v. 5, n. 6, 2025

International Journal of Health Science

Acceptance date: 15/01/2025

RESPONSE OF THE HUMORAL IMMUNE SYSTEM TO COVID-19: EVALUATION OF IMMUNOGLOBULINS WITH EMPHASIS ON SEROCONVERSION AND THE ROLE OF IGG SUBCLASSES

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Abstract: This article aims to discuss the humoral immune system in COVID-19, based on the clinical conditions of infected patients with a focus on seroconversion, immunoglobulin levels and behavioral analysis of IgG subclasses. We used the main scientific searches to elucidate IgG seroconversion and subclasses in the disease, seeking to describe the sequence in the synthesis of antibodies against the viral antigen and how non-seroconversion, absence or presence of a certain immunoglobulin can impact on clinical evolution. In this sense, it was possible to investigate and evaluate the longitudinal changes in antibodies that occur over the course of clinical evolution, taking into account the clinical condition of the patients. Furthermore, the antibody-mediated response seems to be dominated by the IgG isotype, which is considered to be the main antibody present in the body. IgG1 and IgG3 have been shown to act as effectors in mounting the humoral response to the virus, with little detectable response from IgG2-4. The structural aspects of the subtypes, their affinity for binding to Fc receptors, their ability to neutralize and increase the response of cellular immunity are all factors that have been shown to increase the synthesis of these proteins and, consequently, enable viral particles to be eliminated more effectively.
Keywords: COVID-19, SARS-CoV-2, humoral response, antibodies, IgG, subclasses.

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is characterized by mild, flu-like symptoms in most patients, in which about 20% of individuals present with more severe disease, with bilateral pneumonia that can rapidly deteriorate into acute respiratory distress syndrome (ARDS) (Hoepel, et al., 2021). In addition, this infection continues to spread globally in more than 210 countries

and territories, given that the high contagion rate of SARS-CoV-2 leads to its skillful dissemination (Li, et al., 2020).

A disease responsible for more than 2.6 million deaths in the period of approximately one year, whose incidence is estimated at more than 164 million cases, based on the detection of viral RNA (Yates, et al., 2021). The pandemic caused more than six million deaths in March 2022 (Korobova, et al., 2022). Accordingly,

100,000 confirmed cases have been reported daily, which creates a major challenge for public health and medical services around the world, so it is understood that rapid diagnosis and specific treatment for COVID-19 are of serious necessity (Li, et al., 2020).

Coronaviruses (CoVs) have enveloped positive strand RNA and are allocated to the Coronaviridae family, where SARS-CoV-2 belongs to the betacoronavirus genus (Mazzini, et al., 2021). The virion resulting from the synthesis and proliferation of CoV has four basic structural proteins: transmembrane glycoprotein (S) or spike, envelope (E), membrane (M) and nucleocapsid (N) (Tortorici, et al., 2019). These proteins, found on the viral surface, can bind to host receptors and cause infectivity (Robson, et al., 2020).

The S protein, which mediates virus-receptor binding, comprises two subunits (S1- S2). The S1 subunit covers the apex of the S protein trimer, including receptor binding domain (RBD), and stabilizes pre-fusion of the S2 subunit, which is responsible for facilitating viral fusion in the host cell (Tortorici, et al., 2019). Thus, the coronavirus spike glycoprotein, exclusively in its RBD domain, mediates viral entry into host cells, being a potent immunogenic agent and characterizing the main target region of neutralizing antibodies (nAb) after infection (Walls, et al., 2020).

These antibodies are grouped into five main isotypes: IgM, IgD, IgG, IgA and IgE, so that each immunoglobulin (Ig) molecule is the product of a single clone of B cells that differentiate into antibody-producing plasma cells (Smith, K. A., 2018).

The IgA and IgG isotypes can also be grouped into subclasses, IgA (IgA1 and IgA2) and IgG (IgG1, IgG2, IgG3 and IgG4), according to the structural divergences evidenced in the constant (C) regions of the heavy chain of these molecules, thus, all antibody molecules share similar basic structural characteristics, showing variability in regions that bind to the IgG. antigen (Abbas, et al., 2019).

According to the patient's clinical outcome, higher titers of anti-RBD IgG were found in individuals with mild or moderate disease compared to patients who died, in , comorbidities such as diabetes, hypertension, vascular and pulmonary diseases were present in the group of deceased individuals, and it is important to note that individuals recovered from COVID-19, who previously had severe disease, were associated with higher levels of anti-RBD IgG than those with milder disease (Moura, et al., 2021). Similarly, risk factors such as advanced age and comorbidities influence the parameters of IgG subclasses, in view of changes in total IgG (Luo, et al., 2021).

It is clear that there is a tendency to stimulate the severity of COVID-19 and the induction of a humoral immune response, although the correlation between cause and effect is not clear. However, uncontrolled replication of the virus, leading to subsequent hyperinflammation, can cause the overproduction of antibodies, serving as a "biomarker" associated with severity (Garcia-Beltran, et al., 2020).

