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Lectin and alternative complement pathway activation in cutaneous manifestations of IgA-vasculitis: A new target for therapy?

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ABSTRACT

IgA-vasculitis is a systemic small-vessel leucocytoclastic vasculitis and is associated with a high morbidity. The disease can progress to IgA-vasculitis with nephritis (IgAVN) which can result in chronic renal failure. Complement activation is involved in the pathogenesis of IgA-vasculitis. A recent study has shown that cutaneous C3c deposition in IgA-vasculitis is associated with a higher risk to develop IgAVN. In the current study we investigated the different complement pathways that are activated in cutaneous IgA-vasculitis in order to reveal potential targets for intervention. In addition, we analyzed the association of complement factors with IgAVN and the clinical course of the disease.

In this retrospective study, the clinicopathological features of 17 patients with IgA-vasculitis were compared with 25 non-IgA-vasculitis cases. Deposition of immunoglobulins and complement was analyzed by direct immunofluorescence for IgA, IgG, IgM, C1q, C4d, properdin, mannan-binding lectin (MBL), ficolin-2 (FCN2), MBL-associated serine protease 1/3 (MASP1/3), MASP2 and C3c. The vascular intensity and positive area was scored on a nominal scale and cumulative score was calculated by multiplying the intensity x area.

Properdin was positive in 82% of IgA-vasculitis cases, reflecting alternative pathway activation. C4d was positive in 88% of IgA-vasculitis cases reflecting classical and/or lectin pathway activation, although only 12% of cases were positive for C1q. Lectin pathway activation was demonstrated by deposition of MBL (47%), MASP1/3 (53%) and MASP2 (6%) while FCN2 was found negative. Significantly more deposition of MASP1/3 was found in IgA-vasculitis versus non-IgA-vasculitis.

This study demonstrates for the first time activation of lectin and alternative pathways in cutaneous manifestations of IgA-vasculitis. Hence, drugs that intervene in these complement pathways may be an interesting more targeted alternative to the current drugs, in reducing local cutaneous symptoms of the disease, with potentially less side-effects. No association was found between complement activation and IgAVN and/or response to therapy. Therefore, it is unlikely that intervention in complement activation will lead to a better clinical course of the disease.

1. Introduction

IgA-vasculitis, formerly known as Henoch-Schönlein purpura, is a systemic small-vessel leukocytoclastic vasculitis (SVLCV) predominantly affecting children with an annual incidence rate of 3–26,7/100000. Approximately 10% of affected patients are adults. The disease most commonly affects small vessels in the skin, gastro-intestinal tract, joints and kidneys (Heineke et al., 2017). From the patients with IgA-vasculitis, approximately 30–50% of children and 49–83% of adults develop IgA-vasculitis with nephritis (IgAVN), a condition that

Abbreviations: DIF, direct immunofluorescence; EULAR, European League Against Rheumatism; FCN2, Ficolin-2; IgAVN, IgA-vasculitis with nephritis; IgAN, IgA-nephropathy; MASP1, MBL-associated serine protease 1; MASP2, MBL-associated serine protease 2; MBL, mannan-binding lectin; PRES, Paediatric Rheumatology European Society; SVLCV, small-vessel leukocytoclastic vasculitis.

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resembles IgA nephropathy (IgAN), although the latter is restricted to the kidneys (Aleyd et al., 2015; Narchi, 2005; Coppo et al., 2006). If left untreated, 5–15% of children and 25–30% of adults with IgAVN will progress to chronic renal failure and finally require hemodialysis or renal transplantation (Guo et al., 2013; Kanaan et al., 2011). Corticosteroids are the mainstay of treatment but one-third of patients are therapy resistant and/or show recurrence (Saulsbury, 1999). Importantly, early treatment with corticosteroids has little or no effect on the risk to develop nephritis or renal outcome (Weiss et al., 2007; Huber et al., 2004). Dapsone (i.e. diaminodiphenyl sulfone) can be used in therapy resistant cases and can show resolution of purpura but cannot prevent renal manifestations. Furthermore, dapsone is associated with many side effects such as dose-related hemolytic anemia, methemoglobinemia, and agranulocytosis (Roman et al., 2019). For these reasons, there is a need for more personalized therapy, targeting the crucial components involved in the pathogenesis of IgA-vasculitis.

