# Galactose-deficient IgA1 in skin and serum from patients with skin-limited and systemic IgA vasculitis

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***Background:*** IgA vasculitis (IgAV) encompasses a systemic form involving kidneys, gut, skin, or joints, and a skin-limited form. One characteristic feature of systemic IgAV is deposition of galactose-deficient IgA1 (GD-IgA1) in kidneys (as in IgA nephropathy). The relevance of GD-IgA1 for cutaneous vasculitis is unknown.

***Objective:*** We investigated whether GD-IgA1 is deposited perivascularly in systemic and also skin-limited IgAV and whether its serum levels differ between both forms.

***Methods:*** In a case-control study, deposition of GD-IgA1 was analyzed immunohistochemically by KM55 antibody in skin biopsy specimens from 12 patients with skin-limited IgAV and 4 with systemic IgAV. GD-IgA1 levels were compared by enzyme-linked immunosorbent assay in sera from 15 patients each with skin-limited and systemic IgAV and from 11 healthy individuals.

***Results:*** All biopsy samples from systemic IgAV, and also from skin-limited IgAV, revealed perivascular GD-IgA1 deposition. The average GD-IgA1 concentration in serum was significantly higher in systemic IgAV than in skin-limited IgAV, despite overlap between the groups.

***Limitations:*** Although high GD-IgA1 levels may be predictive of systemic IgAV, patient numbers were too low to determine cutoff values for systemic versus skin-limited IgAV.

***Conclusion:*** Perivascular GD-IgA1 deposition is a prerequisite for systemic and skin-limited IgAV; however, high GD-IgA1 levels in some patients with systemic IgAV suggest a dose-dependent effect of GD-IgA1 in IgAV. ( J Am Acad Dermatol 2019;81:1078-85.)

***Key words:*** dermatology; galactose-deficient IgA1; GD-IgA1; Henoch Schönlein purpura; IgA nephropathy; IgA vasculitis; IgA vasculitis with nephritis; IgAVN.

IgA vasculitis (IgAV) is characterized by inflammation of small blood vessels (histologically features of leukocytoclastic vasculitis) and IgA deposition primarily around postcapillary venules. The clinical hallmark of IgAV is palpable purpura with a predilection for the lower limbs.[1] Systemic IgAV, formerly known as Henoch-Schönlein purpura, involves internal organs, such as the kidneys (causing mesangioproliferative IgA nephritis), gut, or joints, in addition to the skin.[2] Vasculitic involvement

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Funding sources: This work was partially supported by grants from "Innovative Medical Research" (IMF) Muenster Medical School, Muenster University (Grant number: PA 1 1 15 15).

Conflicts of interest: None disclosed.

Accepted for publication March 10, 2019.

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Published online March 20, 2019.

0190-9622/$36.00



https://doi.org/10.1016/j.jaad.2019.03.029

of the kidneys is referred to as IgAV with nephritis (IgAVN). Systemic IgAV occurs more often in children than in adults, but renal involvement (IgAVN) is less likely to resolve in adults.[3-6]

There is also a skin-limited form of IgAV, without systemic vasculitis, as recently defined in the interdisciplinary nomenclature of *cutaneous* vasculitides (D-CHCC 2012),[1] a dermatologic addendum to the Chapel Hill Consensus Conference Nomenclature of Vasculitides (CHCC 2012).[7] Skin-limited IgAV does not usually progress into systemic IgAV and is more often seen by dermatologists than systemic IgAV, but there are no studies yet specifically addressing the respective incidence of skin-limited versus systemic IgAV. The reasons for this restriction to the skin are not known, leading to the question whether skin-limited IgAV is an entity with distinct pathophysiologic features or whether it is a variant that shares most of its pathophysiologic features with systemic IgAV, except for clinically detectable manifestation in systemic organs.

