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Exploring the Potential of Monoclonal Antibody Therapeutics for HIV-1 Eradication

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Abstract

The HIV field has seen an increased interest in novel cure strategies. In particular, new latency reversal agents are in development to reverse latency to flush the virus out of its hiding place. Combining these efforts with immunotherapeutic approaches may not only drive the virus out of latency, but allow for the rapid elimination of these infected cells in a ''shock and kill'' approach. Beyond cell-based approaches, growing interest lies in the potential use of functionally enhanced ''killer'' monoclonal therapeutics to purge the reservoir. Here we discuss prospects for a monoclonal therapeutic-based ''shock and kill'' strategy that may lead to the permanent elimination of replication-competent virus, making a functional cure a reality for all patients afflicted with HIV worldwide.

Introduction

HIV CARE HAS EXPERIENCED a dramatic revolution over the past decade due to new evidence that a cure for HIV-infected patients may be possible. Up to now the Berlin patient is the only known instance of functional viral eradication.[1] However, several additional suggestive cases have been reported in Paris[2] and in a cohort of macaques in Portland.[3] However, the specific mechanism(s) by which these unique cases achieved this ''functional cure'' state is incompletely understood, but may hold the key to generalizing this phenomenon globally.

Following acute infection, HIV establishes a latent reservoir in $CD4^+$ T cells and other immune cells. Because latency is linked to transcriptional silencing of the integrated provirus, several classes of latency reversal agents (LRA) have now been tested or considered as a mechanism to potentially derepress the latent reservoir. These include histone deacetylase inhibitors (HDACi) such as vorinostat, panobinostat, and romidepsin[4–6]; disulfiram, involving nuclear factor-κB and the bromodomain-containing protein 4 inhibitor[7]; JQ1, which functions through the positive transcription elongation factor[8]; and protein kinase C (PKC) agonists such as phorbol esters, prostatin,[9] and bryostatin-1.[10–12] In addition, other activators have been considered to draw the reservoir out of hibernation, including T cell activators and TLR agonists.[13]

Interestingly, *ex vivo* treatment of primary peripheral blood mononuclear cells from long-term highly active antiretroviral therapy (HAART)-treated patients with HDAC has shown reliable reactivation of cell-associated viral RNA[4,6,14–19] but a controversial capacity to induce infectious virion release.[6,8,15,20–22] *In vivo*, LRAs have been tested showing more limited effects,[5,6,23] prompting further investigation into different LRAs and combination treatment approaches to enhance viral reactivation. However, despite the accelerating momentum in the field geared toward the discovery of agents able to reactive latent virus, it is less clear how these reactivated viruses/cells can be permanently cleared from the system to make a cure a reality. Specifically, it has become clear that the reactivation of virus alone, even in the presence of HAART to prevent bystander cell reinfection, will not lead to permanent eradication of cells.[24] Thus, additional therapeutic interventions are likely required to rapidly deplete cells that are reactivated. Therefore, eradication efforts are now centered around a ''shock and kill'' strategy[25] aimed at driving the virus out of latency, followed by rapid removal of these infected cells.

Post-treatment Control

Several studies have shown that treatment during acute HIV infection is associated with a lower HIV reservoir size[26–30] as well as a lower viral set-point after cessation of therapy.[31,32] Moreover, isolated cases of post-treatment control have been described with subjects undergoing scheduled treatment interruptions[33] aimed at auto-vaccinating individuals with their own virus, including a case reported in 1999 in which a patient in Berlin was placed on HAART during acute/early infection and gained long-lived control of his

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virus after stopping therapy at 176 days postinfection.[34] However, later analysis showed that this subject possessed the protective human leukocyte antigen B57, naturally associated with spontaneous control, calling into question whether this individual would have controlled viral replication spontaneously even in the absence of a scheduled treatment interruption.[35]

However, the VISCONTI study group showed a more generalizable success. Fourteen patients ($\sim$15%) treated for 36 months following acute HIV infection were identified who controlled their own viremia for 24 months following treatment cessation.[33] Interestingly, none of these subjects exhibited protective HLA-class I alleles, arguing that control was unlinked from any previously defined host-genetic marker associated with natural robust control of viremia. However, unlike HIV controllers, post-treatment controllers had limited HIV-specific CD8 T cell responses and lower levels of CD8 T cell activation, arguing that other arms of the immune response may have collaborated or contributed to the ability of these subjects to gain control over their viral infection.