The pathogenesis of IgA-vasculitis remains poorly understood but it is generally assumed that an external antigen raises an IgA response and IgA-immune complexes are deposited in the capillaries followed by an inflammatory cascade. Rarely, also IgA antibodies directed against the vascular endothelium are found (Heineke et al., 2017). The subsequent immune response is (at least partly) complement mediated, since about 70–90% of patients with IgA-vasculitis also show concomitant C3c deposition which can be detected by direct immunofluorescence (DIF) in the skin (Johnson et al., 2015; Poterucha et al., 2012; Takeuchi et al., 2010). Importantly, a recent study has shown that cutaneous C3c deposition in IgA-vasculitis is associated with a higher risk of renal involvement (Ataeepour et al., 2019). Although the importance of complement activation in the pathogenesis of IgAN is well appreciated, less is known about complement in IgA-vasculitis and IgAVN. In both IgAN and IgANV, there is a growing body of evidence that the lectin pathway is the main driver of complement activation. The lectin pathway can be activated by mannan-binding lectin (MBL), ficolins and/or collectins which activate MBL-associated serine proteases 1 and 2 (MASP1 and MASP2), subsequently cleaving C4 and C2. Activation products of C4 (a.o. C4d) can be extensively found in the kidneys of patients with IgAN and this has also been demonstrated in IgAVN (Endo et al., 2000). IgA is also known to activate the alternative complement pathway, but is unable to activate the classical pathway (Hiemstra et al., 1987). Potentially, IgA could also activate the alternative pathway in IgA-vasculitis, either directly by IgA or as a result of the amplification loop (upon lectin pathway activation). With the advent of new targeted systemic complement inhibitors, there is a growing interest in detailed analysis of the different complement pathways activated in IgA-vasculitis and IgAVN. So far, no in-depth data on the complement pathways activated in cutaneous manifestations of IgA-vasculitis can be found in the public domain. Only one publication from the 1970′s showed absence of C1q, which might be a suggestion for alternative pathway activation in skin biopsies of patients with IgA-vasculitis (De la Faille-Kuyper JSvdM and Kater, 1974), although others failed to find properdin deposition (Sams et al., 1975). As far as we know, at present there are no published reports of studies that investigated the activation of the lectin route in skin biopsies of patients with IgA-vasculitis.

In this study we investigated the intensity and extent of complement activation of all three pathways in comparison with non-IgA vasculitis. Furthermore, we investigated the potential association with IgAVN and the clinical course in terms of remission versus recurrence and therapy resistance. The results of our study will unravel the pathways involved in cutaneous manifestations of IgA-vasculitis and show the importance of complement activation in the course of the disease.

2. Methods

2.1. Patient selection

In this retrospective study, 46 patients underwent a diagnostic skin biopsy for suspicion of IgA-vasculitis between 2013 and 2020 in the Erasmus Medical Center Rotterdam, Rotterdam, the Netherlands. A total of 42 patient were eventually included because there was not enough material left for additional analysis of four patients. The study protocol was approved by the local Medical Ethical committee of the Erasmus Medical Center (MEC-2019–0207). Demographics and clinical features were retrieved from the medical records (Tables 1a 1b). Medical records were reviewed to determine if a patient met the clinical criteria for IgA-vasculitis according The European League Against Rheumatism (EULAR), Paediatric Rheumatology International Trials Organisation (PRIS) and Paediatric Rheumatology European Society (PRES) EULAR/PRIS/PRES criteria (Ozen et al., 2010). These criteria were also found to have a higher sensitivity and specificity when applied to adults compared to the 1990 ACR criteria (Hocevar et al., 2016). Other information that was taken into account was the high clinical suspicion of IgA-vasculitis before biopsy that was included in the pathology report medical information. Patients were only included when the histopathological criteria for small vessel leukocytoclastic vasculitis were met on a formalin-fixed paraffin embedded skin biopsy. These included: perivascular lymphohistiocytic inflammation with neutrophils, karyorrhexis, erythrocyte extravasation and in most cases fibrinoid necrosis. When no fibrinoid necrosis was found, adequate clinicopathological correlation was made for a definitive diagnosis of vasculitis. A (second) frozen biopsy that was stored and analyzed previously was re-analyzed for deposition of immunoglobulins and complement factors by direct immunofluorescence. Direct immunofluorescence was never used as a diagnostic test for vasculitis.

Although DIF IgA positivity is a criterion for the diagnosis of IgA-vasculitis according to the EULAR/PRIS/PRES criteria (based on children <18 years), the sensitivity and specificity are very different for adult patients compared to children. Vascular IgA positive staining has also been found in Churg-Strauss vasculitis, urticarial vasculitis and connective tissue disease-associated vasculitis (Larson and Granter, 2014; Boom et al., 1992). Therefore, although EULAR/PRIS/PRES criteria are helpful in adults, these are not 100% specific for IgA-vasculitis (87% specificity in children and 86% in adults) and other causes of vasculitis have to be considered or ruled out as well. Moreover, careful clinicopathological correlation is crucial for a definite diagnosis of IgA-vasculitis. In our cohort, 17 of 42 patients fulfilled the criteria for IgA-vasculitis based on the EULAR/PRIS/PRES criteria. All 17 patients showed biopsy proven SVLCV with vascular IgA deposits and presented with purpura and one or more of the clinical (abdominal pain, arthralgia

Table 1a
Demographics and disease characteristics of the IgA-vasculitis and non-IgA vasculitis groups.

