Do regulatory antibodies offer an alternative mechanism to explain the hygiene hypothesis?

David N.A. Mekhaiel¹, Claudio T. Daniel-Ribeiro², Philip J. Cooper¹ and Richard J. Pleass¹

¹Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK
²Center for Malaria Research and Training, Instituto Oswaldo Cruz, Flacru, Rio de Janeiro, Brazil

The ‘hygiene hypothesis’, or lack of microbial and parasite exposure during early life, is postulated as an explanation for the recent increase in autoimmune and allergic diseases in developed countries. The favored mechanism is that microbial and parasite-derived products interact directly with pathogen recognition receptors to subvert proinflammatory signaling via T regulatory cells, thereby inducing anti-inflammatory effects and control of autoimmune disease. Parasites, such as helminths, are considered to have a major role in the induction of immune regulatory mechanisms among children living in developing countries. Invoking Occam’s razor, we believe we can select an alternative mechanism to explain the hygiene hypothesis, based on antibody-mediated inhibition of immune responses that may more simply explain the available evidence.

The hygiene hypothesis

In medicine, the ‘hygiene hypothesis’ states that a lack of early exposures to infectious agents, symbiotic microorganisms and parasites increases susceptibility to allergic diseases by suppressing the development of the immune system [1]. The increase of autoimmune diseases and acute lymphoblastic leukemia in young people in the developed world has also been linked to the hygiene hypothesis [2]. Recent research on the molecular mechanisms of the hygiene hypothesis highlights the role of Toll-like receptor ligands in modulating allergic inflammation and the importance of parasite products in directing the development of the immune system of the host [3]. Genetic analysis, by contrast, clearly shows that the response threshold of the immune system to environmental stimuli is controlled by natural genetic variation and gene–environment interactions [1], suggesting that the complex interplay between the organism and the environment might not be regulated by a single mechanism.

Regulatory functions for antibody and immune complexes

The ‘antibody (Ab) theory’, as an alternative mechanism behind the hygiene hypothesis, stems from the observation that significant therapeutic benefit is obtained using

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**Glossary**

**Avidity**: the functional combining strength of an Ab with its receptor, which is related to both the affinity of the reaction with receptor and the valencies of the Abs in the immune complex.

**Bence Jones proteins**: immunoglobulin light chain dimers, normally produced by plasma cells. Bence Jones proteins are sufficiently small to be excreted by the kidney. It is a characteristic protein found in the urine of most patients with multiple myeloma.

**Dendritic cell (IC):** a specialized antigen presenting cell capable of displaying antigen for recognition by T cells and thereby activating them.

**Fab**: the portion of an Ab molecule that contains the antigen-combining site consisting of a light chain and part of the heavy chain.

**Fc**: the portion of an Ab that is responsible for binding to Fc receptors on immune cells and to the C1q component of complement; it is produced by enzymatic digestion.

**Fc receptors**: surface molecules on a variety of cells that bind the Fc region of Ab. They are Ab class specific and iso-type selective. For IgG, these FcRs can be both activating (e.g. FcγRI) or inhibitory (e.g. FcγRIIB). A loss of function variant of FcγRIIB is associated with increased susceptibility to systemic lupus erythematosus (SLE) [46]. It is uncommon in Caucasians but more common in Africa and Southeast Asia suggesting that decreased FcγRIIB function may provide a survival advantage against malaria [46]. This is the first example of an immune polymorphism predisposing to autoimmunity, selected and retained by virtue of its protective effect in malaria, and thus may also provide an alternative explanation for discrepancies in the hygiene hypothesis.

**Guillain-Barre syndrome**: an acute inflammatory demyelinating polyneuropathy affecting the peripheral nervous system. The disease is usually triggered by acute infection. It can be completely cured by prompt treatment with IVIG.

**Hypergammaglobulinemia**: in addition to the increase in specific antibodies, many parasite infections, including malaria, provoke the production of high titers of nonspecific antibody, so-called hypergammaglobulinemia. Much of this increase is probably due to antigens released from parasites acting as polyclonal mitogens (substances that cause cells, particularly lymphocytes, to undergo cell division) for B cells.

**Idiopathic thrombocytopenic purpura** (ITP): a condition of abnormally low platelet counts resulting from the production of Abs against platelets.