**CAPSULE SUMMARY**

- Systemic and skin-limited IgA vasculitis both show perivascular galactose-deficient IgA1 deposition.
- The average galactose-deficient IgA1 level in serum was significantly higher in systemic than in skin-limited IgA vasculitis, suggesting a dose-dependent effect of galactose-deficient IgA1 in IgA vasculitis.

IgA exists in 2 isoforms, IgA1 and IgA2, and as monomers or J chain containing polymers.[8] In serum, 90% of the IgA is IgA1 monomer. Histologically and pathophysiologically, IgAVN resembles IgA nephropathy (IgAN). Both are characterized by the deposition of poorly *O*-galactosylated IgA1 (GD-IgA1)[9] and probably share further common pathomechanisms.[10] Changes in IgA1 *O*-glycosylation occur through dysregulation of post-translational *O*-glycosylation in IgA-committed antibody-secreting cells, predominantly in the mucosa. Genetic and mucosal-microbial interactions have both been implicated in this dysregulation.[11] In both IgAN and IgAVN, elevated average levels of GD-IgA1 are found in serum[12] and urine,[13] and GD-IgA1 is deposited in glomerular capillary walls and the mesangium.[14]

Until recently, studying whether GD-IgA1 is deposited also in cutaneous vessels in IgAV has not been possible. This analysis can now be performed by virtue of a recently described monoclonal antibody, KM55, that specifically recognizes the GD-IgA1 hinge.[15] Because skin-limited IgAV does not present with signs of nephritis, we wondered whether this marked and clinically relevant restriction to the skin could be related to lower serum levels or absence of deposited GD-IgA1.

We therefore investigated whether GD-IgA1 can be detected in skin samples from patients with IgAV and whether (1) deposition of GD-IgA1 in skin tissue and (2) serum levels of GD-IgA1 differ between skin-limited IgAV and systemic IgAV.

## PATIENTS AND METHODS

The Ethical Committee of the University Hospital of Münster, Germany approved this study (study protocol record 2016-243-f-S). The study was part of the "IgA-positive Versus IgA-negative Immune Complex Vasculitis" study registered at ClinicalTrials.gov (identifier: NCT01815190).