Clinical features	IgA-vasculitis (n = 17)	non-IgA vasculitis (n = 25)	P-value
Age	35 (23–46)	29 (41–65)	0.162[2]
Female	7 (41)	11 (44)	1.0[1]
Cause:	17 (100)	8 (32)	
IgA		7 (28)	
Idiopathic		2 (8)	
UV		2 (8)	
GPA		2 (8)	
PNP		1 (4)	
Drugs		1 (4)	
SLE		1 (1)	
PLS		1 (1)	
JIA			
Infection			

Age data is expressed as median (interquartile range), all other data (except for laboratory measurements) is expressed as positive number (%)
*All p-values are two-sided
[1]Fishers exact test
[2]Mann-Whitney test
[3]Chi-square test

Table 1b
Demographics and disease characteristics of the IgA-vasculitis group.

Patient	Age	Gender	Extracutaneous symptoms	IgAVN	Treatment	Outcome
1	35	Male	Abdominal pain	No	None	Recurrences up to 1 year
2	26	Male	Abdominal pain, arthralgia, hematuria (once, normal RF)	No	Dapsone	Remission
3	37	Male	Arthralgia, hematuria (normal RF)	No	None	Recurrences up to 5 years, normal RF
4	46	Male	Arthralgia, diarrea	No	Prednisone	Recurrence up to 1 year
5	16	Female	Abdominal pain, arthralgia	No	Prednisone	Remission
6	8	Male	Abdominal pain	No	None	Remission
7	82	Male	Arthralgia, hematuria, proteinuria	Yes BP	Prednisone Methotrexate Azathioprine Mycofenolate mofetil Cyclosporine Dexamethasone Methylprednisone Cyclofosfamide	Therapy resistant
8	18	Female	Abdominal pain	No	None	Remission
9	33	Male	Arthralgia, hematuria, proteinuria	Yes BP	Prednisone Dapsone	Remission
10	23	Female	Abdominal pain, arthralgia	No	Dapsone	Remission
11	61	Male	Arthralgia, hematuria, proteinuria	Yes BP	Methylprednison	Remission
12	54	Female	Abdominal pain, hematuria, proteinuria	Yes BP	MMF Prednisone	Therapy resistant
13	45	Male	Abdominal pain, hematuria, proteinuria	Yes not BP (BP positive intestine)	Prednisone Azathioprine	Therapy resistant
14	43	Female	Arthralgia, hematuria, proteinuria	No but BP IgA cast nephropathy	Prednisone	Remission
15	28	Female	Abdominal pain	no	Prednisone	Recurrences up to 1 year
16	45	Male	Abdominal pain, arthralgia, hematuria, proteinuria,	Yes Not BP	Prednisone Azathioprine Dapsone	Therapy resistant
17	22	Female	Abdominal pain, arthralgia	No	None	Recurrences up to 5 years

*All IgA-vasculitis patients presented with purpuric lesion.
RF: renal function
BP: biopsy proven

and/or hematuria/proteinuria) criteria for IgA-vasculitis. Eight of 17 cases presented with palpable purpura. In 6/17 cases patients developed IgAVN, 4 of which were biopsy proven and in one case a cast nephropathy was found with renal IgA deposits (Table 1b). In one case, vascular IgA deposits were also found in the nasal mucosa and terminal ileum. Most patients (10/17) were treated after initial symptoms, after which 8 patients went into remission, 5 patient presented with recurrent disease and 4 patients were therapy resistant.

In the other 25 patients with SVLCV, 7 patients also showed vascular IgA deposits but did not fulfill the clinical criteria for IgA-vasculitis. Besides, in 4 of these patients with (after clinicopathological correlation) idiopathic SVLCV, anti-neutrophil-cytoplasmic-antibody (ANCA)-associated vasculitis, urticarial vasculitis or systemic lupus erythematosus (SLE)-vasculitis, concomitant deposition of IgA, IgG and/or IgM was found and IgA deposition was not considered predominant. One patient showed weak IgA deposition and had a clear history of an infectious cause (prostatitis).

