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Is it bad, is it good, or is IgG4 just misunderstood?

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Repeated doses of mRNA vaccines for COVID-19 result in increased proportions of anti-spike antibodies of the IgG4 subclass, which are known to neutralize well and to form mixed immune complexes with IgG1 but, in a pure form, might be less effective than IgG1 or IgG3 antibodies in facilitating opsonization by phagocytes, complement fixation, and NK cell–dependent elimination of infected cells (see related Research Article by Irrgang *et al*.).

In a recent paper published in *Science Immunology*, Irrgang *et al*. (*1*) showed that administering three doses of an mRNA vaccine encoding the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike antigen eventually results in an increased proportion of antigen-specific antibodies of the immunoglobulin G4 (IgG4) subtype, but IgG4 antibodies were not induced after an adenoviral vector vaccine. Similar results were observed in a separate, contemporaneous study (*2*). These results provoke a reconsideration of some fundamental immune mechanisms unique to the human immune system but also raise issues that may (or may not) have practical relevance in terms of vaccinology and public health.

Human IgG4 makes up about 3 to 6% of all human IgG and remains poorly understood from an evolutionary and functional standpoint (Fig. 1). A number of insights about IgG4 have been obtained over the past few decades, largely from the pioneering work of Aalberse and colleagues (*3*, *4*). We now know that structural differences in a loop in the CH2 (second constant heavy chain) domain of IgG4 (when compared with IgG1) impair its binding to C1q and to most activating Fc receptors. This has led to the view that IgG4 may have evolved to dampen inflammation and functions essentially as an antigen sink. In addition, a specific arginine (R409) in the CH3 domain of IgG4 (as compared with IgG1) impairs CH3:CH3 pairing; this impaired pairing combined with the lability of interchain disulfide bridges in the hinge region (because of a serine at position 228 replacing a proline found in IgG1) facilitates the dissociation of IgG4 into half-molecules made up of one heavy chain with the associated light chain. Pairing of IgG4 half-molecules with other half-molecules results in bispecific IgG4 antibodies. It is likely that this process, called "Fab arm exchange," occurs in vivo in endosomes during FcRn-mediated recycling in endothelial cells. IgG4 exists only in primates, but macaque IgG4 lacks the CH2 domain and hinge region changes seen in human IgG4, and, unlike human IgG4, this simian Ig subclass is functionally inflammatory and does not undergo Fab arm exchange (*5*). Human IgG4 molecules whose Fc regions have been artificially stabilized by site-directed mutagenesis have proven to be a useful noninflammatory choice of antibody framework when engineering "blocking" therapeutic antibodies, but this antibody subclass is obviously not suitable for therapeutic monoclonal antibodies that are intended for depletion of specific circulating cells in vivo, because both opsonization by phagocytes and antibody-dependent cellular cytotoxicity (ADCC) by natural killer (NK) cells are poorly facilitated by IgG4.

What generally drives IgG4 class switching? When individuals are exposed for an extended period of time to a protein or glycoprotein antigen (frequently in the absence of an ongoing infection), the humoral immune response eventually switches toward the IgG4 isotype. In a classic study, beekeepers were shown to initially make precipitating IgG1 antibodies specific for a bee venom antigen, phospholipase A2 (*6*). After about 6 months, these IgG1 antibodies began to be replaced by IgG4 antibodies until eventually nonprecipitating IgG4 antibodies dominated the serological response. IgG4 is also the dominant isotype after allergen desensitization, in hemophiliacs treated repeatedly with Factor IX, or after certain monoclonal antibody therapies, to cite but a few examples.

IgG4 class switching (like most class switching other than for high-affinity IgE) occurs extrafollicularly, but switched B cells of all IgG classes can also enter germinal centers and generate high-affinity antibody responses. A recently described $CD4^+$ T cell subset that expresses CXCR5, PD-1, ICOSL, interleukin-10 (IL-10), IL-4, LAG-3, and BATF may drive the IgG4 class switching event (*7*). One can presume that, in certain poorly understood milieus, prolonged activation of $CD4^+$ helper T cells by persistent antigens facilitates the differentiation of the appropriate IL-10–expressing extrafollicular helper T cell subset that drives IgG4 class switching, and this cell type likely collaborates with reactivated B cells that have frequently already switched to other IgG subtypes (*8*). An indolent, gradual, inflammatory (likely autoimmune) process driven largely by infiltrating T and B cells can generate a milieu for the far more exuberant expansion of the appropriate extrafollicular helper T cells that drive IgG4 switching, as seen in IgG4-related disease (IgG4-RD). This disease is likely not caused by IgG4, but the disease process creates the right milieu for IgG4 switching. Perhaps the anti-inflammatory activity of IgG4 helps attenuate, but fails to obliterate, the inflammatory process in IgG4-RD (*9*).

