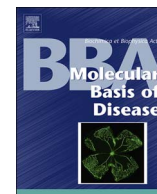




Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadisOn the role of IgG4 in inflammatory conditions: lessons for IgG4-related disease[☆]David C. Trampert¹, Lowiek M. Hubers¹, Stan F.J. van de Graaf, Ulrich Beuers^{*}*Department of Gastroenterology & Hepatology, Tytgat Institute for Liver and Intestinal Research, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands*

ARTICLE INFO

Keywords:

Autoimmune pancreatitis
 Cholangiocarcinoma
 IgG4-associated cholangitis
 Melanoma
 Primary sclerosing cholangitis
 Review

ABSTRACT

The pathophysiology of immunoglobulin G4-related disease (IgG4-RD) and its most common manifestations, IgG4-associated (sclerosing) cholangitis and autoimmune pancreatitis, remains largely unknown, but IgG4 is presumably involved. IgG4 is a promiscuous antibody, which could be directly pathogenic, fulfill a protective role, or could just be a fortuitous marker of an aberrant inflammatory response. IgG4 antibodies possess exclusive structural and functional characteristics suggesting anti-inflammatory and tolerance-inducing effects. By studying the role of IgG4 in other inflammatory conditions, namely hypersensitivity and allergies, autoimmune and immune-mediated diseases, infections and malignancies, new insights can be obtained increasing our understanding of the role of IgG4 antibodies in IgG4-RD. Beekeepers, animal laboratory workers and individuals undergoing allergen immunotherapy possess high serum levels of allergen-specific IgG4, which exhibit immunosuppressive functions, protecting the individual from anaphylactic reactions. In autoimmune/immune-mediated diseases, such as pemphigus vulgaris, pemphigus foliaceus and MuSK-myasthenia gravis, IgG4 auto-antibodies are pathogenic. Regarding malignancies such as melanoma and cholangiocarcinoma or helminthic infections, IgG4 antibodies inhibit clearance of tumor cells or the invader, respectively. Translating these findings to IgG4-RD, IgG4 alone can implement pathogenic effects and structural damage, but may also function as a protective antibody dampening the more harmful effects of IgG1 when directed against the same epitopes. This article is part of a Special Issue entitled: Cholangiocytes in Health and Disease edited by Jesus Banales, Marco Marzoni, Nicholas LaRusso and Peter Jansen.

1. Background: IgG4-related disease of the biliary tract and pancreas

Immunoglobulin G4-related disease (IgG4-RD) is a recently defined immune-mediated disorder of unknown cause [1,2]. Most commonly involved localizations include the pancreas (autoimmune pancreatitis type 1, AIP), biliary tract (IgG4-associated [sclerosing] cholangitis, IAC) and salivary glands, but also the hypophysis, lacrimal glands, orbita, thyroid, mediastinum, lymph nodes, lungs, aorta, retroperitoneum, kidneys, prostate, and testis may be affected. Still, novel organ manifestations are being described sharing the histopathological features characteristic for IgG4-RD: affected organs typically exhibit prominent infiltrates of lymphoplasmacytic cells including high numbers of IgG4-producing (IgG4 +) B and plasma cells, together with storiform fibrosis, obliterative phlebitis and eosinophilia [3]. Correct diagnosis is

frequently delayed or missed, since IgG4-RD may mimic other inflammatory diseases, but also solid and hematopoietic malignancies. This holds particularly true for the diagnosis of IAC and AIP, due to their heterogeneity in clinical presentation and the lack of a highly sensitive and specific diagnostic test in the past. IAC and AIP can mimic cholangiocarcinoma and pancreatic carcinoma, respectively, upon imaging adding a level of complexity to the diagnosis [4,5]. Representative histological biopsies are often difficult to obtain and not without risk of complications. Meanwhile, novel accurate non-invasive diagnostic tests for the diagnosis of IgG4-RD of the biliary tract and pancreas (and possibly other organ manifestations), which showed better diagnostic performance than IgG4 serum levels, are within reach [6,7]. The markers used in these accurate diagnostic tests may eventually be incorporated in the existing criteria for the diagnosis of IgG4-RD, which combine clinical, serological, radiographic and histological

[☆] This article is part of a Special Issue entitled: Cholangiocytes in Health and Disease edited by Jesus Banales, Marco Marzoni, Nicholas LaRusso and Peter Jansen.

^{*} Corresponding author at: Department of Gastroenterology & Hepatology, Tytgat Institute for Liver and Intestinal Research, Academic Medical Centre, University of Amsterdam, Meibergdreef 9, room G4-216, 1105 AZ Amsterdam, The Netherlands.

E-mail address: u.h.beuers@amc.uva.nl (U. Beuers).

¹ Contributed equally.

<http://dx.doi.org/10.1016/j.bbadis.2017.07.038>

Received 28 May 2017; Received in revised form 28 July 2017; Accepted 31 July 2017
 0925-4439/ © 2017 Elsevier B.V. All rights reserved.

parameters [8,9,10]. Once correctly diagnosed, patients with IgG4-RD quickly respond to moderate doses of corticosteroid treatment and (fibro)inflammatory lesions often completely dissolve [11]. In order to keep patients with AIP in remission and prevent relapses, long-term low-dose corticosteroid treatment (5–7.5 mg prednisolone/day) has proven to be beneficial [12]. Nevertheless, a quarter of the patients showed flares of the disease within a period of three years after start of treatment, requiring additional immunosuppressive therapy. Therefore, corticosteroid sparing agents such as azathioprine for maintenance treatment are also widely used [13].

Patients with AIP and IAC are mostly (> 80%) elderly men suffering from obstructive jaundice with or without itch, weight loss and sometimes abdominal complaints and diarrhea. Findings during diagnostic work-up suggestive of IgG4-RD include high serum IgG4 levels (serological), lymphoplasmacytic infiltrate, storiform fibrosis and obliterative phlebitis (histological), narrowing of the pancreatic duct and pancreatic enlargement (radiological) in AIP, cholangiographic findings mimicking primary or secondary sclerosing cholangitis or cholangiocarcinoma (CCA) in IAC, and a good response to corticosteroid therapy [14]. Elevated serum IgG4 is characteristic, but neither very sensitive nor specific for AIP and IAC, helping to distinguish AIP/IAC from other pancreatic and biliary disorders [15,16,17]. Notably, in patients with AIP, pathological changes in combination with infiltration of IgG4 + plasma cells, which are sensitive to corticosteroid therapy, can also be seen in liver biopsies suggesting additional IAC [18].

IAC, the hepatobiliary manifestation of IgG4-RD, is characterized by IgG4-positive lymphoplasmacytic infiltration of the biliary tract. Yet, IgG4 serum levels when $< 4 \times$ the upper limit of normal cannot accurately distinguish between IAC and its mimics, CCA or primary sclerosing cholangitis (PSC), as a fraction of CCA and PSC patients also have elevated IgG4 serum levels [4]. Thus, IAC is diagnosed based on a combination of clinical, biochemical, histological and imaging findings [9]. As seen in other cholestatic disorders, alkaline phosphatase and γ -glutamyltransferase are elevated in the majority of IAC patients [19]. Also serum bilirubin levels are often higher at first presentation when compared to patients with PSC²⁰. The extrahepatic bile duct walls are thickened due to fibrosis and inflammation as can be seen by transabdominal or endoscopic ultrasound or computed tomography. Magnetic resonance cholangiography (MRC) or endoscopic retrograde cholangiography (ERC) are of critical importance for the diagnosis of IAC, but are unable to distinguish IAC from PSC or CCA alone [5]. IAC predominantly, but not exclusively affects large and medium-sized bile ducts, whereas PSC can also affect smaller intrahepatic bile ducts (the extreme being ‘small-duct PSC’, a difficult diagnosis based on histological findings of onion-skin biliary fibrosis only in the presence of a normal cholangiogram), making it in some cases possible to exploit differences upon radiological imaging to differentiate between IAC and PSC [20]. MRC and ERC in IAC patients typically show biliary strictures, which can be classified into 4 types: stenosis of the lower part of the common bile duct (CBD) alone (type I), stenosis throughout the intra- and extrahepatic bile ducts (type II), stenosis of the hilar region of the biliary tree and of the lower part of the CBD (type III), stenosis of the hilar region of the biliary tree alone (type IV) [21,22,23]. IAC type I can resemble pancreatic cancer, whereas IAC type II can resemble PSC and IAC type III and IV can resemble CCA leading to potential misdiagnosis. Intraductal ultrasonography may confirm thickening of the intra- and extrahepatic bile duct wall [24]. As IAC is most often accompanied by AIP, radiological findings characteristic for AIP, such as enlargement of the pancreas, stenosis of the main pancreatic duct and, exceptionally, even pancreatic stone formation (authors’ experience) may contribute to identifying IAC [25]. Histology of pancreatic, biliary or even liver tissue may reveal lymphoplasmacytic infiltrate including (IgG4 +) plasma cells and eosinophil granulocytes, storiform fibrosis and obliterative phlebitis typical for IgG4-RD [24,25,26]. Phlebitis is specifically seen in the adventitial layer of the affected veins [27]. A minimum of 10 IgG4+ cells per HPF (high-power field) upon

immunohistochemical staining of bile duct specimens is required for the diagnosis of IAC [25,26].