2.2. Direct immunofluorescence

Frozen sections were stained with hematoxylin and eosin for light microscopic evaluation. For detection of complement factors, direct immunofluorescence was performed by automated immunofluorescent staining using the Ventana Benchmark Discovery (Ventana Medical Systems Inc.)(Table 2). In brief, wet slides were loaded and incubated with primary antibody of interest for 32 min (Table 2) at 37°C followed by detection with either omnimap-anti rabbit or mouse, labeled with HRP (# and visualized with FAM (#760–243, Ventana). Slides were covered with anti-fading medium (DAKO, S3023). Direct immunofluorescence intensity and distribution was scored by two independent dermatopathologists (J.D. and A.M.). The vascular intensity was scored on a nominal scale of 0–3: none (0), weak (Heineke et al., 2017), moderate (Aleyd et al., 2015), profound/bright (Narchi, 2005). The positive area was scored on a nominal scale in two directions (width and depth) of 0–2: none (0), < 50% of vessels positive (Heineke et al., 2017) and > 50% of vessel positive (Aleyd et al., 2015). A cumulative score was calculated by multiplying the intensity x area.

Table 2
Antibodies and dilutions used in the study.

Antibody	Host and targets species	Supplier	Concentration/ dilution
IgA	Polyclonal-anti-human, FITC labeled	Roche	37.6 μg/ml
IgG	Polyclonal-anti-human, FITC labeled	Roche	163.4 μg/ml
IgM	Polyclonal-anti-human, FITC labeled	Roche	163.4 μg/ml
C3c	Polyclonal-anti-human, FITC labeled	Roche	88 μg/ml
C1q	Polyclonal-anti-human, FITC labeled	Roche	66.6 μg/ml
C4d	polyclonal rabbit-anti-human C4d	Biomedica, Wien, Austria	1:600
Properdin	Polyclonal rabbi-anti-human properdin	Kindly provided by prof. M.R. Daha, Leiden.	1:400
MBL	monoclonal mouse-anti-human MBL, clone 3E7	Hycult, Uden, The Netherlands	1:200
Ficolin-2	Monoclonal mouse-anti-human ficolin-2, clone GN5	Hycult, Uden, The Netherlands	1:150
MASP1/3	Monoclonal mouse-anti-MASP1/3, clone 1E2	Hycult, Uden, The Netherlands	1:100
MASP-2	Monoclonal mouse-anti-MASP2, clone 8B5	Hycult, Uden, The Netherlands	1:50

2.3. Statistics

To compare the demographics between the IgA-vasculitis and other causes vasculitis groups, between IgA-vasculitis with or without renal involvement or IgA-vasculitis with or without therapy resistance, the Mann–Whitney U test was performed for continuous variables and the Fisher's exact test for categorical variables on a nominal scale. For comparison between direct immunofluorescence scores, Fishers exact test was used for categorical variables on a nominal scale while Chi-square was used for categorical variables on an ordinal scale. Statistical analysis was performed using SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) and two-sided p-values of less than 0.05 were considered statistically significant.

Table 3
Direct immunofluorescence intensity and cumulative scores in IgA-vasculitis versus other causes of SVLCV.

	IgA-vasculitis (n = 17)			Non-IgA-vasculitis (n = 25)			P-value	
	Intensity	Cumulative	Total +	Intensity	Cumulative	Total+	Intensity	Cumulative
IgA	0 (0) 1 (71) 2 (29) 3 (0)	0 (0) 1 (59) 2 (18) 4 (18) 8 (5)	100	0 (72) 1 (24) 2 (4) 3 (0)	0 (72) 1 (24) 8 (4)	28	0.000[a]	0.000[a]
IgG	0 (59) 1 (41) 2 (0) 3 (0)	0 (59) 1 (18) 2 (24)	41	0 (56) 1 (32) 2 (12) 3 (0)	0 (56) 1 (20) 2 (12) 4 (8) 8 (4)	44	0.318[a]	0.576[a]
IgM	0 (65) 1 (29) 2 (6) 3 (0)	0 (65) 1 (23) 2 (6) 4 (6)	35	0 (44) 1 (48) 2 (8) 3 (0)	0 (44) 1 (40) 2 (8) 4 (4) 8 (4)	56	0.416[a]	0.650[a]
C1q	0 (88) 1 (6) 2 (6) 3 (0)	0 (88) 1 (6) 4 (6)	12	0 (80) 1 (16) 2 (4) 3 (0)	0 (80) 1 (8) 4 (8) 8 (4)	20	0.598[a]	0.828[a]
C4d	0 (12) 1 (24) 2 (35) 3 (29)	0 (12) 1 (18) 2 (23) 4 (12) 6 (12) 8 (6) 12 (17)	88	0 (24) 1 (8) 2 (16) 3 (52)	0 (24) 2 (12) 6 (24) 8 (12) 12 (28)	76	0.152[a]	0.101[a]
MBL	0 (53) 1 (23) 2 (18) 3 (6)	0 (53) 1 (11) 2 (12) 4 (12) 8 (6) 12 (6)	47	0 (64) 1 (12) 2 (16) 3 (8)	0 (64) 1 (8) 4 (8) 8 (8) 12 (12)	36	0.778[a]	0.562[a]
FCN	0 (100) 1 (0) 2 (0) 3 (0)	0 (100)	0	0 (96) 1 (4) 2 (0) 3 (0)	0 (96) 1 (4)	4	1.000[b]	1.000[b]
MASP1/3	0 (47) 1 (47) 2 (6) 3 (0)	0 (47) 1 (24) 2 (29) 3 (0)	53	0 (72) 1 (20) 2 (8) 3 (0)	0 (72) 1 (8) 2 (4) 4 (8) 8 (8)	28	0176	0047
MASP2	0 (94) 1 (6) 2 (0) 3 (0)	0 (94) 1 (6) 2 (0) 3 (0)	6	0 (92) 1 (8) 2 (0) 3 (0)	0 (92) 1 (4) 2 (4) 3 (0)	8	1.000[b]	0684
Properdin	0 (18) 1 (17) 2 (59) 3 (6)	0 (18) 1 (11) 2 (12) 4 (29) 6 (6) 8 (24)	82	0 (32) 1 (24) 2 (32) 3 (12)	0 (32) 1 (12) 2 (16) 4 (20) 6 (4) 8 (8) 12 (8)	68	0.379[a]	0.617[a]
C3c	0 (35) 1 (41) 2 (24) 3 (0)	0 (35) 1 (41) 2 (12) 4 (12)	65	0 (60) 1 (24) 2 (8) 3 (8)	0 (60) 1 (12) 2 (8) 3 (4) 4 (12) 12 (4)	40	0.153[a]	0.272[a]