Shock and Kill

T cells

Among the potential shock and kill strategies, activated T cells have recently been shown to efficiently kill reactivated cells,[24] suggesting that a therapeutic vaccination approach able to boost cytotoxic T cell activity may help promote the eradication of the pool of latently infected cells. Along these lines, the SIV protein-expressing rhesus cytomegalovirus (RhCMV/SIV) vector drove progressive clearance of the virus, despite initial infection with the pathogenic SIVmac239 strain.[3] This CMV vaccine-mediated eradication was linked to the elevated and durable induction of effector memory T cell responses that were maintained at persistently high levels due to the continual replication of the vaccine vector. These data strongly suggest that a T cell-based ''shock and kill'' strategy will likely require the induction and sustenance of high levels of killer effector T cells. However, T cell-based strategies are limited by potential archived viral escape mutants, with potential irreversible T cell exhaustion resulting in compromised killing activity, qualitative differences in vaccine-induced immunity by polymorphic major histocompatibility complex (MHC) class I alleles, and issues related to T cell homing to sites of viral latency.

Moreover, to date, therapeutic vaccine efforts have focused on inducing cytotoxic T cell responses; however, most have shown a limited capacity to impact viral rebound meaningfully.[36] Previous efforts have included whole inactivated virus or recombinant proteins (gp120). Conversely, more recent approaches included vectors such as DNA, recombinant virus, such as canary pox (ALVAC) or adenovirus, or antigen-loaded dendritic cells; the latter resulted in prolonged viral control of up to 48 weeks and a drop in viral load compared to pre-antiretroviral treatment.[36,37] Thus, with the exception of the DC-based study, the majority of therapeutic vaccine approaches have shown limited prolonged viral control, begging the question as to whether an exhausted immune system will be able to induce *de novo* immune responses able to drive a functional cure.

Natural killer cells

Interestingly, beyond T cells, other innate immune cells have also been considered as potential target effector cells for a ''shock and kill'' strategy, including natural killer (NK) cells, due to their inherent cytolytic capacity in the absence of any requisite antigen sensitization.[38] These strategies aim to take advantage of the natural stress ligands (MHC class I polypeptide-related sequence A-MICA, MICB, or the UL16-binding protein 1—ULBP1, ULBP2, or ULBP3) that activate NK cell killing through a dominant activating NK cell receptor, NKG2D, critically implicated in tumor cell elimination.[39–41] However, as in the setting of tumors, subjects with HIV exhibit high levels of serum MICA, which reduces NKG2D expression on systemic NK cells, resulting in attenuated NKG2D-mediated activation of NK cells, even in the setting of long-term HAART treatment.[42] Thus, HIV infection may result in an irreversible defect in NK cell activity, which may limit the utility of these innate effector cells in direct recognition and lysis of reactivated/infected cells.

Monoclonal antibodies

Conversely, beyond direct cellular-based mechanisms, antibodies (Abs) are also able to induce the rapid destruction of material to which they are bound by directing the cytotoxic and antiviral activity of the innate immune system. Moreover, this immunological activity has been widely exploited by the monoclonal antibody (mAb) therapeutics community for the rapid and effective clearance of tumor[43–45] or autoimmune cellular targets.[46] This large body of literature provides critical strategic insights into how a similar approach can be developed for HIV eradication. Importantly, these monoclonal therapeutics mediate their lytic activity through the recruitment of specific sets of innate immune cells, through Fc receptors, complement, or lectin-like innate immune receptors, aimed at rapidly and effectively eliminating target cells throughout the body. Therefore, a mAb therapeutic strategy may contribute to the ''kill'' in a ''shock and kill'' strategy to support T cell-mediated clearance or offer an alternative strategy to drive a functional cure (Fig. 1).