**Idiotype (Id)/anti-Id**: the idiotype is the antigenic characteristic of the V region of an antibody. Anti-idiotype complexes of Ab (commonly dimers) arise when Abs recognize and bind to the Ag binding site (paratope) of another Ab. The end result can be the production of a chain of autoantibodies that recognize each other and that may modulate the immune system by stimulating or suppressing it. Now the immune system is regulated is of central importance for understanding, treating and eventually preventing many diseases, including allergies and autoimmune diseases, such as diabetes, multiple sclerosis, arthritis and lupus. Neils Jerne, who developed the anti-idiotypic network theory, was awarded the Nobel Prize in physiology and medicine in 1984. IgG dimers of IgG can arise in plasma by noncovalent interactions between the Fab arms of different Ab molecules (see idiotype/anti-idiotype). They may also arise from covalent interactions involving disulfide bond formation between two Fcs from different Abs of the IgG2 class [22,23].

**Immune complexes** (ICs): complexes of antigen bound to antibody and, sometimes, components of the complement system. The number of circulating ICs is increased during chronic infection with parasites (particularly worm infections) and in some autoimmune disorders (particularly SLE) in which they may be deposited in tissues causing inflammation and tissue damage.

**Intravenous immunoglobulin** (IVIG): a preparation of human polyclonal IgG obtained from pooled plasma samples taken from thousands of healthy blood donors.
intravenous immunoglobulin (IVIG) for the treatment of autoimmune inflammatory disorders [4,5]. In fact, 70% of prescribed IVIG is now used for the treatment of autoimmune inflammatory conditions, more than as a replacement therapy in patients with immune deficiency [4,5]. IVIG is typically pooled from ~3000 anonymous donors and has been used successfully to treat an increasing number of autoimmune diseases, including idiopathic thrombocytopenic purpura (ITP), severe rheumatoid arthritis, autoimmune diabetes mellitus, systemic lupus erythematosus (SLE), asthma, Kawasaki disease, Guillain–Barré and Stevens–Johnson syndromes, and Crohn’s colitis (reviewed in [5]). IVIG is also effective in patients with diabetes and chronic inflammatory demyelinating polyneuropathy [5,6], and can reverse diabetes in nonobese diabetic mice [5,7]. Understanding the mechanism of action of IVIG in these autoimmune diseases has vexed investigators for the past three decades, and numerous mechanisms have been proposed for the paradoxical anti-inflammatory effects of Ab (Table 1).

The most compelling of these mechanisms support a role for the Fc portion of Ab and interactions with inhibitory and activating Fcγ receptors (FcγRs) found on monocytes and macrophages, because Fc fragments derived from IVIG can cure children suffering from ITP [7,8], although the exact receptors involved are hotly debated [9–11] [Leontyev, D. et al. (2010) The inhibitory Fcγ receptor is unnecessary for IVIG efficacy. Nature Precedings (http://precedings.nature.com/documents/4635/version/1)] and may vary for the disease for which IVIG is used [12]. It has also been shown that it is the polymeric IgG fraction and more specifically the sialic acid component of IVIG that is responsible for this beneficial effect [9,13,14]; observations are supported by the reversal of ITP in mice by immune complexes (ICs) or IgG Fc fragments enriched for sialic acid (Figure 1 and [10,15–17]).

A mechanism of action to explain the suppressive nature of IVIG (specifically the sialylated Fc) has recently been published [17]. It involves a Th2-dependent pathway involving cytokines and cell types implicated in the control of helminth parasites [17]. Sialylated Fc fragments bind dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) receptor on myeloid cells to drive IL-33 expression, which in turn activates FcεRI+ innate leukocytes (e.g. basophils and mast cells) to produce IL-4. This results in marked expression of inhibitory FcγRIIB on macrophages (Figure 2), and an increased activation threshold required to initiate inflammation [17]. Intriguingly, IL-4 [18], IL-33 [19] and FcεRI+ [20] leukocytes, are all raised during worm infections. Indeed, DC-SIGN can interact directly with fucose residues found on glycosphingolipids found on many helminth parasites [21]. Sialylated Fc fragments derived from IVIG also interact directly with CD22 (Siglec-2) to modulate B cell receptor signaling and promote apoptosis in mature human B lymphocytes [16].
Table 1. Mechanisms for the regulatory effects of immunoglobulins