IgG4-RD can be a challenging disease to identify and extra caution is required for the organ-specific manifestations IAC and AIP. By introduction of a novel costly and labour intensive next generation sequencing technique for detection of IgG4 + B cell receptor clones and, subsequently, an affordable, simple IgG4/IgG RNA qPCR technique, we were recently able to propose novel accurate non-invasive diagnostic tests for the diagnosis of IgG4-RD of the biliary tract and pancreas superior to IgG4 serum levels [6,7]. These accurate markers may eventually be incorporated in the existing criteria for the diagnosis of IgG4-RD of the biliary tract and pancreas, but possibly also other organ manifestations of IgG4-RD.

Most relevant for the understanding of IgG4-RD and its hepatobiliary and pancreatic manifestations, IAC and AIP, is that the role of IgG4 within IgG4-RD is yet to be defined (i.e. pathogenic, protective or a marker of an aberrant inflammatory response). Here, we analyzed the role of IgG4 in other inflammatory conditions, namely hypersensitivity and allergies, autoimmune and immune-mediated diseases, infections and malignancies to obtain more insight into the potential function(s) of IgG4 within IgG4-RD and specifically IAC and AIP.

2. Characteristics of IgG4 antibodies

Unlike other immunoglobulin isotypes and IgG subclasses, IgG4 antibodies possess certain exclusive structural and functional characteristics suggesting anti-inflammatory and tolerance-inducing effects. In serum of healthy individuals, IgG4 antibodies comprise the smallest fraction (< 5%) of IgG antibodies. The IgG4 molecule is composed of two heavy chains (~50 kD) and two light chains (~25 kD) paired together (Fig. 1A). The heavy chain consists of three IgG4 specific constant domains (C_{H1}, C_{H2}, C_{H3}) and one variable domain (V_H), whereas the light chain has one constant domain (C_L) and one variable domain (V_L). The light chain can be classified as either kappa or lambda type based on small peptide sequence differences. The variable domains of the heavy (V_H) and light chain (V_L), with the attached C_L and C_{H1} domains, form the ‘fragment antigen-binding region’ (Fab-region), which is highly specific for one epitope. The molecule contains 12 intra-heavy chain disulfide bridges, two disulfide bonds linking the heavy and light chains, and two inter-heavy chain disulfide bonds in the hinge (Fig. 1A) [28,29].

Due to one amino acid difference between IgG4 and IgG1 at position 228 in the hinge region (serine instead of proline), the two inter-heavy chain disulfide bonds of IgG4 are unstable and may form two intra-heavy chain bonds instead (Fig. 1B) [28,29]. Also, IgG4 and IgG1 differ at amino acid residue 409 (arginine instead of lysine), weakening the non-covalent interactions between the two C_{H3} domains in IgG4 [30]. Due to these structural characteristics the IgG4 heavy chains may dissociate into two half molecules (i.e. a heavy- and light-chain) and form new molecules with another IgG4 half-molecule (Fig. 1C) [31]. This unique property, frequently referred to as “Fab-arm exchange”, results in bispecific antibodies with two antigen-binding sites, which render the IgG4 antibody functionally monovalent and interfere with its ability to form large immune complexes.

The fragment crystallizable region (Fc-region) of the antibody, consisting of two identical C_{H2} and C_{H3} domains, provides a binding site for endogenous Fc-gamma receptors. However, IgG4 antibodies show little binding to activating receptors (except for Fc γ RI) on effector cells. Besides this, IgG4 antibodies have the tendency to interact with other immunoglobulins, acting in a fashion similar to rheumatoid factor antibodies. Furthermore, IgG4 antibodies are incapable of activating the classical complement pathway because of their low affinity for C1q [32]. IgG4 antibodies are even capable of effectively inhibiting the binding of C1q to IgG1 antibodies and thus protecting against the biological effects of the complement-fixing IgG1 subclass.

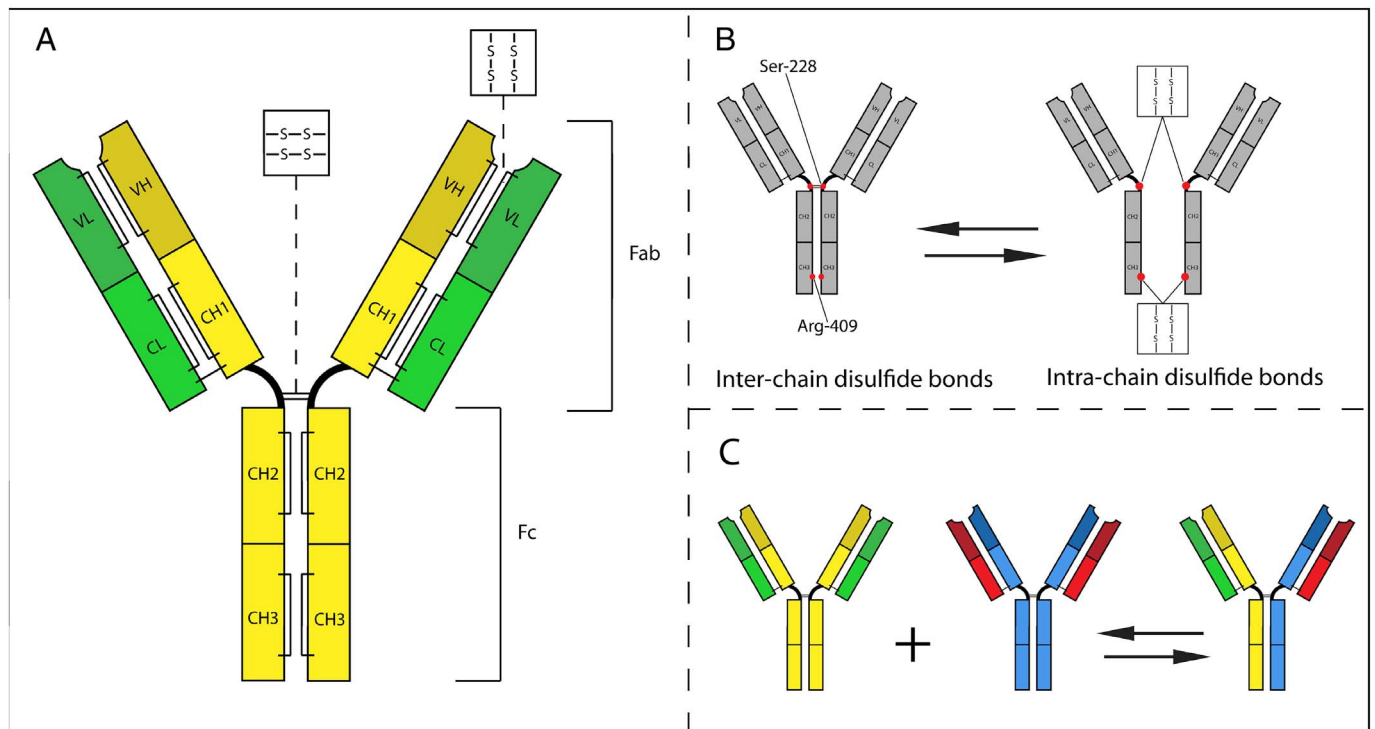


Fig. 1. Structure of IgG4 antibody and Fab-arm exchange. Inter- and intra-chain disulfide bonds give structure to IgG4. Switch of inter-chain disulfide bonds to intra-chain disulfide bonds at amino acids Ser-228 and Arg-409 cause Fab-arms of IgG4 to split [31].

