

C4A Deficiency and Elevated Level of Immune Complexes: The Mechanism Behind Increased Susceptibility to Systemic Lupus Erythematosus

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ABSTRACT. *Objective.* Studies of an Icelandic cohort showed that susceptibility to systemic lupus erythematosus (SLE) in individuals with C4A deficiency was increased only in the presence of increased concentrations of immune complexes. We investigated the interaction of C4A deficiency with elevated concentrations of immune complexes in healthy individuals; i.e., how different levels of C4A affected the activation of C4 and C3 and subsequent binding of increased immune complex load to human red blood cells (RBC).

Methods. Forty-five healthy individuals having different levels of C4A were studied, 8 with homozygous C4AQ0, 12 with heterozygous C4A deficiency, and 25 with normal C4A. For comparison to the complement status present after prolonged disease activity, 5 patients with SLE homozygous for C4AQ0 were also studied.

Results. The results showed that intact immune complex-RBC binding is dependent on the levels of immune complex-bound C3 fragments, which correlate to the levels of IC-bound C4Ad ($R = 0.677$, $p = 0.02$), but not on levels of IC-bound total C4d ($R = 0.451$, $p = 0.16$). Immune complex binding to RBC was also evaluated in increasing immune complex load. C4A deficient sera had less ability to bind the increased immune complex load to RBC than sera with normal C4A. These results are consistent with the presence of increased amounts of poorly opsonized immune complexes in C4A deficiency, leading to increased precipitation in tissues and initiation of a self-perpetuating cycle.

Conclusion. Susceptibility to SLE is increased in individuals with C4A deficiency as C3 opsonization of immune complexes becomes insufficient at elevated immune complex concentrations. (J Rheumatol 2002;29:2359–66)

Key Indexing Terms:

COMPLEMENT C4AQ0
IMMUNE COMPLEX HANDLING

SYSTEMIC LUPUS ERYTHEMATOSUS
IMMUNE COMPLEX DISEASES

Association of C4A deficiency (C4AQ0) with systemic lupus erythematosus (SLE) has been documented in many populations, although some population studies fail to confirm a specific role for C4A deficiency in determining susceptibility to SLE¹⁻⁴. The incidence of C4A deficiency is quite common, but seems to vary according to race and sex^{5,6}. This inconsistency between different populations, as well as the mechanism of the contribution of C4A deficiency to autoimmune pathogenesis, has not been explained. In the Icelandic population, 25% of healthy individuals have deficient expression of either one or both of the C4A genes,

with an increase to 50% in individuals with 4 or more American Rheumatism Association (ARA) criteria for SLE^{6,7}. In contrast, the prevalence of SLE in the Icelandic population is only about 0.06%, indicating that C4A deficiency is an additional but not an isolated risk factor for SLE.

It has been suggested that the association of the increased risk for SLE with the C4A null phenotype might be due to the different roles of C4A and C4B in immune complex handling, although overall reduction of total C4 with the subsequent diminished effector function has also been mentioned⁸⁻¹⁰. Thus the C4A isotype has greater affinity for amino groups, whereas the 99% identical C4B isotype binds more strongly to hydroxyl groups. Immunoglobulins are rich in amino groups, and C4A has been shown to have greater affinity for the immunoglobulin molecules in immune complexes⁸⁻¹¹. In addition, the C4A isotype itself is considered to mediate more effective C4b binding of immune complexes to complement receptor 1 (CR1)^{12,13}. Therefore, defective immune complex clearance has been suggested to be one of the explanations for the association between C4A deficiency and SLE. However, since C4 has experimentally also been associated with generation and

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maintenance of tolerance, and human C4A has been implicated in generation of normal humoral immune response, other mechanisms for C4A deficiency predisposing to SLE may also be of importance^{14,15}.

The clearance of immune complexes from the circulation is dependent on effective opsonization, binding of the immune complex-bound C3b fragment to CR1 on erythrocytes and subsequent transport to the liver and spleen¹⁶. Effective immune complex opsonization with C3d is also necessary for normal functions of B cells through binding to CR2 on their surface¹⁷. Therefore, sufficient binding of immune complex-bound C3b and C3d to cell-bound complement receptors is considered to be essential for maintenance of healthy immunologic balance. The immune complex-bound C3b is subsequently broken down to C3c and C3d. Therefore the level of immune complex-bound C3d directly reflects the amount of the short lived fragment C3b. In the classical pathway, the C4 component is cleaved before the C3 component. Thus, the defective function in C4A deficiency, which enhances the risk of SLE, could be due to either low total C4 or to lack of a specific opsonic property of the C4A isotype, both leading to a limited amount of C3 fragments binding to the immune complexes.

