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Increased Frequency of C4B*Q0 Alleles in Patients with Henoch–Schönlein Purpura

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Abstract

Henoch–Schönlein purpura (HSP) is a vasculitis of unknown aetiology, possibly involving immune complexes. The complement system is essential for the clearance of immune complexes. Our aim was to explore the hypothesis that patients with HSP have abnormal complements, contributing to the development of the disease. The study included 56 patients diagnosed with HSP at the Children's Hospital, Iceland between 1984 and 2000, and 98 blood donors as controls. Serum levels of immunoglobulin A, C4A, C4B and mannan-binding lectin were measured and compared between the two groups. C4 null alleles were significantly more common in HSP patients than in controls ($P = 0.018$) and were carried by 66.1% of the patients compared with 41.2% of the controls. This difference was due to an increased frequency of C4B*Q0 allele in the HSP group (0.25 versus 0.11 in the control group; $P = 0.002$). The fact that the majority of our patients carried a C4 null allele indicates that children with C4 deficiencies may have an increased risk of developing HSP. This may reflect inadequate complement activity and possibly present an opportunity to identify patients at risk of developing serious morbidity associated with HSP.

Introduction

Henoch–Schönlein purpura (HSP) is a vasculitis primarily affecting children. Clinical manifestations are palpable purpuric lesions, typically on the lower extremities, arthralgias and colicky abdominal pain [1]. The course of the disease is often benign, lasting a few days or weeks, but the complications affecting the kidneys or the gastrointestinal tract are well known [1]. The incidence is approximately 10 cases per 100,000 children per year [2–6].

The cause of HSP is unknown. Frequently, an upper respiratory tract infection precedes the onset of the disease, suggesting an infectious trigger in a susceptible individual. The immunopathological mechanism appears to involve circulating immunoglobulin (Ig) A immune complexes [7] and the deposition of IgA in the vascular endothelium of the dermal vessels and the renal mesangium [8]. The deposits found in HSP lesions are exclusively of the subclass IgA1, which represents 90% of serum IgA and are composed of polymeric IgA1 without the secretory component [9–11]. These findings indicate that the source of IgA is in the bone marrow or perhaps circulating lymphocytes. Antigen-specific IgA, which is produced after a primary pathogen contact, is predominantly polymeric which further supports the hypothesis that HSP is triggered by an infection [12]. In one study, patients with HSP were found to have an increased number of circulating IgA-producing B cells [13].

Overproduction of IgA, however, cannot be the sole cause of HSP, as very large quantities of polymeric IgA1 may circulate in other diseases without the clinical symptoms of HSP. The IgA deposits in HSP are of the IgA1 type, which, unlike IgA2, has a unique hinge region with several O-glycosylation sites. Interestingly, an increased prevalence of abnormally glycosylated IgA1 has been demonstrated in patients with HSP and IgA nephropathy [14, 15]. Therefore, the aetiology of HSP may involve abnormal glycosylation of IgA rather than overproduction of IgA.

It has been postulated that abnormal glycosylation leads to the increased deposition of IgA1 immune complexes [15]. The clearance of immune complexes depends on the complement system and therefore defective complement function may contribute to the pathogenesis of HSP. Complement abnormalities in patients with HSP nephritis

have previously been reported [16]. These findings suggest that abnormal clearance of IgA may be crucial in the pathogenesis of HSP and IgA nephropathy. It has been suggested that abnormal O-glycosylation of IgA1 is involved in renal complications of HSP [15], and this could be due to the impaired capacity of the IgA molecule to activate the complement system and thus impaired clearance from the vasculature.

Deficiency of complement 4 (C4) has been suggested as a risk factor for HSP nephritis, which might represent a defective clearance mechanism [16, 17]. Moreover, reports of other vasculitic disorders have been linked to C4 deficiency such as Wegener's granulomatosis and systemic lupus erythematosus [18, 19]. This might reflect a central role for C4 in the mechanism of clearance of immune complexes.

C4 is a highly polymorphic protein, coded for by two tandem-duplicated genes located in the major histocompatibility complex region on human chromosome 6 [20]. It produces two isotypes (C4A and C4B) that can be heterozygous or homozygous. More than 40 allotypic variants are recognized. Null alleles (C4A*Q0 and C4B*Q0) producing no identifiable product are common, and increased frequency of these alleles has been observed in various diseases [20]. C4A is thought to be important in the solubilization of immune aggregates, immunoclearance and opsonization, while C4B is considered important for the propagation of the classical complement-activation pathways, cumulating in the formation of the membrane attack complex against microbes [21].

Mannan-binding lectin (MBL) is thought to bind to complexed IgA followed by complement activation, thereby contributing to the antimicrobial defence and the clearance of immune complexes [12]. MBL activates the complement system via activation of C4, C2 and C3. It has recently been shown that complexed IgA activates the lectin pathway of complement [12]. Defects in the production of MBL are associated with frequent mucosal upper respiratory infections in both children and adults, which might further contribute to increased production of IgA [22].

