathway were sufficient in mice as well as in humans. When crossed with the C1q-deficient mice to generate a strain of mice deficient in C1q, factor B, and C2 (*C1qa/H2-Bf/C2*$^{-/-}$), the spontaneous development of autoimmunity, characterized by the production of ANAs and proliferative glomerulonephritis, was again evident (Mitchell *et al.,* 1999). The only obvious difference between the pathologies of the diseases observed in the *C1qa*$^{-/-}$ and *C1qa/H2-Bf/C2*$^{-/-}$ mice was the complete absence of C3 deposition in the glomeruli of the nephritic *C1qa/H2-Bf/C2*$^{-/-}$ animals, consistent with the inability of these mice to activate C3. These observations supported the existence of a role for C1q, and possibly C4, in protection from autoimmune glomerulonephritis and suggested that activation of complement and deposition of C3 was not required for the expression of the disease. The existence of a hierarchy among the early classical pathway proteins in mice, with respect to their protective role against autoimmunity, recapitulates the findings in humans and shows that they represent valid models for studying the pathogenesis of SLE associated with primary complement deficiency.

C. C3-, C4- and CR1/2-Deficient Mice

So far, there has been no report of a predisposition to the spontaneous development of autoimmunity in mice rendered deficient in C3 (Wessels *et al.,* 1995), C4 (Fischer *et al.,* 1996), or CR1/2 (Ahearn *et al.,* 1996; Molina *et al.,* 1996) in spite of substantial abnormalities in the antibody responses of these mice, which is discussed later. The effect of these deficiencies on the expression of autoimmunity caused by the mutant Fas (CD95) gene, *lpr,* have been studied. Prodeus and co-workers (1998) crossed C3-, C4-, and CR1/2-deficient mice on the mixed genetic back-

ground with Fas-deficient C57BL/6.*lpr/lpr* mice. C57BL/6.*lpr/lpr* mice develop a mild lupus-like disease associated with autoantibody production but not with glomerulonephritis (Izui *et al.,* 1984).

CR1/2- and C4-deficient *lpr/lpr* mice exhibited increased lymphadenopathy (and splenomegaly in the case of the CR1/2-deficient mice), increased levels of ANAs and anti–double-stranded DNA autoantibodies, an increased presence of IgG and C3 in the glomeruli, and increased glomerular hypercellularity compared to the complement-sufficient control animals. Strikingly, the C3-deficient mice did not show a similar phenotype to the C4-deficient animals. Apart from a mild increase in the presence of IgG in the glomeruli of the C3-deficient animals, they were not notably different from the control animals (Prodeus *et al.,* 1998). These results were attributed to a break in the maintenance of B cell tolerance in the CR1/2- and C4-deficient mice, which is discussed in more detail in Section VI,D.

D. Complement Deficiency in Other Nonhuman Species

Colonies of guinea pigs are widely available with C4, C2, and C3 deficiencies (Bitter-Suermann and Burger, 1986). These animals do not display any spontaneous glomerulonephritis (Foltz *et al.,* 1994). However, SLE has not been described in the guinea pig, which seems to be a resistant species with respect to this disease. C4-deficient guinea pigs were found to have increased IgM rheumatoid factor levels and an increased polyclonal antibody response (Bottger *et al.,* 1986a).

The clinical phenotype of C3 deficiency in humans and that of dysregulated C3 consumption, due to deficiency of factor H, have been recapitulated in dogs and pigs with similar inherited deficiencies. A colony of dogs with C3 deficiency developed an extremely similar pattern of MCGN to that seen in some humans with C3 deficiency (Cork *et al.,* 1991). Factor H deficiency was identified among pigs of the Norwegian Yorkshire breed, which suffered from a runting disease caused by early onset of MCGN type II (Hogasen *et al.,* 1995; Jansen *et al.,* 1995). An effective breeding program has eliminated factor H deficiency from the Norwegian pig population, and this model of glomerulonephritis is no longer available for study (Hogasen *et al.,* 1997).

IV. Complement and Inflammation in SLE

Complement activity in sera from patients with SLE is reduced in relation to disease activity (Lloyd and Schur, 1981; Schur and Sandson, 1968; Townes *et al.,* 1963) and increases following treatment (Vaughan *et al.,* 1951). Complement deposition was identified in inflamed tissues (Lachmann *et al.,* 1962; Tan and Kunkel, 1966). Levels of the classical pathway

proteins (C1q, C2, and C4) are particularly reduced (Hanauer and Christian, 1967; Morse *et al.*, 1962; Schur and Sandson, 1968). C3 levels are less frequently abnormal, and low C3 levels are a pointer to the presence of severe disease (Lloyd and Schur, 1981; Weinstein *et al.*, 1983).

However, studies of cohorts of patients with SLE show only weak (albeit highly significant) correlations between disease activity and reductions in the levels of individual complement proteins (Cameron *et al.*, 1976; Valentijn *et al.*, 1985). There are at least two important factors that may confuse the relationship between complement levels in serum and disease activity. The first is the effect of variation in rates of biosynthesis of complement proteins. There have been several studies of the turnover *in vivo* of radiolabeled complement proteins, from which it is possible to estimate synthetic and catabolic rates of turnover of individual complement proteins (Alper and Rosen, 1967; Hunsicker *et al.*, 1972; Sliwinski and Zvaifler, 1972). Protein concentrations are a function of synthetic and catabolic rates. Among patients with SLE, increased catabolic rates for complement proteins were observed, but these were accompanied by great variability in synthetic rates, from reduced to increased. Such variation in synthetic rates may completely mask the effects of increased catabolism secondary to disease activity.

The second important factor confounding the value of simple measurements of complement protein concentrations as a measure of disease activity is the effect of the presence of autoantibodies such as anti-C1q antibodies to complement proteins, discussed in Section V. As seen below, high titers of anti-C1q antibodies are commonly associated with profound reductions in the levels of the classical pathway proteins and C3, and also with severe disease activity.

The presence of complement deposits in tissues is poorly correlated with the presence of tissue injury (Biesecker *et al.*, 1981; Gilliam *et al.*, 1974; Pohle and Tuffanelli, 1968). This raises the key question, Is complement deposition an important cause of inflammatory tissue injury in SLE?

