Immunoglobulin G4 and Related Diseases

Zev Sthoeger MD, Ilan Asher MD, Shira Rosenberg-Bezalel MD and Keren Mahlab-Guri MD

Clinical Immunology, Allergy and AIDS Center, Kaplan Medical Center, Rehovot, affiliated with Hadassah-Hebrew University Medical School, Jerusalem, Israel

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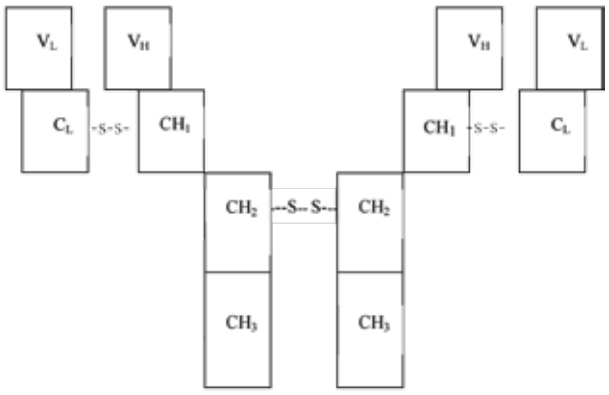
Immunoglobulins, produced by plasma cells, are the effector arm of the humoral immune system [1]. Each immunoglobulin consists of two identical heavy chains (α, β, γ, δ or ε – all encoded by chromosome 14) and two identical light chains (either κ or λ, encoded by chromosome 2 and 22, respectively) [2]. The immunoglobulins have constant (C) regions (similar within the same isotype) and variable (V) regions that define the binding specificity of the immunoglobulins [Figure 1]. There are nine immunoglobulin isotypes: IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD and IgE. All immunoglobulin isotypes are present in the serum of normal individuals [3]. There is a 90% homology between the four IgG isotypes, whereas between IgG and IgM isotypes the homology is about 30% [4]. There are polymorphic variants (single amino acid substitution in the constant region) of certain isotypes. These variants, inherited as a Mendelian trait, define different immunoglobulin allotypes [4]. Thus, since different individuals have different immunoglobulin allotypes (no one person has all allotypes), it can be used in paternity testing. There are four γ-1, two γ-2, thirteen γ-3, two α-2 and three κ allotypes [5]. The clinical significance of the different allotypes has not yet been defined. Some studies have suggested an association between different allotypes and immunoglobulin levels [5]. Idiotypes are the specific antigenic determinants on the hypervariable regions within the variable portion of the heavy and light chains (V_L, V_H) [2].

Figure 1. Schematic structure of immunoglobulin, consisting of two heavy (H) and two light (L) chains



Ig = immunoglobulin

IMMUNOGLOBULIN FUNCTION

Both the variable and the constant regions are important for immunoglobulin activity and function. The variable regions define the antigenic specificity of the immunoglobulins, whereas the constant region (mainly the CH_2 portion) determines the ability to bind complement and Fc receptors which present on various cell types such as monocytes, neutrophils, eosinophils, mast cells, macrophages, natural killer cells, dendritic cells and T cells [4]. The nature of Fc receptor binding affects the levels of phagocytosis, antigen clearance, cytokine release and immune activation generated by the immunoglobulins [4].

In sera of normal individuals there are four IgG isotypes (subclasses): IgG1, IgG2, IgG3 and IgG4 [6]. At age 2 years, serum levels of IgG1, IgG2 and IgG3 reach normal adult levels, whereas IgG4 levels rise gradually, reaching adult levels only at age 13 [6]. As shown in Table 1, IgG4 levels in the serum of normal individuals are quite low (60 mg/dl). IgG4 does not bind complement and therefore does not generate a significant inflammation [7]. IgG4 can bind to Fcγ receptor I (CD64) (though with a lower affinity than IgG1/IgG3), which presents on monocytes, macrophages and neutrophils, but not to the other Fcγ receptors (Fcγ-RII-CD32, Fcγ-RIII-CD16) [8]. The lack of complement and Fcγ receptor binding and the relatively low concentration of IgG4 in the serum suggest that the latter immunoglobulin may have regulatory rather than inflammatory or antigen clearance functions. IgG4 production, like IgE, is controlled by T helper 2 cells (interleukins 4 and 13) [9].