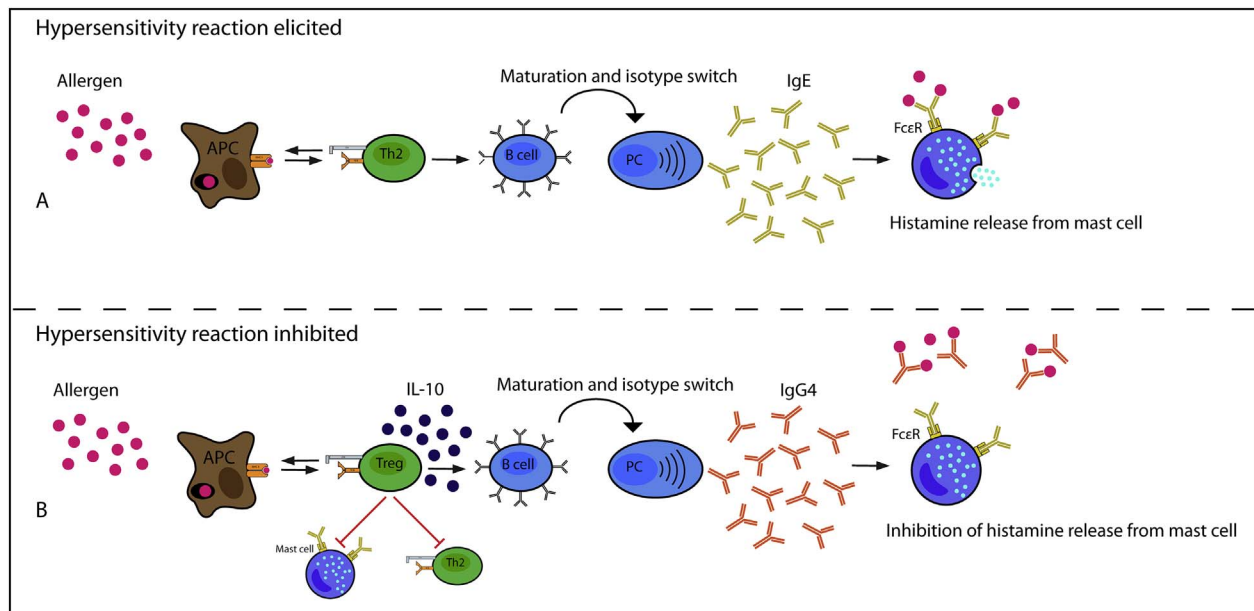


Fig. 2. The role of IgG4 in allergen exposure. While acute allergen exposure (A) may lead to IgE-mediated histamine-induced hypersensitivity reactions, chronic allergen exposure (B) may induce IgG4 formation and allergen binding by IgG4, thereby preventing hypersensitivity reactions and inducing allergen tolerance.

3. IgG4 in allergies and hypersensitivities

IgG4 has been extensively studied in models of chronic allergen exposure. In beekeepers naturally exposed to bee venom, high levels of bee venom-specific IgG4 are found to play a protective role against severe hypersensitivity reactions triggered by the bee venom allergen phospholipase A2 (PLA2) [33,34]. This is in contrast to the situation in bee-venom allergic individuals that exhibit high titers of PLA2-specific IgE and are at risk of IgE-mediated anaphylaxis when stung. Sera from tolerant beekeepers show 1000-fold lower PLA-specific IgE/IgG4 ratios

than sera from allergic subjects [35]. Within days after start of the beekeeping season, Interleukin (IL)-10 producing PLA2-specific regulatory T cells (Tregs) are induced in tolerant beekeepers upon high dose exposure to bee venom [36]. IL-10 is crucial for induction of peripheral tolerance and has several anti-inflammatory effects, including suppression of pro-inflammatory T helper type 2 (Th2) cells and other effector cells of allergic inflammation, such as mast cells, basophils and eosinophils [37]. Also, IL-10 decreases production of allergen-specific IgE, while enhancing the production of allergen-specific IgG4 [38,39].

Although several therapies exist to treat symptoms of allergic patients, the only curative treatment is allergen immunotherapy (AIT). By administration of increasing amounts of allergen, natural immune tolerance is induced. In line with responses in tolerant beekeepers upon high dose bee venom exposure, expansion of IgG4-switched PLA-specific memory B cells and increasing levels of PLA2-specific IgG4 without relevant change of total serum IgG4 are observed in successfully desensitized allergic individuals [40].

Similar to naturally exposed beekeepers and desensitized individuals with bee venom allergy, increased IgG4-specific antibodies correlate with allergen tolerance and can be measured in tolerant animal laboratory workers chronically exposed to rodent allergens [41,42] and in individuals undergoing AIT for severe allergies, including wasp, cat, house dust mite and birch pollen allergies [1,4]. IgG4 are thought to exert their anti-inflammatory effect through competitive binding with IgE to the allergen, thereby preventing formation of IgE immune complexes and IgE-mediated activation of mast cells. Mechanisms for allergen tolerance compared to immediate hypersensitivity reactions and the role of IgG4 are shown in Fig. 2.

4. IgG4 in autoimmune and immune-mediated diseases

In various autoimmune and immune-mediated diseases specific IgG4 antibodies have been identified. Often, the role of IgG4 in the inflammatory process remained unclear. However, in some autoimmune and immune-mediated diseases specific IgG4 antibodies have been shown to cause harm, including pemphigus vulgaris, pemphigus foliaceus and MuSK-myasthenia gravis. The role of IgG4 in these diseases as well as in bullous pemphigoid and idiopathic membranous glomerulonephritis will be discussed.

4.1. Pemphigus

Pemphigus is a family of autoimmune blistering disorders of the skin and mucous membranes. In pemphigus vulgaris (PV) and pemphigus foliaceus (PF) autoantibodies predominantly of the IgG4 subtype target desmogleins 1 and/or 3 [43,44]. The fraction of desmoglein-specific IgG4 in total serum IgG4 seems to differ strongly between patients, and total serum IgG4 mostly seem to be normal. Desmogleins are cell adhesion molecules and components of desmosomes, which are protein linkage structures that keep adjacent epidermal cells physically connected to each other. In pemphigus these linkage structures are masked by IgG4 autoantibodies, causing the epidermal cells to detach from each other, and as a consequence intra-epidermal blistering is thought to occur. The pathogenic role of IgG4 autoantibodies in the pathogenesis of pemphigus was established by passively transferring IgG4 autoantibodies from patients with PF to BALB/c mice, in which blistering and histological features consistent with pemphigus were reproduced [44,45,46,47]. Notably, Fab fragments, derived by pepsin cleavage, were even more potent in causing disease than the full IgG4 antibody, suggesting that Fc-receptor mediated effector functions and complement activation did not directly contribute to the pathogenesis at least in BALB/c mice [48]. Furthermore, the pathogenic potential of desmoglein-reactive autoantibodies seems to be dependent on specific epitope-binding, since both pathogenic and non-pathogenic desmoglein-reactive antibodies have been found in patients with PV and PF [49,50]. A schematic representation of the role of IgG4 autoantibodies in pemphigus is shown in Fig. 3.

4.2. MuSK-myasthenia gravis (MuSK-MG)

Myasthenia gravis (MG) is an autoimmune disease affecting the neuromuscular junction and can be further classified depending on the antigen specificity of the autoantibodies in question. Acetylcholine receptor-MG (AChR-MG) is the most common subtype covering about 80% of MG, and is characterized by autoantibodies against the

acetylcholine receptor (AChR). Muscle-specific kinase (MuSK) MG is a distinct form of MG in which autoantibodies target MuSK. Normally, MuSK ensures the maintenance of the neuromuscular junction through clustering of AChRs on the postsynaptic membrane (Fig. 4). Due to defective MuSK function, MuSK-MG patients suffer from impaired transduction of acetylcholine-mediated synaptic action potentials, leading to typical MG symptoms, such as muscle weakness.

The pathogenic autoantibodies in MuSK-MG, in contrast to AChR-MG, are of the IgG4 subtype and correlate with disease severity [51]. Notably, it has been recorded that a switch in dominance from IgG4 anti-MuSK autoantibodies to IgG1 anti-MuSK autoantibodies results in a significant decrease in disease severity, suggesting that primarily IgG4 anti-MuSK are responsible for inhibition/depletion of MuSK and the clinical symptoms observed in MuSK-MG patients [51]. The pathogenic role of IgG4 autoantibodies in the pathogenesis of MuSK-MG was established by passively transferring purified IgG4, in comparison to IgG1-3, from MuSK-MG patients to NOD/SCID mice, in which characteristics consistent with MuSK-MG were reproduced, such as reduction of postsynaptic acetylcholine sensitivity, and exaggerated depression of presynaptic acetylcholine release during high-rate activity, together causing muscle weakness [52]. In more detail, MuSK antibodies have been shown to block binding of low-density lipoprotein receptor-related protein 4 (Lrp4) to MuSK, thereby inhibiting Agrin-stimulated MuSK phosphorylation (Fig. 4) [53,54]. It is thought that this inhibitory mechanism directly disrupts the structure of the synapse and impairs the synaptic transmission, in contrast to the complement-mediated destruction of the neuromuscular junction observed in AChR-MG [55,56]. Even though IgG1-3 aren't able to block the binding of Lrp4 to MuSK, this fraction is capable of inhibiting agrin-induced AChR clustering in C2C12 cells [54]. Alongside this, it has previously been reported that anti-MuSK autoantibodies block the anchoring of the acetylcholinesterase (AChE)/collagen Q (ColQ) complex to MuSK causing partial endplate AChE deficiency [57]. Surprisingly, further research has revealed that the AChE/ColQ complex is also able to block the binding of Lrp4 to MuSK, however not to the same extent as anti-MuSK IgG4 autoantibodies are able to [58]. In fact, it has recently been demonstrated that anti-MuSK IgG4 autoantibodies in MuSK-MG patients undergo Fab-arm exchange and that both, the mono- and bivalent autoantibodies are pathogenic [59]. Thus, anti-MuSK autoantibodies of the IgG4 subtype seem to be the driving cause of pathology observed in MuSK-MG patients, whilst other contributors seem to play a minor role.