In a family study, we have shown that the increased incidence of SLE (28% of family members) coincided with 2 abnormalities, one being C4A deficiency (47% with homo- or heterozygous C4A deficiency) and the other increased level of immune complexes (56%) independent of the C4A deficiency. Individuals with both abnormalities coexisting had increased risk of SLE (6/8) compared to those who had only one of those abnormalities (2/14 with C4AQ0, and 1/12 with elevated immune complexes)¹⁸. In that study healthy C4A deficient individuals did not show abnormalities in immune complex opsonization and CR1 binding, but the C4A deficient individuals with SLE symptoms did. Those findings suggested our working theory, i.e., when immune complex levels get elevated in C4A deficient individuals, the immune complex handling becomes critical at lower levels in C4A deficient individuals than in C4A normal individuals, thereby increasing the risk of SLE.

In this study we investigate further how these 2 abnormalities, increased immune complex level and C4A deficiency, together might predispose to SLE, and why the majority of C4AQ0 individuals are healthy. For that purpose, the complement opsonic ability was evaluated in both healthy and symptomatic individuals having different amounts of C4A. This was done by measuring by ELISA the deposition of the complement split products C4Ad, C4d, and C3d on surface bound immune complexes. This was then compared to the corresponding immune complex binding to CR1 on normal red blood cells (RBC), under standard conditions and with increasing immune complex load.

MATERIALS AND METHODS

Patient groups. Blood samples were collected from 45 healthy individuals having different levels of C4A, 8 homozygous for C4A deficiency, 12 heterozygous for C4A, and 25 individuals with normal amount of C4A. For comparison to the status of affected sera in C4A deficient individuals with longterm disease activity, 5 homozygous C4A deficient patients with SLE were included in the study. Four had ≥ 4 ARA criteria for SLE¹⁹ and one had < 4 ARA criteria. All the individuals were C4 phenotyped as described. C4 and C3 levels were measured in all the sera by electroimmunodiffusion²⁰. All subjects gave informed consent to participate in this study.

C4 phenotyping. This was done by high voltage agarose electrophoresis on carboxypeptidase and neuramidase treated serum samples, with subsequent immunofixation²¹. Null allele status was confirmed by the relative intensities of C4A and C4B bands. Complement antisera (anti-C4A and anti-C4B) were obtained from Atlantic Antibodies (Scarborough, ME, USA).

ELISA measurements of C4Ad, total C4d, and C3d binding to immune complexes. The binding of complement split products was measured by ELISA as described²². In brief, microtiter plates were coated with 100 $\mu\text{g/ml}$ of heat aggregated human IgG (Pharmacia, Uppsala, Sweden) for 1 h at 37°C. The nonspecific binding sites were blocked with 0.5% bovine serum albumin (BSA) in phosphate buffered saline (PBS). Sera were diluted 1/100 and 1/200 in 0.01% BSA/Veronal buffer with Ca^{++} and Mg^{++} (VBS), and 100 μl added to each well. The plates were incubated overnight at 4°C followed by 1 h incubation at 37°C. After washing 3 times with PBS-Tween (0.05%), the plates were incubated with either 100 μl of monoclonal anti-C4Ad (clone RgD1, J.M. Moulds, Department of Microbiology and Immunology, MCP Hahnemann University School of Medicine, Philadelphia, PA, USA), anti-C4d (Quidel A213, Quidel Corp., San Diego, CA, USA), or alkaline phosphatase (AP) conjugated rabbit anti-C3d (Dako A063, Dako, Glostrup, Denmark) for 4 h at room temperature, followed by 3 washes with PBS/Tween. The monoclonal antibodies have been shown to react with all major C4 allotypes according to their specificity²² (N. Nasser, Quidel Corp., personal communication). The plates with anti-C4d and anti-C4Ad were then incubated overnight at 4°C with AP conjugated rabbit anti-mouse IgG (Dako D314) in 1/1000 dilution. After color development with p-nitrophenyl phosphate (Sigma Diagnostics), C4d, C4Ad, and C3d concentrations were measured by comparing the absorbance readings (Titertec Multiscan) with a standard curve obtained with serial dilutions of normal human serum (in dilutions 1/25 to 1/1800). The values of undiluted normal human serum were set as 100 arbitrary units (AU). A comparative study of C4 and C4A levels assayed by this ELISA to the levels assayed by radial immunodiffusion showed correlation coefficients ranging from 0.78 to 0.98 for both patients and controls²³.