### Patients

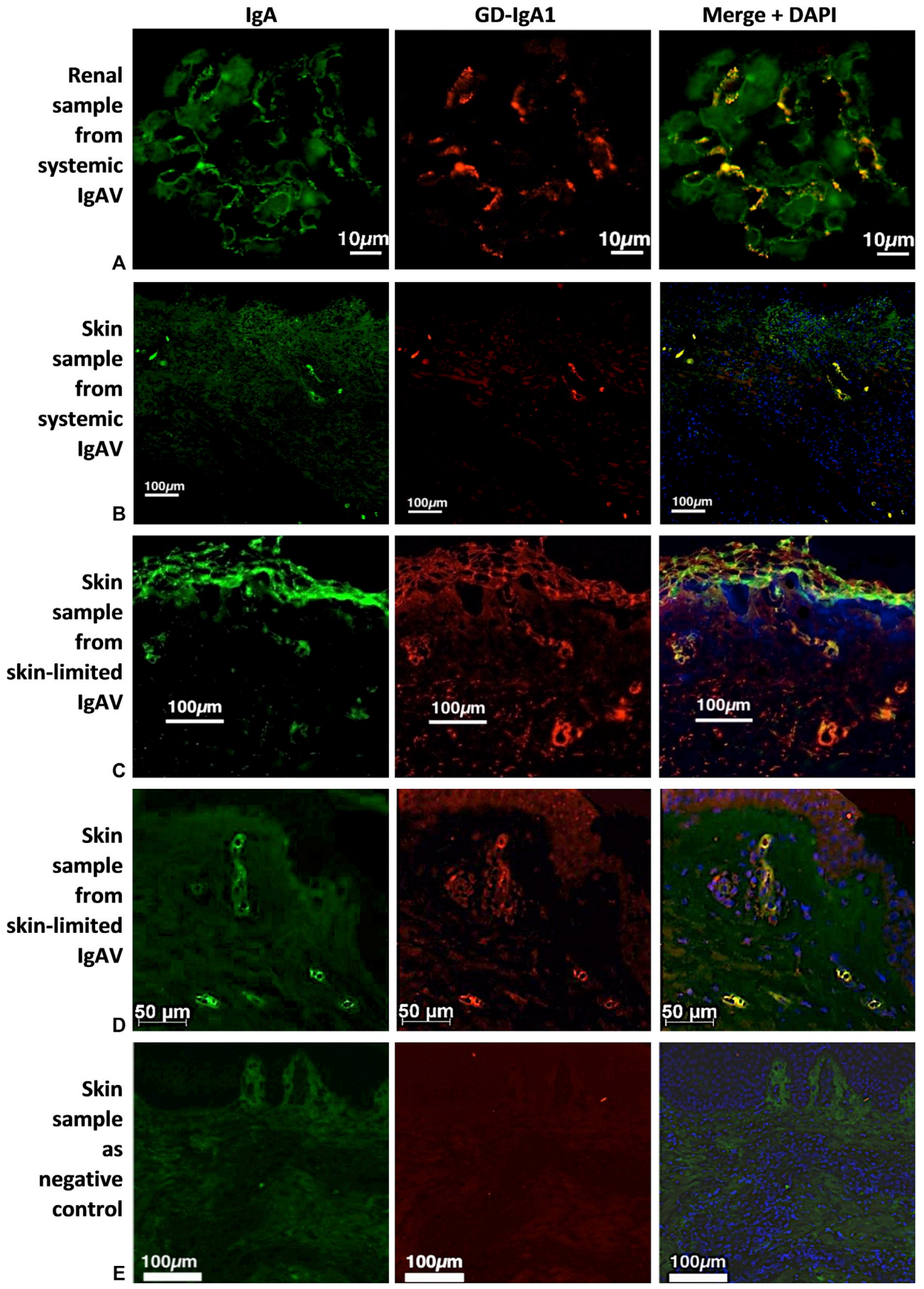
The study enrolled 16 adult patients presenting with a diagnosis of IgAV to the Department of Dermatology at the University of Hospital of Münster, between 2016 and 2018, for analysis of skin and serum samples (mean age, 49.6 years, and 67% were women). All study participants signed an informed consent document approved by the Ethical Committee. The diagnosis was based on biopsy specimen–proven leukocytoclastic vasculitis, deposition of IgA on immunofluorescence staining, and clinical appearance of palpable purpura on dependent parts of the body (ie, with a characteristic predilection for the lower limbs).

A diagnosis of skin-limited (instead of systemic) IgAV was made when (1) urinalysis showed no proteinuria, no dysmorphic erythrocytes, and no erythrocyte casts and the estimated glomerular filtration rate was >90 mL/min/1.73 $m^2$ (no renal biopsy was indicated or performed in these patients) and when there was (2) no abdominal discomfort and no positive fecal occult blood or signs of vasculitis on colonoscopy, (3) no arthritis, and (4) no sign of disturbance of the central nervous system.

A diagnosis of systemic IgAV with renal involvement (IgAVN) was made when patients had biopsy specimen–proven renal involvement with mesangial IgA deposits or >3 g/24 h protein excretion and an estimated glomerular filtration rate of <90 mL/min/1.73 $m^2$.

### Skin biopsy

Biopsy specimens were obtained from early vasculitic lesions that showed partially blanching,


IgA
GD-IgA1
Merge + DAPI
Renal sample from systemic IgAV
A
10μm
10μm
10μm
Skin sample from systemic IgAV
B
100μm
100μm
100μm
Skin sample from skin-limited IgAV
C
100μm
100μm
100μm
Skin sample from skin-limited IgAV
D
50 μm
50 μm
50 μm
Skin sample as negative control
E
100μm
100μm
100μm

slightly palpable round purpura from 12 patients with skin-limited IgAV and from 4 patients with systemic IgAVN. One biopsy sample was obtained from each sample site for cryofixation and formalin fixation. In addition, we obtained biopsy samples from the clinically uninvolved skin in 1 patient with skin-limited and 1 with systemic IgAV. We used renal tissue samples from 2 of these patients as a positive control for KM55 antibody staining[15] and confirmed glomerular deposition of GD-IgA1 in both patients (Fig 1, *A*).