All data is expressed as positive numbers (%). A cumulative score was calculated by multiplying the intensity x area. *All p-values are two-sided.
[a] Chi-square test.
[b] Fishers exact test.

3. Results

3.1. Demographics and clinical features

In general, the majority of included patients with IgA-vasculitis were middle-aged man and women with a median age of 35 years. In contrast with patients who had other causes of vasculitis, patients with IgA-vasculitis presented with more widely distributed lesions. Besides purpura on the lower legs, these patients frequently presented with lesions on the upper legs, arms, abdomen and flanks (Table 3).

3.2. Routes of complement pathways activated in IgA-vasculitis

Direct immunofluorescence staining was performed for complement activation products of all three complement pathways as outlined in Table 3. As a marker of alternative pathway activation, properdin was positive in 82% of IgA-vasculitis. C4d was positive in 88% of IgA-vasculitis reflecting classical and/or lectin pathway activation. C1q was positive in 12% of IgA-vasculitis, reflecting classical pathway activation. Lectin pathway activation in IgA-vasculitis was demonstrated by deposition of MBL (47%), MASP1/3 (53%) and MASP2 (6%) while FCN2 was found negative. Fig. 1 shows representative direct immunofluorescence staining of IgA and the different complement components in a case of IgA-vasculitis. Fig. 2 shows colocalization of MBL and C4d in a case of IgA-vasculitis.

3.3. Routes of complement pathways activated in IgA-vasculitis versus non-IgA vasculitis

IgA intensity and cumulative scores were significantly higher in IgA-vasculitis versus non-IgA vasculitis groups. IgA-vasculitis cumulative scores were significantly higher for MASP1/3 compared to the non-IgA-vasculitis group. No significant differences were found for either intensity or cumulative scores of all other complement factors in IgA-vasculitis versus non-IgA vasculitis groups.

3.4. Association of complement deposition with IgAVN and therapy response

Complement activation (intensity and cumulative scores) was analysed for association with remission versus therapy resistance and remission versus recurrence and therapy resistance. Complement activation was also analysed for association with IgAVN. Subset analyses within the IgA-vasculitis group did not show significant difference of complement deposition pattern in patients with or without therapy resistance (data not shown) and/or renal involvement (Table 4). Significantly less IgG deposition was found in IgA-vasculitis cases with renal involvement.

4. Discussion

IgA-vasculitis is a systemic SVLCV predominantly affecting children and in the minority of cases adults. The disease is associated with a high morbidity and can progress to IgAVN which, if left untreated, can result in chronic renal failure. Importantly, one-third of patients are therapy resistant and/or show recurrence. Complement is involved in the pathogenesis of IgA-vasculitis and is associated with a higher risk to develop IgAVN. In the last few years, the use of therapeutic complement inhibition has expanded significantly and some of these drugs have shown promising results in several preclinical studies. Knowledge and better understanding of the different complement pathways that are triggered in IgA-vasculitis is important, as it yields promising novel treatments options. Although it is known for years that C3c co-deposition can be found in skin biopsies of IgA-vasculitis, no data exists on the nature and extent of the different complement pathways activated in IgA-vasculitis. The current study is the most extensive and complete study on the complement cascade in IgA-vasculitis versus other causes of SVLCV. This study demonstrates that in agreement with IgAN and IgAVN, the lectin and alternative pathways are activated in cutaneous manifestations of IgA-vasculitis, comparable to other causes of SVLCV. Therefore, drugs that intervene in these complement pathways may be an interesting alternative to the current drugs, with potentially less side-effects, in alleviating local cutaneous symptoms of