Evaluation of functional C3 opsonization through immune complex binding to RBC. The binding of immune complexes to RBC was measured by an assay developed in our laboratory²⁴ measuring the ability of complement to mediate binding of AP-anti-AP complexes to CR1 on normal RBC from an ORh+ donor. Briefly, 10 μl AP (Sigma p-5521, 50,000 DEAunits; Sigma Chemical Co., St. Louis, MO, USA) diluted 1/120 in PBS were added to 30 μl of serum diluted in RPMI-1640 (Gibco BRL, Paisley, Scotland), followed by addition of 10 μl of anti-AP (polyclonal goat anti-AP, IgG fraction 55975; Organon Teknica-Cappel, West Chester, PA, USA) diluted 1/12 in PBS. After incubation for 10 min at 37°C, 150 μl RBC solution (20% in RPMI) were added and incubated at 37°C for a further 15 min. Then the reaction was stopped by adding 1 ml of ice cold PBS and the tubes were transferred to an ice bath. After washing, the packed cells were resuspended in 500 μl cold PBS. Fifty microliters of this suspension were transferred to a U-microplate well, and AP enzyme activity assayed with paranitrophenylphosphate (Sigma) in diethanolamine buffer, pH 9.8. After centrifugation, 90 μl of the supernatants were transferred to a 96 well microplate and the absorbance read at 405 nm using a Titertec Multiscan spectrophotometer.

Immune complex binding to RBC with increasing immune complex level. The assay was as described, except for the concentration of antigen and

antibody, which were added in constant volume but decreasing dilution; 10 μ l AP in dilution 1/60 (= $\times 2$), 1/30 (= $\times 4$) mixed with 10 μ l anti-AP in dilutions 1/6 (= $\times 2$) and 1/3 (= $\times 4$). All the serum samples tested in this experiment had low immune complex concentration (< 100 AU).

Immune complex levels in serum. An assay described by Brandslund, *et al* was used²⁵. Briefly, the immune complexes were precipitated from serum by polyethylene glycol (PEG 6000) and resuspended in 260 μ l VBS containing Ca^{++} and Mg^{++} . Seventy-five microliters of the resuspension were mixed with 80 μ l guinea pig serum diluted 1/25 in VBS as the complement source, and incubated at 37°C for 20 min, followed by addition of 125 μ l of sensitized sheep erythrocytes in 16% solution in VBS. After additional incubation for 20 min, the reaction was stopped with 1 ml of ice cold PBS and the tubes were transferred to an ice bath. The resulting hemolysis is inversely proportional to the content of immune complexes. Undiluted standard was set as 500 AU; the 95% upper reference limit derived from measuring 52 healthy individuals was ≤ 105 AU.

Statistical evaluation. Evaluation of significant difference between 2 normally distributed groups was by Student's *t* test, and the possible correlation of 2 groups was investigated by regression analysis. $P < 0.05$ was considered significant.

RESULTS

C4Ad and total C4d deposited on aggregated human IgG. C4 and C3 concentrations were assayed by electroimmunodiffusion in all subjects. The heterozygous C4A deficient individuals and C4A normal individuals had levels within the normal range (C4: 0.19–0.61 g/l, C3: 0.5–1.05 g/l). Mean values for the homozygous C4AQ0 individuals (healthy subjects and patients with SLE) are shown in Table 1, and illustrate that the total C4 levels are fairly similar in these individuals; but although the group is small, the patients with SLE have significantly lower C3 values ($p = 0.02$) than the healthy individuals.

To confirm the C4A deficiency assayed by phenotyping and investigate the opsonization of immune complexes by total C4A and total C4, the deposition of C4Ad and total C4d on heat aggregated IgG were measured. The results show that C4Ad deposition directly reflects subjects' C4A status (Figure 1A). Thus, the C4A heterozygous individuals show values around 50% of the reference serum pool, but C4A normal individuals show normal values up to more than 3 times the value of the reference serum pool, indicating the abundant level in individuals with normal C4A. This was, however, not reflected in the deposition of total C4d, in which all the healthy individuals regardless of C4A status had similar deposition (Figure 1B). The homozygous C4AQ0 patients with SLE had lower values than both the