Evidence from animal models of SLE and glomerulonephritis suggests that immune complex–mediated injury to tissues is mediated mainly by ligation of Fc receptors and that complement may play a much lesser role. For example, Fc–γ chain–deficient NZB/NZW mice were protected from developing severe nephritis, despite the presence of immune complexes in the kidney (Clynes *et al.*, 1998). A reciprocal experiment that supported this result was the finding in C1q-deficient mice that the development of spontaneous glomerulonephritis was independent of C3 activation (Mitchell *et al.*, 1999).

The Arthus reaction is widely used as an experimental model of inflammatory injury mediated by immune complexes. Using this reaction, some

experiments have suggested primacy of Fc receptor ligation in mediating tissue injury, while others have shown a clear role for the complement system. In experiments studying a murine model of the reverse passive cutaneous Arthus reaction, $C3^{-/-}$ and $C4^{-/-}$ mice developed inflammatory responses comparable to those seen in wild-type control mice, whereas the response of Fc–γ chain–deficient mice was significantly reduced (Sylvestre *et al.,* 1996). By contrast, in similar experiments on the reverse passive cutaneous Arthus reaction, although complement depletion did not affect the inflammatory response in wild-type animals, complete abolition of the response in FcγRIII-deficient animals occurred only following depletion of complement (Hazenbos *et al.,* 1996).

Furthermore, using similar experimental models, there were very clear data showing a role for complement in the induction of inflammatory injury. For example, mice deficient in the C5a anaphylatoxin receptor showed marked reduction in inflammation in both peritoneal and cutaneous reverse passive Arthus reactions (Hopken *et al.,* 1997) and were protected from immune complex–mediated lung inflammation (Bozic *et al.,* 1996). In addition, both complement activation and activation of FcγRI on peritoneal macrophages were required to initiate inflammation in a murine model of immune complex peritonitis (Heller *et al.,* 1999).

These findings show that the mechanism of induction of inflammation by immune complexes is, in some situations, complement independent, and in others, complement dependent. They serve to illustrate the complexity of mechanisms of inflammation in disease mediated by immune complexes. The size, composition, and location of immune complexes may each modify whether and how inflammation ensues.

V. Lupus Causes Autoantibody Production to C1q

High titers of autoantibodies to C1q are found in two closely related conditions. The first is the uncommon disease hypocomplementemic urticarial vasculitis syndrome, often abbreviated HUVS. Patients with this condition, as its name implies, suffer from chronic cutaneous vasculitis and urticaria and have very low C1q, C4, and C2 levels. Additional clinical features include glomerulonephritis, chronic obstructive airways disease of the lungs, angioedema, and ocular inflammation (Wisnieski *et al.,* 1995). The serological hallmark of HUVS is the presence of high titers of anti-C1q autoantibodies, first identified as C1q precipitins (Agnello *et al.,* 1971). The second disease in which anti-C1q antibodies are common is SLE, and there is a significant overlap between the clinical features of HUVS and SLE (Trendelenburg *et al.,* 1999).

Approximately one third of patients with SLE have elevated levels of anti-C1q autoantibodies in their serum (Antes *et al.,* 1988; Siegert *et al.,* 1991). The presence of anti-C1q antibodies in lupus is typically accompanied by a number of clinical and serological features. The complement profile is similar to that seen in HUVS, with very low levels of C1q, C4, and C2, and, to a lesser extent, C3. Anti-C1q antibodies tend to remain positive in SLE for prolonged periods, and there is associated prolonged hypocomplementemia. This differs from anti–double-stranded DNA antibody levels, which tend to fluctuate in concentration, together with inverse changes in complement levels. Many studies have shown that anti-C1q antibodies are correlated with the presence of glomerulonephritis and that increases in the titer of anti-C1q antibodies have been associated with flares of disease activity (Siegert *et al.,* 1993). These autoantibodies are typically of the IgG_2 subclass (Prada and Strife, 1992; Wisnieski and Jones, 1992). They react with a neo-epitope in the collagen region of C1q, which is exposed when C1q is bound to a surface, such as an immune complex, and dissociated from C1r and C1s (Antes *et al.,* 1988). One of the most popular assays for the measurement of circulating "immune complexes," the solid-phase C1q binding assay, was discovered to be really a measure of anti-C1q antibodies in the majority of cases. There are two common approaches now for the specific measurement and quantitation of anti-C1q antibodies. The first is a variant of the solid-phase C1q binding assay, in which the addition of 1 *M* NaCl prevents immune complexes from binding to immobilized C1q on a microtiter plate but allows the cognate binding of specific autoantibodies (Siegert *et al.,* 1990). The second method uses pepsin-digested C1q as the antigenic substrate on a microtiter plate, which contains the collagenous region of C1q but lacks the globular heads that might bind immune complexes or Ig aggregates in serum samples to be assayed (Menzel *et al.,* 1991).

The mechanism of the hypocomplementemia associated with the presence of anti-C1q antibodies is not certain. Anti-C1q antibodies do not cause complement activation in the fluid phase when added to fresh serum samples. The most likely mechanism is that they amplify complement activation by immune complexes in tissues, by binding to C1q fixed to immune complexes, enlarging the complexes and promoting further complement activation. This mechanism could also amplify inflammation caused by immune complexes within tissues, accounting for the association of anti-C1q antibodies with the presence of glomerulonephritis (Mannik and Wener, 1997).

The mechanism for the development of anti-C1q autoantibodies in SLE is not understood. A unifying hypothesis that could explain, on the one

hand, how C1q deficiency causes SLE and, on the other, how SLE causes anti-C1q antibodies is presented in Section VI.

Autoantibodies to C1q are also found in a smaller proportion of patients with rheumatoid arthritis and other autoimmune diseases. Among these patients, the presence of these antibodies is not associated with hypocomplementemia and the significance of this autoantibody in these diseases is uncertain.

VI. Hypotheses for the Association between Complement Deficiency and SLE

A. Introduction

Currently, there are three hypotheses to explain the link between complement deficiency and SLE. The first two, which are not mutually exclusive, relate to the role of complement in the physiological "waste disposal" mechanisms for dying cells and immune complexes. The third relates to the role of complement in the humoral immune response. We discuss each of these in turn here. We first consider the links between apoptosis and SLE, which set the stage for exploration of the hypothesis that failures in the pathways for clearance of apoptotic cells may predispose an individual to SLE. We then describe the role of complement in the clearance of immune complexes and how defects in this pathway might promote disease. Finally, we describe briefly the role of complement in the induction of humoral immune responses, reviewed elsewhere in this series (Carroll, 2000), and in the modulation of immune response to autoantigens.

