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REVIEW ARTICLE

Asialoglycoprotein receptor (ASGPR): a peculiar target of liver-specific autoimmunity

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Abstract Asialoglycoprotein receptor (ASGPR) autoantibodies have been considered specific markers of autoimmune hepatitis (AIH). The exact mechanisms responsible for the development of these autoantibodies and leading to autoimmunity to this peculiar liver receptor remain elusive. Furthermore, loss of T cell tolerance to ASGPR has been demonstrated in patients with AIH, but it is poorly understood whether such liver-specific T cell responses bear a pathogenic potential and/or participate in the precipitation of AIH. Newly developed enzyme-linked immunosorbent assays have led to the investigation of the sensitivity and specificity of anti-ASGPR antibodies for AIH. The present review provides an overview of the diagnostic and clinical relevance of anti-ASGPR antibodies. A thorough investigation of the autoreactivity against ASGPR may assist efforts to understand liver autoimmunity in susceptible individuals.

Keywords Autoantibody · Autoimmune hepatitis · Autoimmunity · Liver disease · Primary biliary cirrhosis

Abbreviations

ANA	Anti-nuclear antibody
ASGPR	Asialoglycoprotein receptor
ASMA	Anti-smooth muscle antibody
ELISA	Enzyme-linked immunosorbent assay
LSP	Liver-specific membrane lipoproteins
LKM	Liver kidney microsomal
SLA	Soluble liver antigen

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ASGPR: an introduction to the molecule

Asialoglycoprotein receptor (ASGPR) [1] is a C-type lectin, primary expressed on the sinusoidal surface of the hepatocyte [2–4]. ASGPR was discovered as early as 1965 by Gilbert Ashwell and Anatol Morell in USA [3–9]. These investigators isolated ASGPR from rabbit liver by affinity chromatography employing asialo-orosomucoid sepharose [10].

ASGPR is formed by a major 48 kDa (ASGPR-1) and a minor 40 kDa subunits (ASGPR-2) [11–13]. The major role of ASGPR is the binding, internalization, and subsequent clearance from the circulation of glycoproteins that contain terminal galactose or *N*-acetylgalactosamine residues (asialoglycoproteins) [11, 12, 14]. The binding of

ligands to ASGPR depends on Ca^{2+} [15], the position of terminal galactose residues [16–18], and a pH optimum above 6.5 [19]. Mice lacking ASGPR are characterized by an impaired clearance of injected asialoglycoproteins, but do not accumulate glycoproteins in the serum suggesting that ASGPR is not the only regulator of glycoprotein levels in the blood.

ASGPR has been shown also to be involved in the clearance of IgA from circulation [10, 20–25], in the removal of apoptotic cells, in the clearance of low density lipoprotein (LDL) and chylomicron remnants [26, 27] and in the disposal of cellular fibronectin [28]. Recent data support the assumption that ASGPR is utilized by hepatotropic viruses for hepatocyte entry [29–35]. Furthermore, there is evidence that ASGPR participates in the elimination of activated lymphocytes [36–39].

From a pathophysiological point of view, ASGPR appears to be involved in the clearance of coagulation factors from circulation and to be involved in the establishment of lethal thrombocytopenia seen in sepsis caused by *S. pneumonia* infection [40]. In this disease setting, ASGPR-dependent clearance of platelets, which have been desialylated by the NanA sialidase of *S. pneumonia*, seems to play a pivotal role in sepsis precipitation. This may explain why ASGPR-knockout mice cannot survive inoculation with small doses of *S. pneumonia* [40–43].

ASGPR and liver inflammation

Liver inflammatory diseases irrespectively of the cause alter the expression levels of ASGPR, its synthesis, and binding activity. In normal hepatocytes, ASGPR is expressed in a polar manner on the sinusoidal and basolateral surface of the plasma hepatocyte membrane [11, 12, 44]. However, during liver inflammation, ASGPR's expression shifts towards the canalicular membrane [45]. In end-stage liver disease (cirrhosis), ASGPR is overexpressed and serum levels of asialoglycoproteins are increased [46]. Cytokines appear to have a profound effect on the expression, synthesis, and functionality of the receptor [47, 48].

In immune-mediated liver diseases, ASGPR becomes the target of autoimmune responses, both at the B- and T cell level [49]. Thus, this review will mainly discuss the role of ASGPR as a liver autoantigen.

