Fc-Dependent Immunomodulation Induced by Antiviral Therapeutic Antibodies

Subjects: Immunology

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The multiple mechanisms of action of antiviral monoclonal antibodies (mAbs) have made these molecules a potential therapeutic alternative for treating severe viral infections. In addition to their direct effect on viral propagation, several studies have shown that mAbs are able to enhance the host's adaptive immune response and generate long-lasting protective immunity. Such immunomodulatory effects occur in an Fc-dependent manner and rely on Fc-FcγR interactions. It is noteworthy that several FcγR-expressing cells have been shown to play a key role in enhancing humoral and cellular immune responses (so-called "vaccinal effects") in different experimental settings.

Keywords: antiviral immunity ; antiviral monoclonal antibodies ; immunotherapy ; FcγR ; vaccinal effect ; immunomodulation

1. Introduction

Monoclonal antibodies (mAbs) have gained an important place in the therapeutic arsenal against severe human diseases. More than one hundred mAbs have been approved for human use and several hundred are currently being tested in clinical trials [1], most of them to treat patients suffering from a variety of cancers or inflammatory diseases. The development of powerful antiviral mAbs has provided new therapeutic opportunities to treat severe viral infections [2][3][4][5][6], including emerging viral infections. Indeed, the amount of antiviral mAbs is rapidly increasing, with two treatments developed and authorized for the Ebola virus and five for SARS-CoV2 during the years 2020–2021, while other mAbs are currently in development to fight other variants of concern (VOC) [7]. Several lines of evidence show that Fc-dependent mechanisms are crucial for efficient antiviral activity of neutralizing mAbs. Thus, beyond their neutralization capacity, mediated by their Fab fragment upon binding to "vulnerable" viral antigens, the antiviral effect of mAbs is also mediated by the Fc moiety through interaction with the complement system and with Fcγ receptors (FcγRs) expressed by multiple cells of the immune system. This can lead to viral clearance by various Fc-dependent functional responses. The Fc domain allows the binding of complement on antibody-opsonized virions, inducing direct virolysis. FcγR and complement receptors (CR) can recognize opsonized virions, leading to their phagocytosis by cells of the innate immune system. Infected cells can also be eliminated by complement dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), and antibody-dependent cell-mediated cytotoxicity (ADCC), the latter being mediated by innate immune effector cells expressing the FcγRs. Fc-FcγR interactions can also directly affect viral propagation by other mechanisms, such as antibody dependent cellular viral inhibition (ADCVI) [8]. In addition, FcγR engagement by antiviral mAbs has been shown to have immunomodulatory effects leading to the induction of protective immunity. Indeed, mAbs can form immune complexes (ICs) with different viral determinants (virions or infected cells) that can be recognized by multiple FcγR-expressing cells leading to the induction of stronger antiviral immune responses and the so-called "vaccinal effect" [9][10]. It is worth noting that Fc-FcγR interactions provide a highly versatile system to modulate immune responses (**Figure 1**). On one hand, there are multiple FcγRs, either activating or inhibitory, which display different affinities for different IgG isotypes. On the other hand, FcγRs are differentially expressed in multiple immune cells, with each of them displaying specific functions.