B. Apoptosis and SLE

Research into SLE has been facilitated by discovery that a number of strains of mice develop spontaneous lupus closely resembling its human counterpart. Two strains of mice, MRL/*lpr* (Andrews *et al.,* 1978; Morse *et al.,* 1982) and C3H/*gld* (Roths *et al.,* 1984), revealed a close link between SLE and the apoptotic pathways for physiological cell death. The *lpr* gene was discovered to encode a mutant variant of fas (CD95) protein (Watanabe-Fukunaga *et al.,* 1992) and *gld* encoded a mutant fas–ligand (CD95–ligand) (Lynch *et al.,* 1994). These discoveries provided a satisfying explanation for the similar phenotype of these two strains of mice. As part of their phenotype, both mouse strains develop striking polyclonal lymphadenopathy with massive expansion of a T cell receptor α/β^+, $CD3^+CD4^-CD8^-$ population of lymphocytes. Since ligation of fas triggers death by apoptosis of lymphocytes, it seems likely that dysfunction of this pathway is responsible for the accumulation of lymphocytes, leading to massive lymphadenopathy and splenomegaly. Both MRL/*lpr* and *gld* mice develop many of the phenotypic features of SLE, including the full spec-

trum of specificities of ANAs, anti-C1q antibodies, glomerulonephritis, vasculitis, and arthritis (reviewed in Theofilopoulos and Dixon, 1985). It is important to note that the *lpr* and *gld* genes are not, on their own, sufficient to induce the expression of disease. C57BL/6 mice expressing the *lpr* mutation have a much milder phenotype than do MRL mice expressing the same mutant (Izui *et al.,* 1984; Kelley and Roths, 1985). They develop lymphadenopathy, splenomegaly, and moderate levels of autoantibodies (though very high levels of rheumatoid factor), without glomerulonephritis or other tissue injury. This shows that other gene products are necessary to modify the phenotype associated with null mutations in what are apparently critical cell surface proteins in an important signaling pathway for cell death. Some loci affecting the expression of autoimmunity in MRL/*lpr* mice have been mapped (Vidal *et al.,* 1998; Watson *et al.,* 1992).

Following the discovery of the phenotype and molecular basis of the MRL/*lpr* and *gld* strains of mice, a small cohort of humans has been described with a similar phenotype to that of mice (Sneller *et al.,* 1992). This condition is named Canale–Smith syndrome after the authors who first described the clinical syndrome (Canale and Smith, 1967) or, more descriptively, autoimmune lymphoproliferative syndrome (ALPS) (Fisher *et al.,* 1995). Patients typically have early onset of massive lymphadenopathy and splenomegaly, with an excess of $CD3^+CD4^-CD8^-$ lymphocytes. Three types of mutations have been identified. The first is a homozygous mutation in the *fas* gene, seen in a child with neonatal onset of very severe lymphadenopathy and splenomegaly (Rieux-Laucat *et al.,* 1995). The second and most common mutation is expressed as a dominant disease with very incomplete penetrance within families. A variety of heterozygous mutations in the *fas* gene has been found to cause this syndrome (Fisher *et al.,* 1995). The third is a single example of a mutation in the *fas*–ligand gene reported in a patient with lymphadenopathy and SLE (Wu *et al.,* 1996). The typical pattern of autoimmunity is more restricted than in mice, typically with the expression of autoimmune hemolytic anemia, neutropenia, and thrombocytopenia (Vaishnaw *et al.,* 1999). The main differences between humans and mice are, first, that disease is common in patients with heterozygous mutations, which have a dominant negative effect on signaling following ligation of fas protein and, second, the extreme variability of expression of disease among outbred human families, showing the important influence of modifying genes. In some families, certain individuals with a mutated *fas* allele develop lymphoma rather than autoimmunity (Gronbaek *et al.,* 1998), showing the importance of fas-mediated mechanisms in controlling lymphocyte growth.

The mechanism for autoimmunity has not been firmly established in either mice or humans with mutations in *fas* and *lpr* genes. The most

favored hypothesis has been that they have defects in the mechanisms for negative selection of autoreactive lymphocytes, especially in the periphery (Fatenejad *et al.,* 1998; Herron *et al.,* 1993), due to failure of the apoptotic mechanisms in lymphocytes that are part of the process of negative selection. There is evidence from studies using adoptive transfer of lymphocytes that the fas pathway defect is necessary in both the T cell and B cell compartment for full-blown disease to ensue (Sobel *et al.,* 1994).

A feature of ALPS in humans is the presence of hemophagocytosis with macrophages containing large numbers of phagocytosed blood cells in the liver and spleen (Le Deist *et al.,* 1996). It is of particular note that the autoimmune disease in humans with this syndrome is dominated by hemolytic anemia, neutropenia, and thrombocytopenia, each associated with autoantibodies to the respective cell type. This suggests the hypothesis that the phagocytosed blood cells may be presented as autoantigens.

This brings us to the second hypothesis linking apoptosis and SLE: that apoptotic cells are the source of the autoantigens that drive autoantibody production in genetically susceptible individuals.

1. Apoptotic Cells as a Source of Autoantigens in SLE

The most striking feature of SLE is the spectrum of autoantibodies to ubiquitous autoantigens. These may be categorized into three types. The first are the intracellular autoantigens, typically organized into intracellular clusters of antigens such as chromatin, the spliceosome complex, and the Ro/La small cytoplasmic ribonucleoprotein complex. The second are a series of cell surface antigens, including phospholipids of the cell membrane such as phosphatidylserine. The third category is plasma proteins such as β_2 glycoprotein I and C1q. It is generally accepted that it is the autoantigens themselves that drive the mature autoantibody response. However, it is not known what antigen triggers the autoimmune process in the first place. There are three possibilities. It could be the autoantigen itself, a mimic of the autoantigen (e.g., a viral polynucleotide sequence or cross-reacting viral or bacterial protein), or a complex of the autoantigen with a foreign antigen such as a self protein attached to a viral polynucleotide sequence.

There are two striking puzzles about the lupus autoantigens. The first is that they are ubiquitous and abundant in every cell and every compartment of the body in every individual. It is therefore extremely surprising that tolerance can break down to these autoantigens, which are the very essence of "self." The question that follows is, What are the protective mechanisms that normally prevent autoreactivity to these autoantigens, and how do they fail in lupus? The second puzzle is that, of all the many

thousands of potential intracellular, cell surface, and plasma autoantigens, it is only a particular spectrum of autoantigens that are targeted in SLE. The question that follows is, By what mechanism are these, but not others, selected?