About ten years ago Aalberase et al. [10] reported that IgG4 is breaking the rules of immunoglobulin structure with an in vivo half molecular exchange (one heavy and one light chain) between two IgG4 molecules, generating a new IgG4 with a bivalent reactivity. The new bivalent IgG4 has less affinity to the antigen, suggesting that IgG4 may have an anti-inflammatory rather than antigen clearance effect [10]. Indeed, after immunotherapy in allergic patients, the increase in blocking (most probably bivalent) IgG4 antibodies was shown to correlate with the success of the treatment [11]. More recently, Van der Neut Kolfschoten and co-authors [12] generated two IgG4 murine monoclonal antibodies (one directed against Feld1 antigen and the other against Bet v1 antigen) and two IgG1 murine control monoclonal antibodies directed against the same antigens [12]. Following administration of the two IgG4 monoclonal antibodies to immunodeficient SCID mice, those antibodies (obtained from sera of the SCID mice) revealed bispecific (Feld1/Bet v1) reactivity, indicating an in vivo half molecule (Fab arm) exchange [Figure 2]. The exchange was observed in about 50% of the IgG4 monoclonal antibodies. Administration of the IgG1 control monoclonal antibodies did not result in a similar Fab arm exchange [12]. Furthermore, IgG4 Fab arm exchange was also demonstrated in vitro in the presence of reduced glutathione and with human IgG4 monoclonal antibodies as well [12]. The investigators of that study were able to define the specific domain (located in the IgG4 CH3 region) involved in the Fab arm exchange (mutagenesis experiments of that domain increase or decrease exchange ability) [12]. Moreover, human anti-acetyl choline receptor IgG4 monoclonal antibodies, which degrade AChR on rhabdomyosarcoma TE671 cells, failed to demonstrate such degradation following their administration to rhesus monkeys due to in vivo Fab arm exchange and the generation of bispecific IgG4 antibodies in the monkeys' sera. Those bispecific IgG4 antibodies were shown to block AChR degradation by IgG1 anti-AChR-specific monoclonal antibodies [12]. Taken together, the above experiments demonstrate that IgG4 has the unique ability to exchange Fab arms ("flip-flop" exchange of heavy + light chains) leading to the generation of blocking (protecting) IgG4 antibodies [Figure 2]. In contrast to the view that IgG4 is a protective but not inflammatory immunoglobulin, IgG4 has a central role in the pathogenesis of certain immune mediated disorders. Thus, anti-desmoglein-1 IgG4 antibodies are involved in the generation of cutaneous blisters in patients with pemphigus vulgaris [13,14]. In addition, it was suggested that IgG4 antibodies are involved in the pathogenesis of membranous glomerulonephritis and thrombotic thrombocytopenic purpura [15].

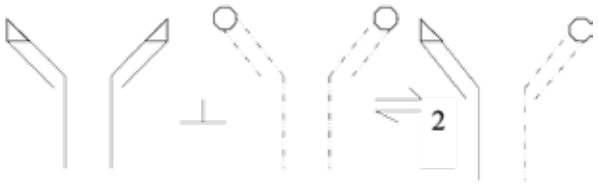
IgG4 is present in small amounts in healthy human serum (about 3% of total IgG levels) but does not fix complement and barely binds to Fcγ receptors

AChR = acetyl choline receptor

Table 1. Concentration and characterization of IgG isotypes

	Concentration (mg/dl)	% of total IgG	Complement fixation	Placental transfer	Main targets
IgG1	910	65	++	++	Proteins, virus
IgG2	510	25	+	+	Polysaccharides, bacteria
IgG3	210	7	+++	++	Proteins, virus
IgG4	60	3	-	++	Polysaccharides, parasites

Figure 2. Fab arm exchange of IgG4 molecules leading to generation of bispecific immunoglobulins



IgG4 DEFICIENCY

Low levels of IgG4 (< 60 mg/dl) are usually observed along with deficiency of other IgG subclasses [16]. Since normal IgG4 levels are quite low, the determination of IgG4 deficiency is a technical laboratory challenge and the clinical value of such determination is questionable [17]. Nevertheless, IgG4 deficiency was reported in patients with IgA deficiency, ataxia telangiectasia, human immunodeficiency virus and chronic mucocutaneous candidiasis (concomitantly with IgG2 deficiency) and in patients with Wiskott-Aldrich disease (concomitantly with IgG3 deficiency) [18-20].

IgG4-RELATED DISEASES

Recently, various clinical disorders were described characterized by high IgG4 (> 135 mg/dl) serum levels and IgG4-bearing plasma cells, dense infiltrates with fibrotic and sclerotic changes, and mild to moderate eosinophil infiltrates in affected organs [21,22]. In glandular organs those infiltrates aggregate around ductal structures. Granulomas and neutrophils are usually absent [21]. The main organs involved in these disorders are the lacrimal glands, salivary glands and the pancreas [22]. Involvement of many other organs has also been reported: namely lungs (pneumonitis, inflammatory pseudotumor), retroperitoneum, periorbital, bile ducts, kidneys, mediastinum, prostate, lymph nodes, thyroid, hypophysis, meninges,

aorta, pericardium, breast and skin [23-27]. The incidence of those disorders is not yet defined. IgG4-related diseases affect males more than females. In Japan an incidence of 2.6–10.6 per 10^6 was reported [28]. Spontaneous remission with regression of organ infiltrates rarely occurs. Nevertheless, in most cases, treatment with corticosteroids (prednisone 0.5–1 mg/kg) is necessary [29]. IgG4-related diseases are responsive to steroids. Thus, in patients who do not respond within 2 weeks to corticosteroid treatment, another diagnosis should be considered [29]. Despite the good response to steroids, disease flares are common [29] and some patients require prolonged treatment. Azathioprine, methotrexate and mycophenolate mofetil are used as steroid-sparing agents although their efficiency was not determined in clinical studies. A few studies have reported a good clinical response to retuximab treatment (accompanied by a decrease in IgG4 levels) [30].