ASGPR as an autoantigen

In the 1970s, liver-specific membrane lipoproteins (LSP) have been found by Roger Williams' group to bear antigenic epitopes for circulating autoantibodies in patients with acute and chronic active hepatitis [50]. Later on, ASGPR was identified as the major antigenic component of LSP by the same group in London and appears to be the main organ-specific autoantigenic target in autoimmune liver diseases reported so far [51]. Subsequent studies, both in human and animal biomaterial, have highlighted the autoimmunogenicity of ASGPR and its relevance to specific disease phenotypes [49, 52–68]. Soon after the experimental data provided evidence that ASGPR-specific monoclonal antibodies can induce liver damage, attempts to study the immunological potential of ASGPR have been accelerating [69]. ASGPR-specific T cell clones have been obtained [67] and ASGPR (or LSP) has been used in immunization experiments to induce liver damage in an animal setting [69–73]. The relevance of anti-ASGPR-specific T cell responses to the pathogenesis of AIH is still elusive [74, 75]. Other liver-specific B and T cell responses against AIH-relevant antigens appear to play an important role in the development of AIH in susceptible individuals, who are characterized by impaired immunoregulatory mechanisms [74–85]. Intriguingly, gastrointestinal receptors, such as the ASGPR with the potential to interact with pathogens appear to play an important role in gastrointestinal autoimmunity as recently demonstrated for the microfold cell-specific glycoprotein 2, the autoantigenic target in Crohn's disease which has even an immunomodulating capacity [86–88].

One of the first efforts to elucidate the humoral loss of tolerance to ASGPR was to develop assays that could reliably detect anti-ASGPR antibody levels in patients with AIH. Due to the peculiar biochemical characteristics of ASGPR, this task proved to be rather difficult, but was nevertheless essential to study the diagnostic and clinical relevance of the tolerance loss to ASGPR.

Anti-ASGPR antibody testing

A variety of assays has been developed in order to detect anti-ASGPR antibody reactivity and such assays included solid-phase enzyme-linked immunosorbent assays (ELISA), liquid-phase radioimmunoassays, immunoblotting and dot blot assays. As an antigenic source, ASGPR purified from rabbit, rat or human liver preparations or recombinant ASGPR subunits have been utilized [52, 58, 60, 62, 89]. One of the major challenges in developing a molecular assay remained the access to highly purified ASGPR [90]. ASGPR obtained through affinity chromatography on galactose–sepharose has been considered a credible source of the antigen [68]. Recombinant antigen has been produced, but its immunogenicity appears poor [58]. In summary, data obtained by several studies produced inconsistent results, raising concerns as to whether a

reliable immunoassay could ever be developed to assess the epitope structure of the antigenic preparation used for ASGPR-antibody testing [89]. As it was expected, the lack of a reliable, standardized assay for the detection of anti-ASGPR antibodies has led to a series of reports with significant variation in the prevalence of these autoantibodies in AIH, and other autoimmune and non-autoimmune liver diseases.

Nevertheless, anti-ASGPR antibodies have been included in the revised criteria of the International Autoimmune Hepatitis Group in 1999, but are missing in recently published diagnostic scores for AIH while other autoantibodies have been incorporated in the routine testing [91–93]. The assumption that ASGPR autoantibodies should not be part of the autoantibody specificities considered to be important for the serological diagnosis in patients with AIH deserves further discussion.

Diagnostic relevance of anti-ASGPR antibodies

Most studies recently published have tested anti-ASGPR antibodies in serum samples from patients with liver diseases, including autoimmune and non-autoimmune disorders of the liver. Anti-ASGPR autoantibodies have been predominantly found in patients with chronic active hepatitis, the term used in the past for what is currently known as AIH. Another key finding was that the level of these autoantibodies sharply decreases during immunosuppressive treatment [68]. This was an intriguing observation with profound implications regarding the broad range of anti-ASGPR seropositivity in various reports published over the years, as most studies included mixed sera from patients at diagnosis and during treatment. Thus, testing of cohorts that include more samples at diagnosis rather than under immunosuppression will most likely provide prevalence rates much higher than those of cohorts mainly containing samples tested after treatment [60].

The prevalence rates in children [94] and adult patients [59] with AIH-1 appear to be comparable, with anti-ASGPR antibodies being detectable in 75–82 % AIH-1 patients. However, in a recent study by Bulgakova et al. reported as an abstract at the 8th International Congress of Autoimmunity in Granada, a significantly higher prevalence of anti-ASGPR antibodies was found in children investigating 18 children and 28 adults with AIH (Fig. 1). However, only 2 out of the 18 children with AIH suffered from AIH-2 which demonstrate in general less prevalent anti-ASGPR antibodies (around 24–40 %) [94, 95].

The diagnostic value of anti-ASGPR antibodies is supported by studies suggesting that up to 80 % of AIH patients, who are seronegative for conventionally tested autoantibodies such as antinuclear antibody (ANA), anti-smooth muscle antibody (ASMA), anti-liver kidney microsomal 1 (LKM1) antibody, and even antibodies to soluble liver antigen (SLA), may have anti-ASGPR antibodies [61, 96]. In practical terms, these findings suggest that the recommended serological profile of patients with this disease may fail, if anti-ASGPR antibodies are not incorporated [97]. This has led some expert reports to underline the importance of anti-ASGPR antibody testing [92, 98]. However, the guidelines of the diagnostic criteria for AIH do not include anti-ASGPR antibodies in the scoring for the probable or definite diagnosis of AIH [93, 99, 100].