The answers to these questions are not known, but there are some good hypotheses. The answer to the question about the nature of the autoantigens targeted in the disease may provide clues for answering the first question, about the mechanisms that protect the vast majority of individuals from the development of SLE.

There is a mounting body of evidence that apoptotic cells are the source of the autoantigens of lupus. This evidence may be divided into three categories. The first is that the structure of apoptotic cells is reorganized such that the lupus autoantigens become superficially accessible to recognition by antibodies. In a series of morphological studies, Rosen and collaborators have found that apoptotic cells express in surface blebs and apoptotic bodies many of the nuclear autoantigens of SLE (Casciola-Rosen *et al.,* 1994; Rosen *et al.,* 1995). The major antigen bound by the antiphospholipid autoantibodies found in approximately one third of patients with lupus is phosphatidylserine. This negatively charged phospholipid is found in the inner lamella of the cell membrane in healthy cells, but is actively translocated to the outer layer as part of the process of apoptosis (Casciola-Rosen *et al.,* 1996; Fadok *et al.,* 1992b). On apoptotic cells, phosphatidylserine acts as one of the recognition molecules for the physiological uptake and disposal of apoptotic cells, which are reviewed below.

The second category of evidence is that many of the lupus autoantigens undergo posttranslational modification during the process of apoptosis and may be cleaved or phosphorylated. This process could generate neoepitopes of autoantigens, which might appear as "non-self" to the immune system (Casciola-Rosen *et al.,* 1999; Rosen and Casciola-Rosen, 1999). However, the putative relevance of the cleavage and posttranslational modification of autoantigens would be more compelling if there were a demonstration that any lupus autoantibodies bound selectively to a neo-epitope in an antigen modified as part of apoptosis.

Third, there have been many experiments trying to induce or accelerate the production of lupus autoantibodies using various immunization protocols. The published results have frequently been equivocally positive. Recent experiments have shown that injection of apoptotic cells into mouse strains not normally susceptible to the development of SLE induces an autoantibody response (Mevorach *et al.,* 1998b).

The discovery that the source of the autoantigens in SLE may be apoptotic cells then leads to a possible answer to the second question above, about the mechanisms that protect against the development of autoimmu-

nity. Despite the massive turnover of apoptotic cells within the body, it is very rare to visualize an apoptotic cell within a section of tissue. This is because there are a large number of complementary mechanisms that result in the rapid removal and destruction of apoptotic cells by phagocytic pathways that do not normally lead to inflammation.

One possible pathway that could promote autoimmunity could be a breakdown of the normal mechanisms of removal of apoptotic cells, which might then drive an autoantibody response. It is hard to think that autoimmunity could be caused by a simple increase in the load of autoantigens. However, if the increased load of apoptotic cells was accompanied also by inflammatory signals, antigen-presenting cells might present autoantigens to T cells, in the context of costimulatory signals, and thereby overcome tolerance to the ubiquitous autoantigens of SLE. In the next section, we briefly consider the normal pathways for the clearance of apoptotic cells before turning to a possible role of the complement pathway in this and related clearance functions.

2. Apoptotic Cell Clearance

Apoptosis, or programmed cell death, is a fundamental process for the removal of damaged and effete cells during development and the maintenance of homeostasis (Savill, 1997). The apoptotic death process is rapid and characterized by cell shrinkage, condensation and fragmentation of the nucleus, cytoplasmic blebbing with maintenance of membrane integrity, and cell fragmentation into discrete apoptotic bodies. Apoptotic cells are rarely detected in healthy tissues, as they are swiftly subject to receptor-mediated ingestion by both professional and nonprofessional phagocytes, followed by intracellular degradation.

The normal processes of clearance of apoptotic cells cause the elimination of cells in a "silent" manner that causes no tissue injury. This is extremely important, because many cells (e.g., neutrophils, which have enormous turnover rates), contain enzymes and other products that could be very harmful in the absence of protective mechanisms for waste disposal. Indeed, the process of phagocytosis of apoptotic cells by macrophages causes the production of anti-inflammatory mediators such as tumor growth factor β_1, prostaglandin E_2, and interleukin 10 (IL-10) while reducing proinflammatory mediators such as TNF-α and IL-1β (Fadok *et al.,* 1998; Voll *et al.,* 1997), ensuring the "safe and quiet" removal of apoptotic cell debris.

As might be expected, in view of the importance of efficient removal of apoptotic debris, many receptor–ligand systems play complementary roles in mediating apoptotic cell clearance *in vitro.* During the process of apoptosis, the cell surface is modified to allow recognition of apoptotic cells by

phagocyte surface receptors. Translocation of phosphatidylserine to the outer lipid layer of the plasma membrane, exposure of altered glycosylation patterns, and the relocation of intracellular molecules to the outer surface of the dying cell may all serve as mechanisms by which a phagocyte can recognize an apoptotic cell from its healthy neighbor.

Receptors that have been implicated in the phagocytic removal of apoptotic cells are the $\alpha_v\beta_3$ vitronectin receptor (Savill *et al.,* 1990), the phosphatidylserine receptor (Fadok *et al.,* 1992a), CD36 (Ren *et al.,* 1995), CD14 (Devitt *et al.,* 1998), scavenger receptor A (Platt *et al.,* 1996), receptors for low-density lipoprotein (Bird *et al.,* 1999; Chang *et al.,* 1999; Sambrano and Steinberg, 1995), and complement receptors 3 and 4 (Mevorach *et al.,* 1998a). These different receptor–ligand systems may function in conjunction with one another, but individual types of phagocyte may show specific receptor–ligand preferences (Fadok *et al.,* 1992a).

3. Immune Response to Apoptotic Cells

There is much interest in the uptake and processing of apoptotic and necrotic cells by dendritic cells and the subsequent presentation to T cells of antigens derived from these dead cells. There are three key messages from the studies of the interactions of apoptotic and necrotic cells with dendritic cells. The first is that immature dendritic cells avidly take up both apoptotic and necrotic cells. The second is that the dendritic cells that have taken up these dead cells then require specific signals in order to mature to active antigen-presenting cells, characterized by up-regulated expression of MHC and costimulatory molecules. The third message is that a number of the specific signals that lead to maturation of dendritic cells have been identified as proinflammatory cytokines and products of infectious agents, such as lipopolysaccharide. From these results, it is possible to speculate how apoptotic cells may, under pathological circumstances, drive autoimmune responses. In this section, we describe experiments on the uptake of apoptotic and necrotic cells by dendritic cells. We also discuss the effects of defects in clearance mechanisms of apoptotic cells on their uptake by macrophages and dendritic cells, and how the presence of autoantibodies to apoptotic cells may perturb their fate. Finally, we speculate how impairment of the mechanisms for the clearance of dying cells may promote the development of autoimmunity.