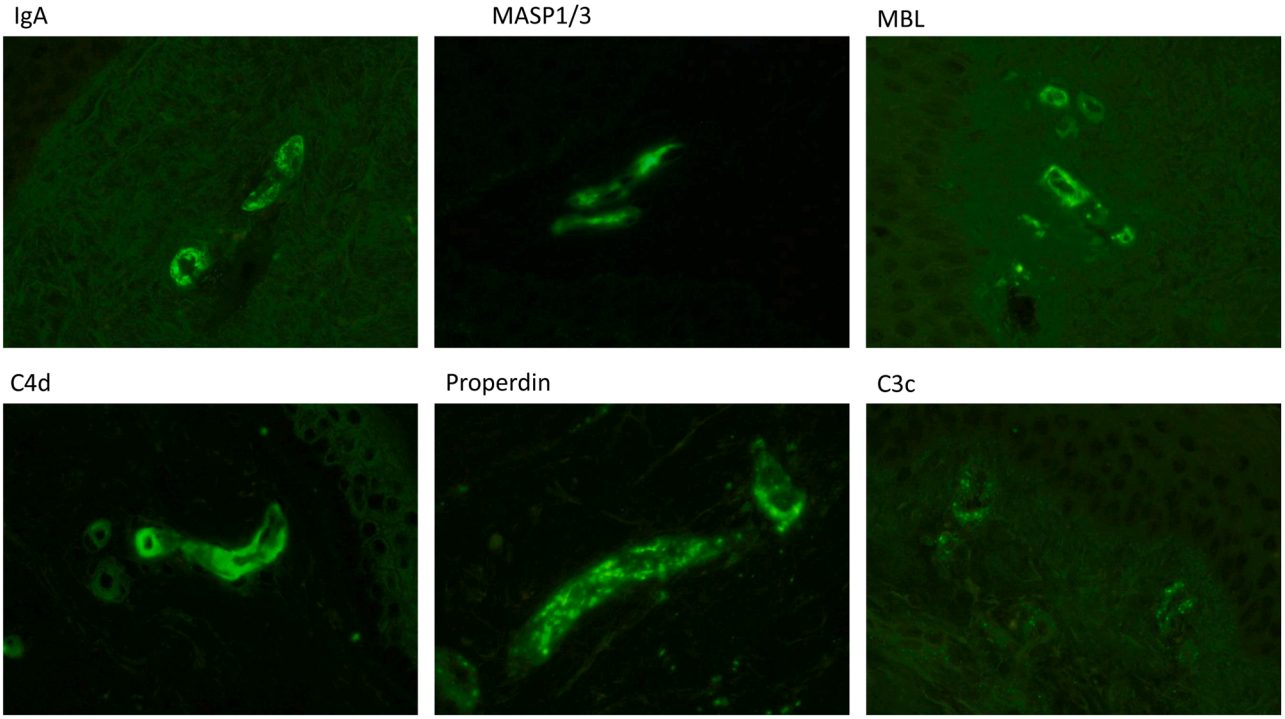

Fig. 1. Representative direct immunofluorescence staining of IgA and the different complement components in a case of IgA-vascuitis.

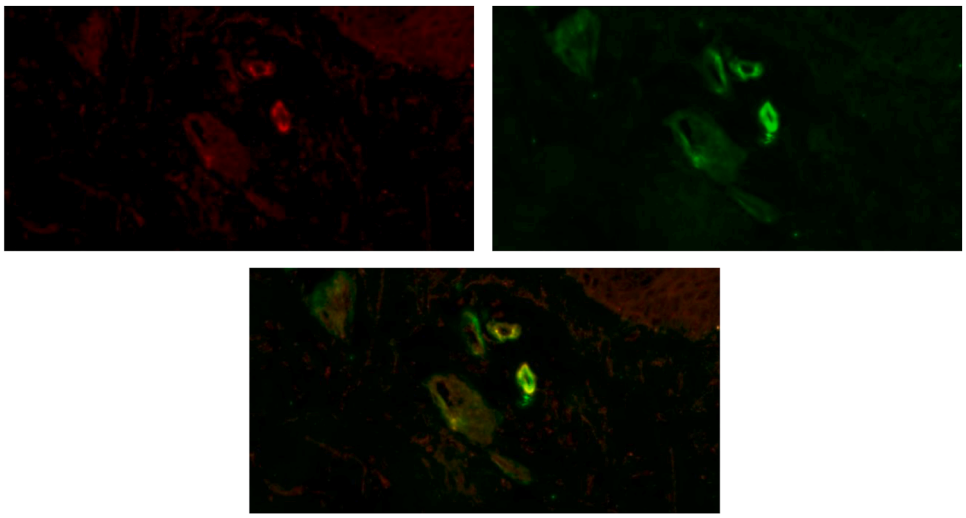

Fig. 2. Colocalization of MBL (red) and C4d (green) in a case of IgA-vasculitis.