In this sense, antibodies represent a potential candidate of the adaptive immune system that could explain the observed worsening of the disease during SARS-CoV-2 infection (Hoepel, et al., 2021).

The serological assays most commonly used to describe and characterize the kinetics and response of these antibodies directed at human coronaviruses HCoVs) include enzyme-linked immunosorbent assays (ELISA), immunofluorescence (IFA), Western Blots and complement fixation (CF) (Huang, et al., 2020). In addition, neutralization assays have been used to identify the functional responses of antibodies, observing their biological activity during viral replication (Patil, et al., 2021).

Real-time polymerase chain reaction (RT--PCR) has been widely used for the diagnosis of COVID-19 (Mazzini, et al., 2021), 2021), however, according to the high false-negative rate evidenced through this technique in the detection viral RNA, the need for alternative methods arises, in view of this, the evaluation of serum IgM and IgG levels was inserted in the "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia in China", on March 3, 2020, presenting itself as a simpler and faster technique compared to the viral RNA test, so that antibodies can provide an important complement for the diagnosis of COVID-19 (Li, et al., 2020).

Understanding the relationship between the varied clinical manifestations of COVID-19 and the serological response that arises as a result of exposure to the virus will contribute to understanding the immunopathogenesis of the disease, including the degree of antigen recognition and antibody subclasses involved in the antiviral response and their role in the protective response (Faustini, et. al, 2021).

This study aims to elucidate the kinetics of the humoral immune response in patients infected with SARS-CoV-2, focusing on seroconversion and the development of IgG antibody subclasses against the viral antigen. The specific objectives are to investigate the levels of anti-SARS-CoV-2 antibodies in infected patients and to clarify the possible positive or negative correlation with clinical outcome. In addition to analyzing the behavior of IgG subclasses, by monitoring Immunoglobulins (Ig) in patients at different clinical stages of COVID-19. And finally, to see if the impact of seroconversion of subclasses or isotypes with low effector activity against viral antigens can trigger the severity of the disease.

Furthermore, monoclonal antibodies (mAbs) targeting a broad spectrum of epitopes present in the spike protein have been developed in the last two years, which favors their use as anti-SARS-CoV-2 antibody-based therapy (Huang, et al., 2022).

METHODOLOGY

The current study in explanatory model has its approach through a narrative review with a focus on observing the state of the art on the immune response in COVID-19, in this way, at first a selection of articles was carried out using keywords such as: ''COVID-19", ''SARS-CoV-2", ''humoral response", ''antibodies", ''IgG" and ''subclasses". Applying the following Boolean operators ''and - or".

Accordingly, a search was carried out in PUBMED, considering articles published in journals with an impact greater than >1.0. These studies were searched for in Portuguese, English and Spanish, and only articles published between 2012 and 2022 were selected.

In this first stage, we verified the existence of studies correlating immunological and clinical aspects of patients during the evolution of COVID-19, being adopted as one of our inclusion criteria for future analyses, obeying as similar as possible of the following parameters of clinical presentations: moderate or without the need for hospitalization; severe with hospitalization; and critical. In addition to studies that address the behavior of immunoglobulins, structural and viral aspects of SARS-CoV-2, as well as longitudinal analyzes of the changes that occur in isotypes and sub-

classes, including antibody dosage and statistical analyzes, thus amplifying the quality of the investigation, above all, the results of the research.

In view of this, after the first selection stage, the summaries all the articles selected will be read, with the aim of optimizing time and selecting only those articles that are relevant to the project and fit the established criteria. Subsequently, a more in-depth reading will take place, with the aim of organizing the ideas, thus allowing the creation of various spheres to be considered and argued in order to clarify the assumptions of the study.

In our initial search, using the respective keywords, we found a total of 329 articles. Of these, 34 studies addressed our criterion 1 (studies focusing on IgG and subtypes during SARS-CoV-2 infection), with 19 being stratified cohort studies that delimited the clinical condition of the patients. Of these 19, 17 made an association between antibodies and disease severity. We also considered 14 studies that addressed structural and molecular aspects of antibodies and the viral structure of SARS-CoV-2.

LITERATURE REVIEW

SARS-COV-2 AND COVID-19

The novel coronavirus-derived pneumonia (COVID-19) emerged in Wuhan, China, where a series of cases were first confirmed in regional patients, and later characterized a pandemic, as the virus began to spread rapidly in several countries, including: Thailand, Japan, South Korea, Singapore, Vietnam, the USA and 24 other countries worldwide (Tian, et al.,2020).

COVID-19, a disease caused by SARS--CoV-2 that can evolve into acute respiratory distress syndrome (ARDS), has spread worldwide, resulting in 21,938,171 confirmed cases and reports of 775,581 patients who died, as of August 19, 2020 (Li, et al., 2020).

Coronaviruses (CoVs) belong to a heterogeneous group viruses that end up infecting some animals and can cause mild or severe respiratory infections in humans (Hu, et al., 2020). Two highly pathogenic coronaviruses of zoonotic origin that cause severe acute respiratory syndrome have been discovered since 2002, named: SARS-CoV and MERS-CoV, the most recent being 2019-nCoV (Sharma, et al., 2021). At the same time, data shows that SARS-CoV-2 infection comes from contact with animals, which subsequently spread from human to human, leading to high infection rates (Chan, et al., 2020). Thus, as a result of the increase in human practices, such as the breeding of wildlife, new pathogens have emerged in recent decades, causing serious public health problems (Mazzini, et al., 2021).