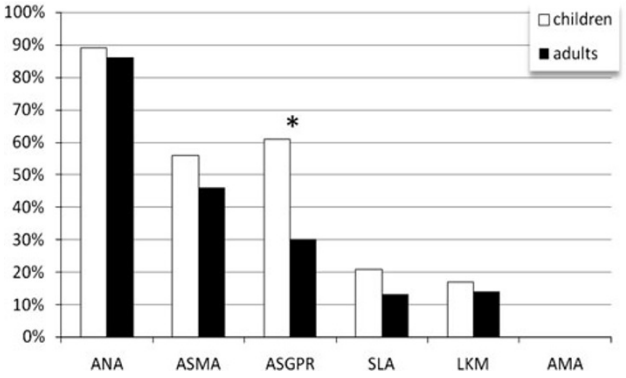

Fig. 1 Prevalence of autoantibodies in children ($n = 18$, median of age 10 years) and adults ($n = 28$, median of age 46 years) with autoimmune hepatitis (AIH). Seropositivity for antinuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA), liver–kidney microsomal type 1 (LKM-1) and anti-mitochondrial (AMA) antibodies were detected by indirect immunofluorescence on respective substrates. Antibodies to asialoglycoprotein receptor (ASGPR) and soluble liver antigen (SLA) were determined by commercial ELISA. 2 of 18 children with AIH suffered from type 2 AIH, whereas 2 of 28 adult patients with AIH demonstrated type 2 disease. All remaining patients had type 1 AIH. $^*P < 0.05$

Considering the lack of commercially available assays, testing for anti-ASGPR antibodies has been limited to few university laboratories using laborious *in house* assays for research purposes. The reports so far published have also indicated that the specificity of anti-ASGPR for AIH is not as good as described for other autoantibodies, like anti-LKM1, anti-SLA, and anti-SMA filamentous actin. Anti-ASGPR antibodies have been reported in more than 10 % of patients with chronic hepatitis B or C, patients with PBC, and patients with alcoholic hepatitis [54, 56, 60, 62, 101, 102]. The inconsistent results may be due to the lack of a standardized assay. In recent years, a new commercially available ELISA based on purified rabbit ASGPR has been developed and a study has been published reporting on the sensitivity and the specificity of this assay. Remarkably, 70 % of naïve (untreated) AIH patients were tested positive for anti-ASGPR antibody, whereas only 30 % patients under immunosuppressive treatment demonstrated elevated anti-ASGPR antibody levels [68].

Detectable anti-ASGPR antibodies were found in approximately 10 % patients with chronic hepatitis B or C [68]. Anti-ASGPR antibodies were practically absent in other pathological controls (including patients with PBC and alcoholic hepatitis) [68]. The interesting finding of this study was the striking specificity of anti-ASGPR for AIH (up to 100 %) as compared to PBC excluding PBC AIH overlap syndromes and such findings have not been reported previously.

Clinical utility of anti-ASGPR antibodies

An early study assessing anti-ASGPR antibodies in AIH-1 patients from USA noted more frequent relapses on treatment cessation in anti-ASGPR antibody positive AIH patients compared to anti-ASGPR antibody negative [59]. Serum immunoglobulin levels appeared higher in those with anti-ASGPR antibodies [59]. The association of disease activity indices or response to treatment and the presence of anti-ASGPR antibodies has been reported by others [53, 59, 94, 100, 103].

The recently published study employing the new anti-ASGPR antibody ELISA has found a correlation between anti-ASGPR and the level of liver transaminases in patients followed for long period of time [68]. A further intriguing result was that anti-ASGPR antibodies may precede the onset of elevated transaminases. That finding and the fact that anti-ASGPR antibodies are decreased over the course of immunosuppression indicate that this autoantibody may be a prognostic marker of disease activity [54, 56, 60]. A recent report has also found that anti-ASGPR antibodies decrease during immunosuppression [91, 104].

Conclusion

In the years to come, the relevance of anti-ASGPR antibodies in the diagnosis of AIH will be understood better with the help of robust and user-friendly anti-ASGPR antibody assays used in routine laboratory analysis. At present, several laboratories over the world are testing the applicability of newly available anti-ASGPR antibody ELISA. The results of these tests will shed a light on the long discussed usefulness of this autoantibody in the serology of AIH. We need to remind ourselves that ASGPR is one of the very few autoantigens that is liver specific in AIH (if it is not the only liver-specific one). It is possible that the understanding of the pathogenesis of AIH lies within the understanding of the immunopathophysiology of this hepatic lectin [91]. We anticipate that we will learn more about the role of ASGPR in the near future.

Conflict of interest Dirk Roggenbuck has a management role and is a shareholder of GA Generic Assays GmbH and Medipan GmbH. Both companies are diagnostic manufacturers. All other authors declare that they have no competing financial interests.

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