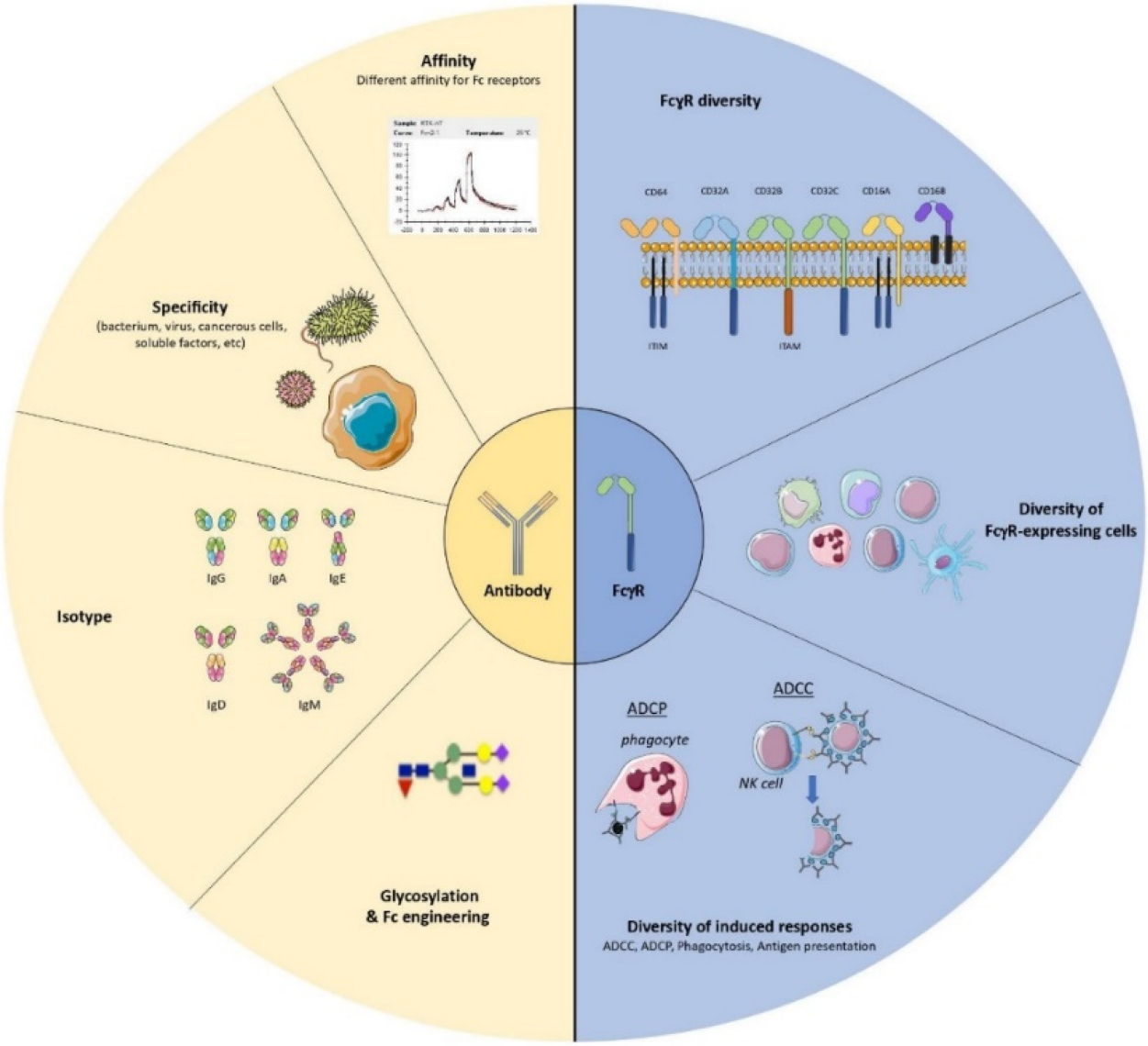

Figure 1. Fc-FcγR interactions constitute a very versatile system to modulate immune responses. The different properties of antibodies (affinity, specificity, isotype, Fc-glyco-engineering, etc.) in addition to the complexity of FcγRs biology (multiple FcγRs, different antibody expression patterns and affinities, multiple FcγR-expressing cells with specific functions, etc.) allow a myriad of immune functions capable of controlling viral spread and modulating immune responses.

2. Multiple FcγR-Expressing Immune Cells Are Involved in the Induction of Vaccinal Effects: Lessons Learned from a Murine Model of Retroviral Infection

The first experimental system to provide mechanistic insight into the generation of protective vaccinal effects by antiviral mAb treatment was the FrCasE retrovirus (a murine leukemia virus, MLV) infection model [9][11]. This model provided the proof of concept that short (five day long) antiviral mAb treatment can induce life-long (more than one year) protective humoral and cellular immunity. This experimental infectious setting has allowed researchers to extensively identify several of the cell types and molecular effectors involved in this process (**Figure 2**).