<table>
<thead>
<tr>
<th>IgG Fc-dependenta</th>
<th>Evidence for role in diseases of the developed world</th>
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<tr>
<td>Blockade of Fc receptors.</td>
<td>Efficacy of Fy fragment therapy in patients with ITP [7,8]. IgG ICs inhibit IgE-mediated anaphylaxis in vivo through Ag interception and FcRRIIB crosslinking [62].</td>
<td>In worm infections IgG2, IgG4 and IgM may block more effective Ab classes such as IgE, IgA and IgG1 [55,63,64].</td>
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<td>Activation of Treg cells.</td>
<td>IVIG expands Treg population and increases the suppressive action of Tregs [65,66]. IgG contains ‘Tregitopes’ for Tregs [67]. ICs from breast milk induce CD25+CD4+ Tregs necessary for protection from allergic airway disease [68]. Fc exerts Treg/-IL-10-dependent anti-inflammatory effects that protect against fatal HSV encephalitis [57].</td>
<td>Natural Treg cells from children infected with geohelminths have strong immune suppressive properties compared with those from uninfected children [69].</td>
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<td>Saturation of FcRn: clearance of parasite-specific antibodies or autoantibodies.</td>
<td>Requirement of FcRn for IVIG in autoimmune skin blistering diseases and thereby the concept of ‘AbDeg’ (antibodies that promote degradation of IgG) designed to bind with high affinity to FcRn have been shown to outcompete native IgG to allow for accelerated catabolism [70].</td>
<td>Unknown.</td>
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<td>Induction of inhibitory FcRRIIB-dependent mechanisms.</td>
<td>ICs enhance tolerogenicity and attenuate SLE [36,37]. IVIG ameliorates ITP and chronic inflammatory demyelinating polyneuropathy [6,13,14,24].</td>
<td>Polymorphic variants of FcRRIIB that predispose to SLE protect from malaria and are more common in African populations [46].</td>
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<td>Induction of FcRRIIA-dependent mechanisms.</td>
<td>IVIG inhibits allergic airway inflammation, ITP and diabetes [7,12,71,72].</td>
<td>FcRRIIA mediates IgG-induced IL-10 and is required for chronic Leishmania mexicana infection [73].</td>
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<td>Induction of alternative inhibitory receptors via glycosylated variants of IgG.</td>
<td>Desialylated IVIG no longer protective via DC-SIGN on macrophages. Mediated by IL-33 and IL-4 from FcRRII+ cells that increase FcRRIIB expression [15–17,74,75]. Desialylated IVIG no longer protective via CD4 on B cells [16]. Agalactosyl IgG raised in autoimmune diseases [48–52]. Breast milk IgG immune complexes induce oral tolerance and prevent asthma development [88].</td>
<td>IL-33, IL-4 and FcRII+ leucocytes protect from helminth infections [18–20]. Agalactosyl IgG raised in tuberculosis patients [49]. IVIG protects against tuberculosis infection in mice [50].</td>
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IgG Fab-dependentc

| Generation of Id/anti-Id dimers. | Dimer fraction of IVIG (5–15% of total) required for efficacy in ITP model [13,76]. | Common in parasitic infections due to crossreactive epitopes on surface Ags and Id paratopes [28,77,78]. |
| Generation of Ig free light chains and light chain dimers (Bence Jones proteins). | Light chains inhibit autonomous signaling capacity of the B cell receptor [79]. | Raised in AIDS patients with Toxoplasma gondii encephalitis [78]. |
| Direct interference with immune cell receptors. | IVIG inhibits differentiation, amplification and function of human Th17 cells by interference with retinoic acid-related orphan receptor C [60]. | Treg generation by helminth parasites dependent on retinoic acid signaling pathways [61]. |

IgG Fc- and Fab-dependentd

| Induction of regulatory IgG4. | Fab arm exchange, IgG4 protects from autoimmune myasthenia gravis [80]. IgG4 can form complexes with the Fc of other IgGs [81]. | Filaria-specific IgG4 can competitively block IgE-mediated basophil activation [55,65]. IgG4 levels are raised in chronic helminth infections and have been negatively associated with pathology in filariasis. IgG4 may therefore protect against lymphoedema in filariasis and allergic diseases [55,66]. |

Non-IgG antibody classes*

| Induction of natural regulatory IgM antibodies. | Mice deficient in IgM at increased risk of autoimmunity and atherosclerosis [82,83]. Low serum IgM associates with increased risk of SLE in humans [84,85]. IgM enriched IVIG suppresses T lymphocyte function [86]. | Raised nonspecific IgM commonly seen in hyperimmunglobulinemia associated with malaria and trypanosomiasis [42,44]. Natural IgM protects from infection (reviewed in [83]). |
| Serum monomeric IgA is inhibitory, whereas IgA complexes are activatory (the opposite to IgG7). | IgA-deficient patients show increased susceptibility to autoimmune and allergic disorders [87]. | Secretory dimeric IgA raised in response to numerous parasitic infections, role of monomeric plasma IgA is less clear [88]. |

Abbreviations: Ig, immunoglobulin; Fc, fragment crystallizable; ITP, immune thrombocytopenic purpura; IC, immune complex; Ab, antibody; Ag, antigen; HSV, herpes simplex virus; IL, interleukin; IVIG, intravenous immunoglobulin therapy; Treg, regulatory T cell; FcRn, neonatal FcR; SLE, systemic lupus erythematosus; Fab, fragment for Ab specificity; Id, idiotypic.