In retrospect, though it was not predicted, the propensity of mRNA vaccination to induce IgG4 antibodies may fit in with what is known about IgG4 induction in general. There is evidence for the continued expression of spike mRNA for many months after mRNA vaccination and for the presumed expression of spike protein and the persistence of germinal centers also for many months. In mRNA vaccines, the mRNA itself is modified to lack immunogenic modifications, and the lipid nanoparticles provide an adjuvant effect that likely declines even while the mRNA apparently

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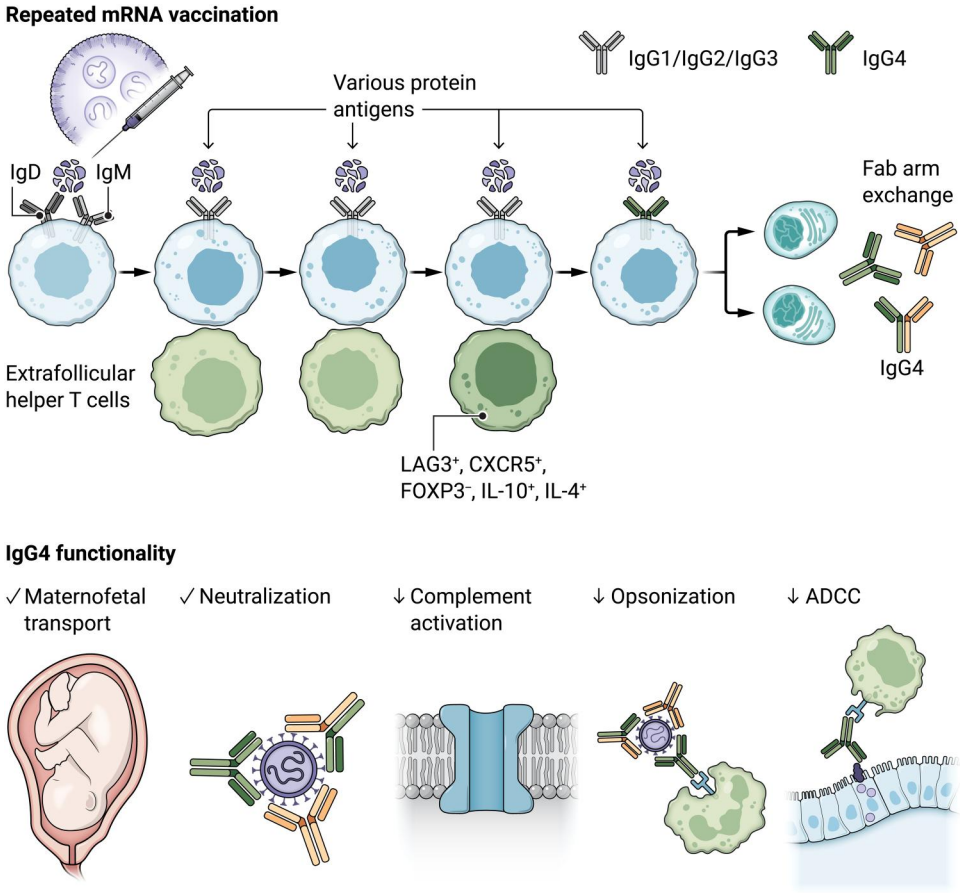

Fig. 1. IgG4 generation and functionality. The top panel depicts the induction of IgG4 by persistent antigen (or repeated doses of a protein or glycoprotein immunogen) and by extrafollicular T helper cells that secrete IL-10 and IL-4. The IgG4 molecule shown at the bottom right of the top panel has undergone "Fab arm exchange." The bottom panel depicts the functionality of human IgG4 relative to other human IgG subclasses. Although IgG4 is efficiently transported across the placenta and neutralizes with high efficiency, IgG4 antibodies do not bind well to activating Fc receptors and to C1q. As a result, they do not activate complement effectively, and opsonization and ADCC are compromised.

continues to be translated. Therefore, perhaps mRNA vaccination, with the passage of time, may facilitate the induction of IL-10–expressing extrafollicular helper T cells and IgG4 switching. Why exactly did a nonreplicating adenoviral vaccine not generate the same result? Most likely the antigen levels achieved so far with two adenoviral vaccine doses have been inadequate to reach a threshold for enough IgG4 switching and accumulation.