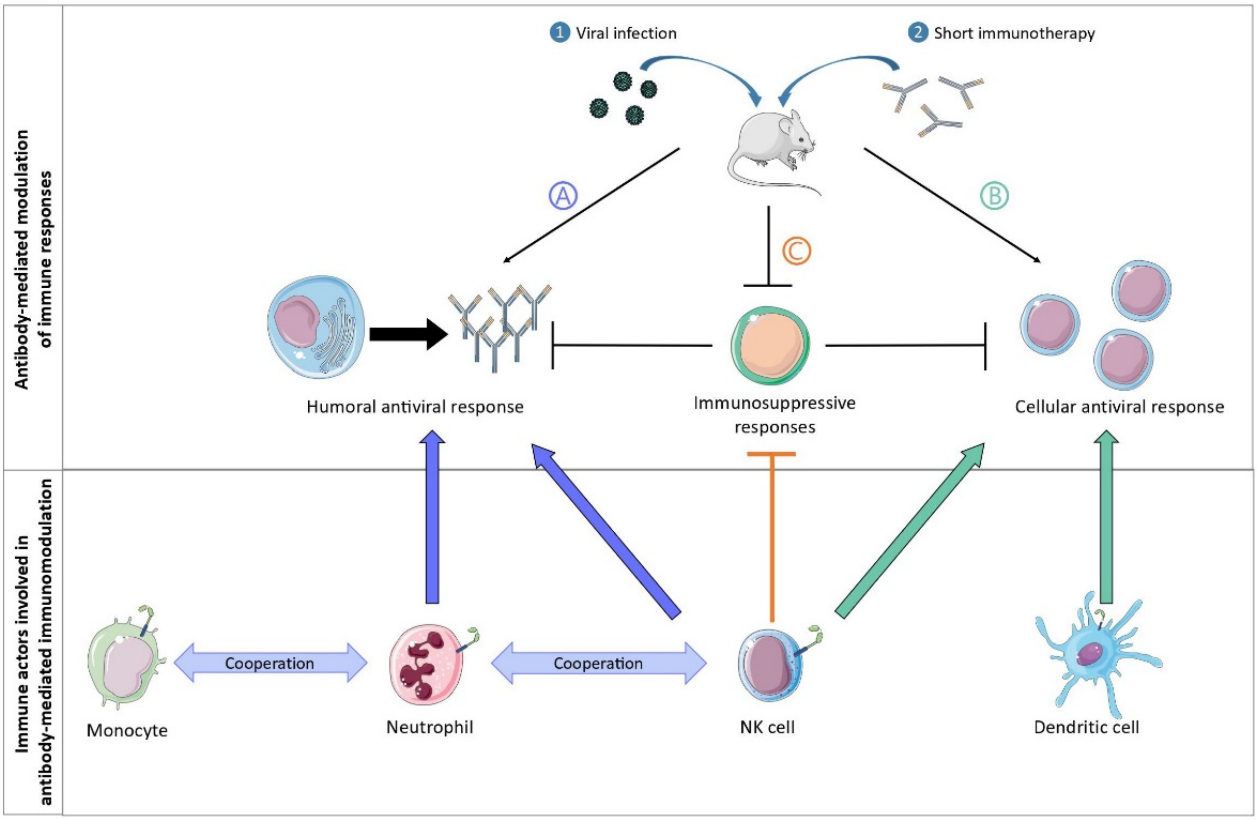

Figure 2. Mechanisms involved in the induction of vaccinal effects. Short treatment of retrovirus-infected mice with a therapeutic monoclonal antibody (mAb) induces a long-term protective response. This is due to (A) the establishment of a humoral antiviral response, (B) the induction of a cellular antiviral response, and (C) the inhibition of immunosuppressive responses (i.e., lack of development of the regulatory T cell response). The mechanisms underlying the induction of protective immunity have been described in this mouse model of a retroviral infection. It has been shown that neutrophils acquire B cell helper functions and are required for the induction of the humoral response (A). Neutrophils also cooperate with monocytes and NK cells to enhance protective immunity. It has also been described that FcγRIV play a key role in the immunomodulatory function of neutrophils and monocytes. Dendritic cells are activated by immune complexes (ICs) formed between the virus and the mAb via their interaction with FcγRs. This results in the enhancement of the antiviral cellular response (B). NK cells are involved in the induction of humoral and cellular responses (A and B), but also, through their ability to control viral spread, they play a role in preventing the development of immunosuppressive immune responses (C) (i.e., the expression of molecules involved in immunosuppressive pathways, such as CD39, PD1, and PD1-L, which are associated with immune cell exhaustion). Thus, several immune cells are involved and may cooperate in establishing a long-term protective immune response.