4.3. Bullous pemphigoid (BP) and idiopathic membranous glomerulonephritis (iMGN)

In other autoimmune/immune-mediated diseases, such as bullous pemphigoid (BP) and idiopathic membranous glomerulonephritis (iMGN), the pathogenic role of IgG4 remains less clear [60,61,62]. Notably, early stages of both BP and iMGN are dominated by IgG1 autoantibodies, whereas IgG4 autoantibodies dominate later disease stages. Moreover, complement activation is present at the site of disease suggesting that complement activating antibodies such as IgG1 may play a significant role in these diseases alongside IgG4 [60,61,62].

5. IgG4 in helminthic infections

IgG4 deficient individuals are a rarity and have been described to be more prone to infections, especially (bacterial) infections of the respiratory tract, urinary tract and skin [63,64].

In helminthic infections, IgG4 is mostly upregulated – as in IgG4-RD – and a strong correlation has been demonstrated for elevated IgG4 serum levels and the presence of helminthic infections [65]. Generally, three types of immune responses have been described after exposure to helminths [65]: individuals who presented with a clinically active disease showed an uncontrolled T helper type 1 (Th₁) cell response, which was associated with relatively high IgE serum levels. A subset of

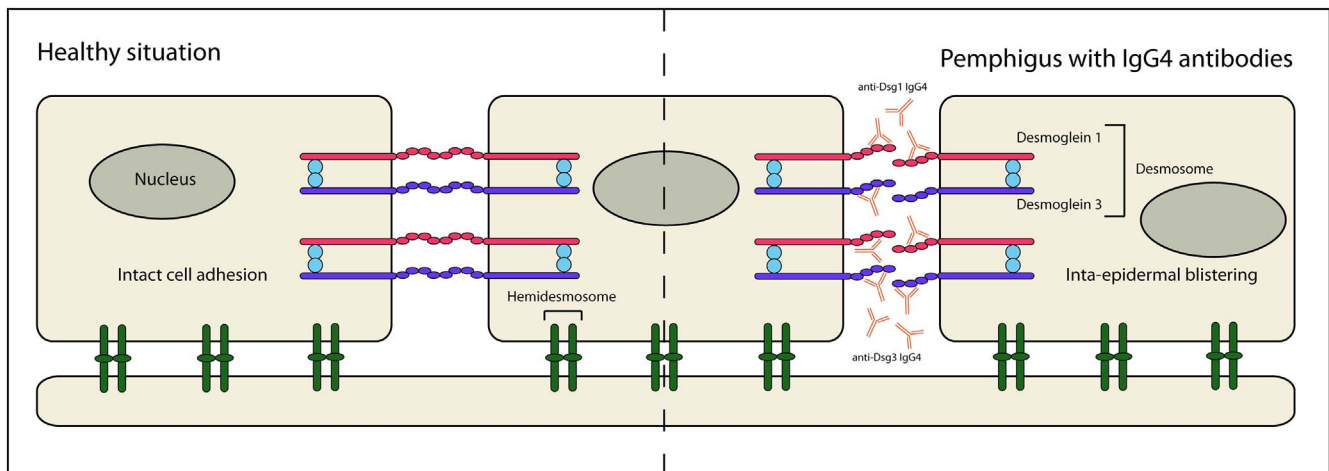


Fig. 3. Schematic model for the pathogenesis of pemphigus. Anti-Dsg1 IgG4 and anti-Dsg3 IgG4 antibodies disrupt binding of the intra-epidermal cell adhesion molecules Desmoglein 1 and Desmoglein 3 respectively causing intra-epidermal blistering.

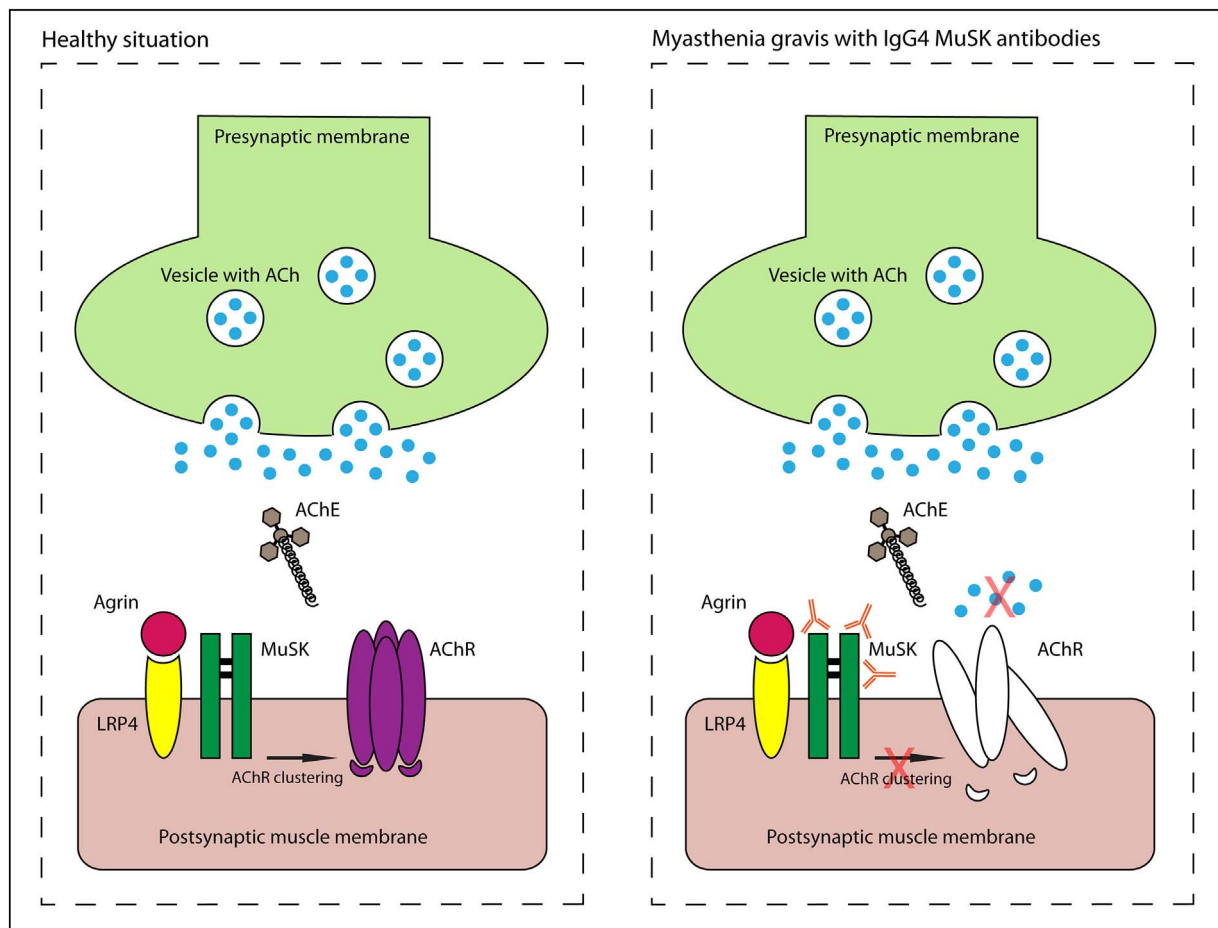


Fig. 4. Schematic model for the pathogenesis of MuSK-MG. Anti-MuSK IgG4 antibodies disrupt MuSK leading to the loss of acetylcholine receptor clustering and subsequent transmission of action potentials. AChE, acetylcholinesterase; AChR, acetylcholine receptor; LRP4, low density lipoprotein receptor-related protein 4; MuSK, Muscle-specific kinase.

people remained free of symptoms and showed a well-balanced Th₁/Th₂ cell immune response. Notably, a modified Th₂ cell response was seen in patients suffering from chronic helminthic infections, which was characterized by high serum levels of IL-10 and IgG4. As IgG4 is “key player” in the modified Th₂ cell response, we will focus here on this immune response.