Coronavirus infections can result in gastrointestinal irritation, cough, fever and shortness of breath, and are generally prevalent in the elderly and immunocompromised (Sharma, et al., 2021). In addition, comorbidities such as obesity, hypertension and diabetes predominate in patients with severe COVID-19, both in recovered patients and in patients who have died (Moura, et al., 2021).

It is worth noting that more than 4,890,000 people were infected with COVID-19 in just five months, as the disease spread to approximately 200 countries, also taking into account that in 24 hours the number of cases exceeded 100,000 notifications and, among these cases, the highest prevalence was in males (Yang, et al., 2020).

It should also be noted that coronaviruses are classified under the order Nidovirales, family Coronaviridae and subfamily Orthocoronavirinae, their genome varies between 26 - 32 kilobases (kb) in length, being considered the largest genome of RNA viruses, in particular, the genome of SARS-CoV-2 is 29.9 kb, based on next-generation sequencing (NGS) analysis, whose phylogenetic analysis shows that SAR-

S-CoV-2 is under the Sarbecovirus subgenus of the betacoronavirus genus (MALIK, A. Y., 2020). Its genome encodes four basic structural proteins: spike protein (S), envelope (E), membrane (M) and nucleocapsid (N), the latter forms the capsid outside the genome which in turn is packaged by an envelope that associates with the M, S and E proteins, in addition, its genome contains sixteen non-structural proteins (nsp1 - 16) (Wang, et al., 2020).

In addition, CoVs have positive (+) strand RNA, and for their replication, they perform a continuous synthesis of negative strand RNA to create a complementary template and subsequent copying of this template in several positive strand genomes (Robson, et al., 2020).

The SARS-CoV-2 genome includes at least ten open reading frames (ORFs) (Malik, A. Y., 2020), with the proximal 5' end containing two ORFs (ORF1a and ORF1b), which encode the polyprotein-replicase and represent two-thirds of the genome (Robson, et al., 2020). The rest of the ORFs encode the structural proteins already mentioned and non-structural proteins that do not take part in viral replication, with functions little known (Malik, A. Y., 2020). Blocking virus-receptor binding and viral neutralization are believed to be primary host protection mechanisms, so antibodies act as effectors in this system (Yates, et al., 2021).

Therefore, viruses infect host cells mainly by interacting with specific receptors expressed on the cell surface (Yates, et al., 2021). In this regard, coronavirus infection resulting from this interaction can trigger neutralizing antibody (nAb) responses with antiviral activities, as already observed in patients infected with SARS-CoV and MERS-CoV (Luo, et al., 2021). Evidently, SARS-CoV and SARS--CoV-2 show 79.6% similarity in their amino acid sequences and use the same entry receptor present in the host, the (ACE2), resulting in subsequent respiratory syndrome (Iwasaki, et al., 2020).

HUMORAL IMMUNE RESPONSE TO SARS-COV-2

The humoral response directed at SARS--CoV-2 is mainly stimulated by the spike protein (S), a trimeric glycoprotein exposed on the viral surface, and the nucleocapsid protein (N) (Yates, et al., 2021). A cohort study that evaluated the serology of convalescent individuals who were previously RT-PCR positive for SARS-CoV-2, showed that 64% of participants had detectable IgG and IgA anti-RBD and anti-nucleocapsid responses, with a trend of increasing antibodies associated with severity, however, 36% represented individuals with undetectable seroconversion, who had younger age and lower amounts of nasopharyngeal viral load (Liu, et al., 2021).

The spike glycoprotein plays an important role in the pathogenesis of SARS-CoV, by binding to target cells through the interaction of the receptor binding domain (RBD) (Wrapp, et al., 2020). As a result, protein S has been found to be a potent immunogenic target that can elicit antibody-mediated responses, particularly anti-RBD antibodies (Hoepel, et al., 2020).

Similarly, the nucleocapsid protein (N) is capable of stimulating high levels of antibodies, being the most abundantly expressed immunodominant protein that interacts with viral RNA (Dwyer, et al., 2021). Despite this, most asymptomatic or mild patients do not develop humoral immunity (Tekeshita, et al., 2021).

The amino acid sequences of the light and heavy chains of immunoglobulins explain much of their structure and function, however, describing antibody sequences is a challenging step due to the heterogeneous nature of an individual's antibodies (Roitt, et al., 2013).

SARS-CoV-2 IgG anti-RBD antibodies have already been correlated with neutralizing antibodies (nAb), with functions directed at the S protein (Iyer, et al., 2020). Thus, it is believed that blocking virus-receptor binding and viral neutralization are primary host

International Journal of Health Science ISSN 2764-0159 DOI https://doi.org/10.22533/at.ed.159562515013

protection mechanisms, so that antibodies act as effectors in this system (Yates, et al., 2021).