The most notable effectors, processes and cells types involved in the induction of vaccinal effects are described below:

(i).The vaccinal effects of mAbs strictly depend on Fc-FcγR interactions. In particular, the formation of ICs composed of the administered mAb and infected cells (rather than with virions) enhances the cytotoxic cellular response via the interaction with FcγRs expressed on dendritic cells (DC). These observations also highlighted that the nature of ICs matters to generate protective immunity, as infected cells display immunodominant peptides that are poorly incorporated into virions [12][13];

(ii).MAb treatment prevents the development of the regulatory T (Treg) response in an Fc-dependent manner, with specific antibody isotypes involved in such Treg inhibition [14]. Thus, whereas the administration of anti-FrCasE mAbs of the IgG2a isotype prevented the development of Treg responses in infected mice, neither anti-FrCasE mAbs of the IgM isotype nor $F(ab')_2$ antibody fragment administration had the same effect. However, the mechanisms involved in this Fc-dependent inhibition of the Treg response by the therapeutic mAbs were not elucidated.

(iii).Neutrophils have a crucial role in the induction of a protective humoral immune response during immunotherapy with neutralizing mAbs [15]. The immunomodulatory potential of neutrophils was evaluated by performing neutrophil depletion experiments. These experiments showed that the absence of neutrophils in infected, mAb-treated mice resulted in a decrease in serum levels of specific anti-FrCasE IgGs as well as a decrease in the frequency of marginal zone B cells and plasma cells in the spleen and bone marrow, respectively. Importantly, neutrophils acquired B cell

helper functions upon FcγR-triggering (i.e., secretion of B cell activating factor; BAFF) leading to the induction of a sustained and protective humoral response that was key for the survival of the mice [15].

(iv).Neutrophils and monocytes cooperate in the induction of a protective immune response [16]. Notably, upon antibody therapy, neutrophils and inflammatory monocytes display distinct functional activation states and sequentially modulate the antiviral immune response by secreting Th1-type polarizing cytokines and chemokines, which occur in an FcγRIV-dependent manner. Notably, mAb-treatment of infected mice led to a strong upregulation of FcγRIV in neutrophils and inflammatory monocytes, as well as an enhanced functional activation of both cell types (i.e., upregulation of several activation markers and enhanced secretion of cytokines/chemokines). Interestingly, neutrophils showed a higher and a wider induction of chemokines and cytokines release than monocytes at day 8 p.i, while monocytes secreted strong quantities of Th1-polarizing cytokines and chemokines at day 14 p.i., suggesting a potential role for neutrophils as early drivers of the induction of vaccinal effects by mAbs. In addition, FcγRIV-blocking in mAb-treated mice led to decreased secretion of cytokines and chemokines by both myeloid cell-types, as well as reduced mAb-mediated protection.

(v).NK cells, in addition to their role in the elimination of infected cells, also have a key immunomodulatory role in the induction of a protective immune response after mAb treatment. This was demonstrated using an NK depletion approach that led to the abrogation of the vaccinal effects induced by mAb therapy (i.e., decreased virus-specific antibody titers and $CD8^+$ T cell responses) [17]. The immunomodulatory effects of NK cells are two-fold. Firstly, control of viral propagation by NK cells prevents immune cell exhaustion and the establishment of immunosuppressive mechanisms (i.e., upregulation of molecules involved in immunosuppressive pathways, such as PD-1, PD-L1, and CD39 on dendritic cells and T cells). Secondly, IFNγ-producing NK cells play a role in the enhancement of the B cell responses through the potentiation of the B cell helper properties of neutrophils [17].

Overall, these findings highlight that multiple FcγR-expressing immune cells with specific and complementary functions cooperate to achieve protective immunity upon antibody therapy. This is all the more important to consider as most studies assessing the mechanisms involved in the induction of the vaccinal effect by mAbs mainly point to a role for IC-mediated activation of DC in the enhancement of antiviral T cell responses (reviewed in [10][18]). However, IC-FcγR interactions are not limited to DC, but also concern other FcγR-bearing effector cells of the innate immune system, such as natural killer (NK) cells, neutrophils and monocytes, which have also been shown to participate in the modulation of the antiviral immune response upon mAb treatment.

3. Fc-Mediated Immunomodulatory Properties of mAbs Directed against Human Viruses: Evidence from Mouse, NHP Preclinical Models and Clinical Studies

Table 1 summarizes vaccinal effects reported in preclinical and clinical studies of human viral infections.

Table 1. Vaccinal effects reported in preclinical and clinical studies of human viral infections.