As a negative control, we used 3 lesional skin samples from patients with the histologic diagnosis of leukocytoclastic vasculitis but no perivascular IgA deposition and from 2 IgA-negative samples of clinically uninvolved skin (Fig 1, *E*).

### Serum samples

Serum GD-IgA1 levels were measured in 15 patients with skin-limited IgAV and for comparison in 15 sex-matched adult patients with systemic IgAVN (proven on renal biopsy specimen and skin involvement) and 11 age- and sex-matched healthy individuals. Serum samples from healthy individuals and patients with IgAVN were obtained from the Glomerular Disease Archive of the Mayer IgA Nephropathy Laboratory, University of Leicester.

### Immunofluorescence staining of skin and renal biopsy specimens

Immunofluorescent staining for GD-IgA1 in renal biopsy samples followed the previously published protocol.[15] Staining for GD-IgA1 in skin biopsy samples was performed using an adjusted protocol based on the lectin-independent method using KM55 antibody on paraffin sections, as previously described. In brief, paraffin-embedded sections were deparaffinized by decreasing ethanol concentrations. Antigen retrieval was performed at room temperature for 2 hours using bacterial protease (0.05% subtilisin A; Sigma-Aldrich, St. Louis, MO). After nonspecific binding sites were blocked (Protein Block; Dako, Glostrup, Denmark), sections were incubated with KM55 (100 $\mu$g/mL) at 37°C for 60 minutes and a secondary antibody Alexa Fluor 555—conjugated goat anti-rat IgG antibody (Thermo Fisher Scientific, Waltham, MA). For staining of IgA, we used anti—human-IgA—fluorescein isothiocyanate (Jackson ImmunoResearch, West Grove, PA), and for visualization of nuclei, we used DAPI. For staining of IgG, IgM, and C3, we used fluorescein isothiocyanate—labeled anti—human-IgG, IgM (both from Dianova, Hamburg, Germany), and C3 (Dako) on corresponding cryopreserved samples. All samples were analyzed using an Axio Observer Z1 microscope (Zeiss, Jena, Germany) at ×20 magnification.

### Measurement of serum GD-IgA1

Serum GD-IgA1 concentrations were measured using a KM55 enzyme-linked immunosorbent assay (ELISA) kit (Immuno-Biological Laboratories, Minneapolis, MN), according to the manufacturer's instructions.

### Statistical analysis

Statistical analysis was performed with GraphPad Prism 7.04 software (GraphPad, La Jolla, CA). Differences among disease groups and healthy individuals were assessed by analysis of variance, followed by Bonferroni correction for multiple comparisons. Data are expressed as mean ± standard error of the mean. $P$ values of $<.05$ were considered to be statistically significant.

## RESULTS

### Cutaneous deposition of GD-IgA1 in skin-limited and systemic IgAV

Immunohistochemical staining with anti—GD-IgA1 antibody KM55 revealed that the 12 patients with skin-limited IgAV and the 4 patients with systemic IgAV had perivascular deposition of GD-IgA1, colocalizing with deposition of IgA (Fig 1, *B* to *D*). C3 was detected immunohistochemically in corresponding cryopreserved sections of 10 of the 16 $IgA^+$ skin samples (in 7 with skin-limited and 3 with systemic IgAV), indicating activation of complement, but additional deposition of IgG was observed in only 2 samples from skin-limited IgAV and additional IgM in 1 sample from skin-limited