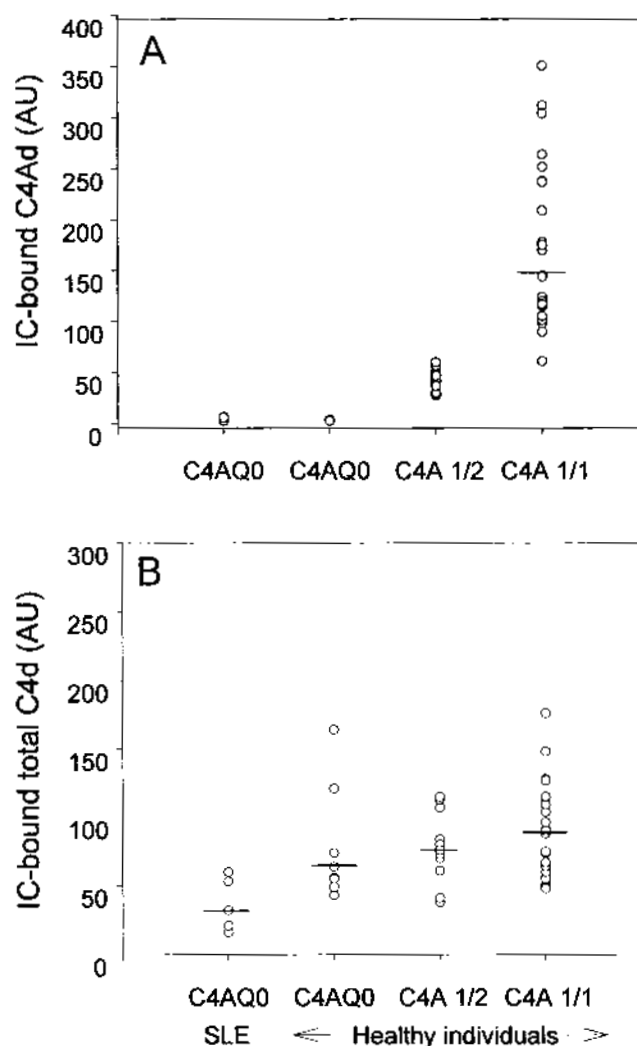


Figure 1. Levels of immune complex-bound C4Ad (A) and C4d (B) in the respective groups measured by ELISA. Homozygous C4A deficient individuals are indicated as C4AQ0, and those with SLE symptoms are marked SLE. All other groups are healthy individuals. The heterozygous-deficient individuals are indicated as C4A 1/2 and C4A normal individuals as C4A 1/1. C4Ad deposition confirms the deficiency status in individuals (A). The groups are small, but indicate that total C4d deposition is similar in all healthy individuals, although significantly lower in SLE symptomatic C4AQ0 individuals ($p = 0.02$ compared to C4A 1/2 and $p = 0.002$ compared to C4A 1/1 individuals)(B). Bars indicate median values.

heterozygous C4A deficient ($p = 0.02$) and C4A normal individuals ($p = 0.002$). The C4d values measured in the SLE patients are lower than in the healthy homozygous C4AQ0 individuals, but the difference is not significant ($p = 0.06$). Although lower, the values show a similar pattern compared to levels measured by immunodiffusion (data not shown).

Level of C4Ad deposited limits the deposition of C3 fragments. To investigate the effect of C4A deficiency on the subsequent steps in the complement cascade, the deposition of C3d on aggregated IgG was measured. The results are illustrated in Figure 2 and show that all the healthy individ-

Table 1. C4 and C3 concentrations in homozygous C4A deficient individuals.

Homozygous C4AQ0 Groups	C4, g/l (0.19–0.61 g/l*)	C3, g/l (0.5–1.05 g/l*)
SLE, n = 5	0.16 \pm 0.04	0.80 \pm 0.18 [†]
Healthy, n = 8	0.18 \pm 0.12	1.20 \pm 0.29

* 95% normal reference interval [†] Mean \pm SD.