Immature dendritic cells have been shown to be able to phagocytose apoptotic cells via the $\alpha_v\beta_3$ vitronectin receptor (Albert *et al.,* 1998a) and subsequently present apoptotic cell–derived antigens to MHC class I– and class II–restricted T cells (Albert *et al.,* 1998a,b; Inaba *et al.,* 1998) in a dose-dependent manner (Rovere *et al.,* 1998). There is a large body of evidence that suggests that the ingestion of apoptotic cells by immature

dendritic cells is not sufficient to cause dendritic cell maturation and effective antigen presentation. Proinflammatory mediators are necessary to induce the maturation of dendritic cells, especially molecules associated with infection, such as lipopolysaccharide and pathogen-derived nucleic acids (Cella *et al.,* 1999). Cytokines produced by the host in response to infection may also play an important role in driving dendritic cell maturation, such as TNF-α and interferon α (Luft *et al.,* 1998). This requirement for inflammatory mediators for the maturation of dendritic cells promotes antigen presentation in the context of inflammation. The corollary of this is that uptake of antigens by immature dendritic cells in the absence of inflammation is less likely to promote effective antigen presentation (Gallucci *et al.,* 1999; Sauter *et al.,* 2000). These mechanisms favor immune responses to infectious agents rather than autoimmune responses.

A number of groups have studied the maturation of dendritic cells that have taken up apoptotic and necrotic cells. The results of one study showed that only necrotic tumor cells induced maturation of human dendritic cells in culture. Neither apoptotic tumor cell lines nor apoptotic or necrotic cells from primary cultures caused dendritic cell maturation. Soluble supernatants from the necrotic cells could also induce dendritic cell maturation, as could monocyte-conditioned medium (Sauter *et al.,* 2000). Similar results were reported using a comparable murine system (Gallucci *et al.,* 1999). Another study casts doubt on even the ability of necrotic cells to induce dendritic cell maturation, as it was found that only necrotic cells from mycoplasma-infected cultures would stimulate dendritic cell maturation. Antibiotic treatment of these lines abolished their ability to induce dendritic cell maturation, even when the cells were rendered necrotic (Salio *et al.,* 2000).

In this chapter, we develop the hypothesis that abnormalities in the physiological clearance mechanisms of autoantigens may promote the development of autoimmune disease. Are the data on the handling of apoptotic cells by dendritic cells compatible with this hypothesis?

A delay in the clearance of apoptotic cells by macrophages may increase the likelihood that apoptotic cell debris could be efficiently captured and presented by dendritic cells. The existence of such a balance between macrophage- and dendritic cell–mediated capture of apoptotic tumor cells, which can influence the outcome of a subsequent immune response, has been demonstrated *in vivo* (Ronchetti *et al.,* 1999).

Ronchetti and colleagues immunized mice intraperitoneally with apoptotic sygeneic tumor cells to protect them from subsequent challenge with live tumor cells. Immunizing mice with bone marrow–derived macrophages and dendritic cells, which had been pulsed with apoptotic tumor cells, showed that only dendritic cells could induce a protective antitumor

cytotoxic T lymphocyte. While the apoptotic cells were not as efficient as nonreplicating live cells at priming an antitumor response, they were rendered more effective at protecting the mice from live tumor cells by pretreatment of the animals with carrageenan to reduce phagocytosis by the peritoneal macrophages. Conversely, enhancement of macrophage phagocytosis with granulocyte–macrophage colony-stimulating factor (GM-CSF) rendered the animals more susceptible to rechallenge with live tumor cells (Ronchetti *et al.,* 1999).

However, it is clear from the data presented in this section that simply increasing the uptake of apoptotic cells by dendritic cells cannot be sufficient to drive dendritic cell maturation and autoantigen presentation. Additional inflammatory signals appear to be required. It has been postulated that ingestion of high numbers of apoptotic cells by dendritic cells may be adequate to instigate dendritic cell maturation and the presentation of antigen derived from apoptotic cells in the absence of additional stimuli (Rovere *et al.,* 1998). The same scientists also found that autoantibodies (anti–β_2-glycoprotein I) bound to apoptotic cells caused dendritic cells to secrete inflammatory cytokines, including IL-1β and TNF-α, that may have autocrine and paracrine effects, enhancing dendritic cell maturation (Rovere *et al.,* 1999). In the next section, we discuss the evidence that complement plays a role in the clearance of apoptotic cells and consider the hypothesis that complement deficiency may promote autoimmunity via a pathway involving impairment of the clearance of apoptotic cells.

4. The Role of Complement in the Clearance of Apoptotic Cells

Complement was implicated first in the clearance of apoptotic cells by the observation by Korb and Ahearn (1997) that C1q could bind specifically and directly to the surface blebs of apoptotic keratinocytes. This interaction is thought to be mediated via the globular heads of the C1q molecule (Navratil *et al.,* 1998). This led to the hypothesis that C1q may promote the clearance of apoptotic cells, and hence exposed autoantigen, preventing stimulation of the immune system.

In vitro studies by Mevorach and colleagues (1998a) using complement-depleted sera and human monocyte-derived macrophages supported a role for both the classical and alternative pathways of complement in the phagocytosis of apoptotic cells. Blockade of complement receptors CR3 and CR4 impaired the phagocytosis of apoptotic cells. The presence of iC3b on the surface of apoptotic cells that had been incubated with serum suggested that the clearance was mediated by interactions between iC3b and CR3 and/or CR4. Binding of opsonized apoptotic cells to CR3-transfected CHO cells confirmed that CR3 had the potential to interact

directly with iC3b-coated apoptotic cells and could hence be an apoptotic cell clearance receptor (Mevorach *et al.,* 1998a).