**Fig 1.** Immunohistochemical staining of renal and skin biopsy tissue for IgA and galactose-deficient IgA1 (*GD-IgA1*). **A**, Glomerular staining demonstrates dual positivity for IgA and GD-IgA1 deposited at mesangial and capillary wall (DAPI staining omitted) in a specimen from a patient with systemic IgA vasculitis (*IgAV*) with nephritis. **B**, Dual positivity for IgA and GD-IgA1 deposition in cutaneous postcapillary venules in a specimen from a patient with systemic IgAV with nephritis. **C** and **D**, Dual positivity for IgA and GD-IgA1 deposition in cutaneous postcapillary venules in 2 representative samples of skin-limited IgAV. **E**, Absence of IgA and GD-IgA1 deposition in a sample of non—IgA-mediated leukocytoclastic vasculitis.

IgAV. We also observed positive staining for GD-IgA1 and IgA in 2 biopsy specimens of clinically uninvolved skin from 1 patient with skin-limited IgAV and 1 with systemic IgAV. Both specimens were also positive for C3, and additional weak positivity for IgG was noted in the sample from systemic IgAV.

As expected, there was positive staining for GD-IgA1 with the KM55 antibody in the renal biopsy specimens from 2 patients with IgAVN (Fig 1, *A*). Both renal samples were also positive for C3, and 1 had additional IgM deposition. Further confirming the specificity of the KM55 antibody, the lesional skin from patients with a histologic diagnosis of leukocytoclastic vasculitis, but no perivascular IgA deposition (e.g. cryoglobulinemic vasculitis), and from $IgA^{-}$ samples from clinically uninvolved skin, did not contain GD-IgA1 (Fig 1, *E*).

## SERUM GD-IgA1 LEVELS IN SKIN-LIMITED AND SYSTEMIC IgAV

Serum GD-IgA1 levels were measured using a commercial KM55-based ELISA (Fig 2). Patients with systemic IgAVN had significantly higher serum GD-IgA1 levels (15.57 ± 3.19 $\mu$g/mL, n = 15) than patients with skin-limited IgAV (7.87 ± 1.13 $\mu$g/mL, n = 15; $P$ = .0499) and healthy individuals (5.39 ± 0.96 $\mu$g/mL, n = 11; $P$ = .0125). It is important to acknowledge that the serum GD-IgA1 levels in several patients with systemic IgAV were similar or only marginally raised compared with levels from patients with skin-limited IgAV or healthy individuals (Fig 2). There was no significant difference in serum GD-IgA1 levels between healthy individuals and patients with skin-limited IgAV, although the mean was slightly higher in skin-limited IgAV.

## DISCUSSION

Our results reveal that skin lesions in systemic IgAV and skin-limited IgAV both contain deposits of poorly *O*-galactosylated IgA1 (GD-IgA1) associated with the endothelium of postcapillary venules, similar to those seen in the glomerular capillary walls and mesangium in IgAN and IgAVN. Thus, deposition of GD-IgA1 is one prerequisite for IgAV, and restriction of vasculitis to the skin in skin-limited IgAV is not due to the absence of GD-IgA1. The presence of dermal GD-IgA1 deposition alone does not, therefore, predict renal involvement or severity of IgAV. We did, however, find quantitative differences in the serum levels of GD-IgA1 between systemic and skin-limited IgAV, with patients with systemic IgAV having a significantly higher average serum level of GD-IgA1 than those patients with the skin-limited form of IgAV.