Table 4
Direct immunofluorescence intensity and cumulative scores in IgA-vasculitis with and without renal involvement.

	With renal involvement (n = 7)			Without renal involvement (n = 10)			P-value	
	Intensity	Cumulative	Total +	Intensity	Cumulative	Total+	Intensity	Cumulative
IgA	0 (0) 1 (86) 2 (14)	0 (0) 1 (72) 2 (14) 4 (14)	100	0 (0) 1 (60) 2 (40)	0 (0) 1 (50) 2 (20) 4 (20) 8 (10)	100	0338[a]	0,75[b]
IgG	0 (100)	0 (100)	0	0 (30) 1 (70)	0 (30) 1 (30) 2 (40)	70	0010[a]	0016[b]
IgM	0 (72) 1 (14) 2 (14)	0 (72) 1 (14) 4 (14)	28	0 (60) 1 (40)	0 (60) 1 (30) 2 (10)	40	0296[b]	0450[b]
C1q	0 (72) 1 (14) 2 (14)	0 (72) 1 (14) 4 (14)	28	0 (100)	0 (100)	0	0198[b]	0198[b]
C4d	0 (14) 1 (29) 2 (43) 3 (14)	0 (14) 1 (14) 2 (44) 6 (14) 8 (14)	86	0 (10) 1 (20) 2 (30) 3 (40)	0 (10) 1 (20) 2 (10) 4 (20) 6 (10) 12 (30)	90	0726[b]	0139[b]
MBL	0 (72) 1 (28)	0 (72) 1 (14) 4 (14)	28	0 (40) 1 (20) 2 (30) 3 (10)	0 (40) 1 (10) 2 (20) 4 (10) 8 (10) 12 (10)	60	0296[b]	0594[b]
FCN	0 (100)	0 (100)	0	0 (100)	0 (100)	0	NA	NA
MASP1/3	0 (42) 1 (58)	0 (42) 1 (29) 2 (29)	58	0 (50) 1 (40) 2 (10)	0 (50) 1 (20) 2 (30)	50	0606[b]	0916[b]
MASP2	0 (100)	0 (100)	0	0 (90) 1 (10)	0 (90) 1 (10)	10	1000[a]	1000[a]
Properdin	0 (14) 1 (14) 2 (72)	0 (14) 2 (28) 4 (29) 8 (29)	86	0 (20) 1 (20) 2 (50) 3 (10)	0 (20) 1 (20) 4 (30) 6 (10) 8 (20)	80	0759[b]	0396[b]
C3c	0 (29) 1 (57) 2 (14)	0 (29) 1 (57) 4 (14)	71	0 (40) 1 (30) 2 (30)	0 (40) 1 (30) 2 (20) 4 (10)	60	0517[b]	0502[b]

All data is expressed as positive numbers (%). A cumulative score was calculated by multiplying the intensity x area. *All p-values are two-sided.
[a] Fishers exact test.
[b] Chi-square test.

the disease. However, no association was found between complement activation and IgAVN and/or response to treatment. Therefore, intervention studies targeting complement activation in IgA-vasculitis are unlikely to prevent the progression to IgAVN or lead to a better therapeutic response compared to current treatment regimens.

An important finding of our study is the activation of lectin and alternative pathways in IgA-vasculitis, comparable to other types of SVLCV. Although the strong deposition of properdin is not unexpected, since IgA is traditionally a well-known activator of the alternative pathway, we are the first to demonstrate this finding in cutaneous IgA-vasculitis. Moreover, we could demonstrate MBL (47%), MASP1/3 (53%) and C4d (88%) deposition in skin biopsies of IgA-vasculitis, a finding that parallels observations in the kidney in IgAVN and IgAN. Comparable to the kidney, C4d deposition is considered a consequence of lectin pathway activation. Indeed we found deposition of MBL and MASP1/3 in IgA vasculitis, although deposition of MASP2 and Ficolin-2 could not be demonstrated. However, only 40% of C4d positive IgA-vasculitis patients were MBL positive, a finding similar to the kidney (Chua et al., 2019). When MBL and MASP 1/3 were combined, 76% of C4d cases were either MBL and/or MASP1/3 positive. In theory, C4d-lectin negative cases might be explained by low-grade classical pathway activation due to (non-codominant) co-deposition of IgG and IgM. However, in our study only 2 cases showed C1q co-deposition and the extent of C4d deposition was not correlated with IgG and/or IgM deposition. Nevertheless, this might be an underestimation since IgG and IgM have a much lower tissue residency time and might already have activated complement despite a negative finding by immunofluorescence. It can also not be ruled out that secondary classical pathway activation occurs due to extensive vessel wall damage in established vasculitis lesions. For example in IgAVN it has been shown by proteomics-bases analyses of laser-captured micro-dissected glomeruli that C1q, C1r and C1s are higher in patients with progressive IgAN (Paunas et al., 2017).