To address the relevance of complement *in vivo* in the phagocytic removal of apoptotic cells, we studied apoptotic cell clearance in complement-deficient mice. We established a model of apoptotic cell phagocytosis during sterile peritonitis. Mice were injected intraperitoneally with thioglycolate to induce sterile peritonitis and the recruitment of inflammatory macrophages. Four days later, the mice were injected intraperitoneally with syngeneic apoptotic thymocytes. The phagocytic uptake of the apoptotic thymocytes by the elicited macrophages was significantly impaired in both C1q- and C4-deficient mice compared to wild-type control animals. However, perhaps more significantly, the defect in phagocytosis was significantly greater in the C1q-deficient animals than in the C4-deficient mice. Furthermore, when apoptotic cells were injected into the peritoneum of untreated mice, only the C1q-deficient mice exhibited a defect in phagocytosis, while C4- and C3-deficient mice showed phagocytic uptake similar to that of control animals (Taylor *et al.,* 2000). These observations indicate the existence of a hierarchy within the classical pathway with regard to the role of the components in the phagocytosis of apoptotic cells, which recapitulates the hierarchy of disease susceptibility in humans with complement deficiency.

Hence, one possible explanation of the link between complement deficiency and the predisposition to the development of SLE may be the degree of impairment of the physiological clearance of apoptotic cells. It is interesting to note that monocyte-derived macrophages from humans with SLE also exhibited impaired phagocytosis of apoptotic cells *in vitro* (Herrmann *et al.,* 1998). We have observed a similar phagocytic defect in macrophages derived from the monocytes of C1q-deficient humans cultured in autologous serum (Taylor *et al.,* 2000). This defect was correctable with purified human C1q.

All of these data are compatible with the hypothesis that C1q deficiency causes SLE by impairment of the clearance of apoptotic cells. These in turn may provide the source of the autoantigens that drive the autoimmune response of SLE. A reduced ability of macrophages to remove apoptotic cells at sites of inflammation may tip the balance of clearance of these cells toward clearance by dendritic cells. As we discussed in Section VI,B,3, it seems likely that increased uptake of apoptotic cells by dendritic cells is not sufficient to induce dendritic cell maturation and antigen presentation. However, if the apoptotic cells are cleared abnormally at sites of inflammation, the necessary cytokine milieu may drive dendritic cell maturation and initiate an autoimmune response.

5. A Hypothesis for the Development of Anti-C1q Autoantibodies in SLE

The binding of C1q to a major particle driving the autoimmune response in SLE could provide an explanation for the high frequency of autoantibodies to C1q, found in ~33% of patients with the disease. The autoantibodies of SLE typically react with several components of a normal physiological complex (e.g., anti–double-stranded DNA and antihistones in nucleosomes, or anti-Ro and anti-La in a small cytoplasmic RNP complex) (Hardin, 1986). Antiphospholipid antibodies are frequently found to be associated with antibodies to phospholipid-binding proteins, including annexin V (Hirata *et al.,* 1981), which binds to apoptotic but not normal cell surfaces and β_2-glycoprotein I (Galli *et al.,* 1990; McNeil *et al.,* 1990).

By analogy, it seems likely that anti-C1q antibodies develop as part of an autoantibody response to C1q complexed with other lupus autoantigens, which could be apoptotic cells or immune complexes. It is then an interesting question whether autoantibodies to C1q or to proteins such as β_2-glycoprotein I might then enhance the expression of SLE by further interfering with autoantigen processing.

C. The Role of Complement in Clearance of Immune Complexes

Fcγ receptors and complement mediate the normal processing of immune complexes. The interaction of complement with immune complexes was first characterized in the 1940s by Heidelberger (1941), who observed increased "particulation" of immune complexes, formed in solution in the absence of complement. Subsequent work has shown that the role of complement in the processing of immune complexes can be divided into two main activities. The first of these is modification of the lattice structure of immune complexes, maintaining immune complex solubility; the second is promotion of the recognition and capture of immune complexes by Fcγ and complement receptors on cells of the mononuclear phagocytic system.

There are several potential final destinations for immune complexes. The first of these destinations reflects the role of antibody in promoting the clearance and killing of pathogens, that is, uptake by a macrophage leading to clearance and catabolism of the immune complex. The second fate reflects another key role of immune complexes in the adaptive immune response, that is, the promotion of immune responses by targeting antigens to B lymphocytes, other antigen-presenting cells, and cells that both present and store antigen, such as follicular dendritic cells within germinal centers. In the absence of complement, the clearance of immune complexes is abnormal and the enhancing activities of immune complexes in adaptive immune responses are muted.

Complement prevents immune complex precipitation at successive steps during complement activation. First, the C1 complex has been shown to delay immune complex precipitation in a dose-dependent manner (Schifferli *et al.,* 1985), an effect that is probably transient, as C1 inhibitor rapidly degrades the complex, leaving just C1q, which is known to promote immune complex precipitation (Muller-Eberhard and Kunkel, 1961). After the initial stages of immune complex formation, classical pathway activation causes the deposition of C4b and C3b. Deposition of C3b on the heavy chain of IgG interferes with Fc–Fc interactions (Hong *et al.,* 1984), which have been shown to promote the aggregation of immune complexes (Moller, 1979). Deposition of C3 on the antigen also blocks sites of antigen–antibody interaction, reducing the effective valency of antigen and antibody (Lachmann and Walport, 1987). The demonstration that sera deficient in C3, C4, or C2 could not prevent immune complex precipitation showed that classical pathway C3b deposition was essential for this process (Schifferli and Peters, 1982; Schifferli *et al.,* 1985). Amplification of C3b deposition by the alternative pathway amplification loop may assist this process; however, the contribution of the alternative pathway to the prevention of immune complex precipitation is much less significant than that of the classical pathway (Schifferli *et al.,* 1982). The alternative pathway can solubilize immune complex precipitates, but this is a very inefficient process compared to the prevention of precipitation in the first place (Miller and Nussenzweig, 1975). Solubilization of immune complexes is accompanied by significant complement activation and the generation of substantial quantities of the anaphylatoxins and membrane attack complex formation.

Complement activity thus maintains immune complexes in a soluble form, which can be carried in the circulation away from the site of formation. In the circulation, there are transport mechanisms for immune complexes that promote their safe delivery to the mononuclear phagocytic system of liver, spleen, and bone marrow. CR1 on erythrocytes is predominantly responsible for the binding of C3b-bearing immune complexes in the circulation of primates. CR1 on the surface of erythrocytes is clustered and this enhances the avidity of binding of immune complexes to these cells (Paccaud *et al.,* 1988).