In addition, several studies have found high levels of neutralizing antibodies (nAb), with a predominance of IgG and an increase in IgG3 specific for subunit 1 of the S protein, especially the RBD domain of SARS-CoV-2, in patients with severe COVID-19, where IgM and IgA specific for the SARS-CoV-2 protein are also found nucleocapsid had lower titers, indicating a high potential IgG-mediated response to SARS-CoV-2 viral proteins, ensuring a protective effect and impact on viral eradication (Yates, et al., 2021).

Therefore, determining the avidity, that is, the strength of antigen-antibody interaction of IgG isotypes and subclasses in COVID-19 can result in correlations between immunological and clinical aspects resulting from SARS-CoV-2 infection, addition to helping to determine protection after immunization (Moura, et al., 2021).

In fact, exposure to the virus generates an antibody-mediated response that changes its kinetics over time and between individuals (Huang, et al., 2020). Therefore, with a view to developing effective vaccines against SARS-CoV-2, it is necessary to evaluate the serum antibody levels generated by exposure to the virus, the duration of the response and the absence or presence of protection against reinfection (Krammer, F. 2020).

ANTIBODY BEHAVIOR

IGM, IGA, IGD and IGE

To combat a wide variety of pathogens, the lymphocytes of the adaptive immune system must recognize a wide range of antigens (Murphy, et al., 2014). Antibodies not only specifically recognize different invading pathogens, but also possess the ability to recruit various components of the immune system (Roitt, et al., 2013). These antibodies are grouped into five main isotypes: IgG, IgM, IgA, IgD and IgE, which have different effector functions in the immune response (Tortora, G. J. 2017).

IgM is the first antibody to be synthesized and can be expressed without somatic mutation of the B cells and subsequent class change (Murphy, et al., 2014). It also makes up around 6% of the antibodies present in the serum and is often synthesized in a primary humoral response, in addition to having a structural which characterizes its potent ability to activate the complement system (Tortora, et al., 2016). As a result, gradual increases in IgM are observed following the development of COVID-19 (Long, et al., 2020).

IgA represents approximately 13% of the antibodies present in serum and is the most frequent isotype found in mucous membranes and secretions (Tortora, et al., 2017), being frequent in the lumens of gastrointestinal and respiratory tracts, possessing the ability to neutralize microorganisms in these environments (Abbas, et al., 2019).

That said, the presence of anti-SARS--CoV-2 IgA is associated with a more rapid reduction of the virus in the respiratory tract, as evidenced by a reduction in viral load in nasopharyngeal swabs (Secchi, et al., 2020).

IgD antibodies represent approximately 0.02% of total serum immunoglobulins and are found in the blood, lymph and mainly associated with membrane molecules present on the surface of B cells (Tortora, G. J. 2017). On the other hand, IgE plays an important role in the degranulation of mast cells in hypersensitivity reactions, as well as providing defense against helminths (Abbas, et al., 2019).

High levels of IgA, IgG and even IgG3 have been found in patients with severe COVID-19 in the second and third week after infection, where IgG1 increased only during the second week and in the third week IgG titers exceed those of IgA, which initially had similar concentrations (Patil, et al., 2021).

REFERENCE	NUMBER OF PATIENTS	SEROCONVERSION TIME	ANTIBODIES ASSOCIATED WITH GRAVITY	MEDICAL CONDITION
Li, et al., 2020	1.850	IgM: 7 Days>IgG: 7 Days	IgG	Patients/serious
Tan, et al., 2020	67	IgM: 7 DaysIgG: 10 days	IgM+/IgG+	Patients/serious
Korobova, et al., 2022	193	IgG: 12 - 14 Days	IgG	Moderate/severe patients
Long, et al., 2020	39	IgM/IgG: 11-13 [90%]	IgM/IgG	Patients/serious
Marklung, et al., 2020	15[Heavy] 29[Light]	IgG: 11 DaysIgG: 22 Days	IgG	Severe/light patients
Garcia-Beltran, et al., 2021	113	NA	IgA+/IgG+	ICU patients [27]
Yan, et al., 2021	409	NA	IgG	Moderate/severe patients
Doke, et al., 2022	4	NA	IgG	Severe patients
Hoepel, et al., 2021	27	NA	IgG	Severe patients

TABLE 1 - Data on the prevalence of antibodies in patients infected with SARS-CoV-2 in severe conditions. Analysis of antibody production in patients who have clinically evolved to a moderate to severe condition, considering the time to detectable seroconversion. NA = data not provided.

Source: created by the authors.

In line with this, it has been possible to observe distinct IgM and IgG seroconversion patterns: IgM before IgG, IgG before IgM or simultaneous seroconversion (Dwyer, et al., 2021). Evidence even shows that deregulated immune responses are related to a worse clinical outcome from COVID-19 (Luo, et al., 2021).

On the other , the IgG-mediated anti-RBD response is not only associated with improved patient survival, indicating one of the main factors that provide protective humoral immunity against COVID-19, but also shows prevalence in cured patients or those with few symptoms, with an additional increase in this isotype for up to 3 months after hospital discharge (Secchi, et al., 2020).