In various helminthic infections, such as schistosomiasis, filariasis and intestinal nematode infections, a protective immune response is

induced, which includes both innate and adaptive immunity [66]. This response is often referred to as a Th₂-type response, which is characterized by upregulation of IL-4 and other Th₂-type cytokines, expansion of CD4⁺ Th₂ cells, plasma cells secreting parasite-specific IgE, eosinophils, mast cells and basophils. However, parasitic helminths attempt to evade the host immune response and create a regulatory environment for long-term survival [67]. Through secretion of different immune modulators into the system of their host, helminths are able to

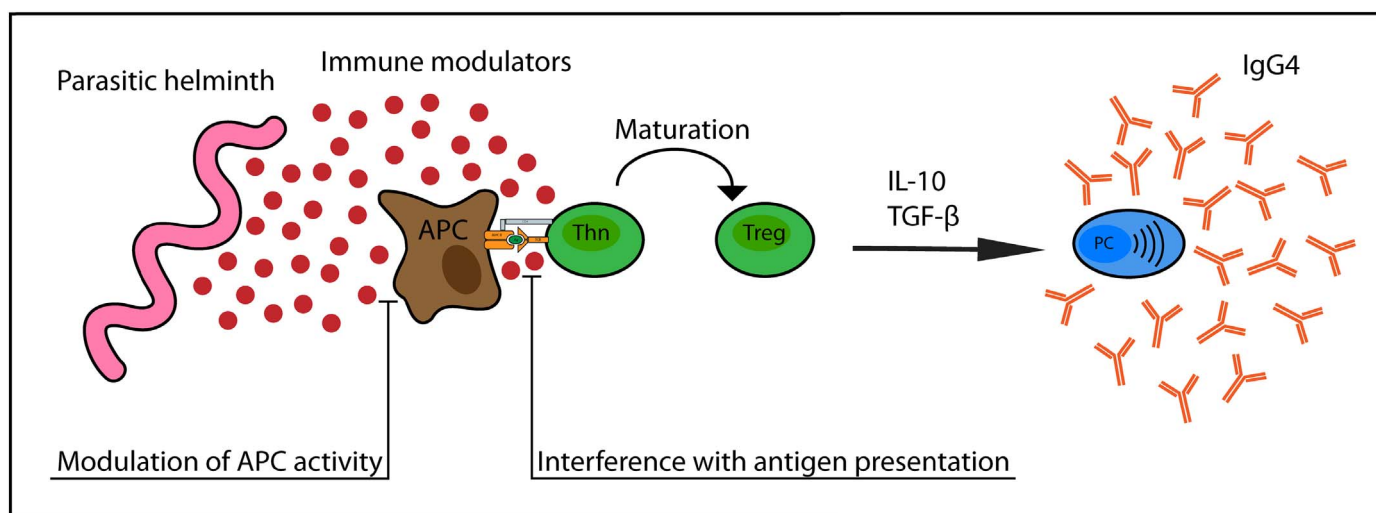


Fig. 5. Helminths modulate the host immune response to establish an immune-tolerant environment. Through secretion of immune modulators into the system of the host, which interfere at different points in the adaptive immune response, helminths are capable of initiating a modified Th₂ cell response ultimately leading to an immune-tolerant environment suitable for long term survival. APC, antigen presenting cell; Thn, naïve T helper cell; Treg, T regulatory cell; PC plasma cell.

shape the immune response (Fig. 5). For instance, helminths may directly or indirectly alter the activity of antigen-presenting cells (APCs) or interfere with antigen presentation between the APC and the T cell. This way T cell polarization is skewed toward Tregs, which secrete IL-10 and TGF- β , while parasite-specific effector T cell populations are kept in a repressed unresponsive state (T cell hypo-responsiveness), also known as a modified Th₂-response. IL-10 and TGF- β among other cytokines have been discussed to be responsible for the antibody class-switching towards IgG4. The predominance of IgG4 supports an immune-tolerant environment for helminths by counteracting helminth-specific IgE responses. Eventually, this may result in a long-term symbiotic relationship between the helminth and the host, causing an asymptomatic carrier state or subclinical chronic infection. Notably, in human filariasis the IgG4 levels were highest in individuals with an asymptomatic chronic infection, whereas symptomatic disease was associated with elevations in IgG1-3, suggesting that helminth-specific IgG4 in helminth-infected patients reflect a regulatory environment [68]. Moreover, IgG4 levels in filariasis showed a rapid decrease upon successful treatment [69,70,71]. Evidence has shown that in the presence of helminthic infections CD4 + Th₂ cells differentiate into CD4 + T follicular helper (Tfh) cells, which are then responsible for the production of IL-4 and IL-21 [72,73]. For example, in active and transitional human cystic echinococcosis (CE) infections, the percentage of circulating Tfh cells and subsequent IL-4, IL21, IgG1 and IgG4 serum levels are significantly increased, whereas in inactive human CE IgG1 and IgG4 levels are decreased [74].

6. IgG4 in malignancies

The involvement of the immune system in the formation, maintenance and progression of malignancies is a topic of growing importance and is particularly under attention in the oncological field regarding novel immunotherapies in cancer treatment. Increasing evidence suggests that IgG4 plays an important role in the pathophysiology of melanoma and possibly also in that of other malignancies such as cholangiocarcinoma.

In melanoma patients tumour-specific IgG4 serum levels are upregulated. Melanoma cells initiate IgG4 production through a modified Th₂ cell-based inflammatory response characterized by the production of IL-4 and IL-10 (Fig. 6) [75]. IgG4, which is a weak activator of macrophages, is incapable of initiating tumour cell death, whilst IgG1, which is a potent activator of macrophages, is capable of initiating tumour cell death. When endogenous IgG1 and IgG4 are both present

throughout the tumour mass, IgG4 is capable of blocking the anti-tumour activity of melanoma-specific IgG1 through competitive binding [76]. Moreover, IgG4 is capable of binding IgG1 via Fc-Fc interactions [77]. Thus IgG4 antibodies, induced by malignant melanocytes, support regulatory mechanisms through which tumours evade a destructive immune response [75]. Of note, in patients with melanoma, tumour-specific IgG4 serum levels negatively correlated with survival rate [78], indicating that IgG4 is a negative prognostic factor especially in early stage melanoma.

Similarly to melanoma, extrahepatic cholangiocarcinoma is often accompanied by the infiltration of IgG4⁺ plasma cells [80]. Intriguingly, cholangiocarcinoma cells were shown to induce an IgG4 response by mimicking the function of APCs or Tregs and inducing IL-10 production. Possibly, the elicited IgG4 response in cholangiocarcinoma may serve a purpose comparable to that in melanoma.

7. Perspectives for the role of IgG4 in IgG4-RD

To date, extensive research has been done on the biochemical and structural characteristics of IgG4. The exact role of IgG4 in IgG4-RD, however, still is unknown: are IgG4 antibodies pathogenic, do they fulfill a protective role, or are they a marker and ‘innocent bystander’ of an aberrant inflammatory response in IgG4-RD? Our short literature review (see above) on the role of IgG4 in various other immune processes teaches us that IgG4 antibodies are capable of regulating immune responses, which can be either beneficial or disadvantageous for the host depending on the context. IgG4 is susceptible to manipulation by other interactants in the immune system, making it a ‘promiscuous’ antibody. In certain situations IgG4 antibodies can be directly pathogenic. The role of IgG4 is summarized in Fig. 7 for different immune processes and associated conditions discussed above.

IgG4 fulfills a protective role in beekeepers and animal laboratory workers by preventing hypersensitivity reactions. This principle is nowadays exploited in allergic individuals undergoing allergen-specific immunotherapy to achieve desensitization. Oppositely, IgG4 has been proven to be directly pathogenic in autoimmune/immune-mediated diseases, such as pemphigus and MuSK-MG. Finally IgG4 production is triggered in the presence of parasitic infections or specific malignancies, such as melanoma and cholangiocarcinoma, by helminths or tumour cells respectively. These findings show the versatility of IgG4 making it a highly interesting and relevant antibody.