The immunopathogenesis of HSP is still largely unknown. In order to find out whether altered levels of complement and/or MBL contribute to the pathogenesis of HSP, we studied the serum from patients, previously diagnosed with HSP, and compared it with healthy subjects.

Materials and methods

Patients, controls and samples. In the year 2001, serum was collected from 56 patients (mean age 5.4 ± 3.3 years) who had been diagnosed with HSP at the Children's Hospital, Landspítali University Hospital, Iceland, between 1984 and 2000 but were healthy at the time of sampling. The serum samples were kept frozen at $-70\,^{\circ}C$ until measured. Blood donors ($n = 98$) served as controls. The study was approved by the National Bioethics Committee and the National Privacy and Data Protection Authority.

IgA measurements. Serum levels of IgA were measured by nephelometry on Beckman Coulter, Array 360 system, using reagents and standards from Beckman (Galway, Ireland).

C4 allotypes. C4 allotypes were determined by visual scoring of protein bands after high-voltage agarose electrophoresis of carboxypeptidase- and neuraminidase-treated samples followed by immunofixation and staining [23]. Null alleles were determined by comparing the relative intensity of the C4A and C4B isotypes.

C4A and C4B measurements. Serum levels of C4A and total C4 were determined by an enzyme-linked immunosorbent assay (ELISA; modified from [23]), using goat polyclonal anti-C4 for catching, and mouse monoclonal anti-C4A (RgD1) or anti-C4d, followed by alkaline phosphatase (AP)-conjugated rabbit anti-mouse immunoglobulins, for developing. C4B values were calculated by subtraction of C4A from total C4. This approach has been validated in the previous studies in our laboratory by simultaneous measurements of C4B in ELISA using mouse monoclonal 99H7. The lower limit of C4 set by the laboratory was 0.14 g/l. The C4 concentrations were analysed in accordance with the carrier state of C4A*Q0, C4B*Q0 or full expression of both isotypes.

MBL measurements. Serum levels of MBL were measured by an ELISA. Mouse monoclonal antibodies (HYB 131-01) were used for catching MBL from patients' serum. The same antibody (biotin labelled), followed by avidin–alkaline phosphatase and *p*-nitrophenylphosphate, was then used to visualize the reaction.

Statistical analysis. The Mann–Whitney *U*-test and the *t*-test were used to compare phenotypes of C4 and MBL. The Chi-square test was used for calculating P-values in frequency numbers. The SigmaStat statistical software, version 2.0 (SyStat Software Inc., Richmond, CA, USA) was used for calculations. The difference was considered significant if $P < 0.05$.

Results

IgA levels

IgA levels were within normal range for all patients.

C4 allotypes

In the HSP group, 66.1% (37 of 56 patients) were found to carry the C4 null allele compared with 41.2% in the control group ($P = 0.018$; Table 1). There was a significant increase of the phenotype C4B*Q0 (allele frequency 0.25 versus 0.11; $P = 0.002$) in the patient group, but no difference was observed between patients and controls with respect to C4A*Q0 (allele frequency 0.15 versus 0.13;

Table 1 Number of patients with complement 4 (C4) deficiency and allele frequencies of C4 nulls in Henoch–Schönlein purpura (HSP) patients compared with controls

	Number of patients			Allele frequency		
	HSP† ($n=56$)	Controls† ($n=98$)	*P*-value	HSP	Controls	*P*-value
C4 null‡	37	40	0.003	0.19	0.13	0.018
C4A*Q0	13 (0)	27 (3)		0.15	0.13	
C4B*Q0	24 (4)	13 (0)	<0.001	0.25	0.11	0.002
C4 normal	19	58		0.81	0.87	

†Some patients had both C4A*Q0 and C4B*Q0 alleles. The number of homozygous patients is given in parentheses.
‡Carrier frequency in HSP is 66% and in controls 41%.

Table 2). Of the 24 patients with the phenotype C4B*Q0, four were homozygous. Forty-three of 224 alleles in the HSP group were C4 null alleles compared with 51 of 394 alleles in the control group (allele frequencies 0.19 versus 0.13, $P=0.018$; Table 1). Of the 22 patients with nephritis, 16 (72.7%) carried a C4 null allele, the corresponding figure being 21 of 34 (61.7%) for patients with no evidence of nephritis (Table 3).

C4 levels

C4 concentrations (Fig. 1) were markedly lower in patients with C4B*Q0 than in patients with either C4A*Q0 or full expression of both isotypes ($P=0.001$), 16/24 showing levels below 0.14 g/l. Patients with C4A*Q0 produced normal levels of total C4.

MBL levels

Serum levels of MBL in patients were not distinguishable from those of the healthy controls.