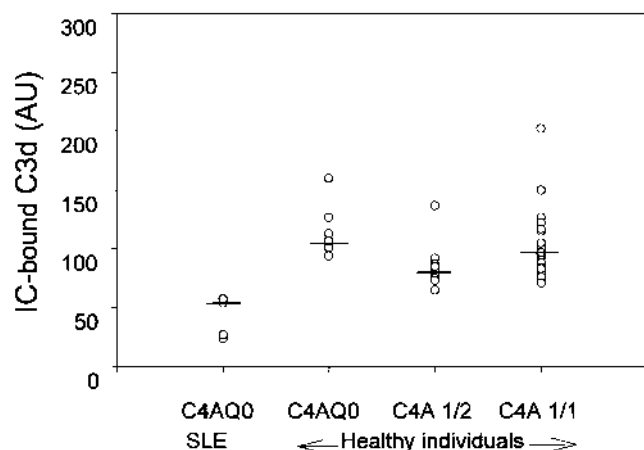


Figure 2. Levels of immune complex-bound C3d measured by ELISA. All healthy individuals have normal levels, but levels in SLE symptomatic C4AQ0 individuals are significantly lower regardless of C4A status ($p < 0.001$). Bars indicate median values.

uals have normal immune complex C3d deposition regardless of C4A status. However, the homozygous C4AQ0 SLE patients have significantly lower immune complex C3d deposition than all the other groups ($p < 0.001$), indicating consumption of C3 due to disease activity.

To investigate whether the deposition of immune complex-bound C3d in healthy individuals reflected immune complex opsonization by different levels of the C4A isotype or different levels of total C4, the immune complex deposition of C3d fragments was compared to the levels of immune complex-bound C4Ad versus levels of immune complex-bound total C4d. This was done in heterozygous C4A deficient individuals, as they have different levels of C4A as well as total C4 (Figure 1). The results are given in Figure 3, and show that C3d deposition is correlated to levels of C4Ad ($R = 0.677$, $p = 0.02$), but not to levels of total C4d ($R = 0.451$, $p = 0.16$).

Immune complex-RBC binding as a function of C3 opsonization. There was a strong relationship between the deposited C3d and the immune complex-RBC binding function ($p < 0.001$) for all individuals (Figure 4), confirming the necessity of immune complex-bound C3 fragments for intact binding to CR1 on RBC. Figure 4 also shows that the healthy C4A deficient individuals all have normal immune complex binding to RBC.

Effect of deficient C4A on the ability to handle increasing immune complex load. To investigate how C4A deficiency affected the ability to handle increasing immune complex load, immune complex binding to RBC from healthy individuals was evaluated with increasing concentration of AP-anti-AP immune complexes in sera from 5 healthy individuals having different levels of C4A: one with intact C4A and one with heterozygous and 3 with homozygous C4A deficiency. These sera were chosen as they all had similar

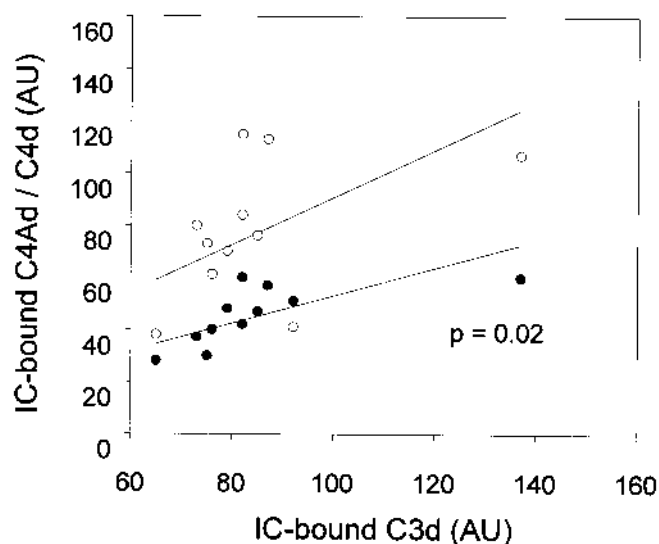


Figure 3. Level of immune complex-bound C3d compared to the level of immune complex-bound C4Ad (●) and total C4d (○), all measured by ELISA. Deposition of C3d is significantly related to immune complex-bound C4Ad ($R = 0.677$, $p = 0.02$), but not to immune complex-bound C4d ($R = 0.451$, $p = 0.16$).

immune complex-RBC binding ability at standard assay procedure (around 100 AU) and they were all found to have low immune complex levels (< 100 AU). To compare these healthy individuals to the situation after longterm disease activity, the test was also done in serum from one patient with SLE. The AP-anti-AP immune complexes have been shown to opsonize human complement²⁶. The results are shown in Figure 5, and indicate that all sera from the healthy individuals mediate similar immune complex-RBC binding ability while the immune complex load is low. However, with increasing immune complex load, proportionally diminished binding is observed in the homozygous C4A deficient sera compared to the C4A normal serum. At 4-fold immune complex concentration, the immune complex-RBC binding ability in homo- and heterozygous C4A deficient individuals can no longer mediate CR1 binding to the same extent as the C4A normal sera, leaving some unbound immune complexes in the solution. To confirm this, C3d activation was measured in these sera with increasing immune complex load. All the samples had similar C3d binding at immune complex load $\times 1$ (66–70 AU), but with 4-fold immune complex load, the most prominent proportional decrease in immune complex-bound C3d was seen in homozygous deficient sera (43–45 AU) as compared to heterozygous (55 AU) and normal C4A (67 AU) (data not shown).