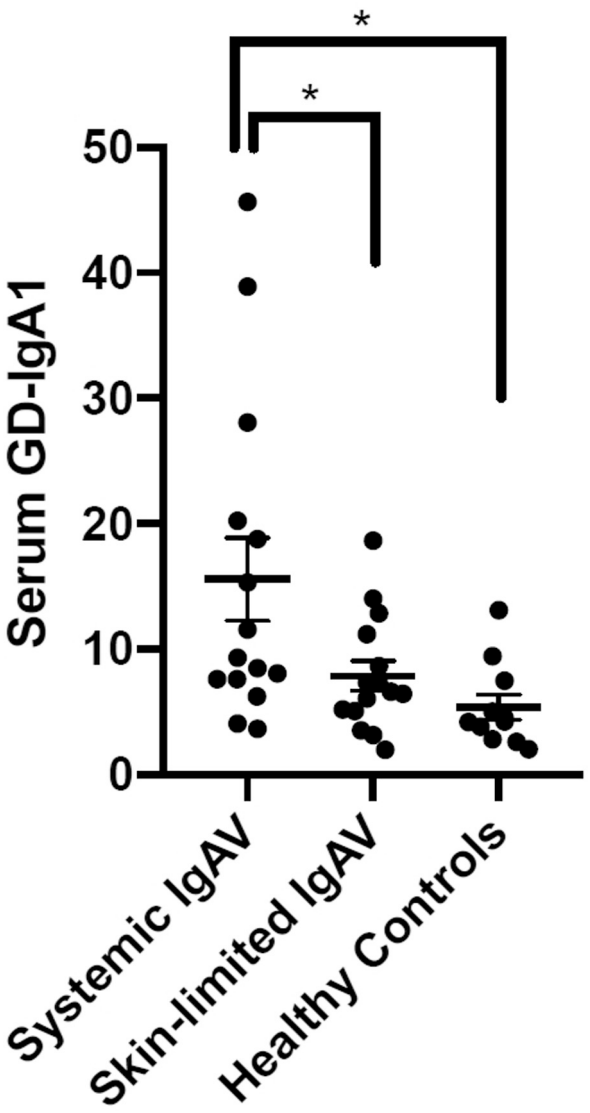



**Fig 2.** Serum galactose-deficient IgA1 (*GD-IgA1*) concentrations ($\mu$g/mL) were measured using a KM55 enzyme-linked immunosorbent assay kit in patients with skin-limited IgA vasculitis (n = 15), systemic IgAV (n = 15), and healthy individuals (n = 11). Serum levels of GD-IgA1 were significantly higher in systemic IgAV compared with skin-limited IgAV ($P < .05$) and healthy individuals ($P < .05$). The difference in GD-IgA1 levels between healthy individuals and those with skin-limited IgAV was not significant. Mean data are presented with the standard error of the mean. *$P < .05$.

We report serum GD-IgA1 levels in precisely phenotyped patients with systemic and skin-limited IgAV using an ELISA based on KM55, a specific monoclonal antibody. Previous studies in adult and pediatric IgAV that have reported serum GD-IgA1 levels have used a lectin-based assay that is known to vary between laboratories.[12,16,17]