Immune complex clearance from the circulation of patients with SLE is abnormal, and there is evidence for defects of both complement- and Fc-dependent clearance mechanisms for immune complexes. Two types of *in vivo* studies have been performed in humans and nonhuman primates. The first type of study was of the clearance of IgG- and C3-coated erythrocytes; these were used as models for the study of the mechanisms of hemolytic anemia. IgG-coated erythrocytes were cleared mainly by the spleen (Frank *et al.,* 1983). Of particular interest, patients with SLE showed

delayed clearance of IgG-coated erythrocytes, which was interpreted to show defects in Fc receptor–mediated activity associated with SLE. By contrast, erythrocytes coated with C3 *in vitro,* using IgM cold agglutinin antibodies as a sensitizing reagent for complement activation, were not cleared permanently from the circulation (Atkinson and Frank, 1974). These cells were removed transiently from the circulation in the liver, thought to be by interaction of C3b and iC3b with complement receptors on Kupffer cells. After a short delay in the liver, during which it is thought that the iC3b on the erythrocytes was catabolized to C3dg, erythrocytes were released back into the circulation with a normal life span, bearing C3dg fragments for which no clearance receptor exists. There was no abnormality of clearance of these cells in patients with SLE.

By contrast with these immune complexes containing erythrocytes as antigen, soluble immune complexes showed different patterns of clearance *in vivo* in both humans (Davies *et al.,* 1992, 1993; Lobatto *et al.,* 1988; Schifferli *et al.,* 1989) and nonhuman primates (Cornacoff *et al.,* 1983; Waxman *et al.,* 1984). Large, soluble complement-fixing immune complexes bound to erythrocytes during their transit through the circulation and were cleared in the liver and spleen. In the presence of complement deficiency, both inherited and acquired, these immune complexes showed absent or diminished binding to erythrocytes. They showed reduced splenic clearance and disappeared from the circulation rapidly in the liver (Schifferli *et al.,* 1989). However, in patients with SLE or complement deficiency, the immune complexes were retained less effectively in the liver and a proportion were released back into the circulation, with the potential to be deposited in many organs throughout the body (Davies *et al.,* 1992, 1993; Schifferli *et al.,* 1989). These results suggest that the efficient removal of soluble immune complexes by the liver requires ligation of both complement and Fc receptors on Kupffer cells.

In the absence of efficient complement fixation, immune complexes may escape efficient clearance, deposit in tissues, and cause tissue injury via ligation of Fc receptors on neutrophils and other leukocytes. The resulting tissue injury may cause the release of autoantigens in an inflammatory milieu and augment the lupus autoimmune response.

D. The Role of Complement in the Humoral Immune Response

Pepys (1974) first showed a link between the complement system and antibody responses. He depleted mice of C3 using cobra venom factor and found that the antibody response to T cell–dependent antigens was suppressed, as was the generation of B cell memory in germinal centers (Papamichail *et al.,* 1975). However, the use of cobra venom factor only allows transient depletion of C3. The identification of animals and patients

genetically deficient in complement offered further opportunities to investigate the role of the complement system in the induction of humoral immune response. Guinea pigs genetically deficient of C2, C4, and C3 were investigated for their ability to mount an antibody response to bacteriophage ϕX174, an experimental thymus-dependent immunogen. When only limited amounts of antigen were used for immunization, all three types of complement-deficient animals showed markedly impaired antibody formation. This reduced antibody response was strictly dose dependent; when the antigen dose was increased, the immune response returned to normal (Bottger *et al.,* 1986b).

A similar role for the classical pathway complement in enhancing antibody responses has also been shown in mice genetically deficient in C3, C4 (Fischer *et al.,* 1996), and C1q (Cutler *et al.,* 1998). Comparison of the primary and secondary immune responses of these mice with wild-type controls following challenge with T cell–dependent antigens (bacteriophage ϕX174, sheep erythrocytes, or keyhole limpet hemocycianin-dinitrophenyl [KLH-DNP]) demonstrated that while their T cell response was normal, their B cell response was impaired. Splenic B cells of the complement (C1q-, C3-, and C4-)–deficient mice responded normally in proliferation assays *in vitro* following cross-linking of the B cell antigen receptor or CD40 and in response to stimulation with lipopolysaccharide. These results show that the B cells showed normal responses to pathways of stimulation independent of the complement system.

The obverse of these experiments showing defective antibody responses in the absence of complement is the study of Dempsey and colleagues (1996), who demonstrated that oligomers of C3dg coupled to antigen may act as an adjuvant, markedly enhancing antibody response and lowering the threshold of B cells for activation by ligation of the antigen receptor.

A similar phenotype to that of mice deficient in C3 and C4 was seen in mice deficient in CD21 and CD35 ($Cr2^{-/-}$) (Ahearn *et al.,* 1996; Molina *et al.,* 1996), confirming the critical role of the complement system in enhancing antibody responses.

Can the results obtained in experimental animal models be extrapolated to humans with complement deficiency? A C4-deficient patient showed an impaired humoral immune response to bacteriophage ϕX174 and failed to class switch the antibody response (Jackson *et al.,* 1979). However, antibody responses to rubella, Epstein–Barr virus, and tetanus toxoid were normal in another C4-deficient individual (Mascart-Lemone *et al.,* 1983). This discrepancy could be attributed to the antigen dose dependency that was observed in guinea pigs, described above. A patient with C3 deficiency was shown to have a reduced primary immune response to hemocyanin (Roord *et al.,* 1983). However, the same subject showed normal titers of

antibody following secondary immunization with tetanus, diphtheria, and pertussis vaccine (Roord *et al.,* 1983). Two C3-deficient patients who developed normal titers of primary and secondary antibody after immunization with ϕX174 failed to switch the antibody response from IgM to IgG, a result similar to that seen in complement-deficient animals (Ochs *et al.,* 1986). The majority of C3 patients have had total IgG levels within the normal range. However, it has been reported that patients with deficiency in the classical pathway proteins have reduced levels of IgG_4 and, to a lesser extent, IgG_2 (Bird and Lachmann, 1988).

From these observations, it is clear that the complement system plays an important role in the generation of humoral responses to low doses of antigens. Two mechanisms to explain the increase in immunogenicity have been proposed. First, opsonization of the antigen by complement enhances the targeting of antigen to follicular dendritic cells, which express CD35 (CR1) and CD21 (CR2), leading to more efficient antigen presentation to B cells and germinal center formation (Klaus *et al.,* 1980; Papamichail *et al.,* 1975). Second, the binding of cognate antigen carrying complement (in the form of an immune complex) to B cells lowers the threshold of activation of the cell by co-ligation of CD21 and the B cell IgM antigen receptor (Fearon and Carter, 1995).