Immune complexes. To investigate how immune complex levels in the study group would reflect their interaction with C4A deficiency and the increased risk of SLE symptoms, the immune complex level was evaluated in all the individuals. The results are consistent with interplay of elevated

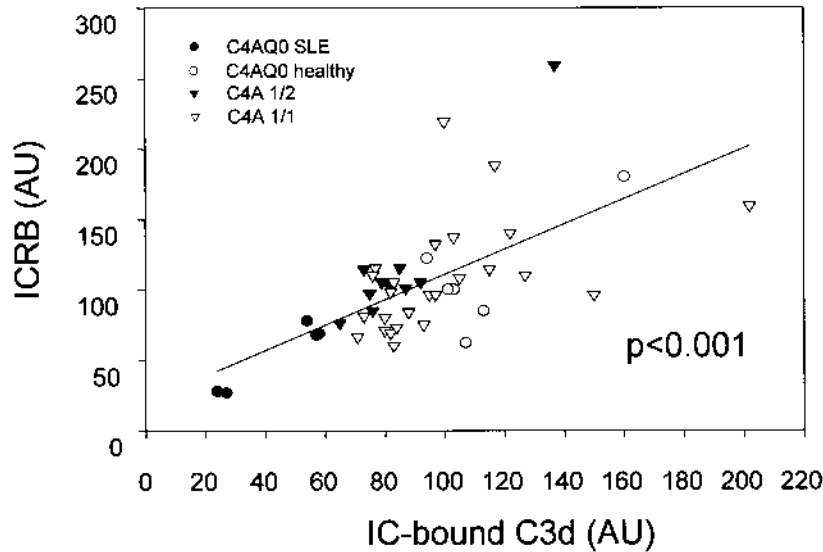


Figure 4. Immune complex-bound C3d measured by ELISA compared to immune complex-RBC binding (ICRB) function in patients with C4AQ0 SLE, C4AQ0 healthy individuals, heterozygous C4A deficient (C4A 1/2) and C4A normal (C4A 1/1) individuals ($R = 0.708$, $p < 0.001$). Healthy C4AQ0 individuals all have normal values of immune complex-bound C3d and ICRB, but patients with SLE have low values of both variables.

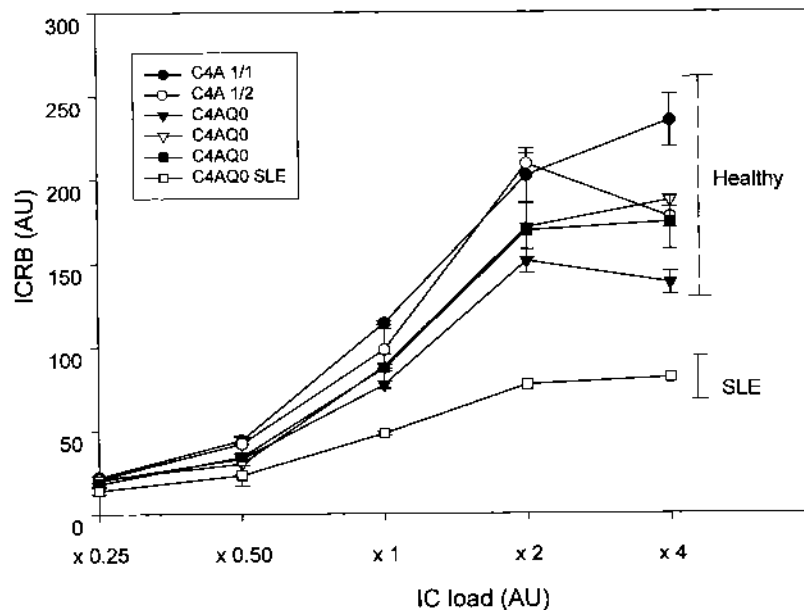


Figure 5. The effect on immune complex-RBC binding (ICRB) of increasing the immune complex load in C4A deficiency. Samples were from 5 healthy individuals with different C4A status: one with normal C4A, one with heterozygous C4A deficiency, and 3 with homozygous deficiency were assayed in duplicates with standard and increasing immune complex load, and compared to one representative sample from a homozygous C4A deficient patient with SLE. The term $\times 1 \dots \times 4$ indicates the increasing concentration of immune complex used, where $\times 1$ represents the immune complex level normally used in the ICRB test and $\times 0.25$, $\times 0.5$, $\times 2$, $\times 4$ are multiples, with no change in volume. The figure represents one of 3 experiments done, with identical results.