Disease-specific autoantibodies of the IgG4 subtype had not been identified until most recently in IgG4-RD. Furthermore, affected tissues

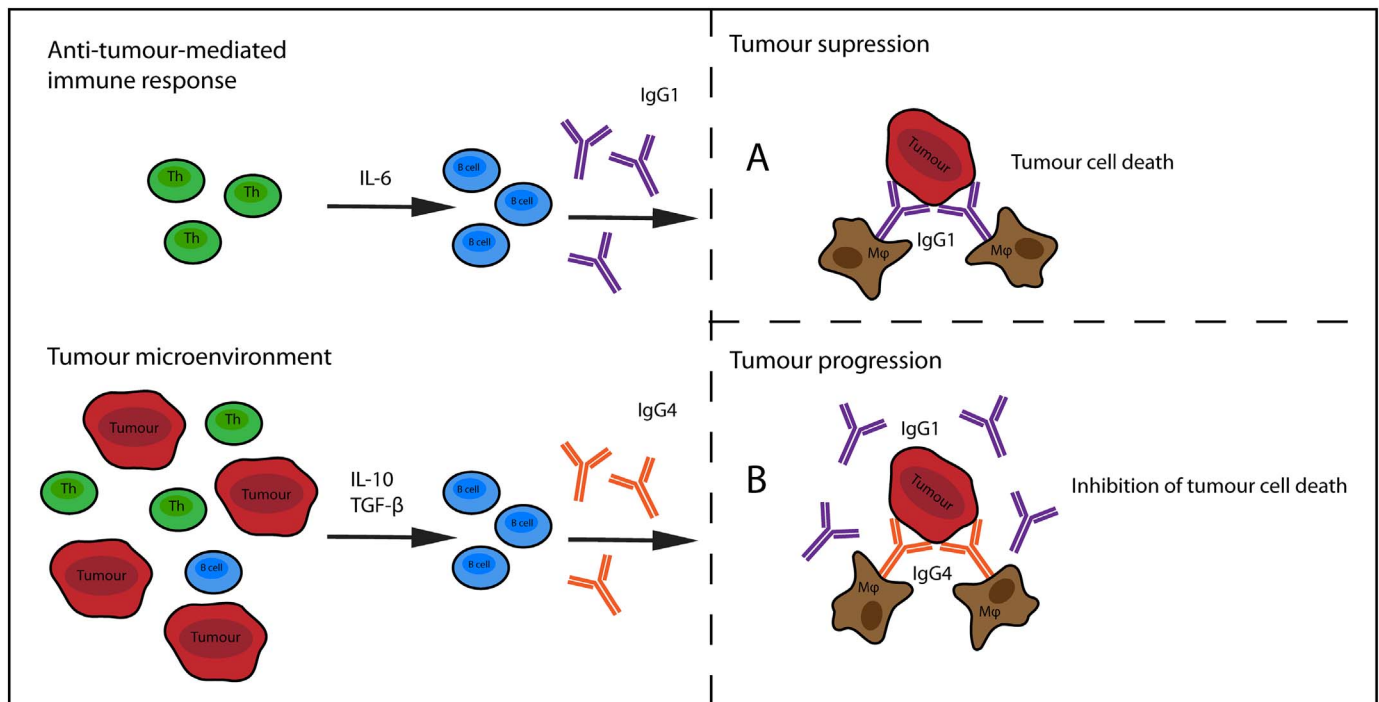


Fig. 6. Mechanism by which IgG4 inhibits the antitumour actions of IgG1. IgG4 inhibits tumouricidal IgG1 (A) through competition (B) preventing tumor cell death [76,79].

in IgG4-RD typically show a dense inflammatory infiltrate with all sorts of immune cells, including large quantities of T and B cells. This is in contrast to conditions where IgG4 antibodies seem to play a direct pathogenic role, such as in pemphigus and MuSK-MG. Taken together, these findings could indicate that IgG4 is upregulated to dampen a chronic immune response in IgG4-RD, but it does not exclude that IgG4 antibodies could in that context be pathogenic themselves. In a recent study, BALB/c mice were subjected to injections with IgG of patients with AIP, similarly to passive transfer studies in pemphigus vulgaris, which resulted in both pancreatic and salivary tissue injury, while injections with control IgG did not. In particular, injection with IgG1 alone caused damage, which could be partly abrogated by simultaneous injection with IgG4 [81]. Of note, in this study IgG4 antibodies themselves were not completely harmless.

Previously, we showed that the IgG4-producing B and plasma cells in patients with IgG4-RD of the biliary tract and pancreas are derived

from a limited number of clonally expanded B cell receptor clones [6,7]. This suggests that specific antigenic stimuli are involved in the immune process, and that elevated IgG4 serum levels are not just an ‘innocent bystander’ effect. In this context, our observation may be of interest that ‘blue collar work’ with long-term exposure to solvents, oil products or industrial gases was found in the history of > 70% of patients from two independent European cohorts with IgG4-RD of the biliary tract and pancreas [6,82]. Notably, although only a low titer of anti-nuclear antibodies (ANA) is detectable in IgG4-RD patients, recent work has shown that IgG4-RD patients, who are ANA-positive, do not have ANA of the IgG4 subclass but do have high serum IgG4 levels [83].

Identification of (auto-)antigens that are responsible for the clonal expansion of IgG4 + B cells in IgG4-RD may help to better understand the role of IgG4 in IgG4-RD. We just succeeded to unravel the first IgG4 auto-antigen in IgG4-RD being the calcium-dependent phospholipid-binding protein, Annexin A11, a 56 kDa protein against which IgG4 and

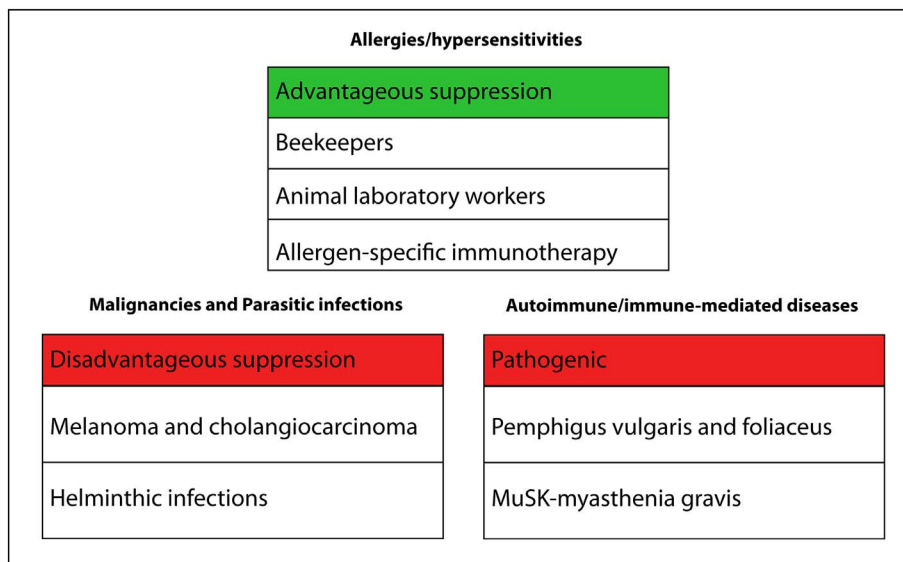


Fig. 7. The role of IgG4 in different immune processes and diseases.

IgG1 antibodies were directed in patients with IgG4-RD of bile ducts, pancreas and salivary glands, but not in disease controls [84]. Remarkably, epitope analysis disclosed that two Annexin A11 epitopes targeted by IgG1 and IgG4 autoantibodies of our patients were shared whereby IgG4 antibodies blocked binding of IgG1 antibodies to the shared Annexin A11 epitopes [84]. Identification of more IgG4 autoantigens will follow. Furthermore, IgG4 is merely a single component of the inflammatory process of IgG4-RD.

Future research should focus on the various players, such as T cell subsets [85], that all together orchestrate the inflammatory response in IgG4-RD. Especially T follicular helper (Tfh) cells have been recently investigated revealing that the number of T follicular helper type 2 (Tfh2) cells is significantly higher in IgG4-RD patients compared to the control groups and also correlates with serum levels of IgG4, IL-4 and plasmablast counts [86]. *In vitro* experiments showed that Tfh2 cells were able to induce the differentiation of naïve B cells into plasmablasts [87]. Moreover, IgG4 production in culture supernatants of Tfh2 cells was significantly higher with Tfh2 cells from IgG4-RD patients compared to healthy controls. Also T follicular helper type 1 (Tfh1) cells are elevated in IgG4-RD patients and seem to correlate with disease activity but not with serum IgG4 levels, suggesting a prominent role for Tfh2 cells in the production process of IgG4 and a yet to be established role for Tfh1 cells in IgG4-RD.

In summary, the role of IgG4 in inflammatory conditions may vary: IgG4 alone can cause beneficial and pathogenic effects. In IgG4-RD, we favour the view that IgG4 rather functions as a protective antibody dampening the more harmful effects of IgG1 when directed against the same epitopes.

Transparency document

The [Transparency document](#) associated with this article can be found, in online version.

Acknowledgement

This work was supported by German (Deutsche Crohn und Colitis Vereinigung, Sektion PSC), American (PSC Partners) and South African PSC Patient Foundations (to UB).