IGG PROPERTIES AND SUBCLASSES

IgG is a monomeric antibody capable of inactivating pathogens through interactions with activating effector molecules, such as Fc receptors and complement molecules, and is the main antibody found in serum (Roitt, et al., 2013).

Viral infections generally require the action of cellular immunity to eradicate the virus, where antibodies can stimulate cell populations such as natural killer (NK) cells and macrophages through antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) (Garcia-Beltran, et al., 2020), In addition, data show increased expression of FcyRllla in hospitalized patients with severe COVID-19, indicating a possible correlation with increased antibody-mediated NK cell cytotoxicity (Yates, et al., 2021).

Viruses can be neutralized by a single antibody that binds to a viral particle that has many receptor binding sites or epitopes on its surface. This mechanism can contribute to viral containment by binding to and disrupting viral structures, preventing virus-receptor interaction or even interfering with the fusion of the virus with the cell membrane (Murphy, et al., 2014).

These potential antibodies have two main compartments, the variable region or Fab (antigen-binding fragment) and the constant region or Fc (crystallizable fragment) which have great similarity between antibody isotypes and

mediate different effector functions, such as: activation of the complement system (classical pathway, dependent on IgM or IgG), increase the activity of phagocytic cells by coupling antibodies to receptors (FcRs) present on the membrane of host cells, stimulate the chemotactic attraction of phagocytes and increase vascular permeability (Roitt, et al., 2013).

The structure of IgG consists of four polypeptide chains, linked together by disulfide bridges, with two heavy chains (H) and two light chains (L), so that each heavy chain has an N-terminal variable domain (VH) and three constants (CH1. CH2 and CH3), plus an additional ''hinge region" between CH1 and CH2 and, in turn, the light chains consist of an N-terminal variable domain (VL) and a constant domain (CL), where the light chain associates with the VH and CH1 domains to form a Fab arm and bind to the antigen (VIDARSSON, et al., 2014)

In addition, in the changes that occur when B lymphocytes become plasma cells, it is important to highlight the increase in secreted Ig compared to the membrane form and the expression of heavy chain isotypes, unlike IgM and IgG, due to class switching (Abbas, et al., 2019).

That said, although the IgG subclasses have 90% similarity in their amino acid sequences, they have unique and distinct response profiles in relation to antigen-antibody binding, immune complex formation, activation of effector cells and the complement system, half-life and placental transport, but with significant and important differences, which affect their ability bind to molecules or receptors and can consequently affect their functionality (Vidarsson, et al., 2014).

Thus, some of the greatest divergences evidenced between the C regions of these subtypes are allocated in the hinge region, characterizing different general forms of IgG subclasses, so that they share similar basic structural characteristics, although they show variability in regions that bind to the antigen (Abbas, et al., 2019).

The four IgG subclasses have different concentrations, the most prevalent being IgG1 (60-70%), followed by IgG2 (20-30%), IgG3 (5-8%) and IgG4 (approximately 5%), which is the least abundant (Napodano, et al., 2020).

The residues found in the CH2 domain, close to the hinge region in the Fc portion, give them effector activity by presenting a binding site with affinity for the C1q complex, which activates complement, as well as affinity for FcyR, where IgG-Fc can increase immune responses (Vidarsson, et al., 2014).

The molecules that bind to the Fc portion of IgG are C1q, important for activating cascades of the complement system, Fc gamma receptors (FcyR), present in several host cells, neonatal Fc receptor (FcRn), which can transport maternal IgG to the fetus and bacterial receptors such as protein A and protein G (Lefranc, et al., 2020).

In general, in terms their main functional activities, IgG1 and IgG3 are characterized by their moderate neutralization capacity and sensitization of NK cells to recognize target cells (Murphy, et al., 2014). IgG3 has a high effector function, affinity with complement proteins and activation of Fc type 1 receptors (Yates, et al., 2021).

IgG1 is generally stimulated by soluble protein antigens and membrane proteins, and its absence causes hypogammaglobulinemia, indicating that IgG1 deficiency leads to a consequent decrease in total IgG, thus deficiencies in its synthesis are associated with recurrent infections (Vidarsson, et al., 2014). It is notable that IgG1 deficiency can result in an increase in infections, as well as being similarly associated with reduced levels of IgG3, however, patients with a deficiency in IgG2 synthesis may have a decrease in other subtypes (Sigal, et al., 2012).

IgG3 has an extended hinge region, which consists of 62 amino acid residues already identified (Sigal, et al., 2012), as well as 21 proline and 11 cysteine residues (Korobova, et al., 2022). This hinge region forms a polyproline double helix containing 11 disulfide bridges (Damelang, et al., 2019). While IgG2 has 4 bridges, followed by IgG1 and IgG4, both of which have two bridges in the same region (Vidarsson, et al., 2014). Notably, the larger hinge region in IgG3 grants it the ability to expand and increase its flexibility, increasing the reach of the Fab region (Damelang, et al., 2019.