immune complex levels and C4A deficiency having a role in SLE, as all the healthy individuals with homozygous C4A deficiency had low immune complex levels (Figure 6).

DISCUSSION

The role of defective immune complex clearance in the pathogenesis of SLE has been well documented^{27,28}.

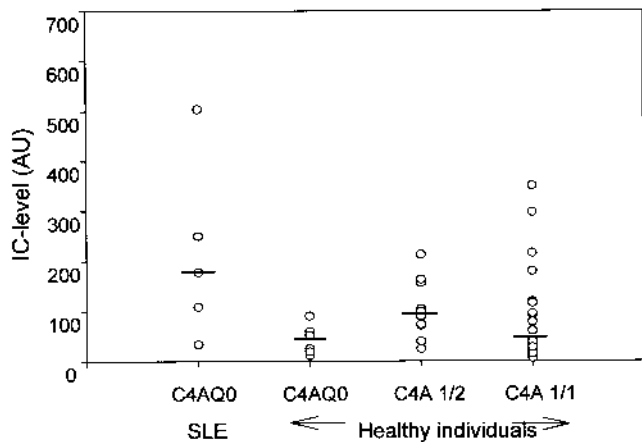


Figure 6. The level of immune complex in all the individuals, measured by complement consumption. Bars indicate median values. All the healthy C4AQ0 individuals have low immune complex levels. Reference value for normal immune complex concentration is < 105 AU, as evaluated in 50 healthy blood donors.

Insufficient complement opsonization of immune complexes is regarded as the main cause, leading to defective binding of immune complex to red blood cells and defective uptake in the liver and spleen^{29,30}. Since deposition of sufficient C4b and C3b on immune complexes is essential for normal immune complex handling, this deposition might be critical in C4A deficiency and explain to some extent the association of this deficiency with SLE. However, this has not been elucidated in detail¹⁶. Our group has shown that C4A deficiency, independent of the associated MHC alleles, contributes to susceptibility to and pathogenesis of SLE in Iceland^{6,7}. In a separate study, we have also shown that the disease susceptibility of C4A deficient individuals is greatly enhanced when coinciding with elevated immune complexes. Interestingly, the elevated immune complex level was not only present in SLE patients with C4AQ0, but also among healthy family members who had normal levels of C4A, showing that elevated immune complex level was an isolated risk factor that enhanced the risk of SLE when coinciding with the C4A deficiency¹⁸.

To investigate how C4A deficiency affects immune complex opsonization in healthy individuals, deposition of the complement fragments C4Ad, C4d, and C3d on heat aggregated IgG was measured in ELISA, as they reflect directly the short-lived active fragments produced during complement activation (C4Ab, C4b, and C3b). The C4Ad measurements confirmed the C4A deficiency in the respective groups. The deposition of total C4d can be regarded to reflect the actual C4 levels in individuals²³. However, the fact that patients with SLE had lower immune complex-bound C4d values than healthy homozygous C4AQ0 individuals (although they were found to have similar total C4 levels by immunodiffusion) suggests that using C4d ELISA is probably a more sensitive method to evaluate the total C4

available for immune complex opsonization in affected individuals with complement consumption. Total C4 opsonization would then depend on other factors, such as the levels of C1q and Mannan binding lectin, which might decrease with longterm disease activity. These factors were not measured in this study as we focused on immune complex handling in healthy individuals. That C4 (C4A + C4B) levels and immune complex-bound C4d levels in all the homozygous C4A deficient individuals regardless of SLE symptoms were not significantly different ($p = 0.06$) suggested that, even though the groups were small, deposition of total C4d would not be the critical factor to discriminate between healthy and symptomatic C4AQ0 individuals.