Viral Protein Targets

Like T cell escape, which may limit the utility of $CD8^+$ T cell-mediated viral eradication strategies, the virus may have historically also escaped antibody (Ab)-mediated immune pressure. However, a mAb footprint is remarkably different from one targeted by a T cell, which may be much larger, broader, and more flexible in binding to its epitope even in the setting of escape. Many neutralizing and non-neutralizing HIV-specific monoclonals, targeting diverse areas of the virus, have now been cloned that cover HIV strains with remarkable breadth, via the targeting of highly conserved epitopes across global viral quasispecies. Importantly, the selection of the best mAb or mAb cocktail may not depend only on the mAb's capacity to recognize HIV broadly, but may also relate to the kinetics of the mAb's epitope expression on infected cells.

The mature HIV-1 envelope (Env) spike is composed of trimeric surface gp120 that is noncovalently bound to trimeric transmembrane gp41. Importantly, while the emerging Env spike first appears on the cell surface as a trimer,[47]

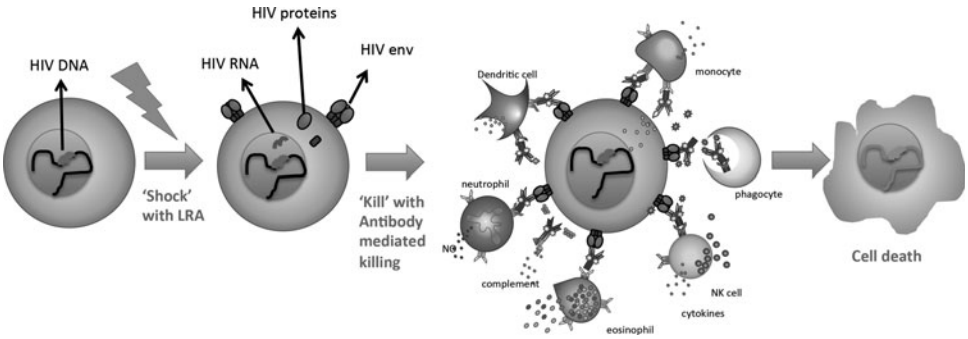

FIG. 1. Shock and kill approach to eliminate latently infected cells using antibody-targeted killing. Resting, latently infected $CD4^+$ T cells can be ''shocked'' by various latency reversal agents (LRA). This will lead to the expression of viral RNA and proteins, including expression of Env on the infected cell surface. Exogenously administered antibodies directed against epitopes on Env will then recruit various innate effector cells to kill off infected cells in the presence of HAART to prevent further infections.

several lines of evidence suggest that Env epitopes are exposed at different times during Env maturation on the surface of virion-producing cells and on the surface of released virions.[47–49] These changes in exposed structures are related to the transient association of the trimeric complex as well as the transient nature of the association between gp120 and gp41, resulting in the production of variable amounts of trimer, monomer, and gp41 alone on the surface of infected cells. Thus, it is likely that differences in epitope exposure on the surface of virion-producing cells are directly related to the kinetics of the structure of the HIV Env expressed on the surface of infected cells over the course of cellular infection.[50–52] Specifically, early after infection, as virions begin to assemble and release from the surface of cells, surface-exposed Env proteins are likely mostly present in a trimeric form prior to virion release.[47]

By contrast, as cells become exhausted, due to large-scale virion production, the remaining unincorporated Envs likely dissociate into monomeric Envs and gp41 alone, resulting in an accumulation of nonfunctional ''spikes'' on the surface of the cell (Fig. 2). Furthermore, the natural ligand for Env is CD4, which when bound results in conformational changes in the epitopes exposed on gp120.

The CD4 expression levels, in cis, can therefore profoundly modify the epitopes expressed on a given target cell, resulting in the exposure of CD4-inducible epitopes (CD4i).[52,53] Thus, while the most potent neutralizing mAbs will recognize the earliest infected cells, mAbs that recognize gp41 alone may mark a greater number of cells for longer periods of time, as gp120 molecules dissociate readily, leaving gp41 stumps on the surface of cells. Therefore the ultimate selection of the optimal mAb(s) for eradication will likely be guided by the HIV-envelope targets that may be differentially enriched on the surface of cells following reactivation.