IgG4 is unique in engaging in Fab-arm exchange between two unrelated IgG4 molecules and is therefore probably an immune regulatory protective rather than inflammatory antibody

Previously, these disorders were called IgG4 multiorgan lymphoproliferative syndrome (IgG4 MOSLP), IgG4 sclerosing disease, or IgG4-related systemic plasmocytic syndrome (SIPS) [31]. Currently the term IgG4-related diseases is accepted as the correct definition of those disorders [31].

IgG4-related diseases are defined by high serum IgG4 levels and IgG4-bearing plasma cell infiltrates with fibrosis/sclerosis involving mainly the parotid glands and the pancreas

Recently, Umehara et al. [32] defined the diagnostic criteria for the diagnosis of IgG4-related diseases. These included elevated serum IgG4 levels (> 135 mg/dl) and the presence of IgG4-bearing plasma cell infiltration (IgG4-expressing plasma cells > 40% of total IgG-expressing plasma cells) with tissue fibrosis and sclerosis [32]. More recently, Sah and colleagues [33] reported that 20–30% of patients with typical IgG4 histopathological infiltrates have normal serum IgG4 levels. Thus, normal (not high) levels of serum IgG4 do not exclude IgG4-related disease [34]. In most but not all patients, serum IgG4 levels correlate with disease activity and with the response to treatment [29,33]. In addition to high levels of IgG4, sera of those patients revealed eosinophilia and high IgE levels [35]. Interestingly, about 40% of the patients also have allergic diseases, mainly allergic rhinitis or asthma [35]. It should be noted that the organ infiltration in IgG4-related disease demonstrates high uptake (signal) on gallium scan and PET-CT (positron emission tomography-computed tomography) [36].

The two most common IgG4-related diseases are IgG4 Mikulicz disease and type 1 autoimmune pancreatitis [26]. IgG4 Mikulicz disease usually presents as a bilateral swelling of lacrimal, parotid and submandibular glands but without complaints or signs of dryness. A unilateral involvement was also reported [37]. About 50% of the patients are males (40–60 years old). Laboratory abnormalities include: high serum IgG4 and IgE levels, eosinophilia and the absence of anti-Ro/anti-La autoantibodies [37]. Low titers of antinuclear antibodies are rarely observed [38]. Biopsy of the involved gland, which is mandatory for diagnosis, demonstrates dense infiltration of IgG4-expressing lymphocytes and plasma cells with fibrosis and sclerosis [38]. The differential diagnosis should include Sjogren syndrome (mainly females with dryness, high serum levels of IgG1/IgG3 autoantibodies), sarcoidosis (high angiotensin-converting enzyme levels) and lymphoma, especially in patients with unilateral involvement [37]. IgG4 Mikulicz disease responds dramatically to steroid treatment, although some patients need long maintenance treatment with 5–10 mg/day of oral prednisone [38].

Type 1 autoimmune pancreatitis, also called lymphocytis sclerosing pancreatitis (LPSP), is a disease affecting elderly males [37]. It should be differentiated from other types of pancreatitis (e.g., biliary, alcoholic, drug induced), type 2 AIP (young adults with inflammatory bowel disease) [27] and pancreatic tumors. Determination of serum IgG4 levels (high in type 1 AIP) in such patients is essential for making the correct diagnosis. The characteristic IgG4-bearing plasma cell infiltration with fibrosis and sclerosis differentiates type 1 AIP from pancreatic tumors [27]. Upon diagnosis of type 1 AIP corticosteroid treatment should be initiated [29].

IgG4-related diseases can involve other organs (as mentioned above) [32]. A high level of suspicion and the determination of serum IgG4 levels are mandatory for diagnosis. Early diagnosis is beneficial, since the initiation of steroid treatment following the diagnosis of IgG4-related disease results in clinical improvement and obviates unnecessary investigations or treatments.

CONCLUSIONS

IgG4 is a unique bivalent blocking immunoglobulin. IgG4 does not fix complement and does not bind efficiently to Fcγ receptors, suggesting its role as an immunomodulator immunoglobulin. IgG4-related diseases can affect any organ but are most common in the pancreas, and lacrimal, submandibular and parotid glands. Patients with an IgG4-related disease demonstrate high levels of IgG4 in their serum and organ infiltration of IgG4-bearing plasma cells with fibrosis and sclerosis. IgG4-related diseases respond to corticosteroid treatment, with a decrease in IgG4 serum levels and tissue infiltrates.

AIP = autoimmune pancreatitis

Corresponding author:
Dr. Z. Sthoeger
Head, Dept. of Medicine B and Clinical Immunology, Allergy and AIDS Center, Kaplan Medical Center, Rehovot 76100, Israel
Phone: (972-8) 944-1403
Fax: (972-8) 944-1826
email: Zev_s@clalit.org.il, Sthoeger@gmail.com

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A likely impossibility is always preferable to an unconvincing possibility

Aristotle (384-322 BC), Greek philosopher whose writings cover physics, metaphysics, poetry, theater, music, logic, rhetoric, linguistics, politics, government, ethics, biology and zoology. Together with Plato and Socrates, Aristotle is one of the most important founding figures in Western philosophy