*aMechanism requiring the Fc portion of IgG (Figure 1).

*bDimers of IgG can arise through interaction between Fab or Fc arms of two different IgG molecules (Figure 1 and Glossary).

*cMechanism requiring the Fab portion of IgG (Figure 1).

*dMechanism requiring both Fc and Fab portions of Ab (Figure 1).

*eMechanisms requiring alternative antibody classes to IgG (e.g. IgA and IgM).
Because the proportion of active multimeric IgG and/or sialylated IgG in IVIG is extremely low (<1% and 5%, respectively), very large doses of IVIG have to be given to patients, typically 2 g/kg body weight, and this may cause adverse reactions in a very small number of patients. The exact nature and molecular make-up of the polymeric fraction of IgG in human IVIG preparations remains unclear. Although many IVIG preparations contain dimers that arise from idiotype (Id)/anti-Id complexes associating noncovalently through Fab interactions [22], they may also contain covalent IgG2 dimers [23]. Although larger (>350 kDa) multimeric IgG and ICs are mostly removed in the preparation of IVIG, these are common in normal healthy individuals, where they can be found at levels up to 15 μg/ml, suggesting a physiological role in maintaining immune homeostasis [24]. However, levels of multimeric IgG are commonly higher in the plasma of West African Gambians, whose mean IgG concentration is approximately twice that of UK controls [25]. A very important increase in immunoglobulin levels seems to result from a remarkable polyclonal B and T cell activation associated with protozoal infections such as malaria and African trypanosomiasis. During an immune response to chronic infections, for example with long-lived helminth parasites, circulating ICs increase dramatically and are maintained at high levels for long periods [26]. These ICs will interact with a greater number of FcγRs by nature of their higher-avidity binding (Figure 2). Quantitative variations in the subclass composition and glycosylation status of ICs will therefore determine both their affinity and specificity for either activating or inhibitory Fc receptors (that are themselves extremely polymorphic in different populations), thus generating qualitative differences in protection from autoimmune disease (Figure 2). Such valence-dependent signaling by ICs to highly polymorphic receptors may explain why human epidemiological studies do not consistently support a protective role for helminths in allergy and autoimmunity [1].

Affinity-purified anti-ovalbumin ICs can severely suppress resistance to infection, for example with Listeria monocytogenes [27]; ICs, but importantly not monomeric IgG or F(ab)2, can suppress granulomatous hypersensitivity to soluble egg antigens (Ags) from Schistosoma mansoni [28]. Indeed, elevated levels of circulating ICs are characteristic of infections with S. mansoni [29] and have even been detected in the cerebrospinal fluid of patients, although their role in this location is unclear [30].

Although several studies have shown monoclonal antibodies and ICs to be potent activators of dendritic cells (DCs), able to prime stronger immune responses than Ag alone [31–33], several studies have shown them to be inhibitory [33–35]. This paradox may arise from difficulties in defining the exact nature of ICs used in these studies, for example the ratios of Ab:Ag or individual IgG subclass found within any single IC [36]. Monoclonal IgG antibodies of all murine subclasses can suppress Ab responses, particularly to large particulate Ags such as erythrocytes [33]. Factors including affinity, avidity, specificity, subclass composition, glycosylation and size of the IC can contribute to determining which FcγRs and/or carbohydrate receptors (e.g. DC-SIGN or CD22 for sialic acid) are engaged, and thereby the fate of an IC as activating or inhibiting (Figure 2).
Opinion

Strong evidence for the regulatory nature of Abs comes from the observation that maternal Abs can inhibit protection mediated by vaccination of neonates against malaria [37], measles [38] and poliomyelitis [39]. In the cotton rat model of measles virus vaccination, this inhibition by maternal Abs results from crosslinking of the B cell receptor with FcγRIIB [40]. Intriguingly, maternal inhibition by IgG could be partially overcome by injection of specific monoclonal IgM, a finding that has also been demonstrated in rodent malaria models [41].

Taken together, it is clear that antibodies and ICs can be highly inhibitory and/or regulatory. Thus, could they offer an alternative explanation for the hygiene hypothesis?