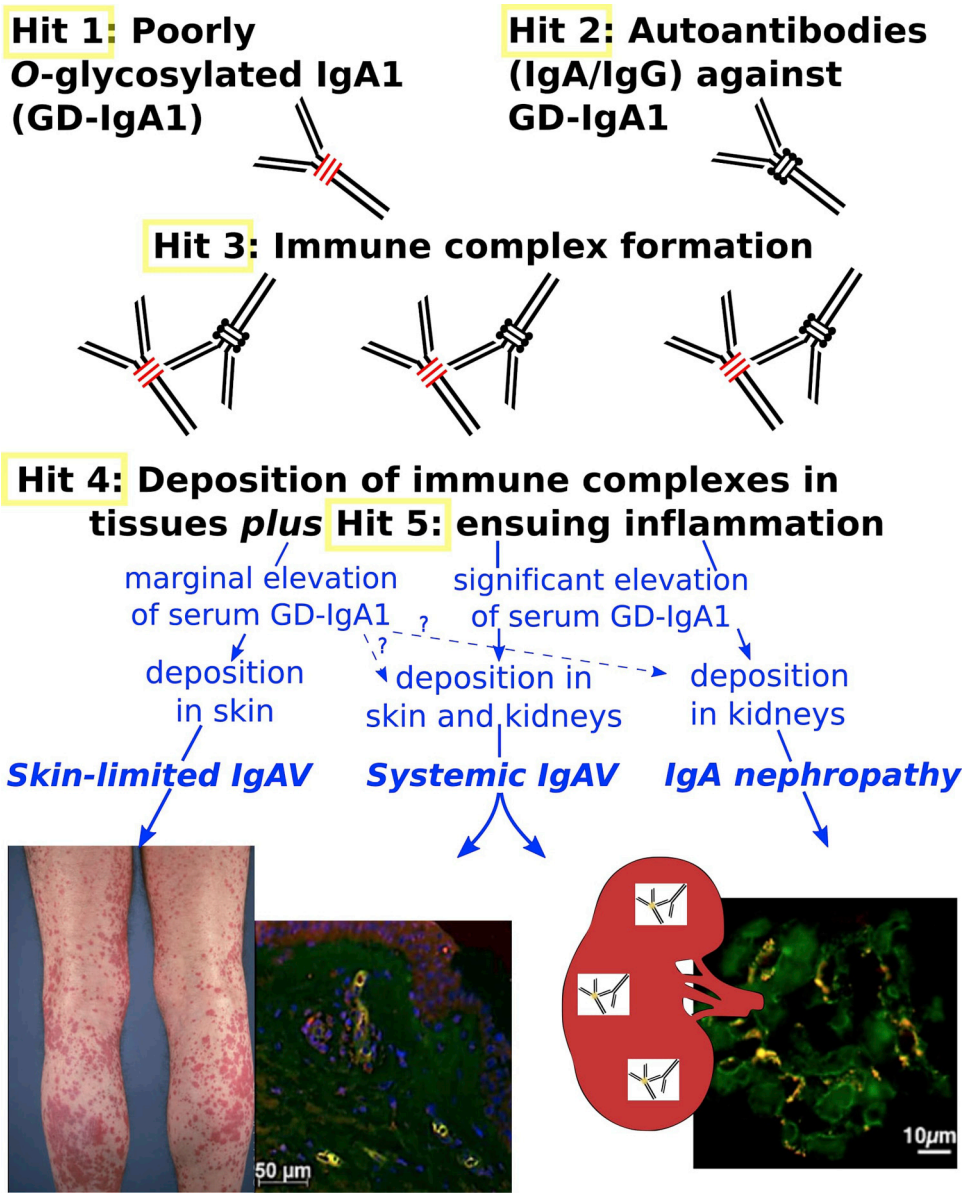



**Fig 3.** Simplified scheme of the multihit hypothesis for deposition of immune complexes in skin-limited IgA vasculitis, systemic IgAV, and IgA nephropathy (*IgAN*). Synthesis of galactose-deficient IgA1 (*GD-IgA1*)-IgA1 (**Hit 1**), which is recognized by circulating anti-glycan autoantibodies (IgG or IgA1) (**Hit 2**), resulting in formation of large pathogenic circulating immune complexes (**Hit 3**). The target antigen(s) of GD-IgA1 and possibly other components of these immune complexes are unknown. Under certain circumstances, they deposit at walls of postcapillary venules in skin and— especially when present at higher serum levels—in glomerular capillary walls and mesangium (**Hit 4**). They activate complement and neutrophils, but the other elicitors and the exact processes leading to ensuing vascular or renal injury (**Hit 5**) are not yet completely known.

In 1 study in children with IgAV, with and without nephritis, no significant difference in the median serum GD-IgA1 levels was seen at the onset of IgAV,[18] whereas Berthelot et al[12] found (1) higher serum levels of GD-IgA1 in 60 adult patients with IgAVN compared with 25 patients with skin-limited IgAV (they did not explicitly use this term and definition), and (2) slightly but not significantly increased serum levels in skin-limited IgAV compared with healthy individuals (as in our study).