Table 2 Frequency of C4 allotypes in Henoch–Schönlein purpura (HSP) and controls

	Control		HSP		
Allotype	Number	Frequency	Number	Frequency	*P*-value
C4A*6	6	0.03	7	0.06	
C4A*5	2	0.01	1	0.01	
C4A*4	26	0.13	10	0.09	
C4A*3	121	0.62	78	0.70	
C4A*2	8	0.04	1	0.01	
C4A*Q0	30	0.15	15	0.13	
C4A-rare	5	0.03			
Total	196		112		
C4B*6	1	0.01			
C4B*5	2	0.01			
C4B*4					
C4B*3	10	0.05	2	0.02	
C4B*2	30	0.15	14	0.12	
C4B*1	129	0.66	68	0.61	
C4B*Q0	21	0.11	28	0.25	0.002
C4B-rare	3	0.02			
Total	196	1.00	112	1.00	

Discussion

HSP appears to be an immune-mediated disease. Purpuric lesions of the skin and the kidneys contain deposition of IgA complexes, and it can therefore be postulated that the pathogenesis of HSP involves either increased production of these complexes or, more likely, defective clearance.

Our findings that C4 null alleles were significantly ($P=0.018$) more common in HSP patients than in controls suggest that subtle abnormalities in complement function may be a predisposing factor for HSP. The fact that the IgA complexes are mostly polymeric indicates that production of monomeric IgA is not responsible for the defective clearance. The complement system is essential for the clearance of immune complexes. It is interesting in this context that 66.1% of the patients in our study group carried a C4 null allele and 24% a C4B*Q0 phenotype. C4 null alleles were significantly more common in patients with HSP than in the control group ($P=0.018$). This is consistent with the findings of McLean *et al.* [24], indicating that an abnormality in the complement system may predispose an individual to develop an immune complex-mediated disease such as HSP. Similar findings have previously suggested that complement deficiencies may be involved in other immune complex-mediated diseases such as systemic lupus erythematosus [19, 25].

Our results reveal that children with C4 deficiencies may be at increased risk of developing HSP. This may be due to a diminished capacity to activate the complement system and thus diminished clearance of immune complexes. The MBL system also plays a part in this activation. It has recently been demonstrated that IgA-containing immune complexes activate C4 and C2 through the lectin pathway [26]. However, in our study, the patient group

Table 3 C4 null alleles in Henoch–Schönlein purpura patients with and without nephritis

	Nephritis	Normal urine analysis
C4 null allele	16	21
Normal C4	6	13
Total	22	34

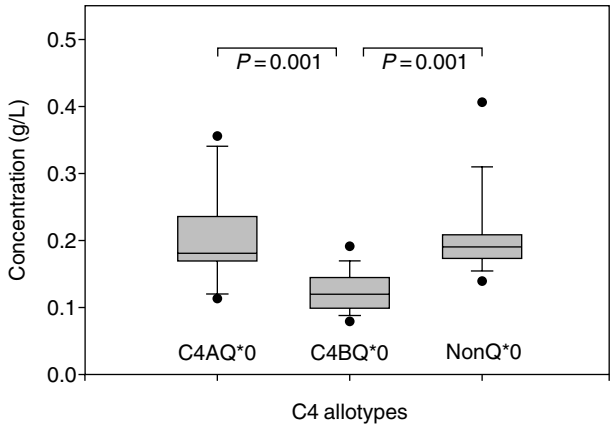

Figure 1 C4 levels in Henoch–Schönlein purpura patients with different allotypes.

had similar concentrations of MBL compared to the control group. The incidence of MBL mutation in the population is about 30% with one variant allele and 5% with homozygosity for MBL gene mutation [27, 28]. Noteworthy, a subgroup of patients who had low levels of C4 also had low levels of MBL. It is possible that any fault in this clearance mechanism of MBL and the complement system increases the risk of various diseases caused by depositions of circulating immune complexes.

Individuals with abnormal glycosylation of IgA have been shown to carry an increased risk of HSP nephritis [15]. Abnormal glycosylation of IgA is thought to result in diminished clearance and perhaps altered function of the molecule [26]. This might also reflect diminished binding with MBL and thus a lack of activation of the complement system. It is also quite possible that this abnormal glycosylation of IgA and its defected clearance, increase susceptibility not only to HSP nephritis, but more importantly to HSP itself.

In our earlier study, 37 of 101 patients (36%) diagnosed with HSP had clinical evidence of nephritis [2]. Interestingly, these patients were slightly more likely to carry a C4 null allele (72.7%) than the HSP patients without evidence of nephritis (61.7%). The difference was, however, not significant.

Our results indicate that children with C4 deficiencies are at increased risk to develop HSP. This may be because of a diminished capacity to activate the complement system and thus diminished clearance of immune complexes. These results may cast some light on the pathophysiology of HSP but also further underline the role of the complement system and immune complex clearance in autoimmune disorders. The fact that HSP patients with C4B*Q0, unlike patients with C4A*Q0, were not able to compensate for the lack of productive alleles and thus had reduced serum C4 may be important for the pathophysiology of the disease. Perhaps, the risk of developing HSP is increased only in a subpopulation of C4B*Q0 carriers with low C4 production from the remaining functional C4B allele in heterozygotes.

Although HSP usually follows a benign course, it can be a debilitating disease, sometimes associated with considerable morbidity and even mortality. It is possible that the complement abnormalities can be linked to various clinical expressions of the disease and that patients at risk of developing nephritis can be identified early. Our findings therefore may facilitate better diagnostics of the disease and possibly improve its treatment and outcome.

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