References

- [1] T. Kamisawa, Y. Zen, S. Pillai, J.H. Stone, IgG4-related disease, *Lancet* 385 (2015) 1460–1471.
- [2] L.M. Hubers, et al., IgG4-associated cholangitis: a comprehensive review, *Clin Rev Allergy Immunol* 48 (2015) 198–206.
- [3] V. Deshpande, et al., Consensus statement on the pathology of IgG4-related disease, *Mod. Pathol.* 25 (2012) 1181–1192.
- [4] A.M. Oseini, et al., Utility of serum immunoglobulin G4 in distinguishing immunoglobulin G4-associated cholangitis from cholangiocarcinoma, *Hepatology* 54 (2011) 940–948.
- [5] E. Kalaitzakis, et al., Endoscopic retrograde cholangiography does not reliably distinguish IgG4-associated cholangitis from primary sclerosing cholangitis or cholangiocarcinoma, *Clin. Gastroenterol. Hepatol.* 9 (2011) 800–803.
- [6] M.E. Doorenspleet, et al., Immunoglobulin G4 + B-cell receptor clones distinguish immunoglobulin G 4-related disease from primary sclerosing cholangitis and biliary/pancreatic malignancies, *Hepatology* 64 (2016) 501–507.
- [7] Maillette de Buy Wenniger, L. J., et al., Immunoglobulin G4 + clones identified by next-generation sequencing dominate the B cell receptor repertoire in immunoglobulin G4 associated cholangitis, *Hepatology* 57 (2013) 2390–2398.
- [8] S.T. Chari, Diagnosis of autoimmune pancreatitis using its five cardinal features: introducing the Mayo Clinic's HISORt criteria, *J. Gastroenterol.* 42 (2007) 39–41.
- [9] A. Ghazale, et al., Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy, *Gastroenterology* 134 (2008) 706–715.
- [10] J.H. Tabibian, K.D. Lindor, Distinguishing immunoglobulin G4-related disease from its pancreatobiliary mimics: are we there now? *Hepatology* 64 (2016) 340–343.
- [11] J. Buijs, et al., Comparable efficacy of low- versus high-dose induction corticosteroid treatment in autoimmune, *Pancreatitis* 43 (2014) 261–267.
- [12] A. Masamune, et al., Randomised controlled trial of long-term maintenance corticosteroid therapy in patients with autoimmune pancreatitis, *Gut* 66 (2017) 487–494.
- [13] K. Okazaki, et al., International consensus for the treatment of autoimmune pancreatitis, *Pancreatology* 17 (2017) 1–6.
- [14] K. Kawaguchi, et al., Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas, *Hum. Pathol.* 22 (1991) 387–395.
- [15] H. Hamano, et al., High serum IgG4 concentrations in patients with sclerosing pancreatitis, *N. Engl. J. Med.* 344 (2001) 732–738.
- [16] T. Tabata, et al., Serum IgG4 concentrations and IgG4-related sclerosing disease, *Clin. Chim. Acta* 408 (2009) 25–28.
- [17] S.T. Chari, et al., A diagnostic strategy to distinguish autoimmune pancreatitis from pancreatic cancer, *Clin. Gastroenterol. Hepatol.* 7 (2009) 1097–1103.
- [18] T. Umemura, et al., Immunoglobulin G4-hepatopathy: association of immunoglobulin G4-bearing plasma cells in liver with autoimmune pancreatitis, *Hepatology* 46 (2007) 463–471.
- [19] L. Maillette De Buy Wenniger, E.A. Rauws, U. Beuers, What an endoscopist should know about immunoglobulin-G4-associated disease of the pancreas and biliary tree, *Endoscopy* 44 (2012) 66–73.
- [20] T. Nakazawa, et al., Clinical differences between primary sclerosing cholangitis and sclerosing cholangitis with autoimmune pancreatitis, *Pancreas* 30 (2005) 20–25.
- [21] Y.A. Alderlieste, B.D.J. Van Den Elzen, E.A.J. Rauws, U. Beuers, Immunoglobulin G4-associated cholangitis: one variant of immunoglobulin G4-related systemic disease, *Digestion* 79 (2009) 220–228.
- [22] T. Nakazawa, H. Ohara, H. Sano, T. Ando, T. Joh, Schematic classification of sclerosing cholangitis with autoimmune pancreatitis by cholangiography, *Pancreas* 32 (2006) 229.
- [23] T. Nakazawa, et al., Diagnostic procedures for IgG4-related sclerosing cholangitis, *J. Hepatobiliary Pancreat. Sci.* 18 (2011) 127–136.
- [24] T. Smyrk, Pathological features of IgG4-related sclerosing disease, *Curr. Opin. Rheumatol.* 23 (2011) 74–79.
- [25] V. Deshpande, et al., IgG4-associated cholangitis: a comparative histological and immunophenotypic study with primary sclerosing cholangitis on liver biopsy material, *Mod. Pathol.* 22 (2009) 1287–1295.
- [26] H. Kawakami, et al., IgG4-related sclerosing cholangitis and autoimmune pancreatitis: Histological assessment of biopsies from Vater's ampulla and the bile duct, *J. Gastroenterol. Hepatol.* 25 (2010) 1648–1655.
- [27] E. Björnsson, S.T. Chari, T.C. Smyrk, K. Lindor, Immunoglobulin G4 associated cholangitis: description of an emerging clinical entity based on review of the literature, *Hepatology* 45 (2007) 1547–1554.
- [28] J. Schuurman, G.J. Perdok, A.D. Gorter, R.C. Aalberse, The inter-heavy chain disulfide bonds of IgG4 are in equilibrium with intra-chain disulfide bonds, *Mol. Immunol.* 38 (2001) 1–8.
- [29] S. Angal, et al., A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody, *Mol. Immunol.* 30 (1993) 105–108.
- [30] A.F. Labrijn, et al., Species-specific determinants in the IgG CH3 domain enable Fab-arm exchange by affecting the noncovalent CH3-CH3 interaction strength, *J. Immunol.* 187 (2011) 3238–3246.
- [31] M. van der Neut Kolfschoten, et al., Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange, *Science* 317 (2007) 1554–1557.
- [32] M.H. Tao, R.I. Smith, S.L. Morrison, Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation, *J. Exp. Med.* 178 (1993) 661–667.
- [33] E.-M. Varga, et al., Tolerant beekeepers display venom-specific functional IgG4 antibodies in the absence of specific IgE, *J. Allergy Clin. Immunol.* 131 (2013) 1419–1421.
- [34] J.C. Garcia-Robaina, F. de la Torre-Morin, C. Vazquez-Moncholi, J. Fierro, C. Bonnet-Moreno, The natural history of Apis-specific IgG and IgG4 in beekeepers, *Clin. Exp. Allergy* 27 (1997) 418–423.
- [35] W. van de Veen, et al., IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses, *J. Allergy Clin. Immunol.* 131 (2013) 1204–1212.
- [36] F. Meiler, et al., In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure, *J. Exp. Med.* 205 (2008) 2887–2898.
- [37] S.M. Coomes, et al., CD4 + Th2 cells are directly regulated by IL-10 during allergic airway inflammation, *Mucosal Immunol.* 10 (2017) 150–161.
- [38] F. Meiler, S. Klunker, M. Zimmermann, C.A. Akdis, M. Akdis, Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors, *Allergy* 63 (2008) 1455–1463.
- [39] P. Jeannin, S. Lecoanet, Y. Delneste, J.F. Gauchat, J.Y. Bonnefoy, IgE versus IgG4 production can be differentially regulated by IL-10, *J. Immunol.* 160 (1998) 3555–3561.
- [40] T. Boonpiyathad, et al., High-dose bee venom exposure induces similar tolerogenic B-cell responses in allergic patients and healthy beekeepers, *Allergy Eur. J. Allergy Clin. Immunol.* 72 (2017) 407–415.
- [41] E.C. Matsui, et al., Mouse allergen-specific immunoglobulin G and immunoglobulin G4 and allergic symptoms in immunoglobulin E-sensitized laboratory animal workers, *Clin. Exp. Allergy* 35 (2005) 1347–1353.
- [42] M. Jones, et al., Rat-specific IgG and IgG₄ antibodies associated with inhibition of IgE-allergen complex binding in laboratory animal workers, *Occup. Environ. Med.* 71 (2014) 619–623.
- [43] M. Amagai, V. Klaus-Kovtun, J.R. Stanley, Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion, *Cell* 67 (1991) 869–877.
- [44] B. Rock, et al., The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem), *N. Engl. J. Med.* 320 (1989) 1463–1469.
- [45] B. Rock, R.S. Labib, L.A. Diaz, Monovalent Fab' immunoglobulin fragments from endemic pemphigus foliaceus autoantibodies reproduce the human disease in neonatal Balb/c mice, *J. Clin. Invest.* 85 (1990) 296–299.
- [46] G.J. Anhalt, R.S. Labib, J.J. Voorhees, T.F. Beals, L.A. Diaz, Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease, *N. Engl.*