However, these findings do not lead to a good hypothesis to explain how complement deficiency is associated with SLE. In summary, they show that a normal physiological activity of complement is to lower the threshold of activation of B cells and promote B cell memory. In the absence of complement, antibody formation is impaired and this should ameliorate, rather than promote, autoimmunity.

Another approach to the study of the role of complement in self-tolerance is the use of transgenic models of self-tolerance. Using such models, the expression of a transgenic autoantibody, either to a natural autoantigen or to a transgenically expressed "pseudo-autoantigen" can be examined in the context of other mutations. Prodeus and colleagues (1998) used such a system, comprising soluble hen egg lysosyme (sHEL) as autoantigen and anti-HEL as autoantibody, studied in the presence and absence of C4, C3, or CD21/CD35. They found evidence that "self-tolerance" to the sHEL was depressed in the presence of C4 or CD21/CD35 deficiency but not in the presence of C3 deficiency. They argued that this showed a role for complement in self-tolerance and proposed that deficiency of this activity may promote SLE. However, we interbred C1q-deficient mice with the same transgenic model and found no defect in self-tolerance to the pseudo-autoantigen, sHEL in the absence of C1q (Cutler *et al.,* 1999). sHEL does not represent a typical autoantigen of SLE, as a soluble secreted protein. It will be of interest to study the expression of a more representative

transgenic autoantibody, such as an anti-Sm or anti-DNA autoantibody in the context of complement deficiency.

VII. What Lessons Can Be Learned from Other Murine Models of Autoimmunity?

The existence of other models of murine SLE generated by both gene-targeting and natural mutations has given substantial insight into different mechanisms by which self-tolerance is broken, resulting in the spontaneous development of an SLE-like phenotype. These mechanisms can be divided into at least three different categories, each of which may require a mixture of genetic and environmental factors for full expression. The first of these is defects in the clearance of autoantigen, on which this review has focused. The second is abnormalities in the regulation and thresholds of B and T cell activation. The third category are the determinants, as yet unidentified, which cause susceptibility to particular patterns of organ injury (e.g., glomerulonephritis). SLE is a syndrome in mice and humans in which a series of different predisposing genes and environmental stimuli end in a final common pathway of autoantibody production and tissue injury.

Additional support for the hypothesis that defects in autoantigen clearance may stimulate the development of SLE comes from two further transgenic models of disease, mice with targeted deletions in serum amyloid P component (SAP) and those with isolated IgM deficiency. An *in vivo* demonstration linking spontaneous autoimmunity and the failure of the physiological clearance of chromatin was shown in mice deficient in SAP (Bickerstaff *et al.,* 1999). SAP is known to be able to bind to chromatin and displace H1-type histones, aiding in chromatin solubilization (Butler *et al.,* 1990). SAP has also been shown to bind *in vivo* to the surface of apoptotic cells and to nuclear debris released during cell necrosis (Hintner *et al.,* 1988). Mice deficient in SAP spontaneously developed high levels of autoantibodies against chromatin, histones, and DNA and also developed glomerulonephritis.

Mice have been developed with a targeted deletion of part of the secretory μ tailpiece, which prevents the secretion of IgM, though allowing the production of normal IgG levels (Boes *et al.,* 1998; Ehrenstein *et al.,* 1998). These mice are an important model to explore the physiological role of secreted IgM *in vivo.* When crossed with the spontaneous autoimmune mouse strain, MRL/*lpr,* the progeny developed a higher frequency of ANAs and glomerulonephritis than in the parental strain (Boes *et al.,* 2000). Mice lacking secreted IgM expression alone developed higher titers of anti–double-stranded DNA antibodies (Ehrenstein *et al.,* 2000). The investigators speculated that the development of autoimmunity in these mice was compatible with a role for natural autoreactive IgM in the clearance of autoantigens.

A series of mouse strains illustrate that particular mutations that affect thresholds for activation of B and T cells may cause the development of SLE. An autoimmune phenotype was reported in mice with genetic deficiency of CD22 (O'Keefe *et al.,* 1996, 1999), Lyn (Hibbs *et al.,* 1995), and SHP-1 (Shultz *et al.,* 1993; Tsui *et al.,* 1993). Of particular interest was a study showing that a combination of heterozygous deficiencies of CD22, Lyn, and SHP-1 behaved as a complex quantitative trait, causing in combination a reduction in B cell threshold for activation by a transgenic autoantigen (Cornall *et al.,* 1998). In addition, a break in self-tolerance has been observed in mice with defects in cell cycle regulation (Balomenos *et al.,* 2000) and in genes involved in Fas-mediated cell death (Di Cristofano *et al.,* 1999).

VIII. Conclusions

The evidence is overwhelming that deficiency of classical pathway complement proteins causes the development of SLE in humans and transgenic mice. The most important complement proteins for protection against SLE are C1q, C1r and C1s, and C4. It is an important challenge to understand the mechanism of this protective effect.

Complement plays a key role in the clearance of immune complexes. Recent evidence supports a role for complement in the clearance of apoptotic cells. These data support the hypothesis that complement deficiency causes lupus by the impairment of physiological disposal of autoantigens, and apoptotic cells may be an important source of the autoantigens that drive the characteristic spectrum of autoantibodies found in SLE.

The events needed to break tolerance to ubiquitous autoantigens in SLE must be more than a simple increase in autoantigen levels. It seems likely that there must be concomitant proinflammatory signals that induce dendritic cells to provide the necessary costimulatory signals to drive T cell responses, which in turn provide the necessary help to B cells to produce an autoantibody response. There is good evidence, at least in murine models of disease, that genetic abnormalities modifying lymphocyte activation thresholds may increase disease susceptibility.

One of the major paradoxes in the relationships between the complement system and SLE is that C1q deficiency causes SLE, yet SLE causes autoantibodies to C1q in approximately one third of patients. This paradox might be explained if C1q binds to lupus autoantigens and promotes their physiological clearance. An absence of C1q could then modify the clearance pathways of autoantigens and trigger an autoimmune response. On the other hand, complex formation between C1q and autoantigens might ex-

plain the prominent autoantibody response to C1q seen in patients with SLE, who are not C1q deficient.

It is clear that the traditional view of the role of complement in SLE needs revision. Complement activation in SLE has been viewed as a major cause of tissue injury. Instead, evidence is emerging that complement may play a protective role rather than an exclusively proinflammatory role in tissue injury. There is much work still to be done in order to understand fully the immunobiology of complement in relation to SLE.

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