Hypergammaglobulinemia and immune complexes are commonly associated with infection

One of the consequences of chronic viral, bacterial and parasitic infections is the presence of circulating Ags, persistent antigen stimulation and the formation of ICs [42]. The nature of Ig-containing complexed material has not been fully characterized in any infection but may represent Ag–Ab or Ab–Ab interactions [38]. Indeed, such is their ubiquity that circulating ICs have even been used as diagnostic markers of infectious disease [43]. In addition to the increase in specific Ab, many bacterial, viral and the majority of parasitic infections provoke a nonspecific hypergammaglobulinemia, particularly IgG and IgM [25,29,42,44]. Wild rodents matched for age, gender and strain show significant increases in levels of autoreactive and polyreactive IgG compared with laboratory rats, and these have been proposed to contribute to inhibitory feedback mechanisms that protect from autoimmune disease [45].

During malaria infections, clinically immune West Africans produce approximately seven times as much IgG per day [25,44], much of it nonspecific when compared with uninfected Europeans, and the level of synthesis falls after antimalarial drug treatment. Intriguingly, a polymorphic variant of inhibitory FcγRIIB\(^{229}\), that predisposes African or Asian populations to SLE, is associated with substantial protection from malaria [46], and epidemiological and experimental data have also shown that malaria may protect individuals from developing autoimmune disease [47]. Because sialylated IgG and/or ICs are responsible for anti-inflammatory effects, then affinity-purified IgG from these West Africans may be more effective at treating autoimmune diseases rather than IVIG purified from European donors whose immune systems are not persistently being stimulated by parasites.

**Fc glycosylation modifies IgG function**

Human IgG antibodies share a conserved N-glycosylation site at asparagine 297 (Asn297) within the CH2 domain of their Fc moieties (Figure 1). Sugars attached at Asn297 have a common biantennary glycan structure of four N-acetylgalactosamine (GlcNAc) and three mannose residues, with variable additions of fucose, galactose and sialic acid residues that are crucial for the biological activity of IgG (Figure 1). The glycoform profile of IgG varies with age, over the term of pregnancy and during disease [48]. IgG from patients with multiple myeloma reveal unique glycoform profiles, including major differences in galactosylation, fucosylation and the addition of bisecting N-acetylglucosamine residues [48]. It is therefore not surprising that IgG responses to some pathogens also comprise predominant glycoforms. For example, galactosyl IgG, which also lacks terminal sialic acid, is raised in patients with tuberculosis [49], and this increase is not part of the acute phase response, as most viral infections do not alter levels of galactose on IgG [49]. Intriguingly, high dose IVIG also protects mice from *Mycobacterium tuberculosis*, in a manner independent of Fab specificity [50].

Ex vivo studies with B cells have shown that CpG oligodeoxynucleotide and IL-21 increase Fc-linked galactosylation and reduce bisecting N-acetylgalactosamine levels, whereas all-trans retinoic acid significantly decreases galactosylation and sialylation levels [51]. Although the glycosylation status of IgG, especially with regard to sialic acid, fucose and galactose, has not been determined for individuals infected with parasites and in whom autoimmune and atopic conditions are rare, it clearly would be a worthwhile exercise, because alterations in the glycosylation status of IgG significantly alters its ability to interact with either activating and inhibitory Fc receptors [52]. It is therefore not surprising that pathogens have evolved glycosidases that are exquisitely specific for the sugars on IgG [53]. These significantly alter the effector functions of IgG and can even inhibit the development of autoimmune disease when administered in vivo [53,54].

Polyparasitism is the rule rather than the exception in underdeveloped countries. In these scenarios, regulatory mechanisms will need to be fine-tuned to deal with coinfections, and the increased likelihood of an activated immune response leading to higher levels of Abs (with altered glycosylation profiles) and more elaborate ICs. Taken together with the polymorphic nature of the Fc receptors to which ICs bind, this may explain why human epidemiological studies do not consistently support a protective role for any individual helmhint to protect against allergy and autoimmune disease [1].

**Concluding remarks**

In this review, we have laid bare the evidence supporting the immunomodulatory and regulatory capacity of antibodies, and why it may favor parasite immune evasion tactics to drive such responses. Determining how ingested or injected parasite products are regulatory may lead to the development of reagents that simulate these effects in vivo, without recourse to using ill-defined parasite material, or self-infection with potentially harmful organisms. In any event, understanding the molecular basis behind the regulatory effects of antibodies will lead to the development of novel therapies for treating inflammatory autoimmune and allergic disease. The flip side to understanding regulation may also allow immunologists to design better vaccines, for example by using IgM-based adjuvants to overcome maternal inhibition during vaccination.

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