Type of Study	Infection	Ab	Animal Model/Patients	Immune Outcome (Observed Vaccinal Effect)	Mechanism	Reference
Preclinical	Henipaviruses	m102.4	African green monkeys	Humoral responses		[19][20]
Preclinical	Influenza virus	3C05 (GAALIE variant)	Transgenic FcγRs humanized mice	$CD8^+$ T cell responses	Dendritic cell activation	[21]

Type of Study	Infection	Ab	Animal Model/Patients	Immune Outcome (Observed Vaccinal Effect)	Mechanism	Reference
Preclinical	SARS-CoV-2	COV2-2050	Mice and hamsters	Increased numbers and more activated $CD8^+$ T cells. Decreased inflammation	Potential monocyte involvement in decreasing inflammation	[22]
Preclinical	SHIV-SF162P3	PGT121/3BNC117/b12 mAb cocktail	Rhesus macaques *(Macaca mulatta)*	Increased frequencies and decreased exhaustion of Gag-specific $CD8^+$ and $CD4^+$ T cells		[23]
Preclinical	$SHIV_{AD8-EO}$	3BNC117 and 10–1074	Rhesus macaques (*Macaca mulatta*)	Polyfunctional $CD8^+$ T cells		[24]
Clinical	HIV	3BNC117	Viremic and aviremic subjects on antiretroviral therapy (ART)	Humoral response		[25]
Clinical	HIV	3BNC117 and 10–1074	HIV-1-infected individuals and ART interruption	Virus-specific T cell immunity		[26]

4. Induction of Vaccinal Effects by mAbs in HIV-Infected Patients

The development of powerful anti-HIV-1 bNAbs, able to efficiently control viral propagation and to enhance adaptive immune responses in multiple preclinical models of HIV-1 infection, provided the rationale for studying their capacity to induce vaccinal effects in HIV-1 infected patients. Importantly, the enhancement of both humoral and cellular immune responses by bNAbs has been reported in HIV-1 infected patients [25][26] (**Table 1**). Schoofs et al. [25] demonstrated that the administration of the therapeutic antibody 3BNC117 to HIV-1 infected patients enhanced infected individuals' humoral response against the virus during a six-month observation period. Most bNAb-treated, HIV-1-infected patients showed improved antibody responses (with increased breadth and/or potency) to heterologous tier 2 viruses. Furthermore, the elicitation of anti-HIV-1 antibodies occurred in both viremic and aviremic subjects on antiretroviral therapy (ART). By contrast, untreated individuals showed no consistent improvement in their neutralization capacity, neither qualitatively nor quantitatively.

More recently, Niessl et al. [26] showed that anti-HIV-1 antibody therapy is associated with increased virus-specific T cell immunity. In a phase 1b clinical trial, HIV-1-infected individuals on ART were infused with a combination of two bNAbs (3BNC117 and 10–1074) at zero, three and six weeks, followed by temporarily stopping ART (analytical treatment

interruption; ATI) two days after the first antibody infusion. Individuals who were infected with HIV-1 and on ART without antibody therapy showed stable or decreasing levels of HIV-1-specific $CD8^+$ and $CD4^+$ T cell responses over time. In contrast, bNAb-treated patients under ATI showed improved HIV-1 Gag-specific $CD8^+$ T cell responses (with significantly increased frequency of antigen-specific $CD8^+$ T cells expressing IFN-γ, TNF-α, MIP1-β and/ or CD107A) as well as enhanced $CD4^+$ T cell responses (with increased frequency of $CD4^+$ T cells expressing IFN-γ, CD40L, TNF-α and/ or IL-2 in response to Gag). Importantly, enhanced $CD8^+$ and $CD4^+$ T cell responses to Gag were associated with viral suppression for at least fifteen weeks following ATI. However, whether or not these augmented T cell responses contributed to bNAb-mediated viral control was not elucidated.

These results highlight the potential of HIV-1 bNAbs in boosting adaptive immune responses. The elucidation of the mechanism involved and whether such enhanced immune responses might lead to long-term protection in HIV-1 infected patients is now an important issue to address, as it will be key to enhancing the therapeutic efficiency of bNAbs. Several hypotheses have been put forward to explain how antibody therapy boosts the emergence of humoral and cellular immune responses. In particular, it has been hypothesized that the formation of ICs with the therapeutic antibodies and viral determinants, via the engagement of FcγRs on DCs, can lead to enhanced antigen uptake and presentation resulting in the induction of improved antiviral responses. While this hypothesis has been confirmed in in vitro and in vivo mouse studies as detailed above, whether or not this mechanism is involved in bNAbs-treated, in HIV-1-infected patient is still not known.

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