Our findings also shed new light on the contribution of complement in the proposed pathogenesis of IgA-vasculitis. C4d appeared to be a more sensitive marker for complement activation and revealed 88% of patients showed complement activation in contrast to 65% (comparable to published reports) revealed by C3c. These results indicate a more prominent role for complement than previously thought, next to other complement independent effector functions of IgA such as FcαRI activation. Also, activation of the lectin pathway next to the alternative pathway reveals the possible triggers of complement mediated inflammation in IgA-vasculitis. MBL can be activated by terminal mannose and N-acetylglucosamine moieties present on various pathogens including bacteria, yeast, fungi and viruses (Thiel et al., 1997; Stover et al., 1999). This hypothesis can be easily integrated in the well accepted hypothesis that IgA-vasculitis is commonly preceded by upper respiratory infection. Another cause could be a difference in IgA subtypes and/or abnormal glycosylation pattern of galactose deficient IgA1 that triggers the lectin pathway. On the one hand, IgA1 is heavily glycosylated and likely to be more effective activator of MBL. On the other hand. in IgANV, MBL/MASP1 are codeposited with IgA1/IgA2 and C4 but not with IgA1 alone (Endo et al., 2000; Hisano et al., 2001, 2005). These results indicate that the lectin pathway in IgANV might be triggered by IgA2 and that the alternative pathway is activated via IgA1 (Hisano et al., 2005). Whether IgA2 also plays a role in dermatological manifestations of IgA-vasculitis remains to be investigated. However, an exclusive role for MBL in IgA-vasculitis in the skin is unlikely since MBL deposition was also found in other types of SVLCV.

In the current treatment of IgA-vasculitis steroids and dapsone are the mainstay of treatment. Unfortunately, these drugs are associated with many side-effects and one-third of patients are therapy resistant and/or show recurrence. Therefore, targeting the complement system in IgA-vasculitis could be an interesting novel approach in reducing local cutaneous symptoms and/or to prevent the progression to IgAVN, with potentially less side-effects. Ongoing trials targeting complement in IgAN could potentially also be beneficial in the treatment of cutaneous IgA-vasculitis with or without IgAVN. Clinical (ongoing) studies addressing the complement system in IgAN are drugs targeting MASP2 (OMS721, Omeros), Factor B (LPN023, Novartis), C3 (APL2, Apellis), C5 (Cemdisiran) and C5aR1 (Avacopan, Chemocentryx) (Zipfel et al., 2019). Although complement is highly activated in the skin in patient with IgA-vasculitis, we did not find association of complement activation with progression to IgAVN or response to therapy. Although complement could still be a therapeutic target for intervention, based on our study, prompt treatment with complement blocking agents is unlikely to prevent the development of IgAVN or will lead to a better therapeutic response in therapy resistance or diseases recurrent cases. In contrast to our results, Ataeepour et al. (2019) did find an association of C3c deposition in the skin with renal involvement in a pediatric population. The difference between studies might be explained by the difference in study population (pediatric versus adults) or the low number of patient ($n = 17$) in our study compared to the study by Ataeepour et al. ($n = 95$). In the future, larger multicentre studies are needed to confirm the findings by Ataeepour et al. in the pediatric and adult population.

In conclusion, the current study demonstrated activation of lectin and alternative pathways in the pathogenesis of cutaneous manifestations of IgA-vasculitis. Hence, drugs that intervene in these complement pathways may be an interesting alternative to the current drugs in reducing local cutaneous symptoms of the disease, with potentially less side-effects. No association was found between complement activation and IgAVN and/or response to therapy. Therefore, it is unlikely that intervention in complement activation will lead to a better clinical course of the disease.

CRediT authorship contribution statement

Jeffrey Damman, Antien L. Mooyaart and Thierry P.P. van den Bosch designed and performed experiments, analyzed data, and wrote the manuscript; Marc AJ Seelen and Martijn B. van Doorn edited and approved the final manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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