Extended expression of protein antigens in other vaccination regimens has also led to IgG4 expression, but why certain strategies create an IgG4 response and others do not is not that clear. The VAX003 HIV vaccine trial involved seven doses of a recombinant gp120 Env protein adjuvanted with alum. In the six-dose RV144 HIV vaccine trial, the first four doses used a canarypox-based vaccine expressing Env (along with Pol and Gag), whereas the last two doses included the gp120 Env recombinant protein vaccine used in the VAX003 trial. VAX003 generated IgG4 antibodies prominently, whereas RV-144 generated few IgG4 but more IgG3 antibodies. The IgG3 antibodies contributed to better Fc receptor–related functionality (*10*).

Evolution is best explained in the context of reproductive fitness at a species level, so how IgG4 evolved is not at all clear. It is difficult to square an evolutionary advantage potentially linked to the anti-inflammatory function of IgG4 with the absence of conservation of this function in other vertebrates, even in other primates. Currently, there is no convincing evidence to explain why bispecificity or "functional monovalency" of an antibody isotype could be advantageous from a host-defense perspective.

Nevertheless, in general, even if this IgG subtype evolved in humans "by accident," IgG4 antibodies in many contexts are clearly "good." They can neutralize effectively, they are transferred across the placenta, and they can contribute to dampening immunity. They can compete with IgE and prevent anaphylaxis. They may even form small immune complexes and can certainly be a part of mixed immune complexes with IgG1. Indeed, when the concentration of IgG4 is not particularly high, the likelihood that one antigen-specific IgG4 half-molecule will frequently meet other half-molecules of IgG4 directed against other antigens in endosomes and undergo Fab arm exchange with them is likely low. It is possible that when IgG4 levels rise because of persistent activation by a specific protein antigen, many bispecific IgG4 molecules

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might well bind to two different epitopes on the same target protein.

Other than their ability (at least in pure form) to compromise opsonization by both Fc receptors and complement receptors, is there anything else potentially problematic about IgG4 per se? Is functional monovalency something we should have concerns about? IgG4 autoantibodies against desmoglein 3 in pemphigus vulgaris and against MUSK (muscle-specific kinase) in the MUSK form of myasthenia gravis cause disease by binding to and functionally disrupting cellular function. In fact, "functional monovalency" of IgG4 has been suggested to be necessary for pathogenicity in the anti-MUSK form of myasthenia gravis, wherein IgG4 antibodies with a single Fab against MUSK functionally antagonize the enzyme and disrupt neuromuscular synapses, whereas bivalent antibodies can cross-link neighboring MUSK molecules and thus activate neuromuscular synapse formation (*11*). Functional monovalency of IgG4 anti-spike antibodies should not compromise the neutralization of SARS-CoV-2, but it could compromise immune complex formation if IgG4 were to dominate the immune response.

Innumerable large studies from across the world have established that mRNA and adenoviral coronavirus disease 2019 vaccines protect immunized participants in a major way from hospitalization and severe disease, but boosting is required to extend the duration of protection (*12*). Because SARS-CoV-2 has effectively generated numerous variants that have compromised vaccine-induced neutralization, vaccines probably provide protection today via $CD8^+$ T cell activation, ADCC involving antibodies and NK cells, and the attenuation of viral load by opsonization of the virus and clearance by phagocytes. However, neutralizing antibodies remain the most rigorous correlate of immune protection after vaccination, so the relative importance of non-neutralizing antibodies, opsonization, and ADCC remains uncertain. It should be kept in mind that repeated boosting with mRNA vaccines has been protective (*12*). Accurately deciphering the negative consequences, if any, of increased IgG4 levels will be difficult. IgG4 antibodies constitute a relatively small proportion of total anti-spike IgG after vaccination, will also likely be of higher affinity because they emerge late, and can form mixed immune complexes with IgG1; in practical terms, they are unlikely to compromise immunity in vaccinated patients at this time.

Nonetheless, on the basis of the results of the studies discussed here and other theoretical considerations, future clinical studies need to evaluate the effectiveness of temporal spreading out of mRNA vaccine boosts—possibly no more than once a year. Other approaches worth investigating would be the use of smaller quantities of mRNA for booster doses and, separately, the use of mRNA vaccines for priming only, with heterologous boosts with adjuvant-free recombinant spike proteins because, theoretically, adjuvants are most relevant during priming and may not be necessary for boosting. Hybrid immunity, as generated by breakthrough infections after vaccination, can also induce anti-spike IgG4 (*1*), so there is a need for ongoing evaluation and possibly tweaking of mRNA vaccination strategies going forward.

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