Similarly, 2 other studies reported that IgA1 from 24 and 33 children with renal involvement showed

significantly higher lectin binding (indicating high GD-IgA1 levels) than IgA1 from 22 and 17 children lacking renal involvement,[16,17] whereas lectin binding of IgA1 from children with IgAV without renal involvement did not differ from healthy individuals.[16] It is noteworthy that despite the significantly higher average serum GD-IgA1 levels, many individual patients with IgAVN had no raised levels (compared with IgAV or healthy subjects).[17,18]

Our cohorts of skin-limited IgAV, systemic IgAVN, and healthy individuals also had overlapping serum GD-IgA1 concentrations in the lower range values. Yet, only in the cohort of systemic IgAVN did we find concentrations of 20 $\mu$g/mL and higher. It is tempting to speculate that as serum levels of GD-IgA1 increase, it may tip the balance in susceptible patients from a skin-limited disease to systemic IgAV and whether a threshold of 20 $\mu$g/mL could be used in the future to indicate systemic IgAV. Concentrations below this threshold do not allow a clear designation of skin-limited or systemic IgAV. Therefore, it will be interesting to perform standardized measurements of serum GD-IgA1 using the KM55 ELISA (in place of lectin-binding assays) in large cohorts of patients with IgAV to determine whether there is a threshold or cutoff value above which systemic vasculitis can be confidently diagnosed (high predictive value).

When we related serum levels of GD-IgA1 with total IgA levels in our cohorts, GD-IgA1 was only approximately 0.2% of the total serum IgA, consistent with the widely held belief that the pathogenic fraction of serum IgA is limited to a highly specific subset of circulating IgA molecules. This is similar to previous studies which have also reported that higher GD-IgA1 levels are associated with worse renal outcomes and are independent of total IgA levels.[15,17,19]

Deposition of GD-IgA1 in cutaneous blood vessels appears to be mandatory but alone is not sufficient to induce vascular damage, because clinically uninvolved skin from patients with IgAV also showed GD-IgA1 deposits and since in previous studies (and reflected by our unpublished data) IgA deposition is often found in biopsy samples from clinically uninvolved skin from patients with IgAV.[20-24] The dermal deposition of GD-IgA1 in both skin-limited and systemic IgAV and increased serum GD-IgA1 levels in IgAVN suggests that skin-limited IgAV, systemic IgAV, and IgAN (which is not associated with cutaneous vasculitis) may be variants of the same disease.

After we have provided evidence that Gd-IgA1 is deposited at vessels of systemic and skin-limited IgAV, we believe it is reasonable to extrapolate the IgAN multihit hypothesis[25-28] to IgAV, as shown in Fig 3. We detected codeposition of IgG (as in IgAN) in 2 samples of skin-limited IgAV, consistent with the presence of IgA-IgG immune complexes.[29] In those samples with no IgG codeposition, IgA1 autoantibodies to GD-IgA1 (as has been described in IgAN), which cannot be definitively identified with current staining techniques, likely developed in these patients. Immune complex deposition leads to activation of neutrophils in the vessel wall, which is one of the initiating steps in vascular injury.[30,31] Immune complex, rather than monomeric IgA, deposition is essential for this process because only IgA immune complexes are capable of cross-linking neutrophil Fc receptor I for IgA (Fc$\alpha$RI) and thus of inducing production of reactive oxygen species, degranulation, cytokine secretion, and release of neutrophil extracellular traps.[30,32] The additional factors that lead to aberrant activation of transmigrating neutrophils and vessel damage are not completely known.

The presence of perivascular C3 in 63% of $IgA^+$ skin samples indicates that complement activation plays a part in the IgA-induced tissue injury, both in the skin and at systemic sites in IgAV, similarly as in IgAN, where it is thought to contribute to the formation and nephritogenic activities of the complexes.

A limitation of our study is the low number of skin biopsy samples and serum samples from patients with skin-limited IgAV and systemic IgAVN. Yet, on the basis of our provisional data, it will now be possible to undertake longitudinal studies on more diverse and larger patient cohorts to evaluate the prognostic utility of serum GD-IgA1 levels and their predictive value for renal involvement in IgAV.

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