Opsonization of immune complexes with C3 fragments is critical in maintaining a healthy immunologic balance, and this was investigated in all the individuals¹⁶. The results illustrate the critical role of C3 opsonization in SLE, as patients have significantly lower values of immune complex-bound C3d ($p < 0.001$) than all the healthy individuals, regardless of their C4A status. These results reveal the dual role of C3 in SLE, i.e., the necessity of C3 opsonization of immune complexes for sufficient immune complex clearance, and the fact that SLE symptoms are more likely to appear in the absence of C1q, C2, and C4 (C4A), but not C3³¹. Our results confirm again, however, that C3 opsonization is the crucial factor for effective immune complex handling and decrease of susceptibility to SLE. Thus, the necessity of C2 and C4 in the pathogenesis of SLE would mainly be through limiting the possible C3 opsonization. This also holds true for C1q, which in addition has been reported to mediate uptake of apoptotic material by directly binding to CR1^{32,33}. The lack of association of C3 deficiency with SLE could, however, be explained by the absence of the main mediator of inflammation and tissue damage^{34,35}.

The additional finding that the immune complex-bound C3 fragments correlate to the levels of C4Ad, but not to total C4d, in individuals with heterozygous deficiency of C4A (Figure 3) suggests that the activation of C4A serves as a functional threshold for immune complex-bound C3 opsonization. This is observed even with normal concentrations of C4B, and is consistent with the reported preferential binding of C4A to immune precipitates^{8,10,11}. This could suggest that the different affinity of the C4 isotypes for immunoglobulins also extended to their affinity for C2 and thereby their ability to form the C3 convertase C4bC2a on immune complexes. This has been investigated to some extent, with no difference reported in the kinetics of C3 convertase formation *in vitro* between homozygous C4A null and C4B null individuals³⁶.

To investigate the functional ability of immune complex-bound C3 fragments in C4A deficiency, an assay for immune complex binding to RBC was chosen since it relies on C3 opsonization²⁴. The observed relationship of IC-

bound C3 fragments and the immune complex-RBC binding function ($p < 0.001$) confirms the reliability of the assays measuring these variables. Also, the fact that all the healthy individuals with homo- or heterozygous deficiency of C4A have normal functional ability, in contrast to the low values in the C4A deficient SLE patients, emphasizes that C4A deficiency alone does not mediate defective IC binding to normal RBC.

In our previous study, it was clear that the increased immune complex load was an isolated risk factor that together with C4A deficiency enhanced the susceptibility to SLE¹⁸. In our hands, evaluation by complement consumption assay (CCA) has been a reliable tool for determining immune complex levels³⁷. The increased immune complex load could be mediated by viral infections or expression of autoantigens that could induce extensive antigen load and antibody production. This might disturb the balance of immune complex formation and handling, although the individuals had normal immune complex handling ability under normal conditions³⁸. Thus, to investigate the mechanism of how the interaction of C4A deficiency and immune complexes could predispose to SLE, immune complex-RBC binding ability was assayed with increasing amounts of immune complexes in sera with different amount of C4A. The results show that increasing the total immune complex load by 4-fold results in competition between the immune complexes for the necessary C3 opsonization, providing limited immune complex binding to CR1. The proportional reduction in immune complex binding is more prominent in C4A deficient sera than in C4A normal serum. This indicates that the immune complexes become less opsonized in C4A deficient sera, leaving complexes available for tissue deposition. The subsequent tissue damage could initiate a self-perpetuating cycle that is one of the suggested causes of the pathogenesis and chronicity of SLE²⁹. Evaluation of the immune complex level in our study group confirms the critical effect of elevated immune complex levels, as all the healthy C4A deficient individuals (who formerly had intact immune complex-bound C3d and RBC) had low immune complex levels.

C4A deficiency has also been linked to other autoimmune diseases in which autoantibodies are thought to be the main mediator^{39,40}. Thus the finding of the association of C4A deficiency with SLE in only some populations emphasizes the necessity of evaluating this association in the presence or absence of elevated immune complex levels in the deficient individuals.

The finding that C4 has a role in maintaining B cell tolerance in mice suggests that in humans the C4A isotype could be mediating this necessary function of C4^{14,15}. However, in this study group, there is no indication that the C4A deficiency itself predisposes to elevated level of immune complexes or the development of autoantibodies.

These results indicate that C4A deficiency alone does not

increase susceptibility to SLE, as sera with C4A deficiency have normal immune complex handling as long as their immune complex level is low and their C3 opsonization ability remains sufficient. However, increased immune complex load *in vivo* leads to insufficient immune complex-bound C3 opsonization, which can disturb the delicate immunologic balance with initiation of the cycle and the pathogenesis of SLE.

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