IgG3 is a potent pro-inflammatory antibody and is often stimulated by viral antigens; in contrast, IgG4, in the presence of IL-10, is important for inducing tolerance (Vidarsson, et al., 2014). In addition, there are reports of isolated IgG3 deficiencies associated with lung diseases, generating damage for prolonged periods (Sigal, et al., 2012) and initial IgG3 responses may have a beneficial effect on the elimination of pathogens that express large concentrations of proteins, including viruses (Damelang, et al., 2019).

IgG1 and IgG3 have high affinities with receptors (FcyR) and complement molecules (Napodano, et al., 2020). These receptors comprise a family of molecules expressed on the surface of different cell populations that bind to the Fc region of antibodies, in humans, the following Fcs receptors are documented: Fc-RIa (CD64a), Fc-RIb (CD64b), Fc-RIIa (CD32a), Fc-RIIb (CD32b), Fc-RIIc

(CD32c), Fc-RIIIa (CD16a) and Fc - RIIIb (CD16b) (Kapur, et al., 2014). While IgG2 is a weaker mediator of these receptors (FcyR) and complement functions and IgG4 has a low capacity to activate effector cells or fix complement (Napodano, et al., 2020).

It is important to note that Fc-llb has an inhibitory function and can regulate the activating Fc-R. However, the other receptors in this family have activation functions, impacting on phagocytosis, ADCC and the release of pro-inflammatory mediators, for example, in addition to having a varied distribution among cells such as macrophages, monocytes and activated granulocytes that are restricted to Fc-Rl, myeloid cells restricted to Fc--Rlla, Fc-Rlllb expressed only in granulocytes (neutrophils, basophils, eosinophils) and Fc--Rllla expressed in NK cells and macrophages (Kapur, et al., 2014).

In contrast, IgG2 is activated when the body is exposed to enveloped bacteria and has important functions against polysaccharide antigens (Napodano, et al., 2020). IgG4 is generally stimulated by allergens and is frequent after long or repeated exposure (Vidarsson, et al., 2014). In addition, IgG4 has a lower capacity than the other isotypes to activate the complement system (Roitt, et al., 2013).

It is important to note that, although IgG4 is described as the least abundant subclass, these molecules have the ability to form hybrid antibodies, in this way, the heavy and light chain of IgG4 separate from the original heavy chain dimer and associate with a different chain pair, transforming into a bivalent antibody with two distinct specificities (Murphy, et al., 2014).

IgG2, among the subtypes, is the one with the smallest hinge region, which restricts its flexibility, a deficiency in its synthesis can result in the absence of IgG anti-carbohydrate antibodies, illustrating its role and potential activity against these molecules, however, its activity can be compensated by activation of IgG1 and IgG3 (Vidarsson, et al., 2014).

An in-depth understanding of the mechanisms involved in the expression and synthesis of IgG subclasses can contribute to the rational design of immunogens for therapeutic purposes, so that the evaluation of IgG subclasses can be introduced into the diagnostic routine and improve patient management; however, there is still a lack of reference material and standardization to ratify the importance of its usefulness (Napodano, et al., 2020).

International Journal of Health Science ISSN 2764-0159 DOI https://doi.org/10.22533/at.ed.159562515013

Two other receptors make a notable contribution to the IgG response: neonatal Fc (FcRn) and the tripartite motif-containing protein 21 (TRIM21), both of which are expressed inside cells and have important activities: the transport of FcRn through cellular barriers is responsible for facilitating the transmission IgG from mother to child through mucosal barriers, on the other hand, TRIM21, expressed in the cytoplasm, binds with great affinity to human IgGs, having the capacity to recognize viruses and stimulate immune signaling via transcription factors such as NF-B, AP-1, IRF3, IRF5 and IRF7, driving a pro-inflammatory response and inducing antiviral response (Kapur, et al, 2014).

EVALUATIONS OF SUBCLASSES E THEIR BEHAVIOR AT DIFFERENT STAGES OF SARS-COV-2 INFECTION

IgG subtypes have been described as molecules that can provide antiviral activity, especially IgG1 and IgG3, this is mainly due to the broad binding capacity of these subclasses in the FcyR receptor family and being associated with a pro-inflammatory response mediated by Th1 response (Patil, et al., 2021). Thus, IgG subclasses synthesized specifically to combat SARS-CoV-2 are the key to a better clinical condition (Moura, et al., 2021).

Evaluating IgG parameters and subclasses during SARS-CoV-2 infection, 94.32% of anti-RBD IgG positivity is observed in 166 samples from infected patients, however, no reactivity is seen in 118 uninfected individuals (Moura, et al., 2021), so that the anti-RBD antibody also has a tendency to remain in the body for up to 500 days after the onset of infection (Korobova, et al., 2022).

During the third week of SARS-CoV-2 infection, it is already possible to see significantly higher levels of IgA, IgG and IgG3 in patients with severe clinical condition, this increase is associated with comorbidities and advanced age (Patil, et al., 2021).

In this regard, when analyzing the anti--N IgG subclasses, a higher concentration of IgG3 is observed in response to COVID-19, and together with IgG1, they are associated with an effective antiviral response (Korobova, et al., 2022).