- J. Med. 306 (1982) 1189–1196.
- [47] X. Ding, L.A. Diaz, J.A. Fairley, G.J. Giudice, Z. Liu, The anti-desmoglein 1 auto-antibodies in pemphigus vulgaris sera are pathogenic, *J. Invest. Dermatol.* 112 (1999) 739–743.
 - [48] J. Yamagami, et al., Homologous regions of autoantibody heavy chain complementarity-determining region 3 (H-CDR3) in patients with pemphigus cause pathogenicity, *J. Clin. Invest.* 120 (2010) 4111–4117.
 - [49] K. Bhol, et al., Correlation of peptide specificity and IgG subclass with pathogenic and nonpathogenic autoantibodies in pemphigus vulgaris: a model for auto-immunity, *Proc. Natl. Acad. Sci. U. S. A.* 92 (1995) 5239–5243.
 - [50] D. Kricheli, et al., The distribution of pemphigus vulgaris-IgG subclasses and their reactivity with desmoglein 3 and 1 in pemphigus patients and their first-degree relatives, *Br. J. Dermatol.* 143 (2000) 337–342.
 - [51] E.H. Niks, et al., Clinical fluctuations in MuSK myasthenia gravis are related to antigen-specific IgG4 instead of IgG1, *J. Neuroimmunol.* 195 (2008) 151–156.
 - [52] R. Klooster, et al., Muscle-specific kinase myasthenia gravis IgG4 autoantibodies cause severe neuromuscular junction dysfunction in mice, *Brain* 135 (2012) 1081–1101.
 - [53] M.G. Huijbers, et al., MuSK IgG4 autoantibodies cause myasthenia gravis by inhibiting binding between MuSK and Lrp4, *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013) 20783–20788.
 - [54] I. Koneczny, J. Cossins, P. Waters, D. Beeson, A. Vincent, MuSK myasthenia gravis IgG4 disrupts the interaction of LRP4 with MuSK but both IgG4 and IgG1-3 can disperse preformed agrin-independent AChR clusters, *PLoS One* 8 (2013) 1–16.
 - [55] S. Mori, K. Shigemoto, Mechanisms associated with the pathogenicity of antibodies against muscle-specific kinase in myasthenia gravis, *Autoimmun. Rev.* 12 (2013) 912–917.
 - [56] A.M. Gomez, et al., Antibody effector mechanisms in myasthenia gravis-pathogenesis at the neuromuscular junction, *Autoimmunity* 43 (2010) 353–370.
 - [57] Y. Kawakami, et al., Anti-MuSK autoantibodies block binding of collagen Q to MuSK, *Neurology* 77 (2011) 1819–1826.
 - [58] K. Otsuka, et al., Collagen Q and anti-MuSK autoantibody competitively suppress agrin/LRP4/MuSK signaling, *Sci Rep* 5 (2015) 13928.
 - [59] I. Koneczny, et al., IgG4 autoantibodies against muscle-specific kinase undergo Fab-arm exchange in myasthenia gravis patients, *J. Autoimmun.* 77 (2017) 104–115.
 - [60] S. Mihai, et al., IgG4 autoantibodies induce dermal-epidermal separation, *J. Cell. Mol. Med.* 11 (2007) 1117–1128.
 - [61] P.M. Lamb, T. Patton, J.-S. Deng, The predominance of IgG4 in prodromal bullous pemphigoid, *Int. J. Dermatol.* 47 (2008) 150–153.
 - [62] E.J. Filippone, Idiopathic membranous nephropathy and IgG4: an interesting relationship, *Clin. Nephrol.* 82 (2014) 7–15.
 - [63] R.B. Moss, M.A. Carmack, S. Esrig, Deficiency of IgG4 in children: association of isolated IgG4 deficiency with recurrent respiratory tract infection, *J. Pediatr.* 120 (1992) 16–21.
 - [64] J.A. Freeman, et al., Immunoglobulin G subclass deficiency and infection risk in 150 patients with chronic lymphocytic leukemia, *Leuk. Lymphoma* 54 (2013) 99–104.
 - [65] R. Muller, *Worms and Human Disease*, CABI publishing, 2002.
 - [66] R.M. Anthony, L.I. Rutitzky, J.F. Urban, M.J. Stadecker, W.C. Gause, Protective immune mechanisms in helminth infection, *Nat. Rev. Immunol.* 7 (2007) 975–987.
 - [67] H.J. McSorley, R.M. Maizels, Helminth infections and host immune regulation, *Clin. Microbiol. Rev.* 25 (2012) 585–608.
 - [68] P. McGuirk, C. McCann, K.H.G. Mills, Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by *Bordetella pertussis*, *J. Exp. Med.* 195 (2002) 221–231.
 - [69] D. Van der Kleij, et al., A novel host-parasite lipid cross-talk. Schistosomal lyso-phosphatidylserine activates toll-like receptor 2 and affects immune polarization, *J. Biol. Chem.* 277 (2002) 48122–48129.
 - [70] A. Kurniawan, et al., Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis, *J. Immunol.* 150 (1993) 3941–3950.
 - [71] A. Atmadja, et al., Differential decline in filaria-specific IgG1, IgG4, and IgE antibodies in *Brugia malayi*-infected patients after diethylcarbamazine chemotherapy, *J. Infect Dis* 172 (1995) 1567–1572.
 - [72] I.L. King, M. Mohrs, IL-4-producing CD4⁺ T cells in reactive lymph nodes during helminth infection are T follicular helper cells, *J. Exp. Med.* 206 (2009) 1001–1007.
 - [73] A.G. Zaretsky, et al., T follicular helper cells differentiate from Th2 cells in response to helminth antigens, *J. Exp. Med.* 206 (2009) 991–999.
 - [74] F. Zhang, et al., CCR7^{hi}PD-1^{hi} CXCR5⁺ CD4⁺ T cells are positively correlated with levels of IL-21 in active and transitional cystic echinococcosis patients, *BMC Infect. Dis.* 15 (2015) 457.
 - [75] P. Karagiannis, et al., IgG4 subclass antibodies impair antitumor immunity in melanoma, *J. Clin. Invest.* 123 (2013) 1457–1474.
 - [76] P. Karagiannis, A.E. Gilbert, F.O. Nestle, S.N. Karagiannis, IgG4 antibodies and cancer-associated inflammation: Insights into a novel mechanism of immune escape, *Oncoimmunology* 2 (2013) e24889.
 - [77] S. Crescioli, et al., IgG4 characteristics and functions in cancer immunity, *Curr Allergy Asthma Rep* 16 (2016) 7.
 - [78] P. Karagiannis, et al., Elevated IgG4 in patient circulation is associated with the risk of disease progression in melanoma, *Oncoimmunology* 4 (2015) e1032492.
 - [79] Y. Kawano, T. Noma, J. Yata, Regulation of human IgG subclass production by cytokines. IFN-gamma and IL-6 act antagonistically in the induction of human IgG1 but additively in the induction of IgG2, *J. Immunol.* 153 (1994) 4948–4958.
 - [80] K. Harada, et al., Significance of immunoglobulin G4 (IgG4)-positive cells in extrahepatic cholangiocarcinoma: molecular mechanism of IgG4 reaction in cancer tissue, *Hepatology* 56 (2012) 157–164.
 - [81] M. Shiokawa, et al., Pathogenicity of IgG in patients with IgG4-related disease, *Gut* 65 (2016) 1322–1332.
 - [82] Maillette de Buy Wenniger, L. J., L.E. Culver, U. Beuers, Exposure to occupational antigens might predispose to IgG4-related disease, *Hepatology* 60 (2014) 1453–1554.
 - [83] K. Kiyama, et al., Screening for IgG4-type anti-nuclear antibodies in IgG4-related disease, *BMC Musculoskelet. Disord.* 16 (2015) 129.
 - [84] L.M. Hubers, et al., Annexin A11 is targeted by IgG4 and IgG1 autoantibodies in IgG4-related disease, *Gut* (2017) PMID: 28765476 (in press).
 - [85] M. Akiyama, et al., Follicular helper T cells in the pathogenesis of IgG4-related disease, *Rheumatology (Oxford)* (2017).
 - [86] M. Akiyama, et al., Number of circulating follicular helper 2 T cells correlates with IgG4 and interleukin-4 levels and plasmablast numbers in IgG4-related disease, *Arthritis Rheumatol.* 67 (2015) 2476–2481.
 - [87] M. Akiyama, et al., Enhanced IgG4 production by follicular helper 2 T cells and the involvement of follicular helper 1 T cells in the pathogenesis of IgG4-related disease, *Arthritis Res. Ther.* 18 (2016) 167–177.