Data show an increase in IgG1 and IgG3 from the 8th day after symptom onset, with low detection levels for IgG4 during the same period of analysis, so that serum levels of these subclasses gradually increased and showed variations: IgG1 ranged from 66.6 to 100%, with a peak reached in the third and fourth week after infection and IgG3 anti-RBD ranged from 66.6 to 90.9%, however, IgG4 ranged from 66.6 to 46.1%, also showing a weak correlation with the progression of the disease (Moura, et al., 2021).

Different analyses show low induction of IgG2 and IgG4, and high induction of RBD-specific IgG1 and IgG3, so that they are considered the predominant subtypes, where it is possible to observe the production of IgG1 and IgG3 even in the acute phase of the infection (Korobova; Moura; Suthar., 2022). Also, in relation to the severity of the disease, higher titers are observed in severe patients compared to patients classified as moderate, in addition to the high induction of IgG1, IgG2 and IgG3 (Patil, et al., 2021).

In line with these observations, IgG-mediated anti-RBD responses are slightly stimulated in patients hospitalized for COVID-19, this rapid seroconversion occurs early in the infection and is dominated by RBD-specific IgG1 and IgG3, indicating that there is indeed a robust induction of humoral immune response in severe SARS-CoV-2 infections (Suthar, et al., 2020). At the same time, evidence shows that, in the acute phase, the risk of developing critical illness is higher in elderly patients, in addition to associating disease progression with impaired CD8+ T lymphocyte responses (Zhu, et al., 2021).

IgG1 proves to be the most prominent subtype detected in the majority of samples analyzed in a study focusing on the behavior of the subclasses, with positivity in 94/135, followed by IgG3 (80/135), both suggesting a Th1 response profile and were activated simultaneously in 74 samples, however, a contrary activation is observed in the other subtypes, where IgG2 and IgG4 had less detectable levels, 19/135 samples and 8/135, respectively, both suggestive of a Th2 profile (Patil, et al., 2021).

IgG3 is largely related to primary responses, while IgG2-G3 increase in later phases or even, as reported, in the post-recovery period (Korobova, et al., 2022; Chen, et a., 2022.).

It is worth noting that factors such as advanced age, higher levels of chemokines such as IL-8 and IP-10, nAbs response, SARS-CoV-2-specific IgG1 and IgG3 activity, T-lymphocyte-mediated response and multiple cytokines are associated with severe cases of the disease, including IL-1b being positively related to most isotypes, subclasses and neutralizing antibodies (Luo, et al., 2021).

An unusual finding is that patients who died had higher levels of RBD-specific IgG4 in their serum, while those who survived had lower titers of these molecules (Moura, et al., 2021). In , patients with mild COVID-19 have significantly lower levels of IgG subtypes compared to severe patients (Korobova, et al., 2022).

In combination with this, patients with severe often have cytokine storm syndrome (Moura, et al., 2021), so the strong humoral response induced by SARS-CoV-2 may be related to exacerbated immune responses, including cytokine response and increased interleukin 1 (IL-1), IL-6 and interferon-y (IFN-y) (Yan, et al., 2021). On the other hand, the production of IL-10 may be associated with the induction of IgG4 in critically ill patients, since its synthesis is increased through Th2 responses, the action of regulatory T cells (Treg) or even B cells with regulatory functions (Breg) (Moura, et al., 2021).

It is important to note that COVID-19 patients negative for IgG-anti-S do not show positive regulation of cytokines with pro--inflammatory activity, which indicates that other cellular components involved in the immune response can be induced through IgG stimulation (Hoepel, et al., 2021).

In an observational study that investigated the presence of anti-SARS-CoV-2 IgG in 473 survivors who recovered from COVID-19, antibody levels were observed with notable variations, in addition to 78 survivors who had no detectable seroconversion and asymptomatic carriers of the disease who also recovered and were negative for specific IgG, on the other hand, survivors of severe disease had high IgG titers (Yan, et al., 2021).

In contrast, another study showed similar IgG and IgM seroconversion in patients between the third and fourth week, but with different prevalence: the clinical manifestations of the patients were expressive of the disease, but IgM declined more rapidly than IgG at around 5 weeks, while IgG was more abundant and persistent, with high levels at more than 7 weeks (Sethuraman, et al., 2020).

Comparing non-severe patients with severe patients, it can be seen that the latter have higher levels of anti-S IgG for the different domains found in its structure: RBD, RBM, NTD and CTD, with a notable presence of IgG1 and IgG3, as well as a high capacity to bind to receptors such as FcyRlla and FcyRllb (Wang, et al., 2022).

Similarly, stratifying patients based on the severity of the disease, including: hospitalized, ICU patients and non-hospitalized, it is possible to denote that individuals who develop IgG RBD antibodies have a higher chance of survival and also show a tendency to develop IgG specific to the peak protein (S1 and S2) (Secchi, et al., 2020).

International Journal of Health Science ISSN 2764-0159 DOI https://doi.org/10.22533/at.ed.159562515013

REFERENCE	NUMBER OF PATIENTS	ACTIVATION OF SUBCLASSES	CLINICAL CONDITION
Faustini, et al., 2021	6	>IgG1/IgG3	Severe patients
Korobova, et al., 2022	193	IgG1++/IgG3+++	Moderate/severe patients
Luo, et al., 2021	63	IgG1/IgG3	Severe patients
Moura, et al., 2021	37	>IgG1/IgG3<IgG2/IgG4	Critically ill patients [ICU]
Wang, et al., 2022	9	IgG1/IgG3	Severe patients
Yates, et al., 2021]	48	>IgG3 Anti-S	Severe patients
Patil, et al., 2021	46	>IgG1/IgG3<IgG2/IgG4	Severe patients
Suthar, et al., 2020	44	IgG1/IgG3	Severe patients
Wasiluk, et al., 2022	36	>IgG1/IgG3	Convalescent patients
Tandhavanant, et al., 2021	27	IgG1/IgG3	Convalescent patients
Chen, et al., 2022	174	IgG1/IgG3	Vaccinated patients

Studies that evaluated the prevalence of IgG subclasses in patients who developed acute respiratory distress syndrome (ARDS).

TABLE 2 - Activation of IgG subclass prevalence in critically ill patients.

Source: created by the authors.

CONCLUSION

The response mediated by B cells, through their differentiation into antibody-producing plasma cells, has provided a high level of complexity in the fight against different invading pathogens, through the recognition of a wide range of antigens and the activation of various molecules that make up the immune system. In this sense, it is important that the humoral response directed at SARS-COV-2 is effective, ensuring rapid seroconversion and in balance with other immune responses, so that antibodies can prevail in cases of reinfection, as well as establishing an immunological memory.

COVID-19 has spread rapidly around the world, which has increased the number of deaths from the disease. In view of this, it should be noted that testing and evaluating serum antibodies can provide alternative diagnoses, as well as serving as a measure to monitor the humoral immune response. It can also indicate previous exposure and act as a protective measure.

Our hypotheses point to the modification and prevalence of the levels antibody isotypes and subclasses, which could be predictive of the worsening of the disease, as well as the increase in neutralizing antibodies. Antibodies with a neutralizing function, in turn, can block the binding of the virus to the hACE2 receptor, or even promote the premature elimination of S1 and decrease the functionality Spike (Huang, et al., 2022). In this study, we also found that IgG prevalence is associated with severity and remains at high levels in critical conditions (Table 1).

We also suggest that the decrease in IgG1 and IgG3 potentiates viral escape, while their high levels may correlate with severity; on the other hand, the activation of IgG2 and IgG4 could mitigate their effector functions, generating less neutralizing activity and recognition of viral antigen epitopes, facilitating the invasion and proliferation of SARS-CoV-2. Apparently, seroconversion to IgG2 and IgG4 subclasses is weaker and less pronounced, but IgG1 and IgG3 activation prevails in patients infected with SARS-CoV-2 (Table 2). So , there have been several correlations between the persistence of IgG1 and IgG3 in critically ill pa-

tients, supporting the idea of using the monitoring of subclasses as a follow-up measure, considering that their serum levels increase as the virus spreads and the immune system tries to keep up with and slow down viral proliferation.

Through our analysis, we were able to show that the increased IgG1 and IgG3 responses are due to their ability to bind to FcyR-type receptors more extensively. A strong anti-RBD IgG response to SARS-CoV-2 is observed, providing an important therapeutic target. In addition, there is a persistence of these antibodies for long periods, denoting the generation of specific immunological memory of the viral antigen. IgG3 seems to have a primary response and rapid activation in the acute phase of the disease, while, together with IgG1, they persist during the following phases, including the convalescence period. In addition, we must consider the heterogeneous nature of humoral responses in different individuals, also taking into account other aspects that may influence the synthesis of these antibodies.

It is important to note that some differences in the structure of IgG can be directly associated with a broader response. Accordingly, IgG3 has a greater number of amino acids present in the hinge region, which ensures greater flexibility for these molecules that act as a potent antibody with pro-inflammatory activity. It is possible to observe the dynamics of a higher concentration of IgG3 in response to COVID-19.

Finally, although IgG1-3 are dominant in the acute phase of infection, in convalescent plasma it is possible to observe a pool of IgG antibodies with a predominance of IgG1, which leads us to take into account the time of the disease when assessing total IgG (Wasiluk, et al., 2022). In addition, vaccinated patients followed up for 15 months show an increase and abundance of IgG1-3, with low detection IgG2-4 (Chen, et al., 2022).

The data obtained in this study corroborates the hypotheses put forward, leading us to understand the prevalence of these antibodies, which could provide alternative tools for better patient prognosis.

The response mediated by immunoglobulin G subclasses is still poorly understood, but it is known that these molecules can have a major influence on viral eradication. They can therefore be used as a metric for monitoring the progression of the disease in clinical practice. Finally, new studies focusing on these antibodies are needed to complement our analysis and make contributions to elucidating the antibody response to SARS-CoV-2 viral proteins.

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