Beyond Env-specific targeting approaches, a few studies have observed an enrichment of HIV Gag-specific[54] and other regulatory/accessory protein-specific antibodies[55] in subjects who durably control HIV infection in the absence of therapy. These antibodies against non-Env targets have been shown to mediate ADCC.[55–58] Yet, because Gag is not expressed on the surface of infected cells, it is unclear whether these antibodies contribute to direct lysis of infected cells or whether these antibodies act as surrogates of a more potent humoral immune profile. Along these lines, subjects who selectively generate elevated Gag-specific antibodies also generate the more abundant gp120- IgG3 antibody subclass, known to have the greatest antiviral activity. Nevertheless, if expressed on the infected cell surface, additional viral targets may represent additional targets for the eradication of the reactivated reservoir.

Interestingly, HDACi-mediated reactivation is associated with the transcription of detectable levels of RNA within primary cells[14] and the release of infectious virions.[6,8,15,20] Because the production of virions requires viral protein

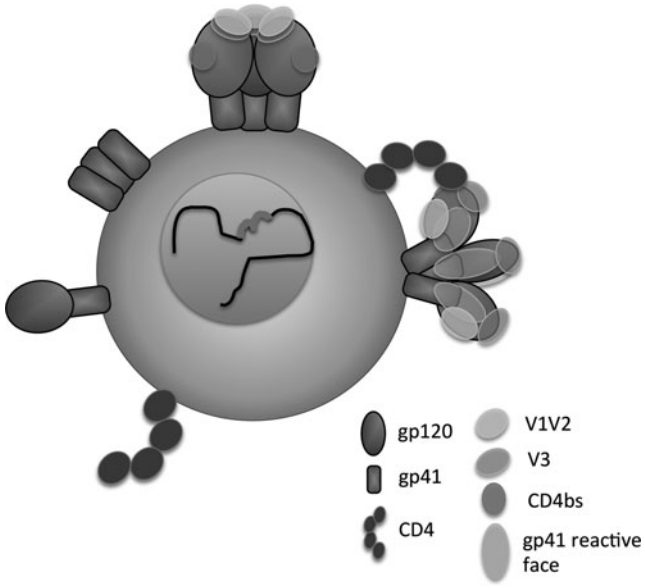

FIG. 2. Potential forms of Envelope on the HIV-1-infected cell membrane. Gp41 and gp120 are shown. From *left to right*: gp120/gp41 monomers begin to form less trimers in mid-infection; naked gp41 after gp120 shedding may occur during late stage infection; functional Env trimers form early in cellular viral infection exposing epitopes for neutralizing antibodies; Env trimers interact with CD4 on virally infected cells that change the conformation of the trimer exposing CD4-induced nonneutralizing epitopes; V1V2 move to the side, exposing V3 and the HR1 region from gp41.

production for the formation of virions, we speculate that newly translated Env proteins could serve as targets for HIV-1-specific mAbs if expressed on the cell surface. Along these lines, previous studies using anti-CD3/28 and interleukin (IL)-7 treatment resulted in Env expression,[59] supporting the possibility that this target can be induced. However, it remains unclear to what extent Env may be expressed on LRA-reactivated cells, and specifically which Env epitopes may be expressed most broadly on reactivated cells. Interestingly, in *an in vitro* resting latency model, negligible levels of Env were detectable in resting cells[59] and *ex vivo* virion production with LRAs remains elusive,[22] suggesting that much work is necessary to define whether Env is a viable target and consequently which epitopes may serve as the best targets.

Yet, given the remarkable affinity of many of the most potent HIV-specific mAbs, their breadth of viral quasispecies recognition, and the emergence of novel LRAs, it is likely that even low level Env production may be sufficient to rapidly label and mark a cell for rapid destruction upon reactivation. Thus, collectively, just as the antiretroviral therapy (ART) field worked toward the optimal combination of drugs to block viral replication, great strides are anticipated in the cure field to define the most effective LRA combinations to reverse latency *in vivo.*

Monoclonal Antibodies to HIV

Current research efforts in identifying new epitopes for both neutralizing[60,61] and nonneutralizing antibodies[62] against viral surface proteins have produced a plethora of monoclonal antibodies. These antibodies are directed against various conserved epitopes covering the vast array of circulating Envs with varying genetic diversity among different HIV clades. These sites include the CD4-binding site, the V1V2 region, glycans on the V3 loop, the membrane proximal external region (MPER) on the gp41, a newly identified glycan site on gp41,[63,64] and a recently identified trimer-specific epitope spanning the gp120 and gp41 proteins.[65] As discussed above, depending on the stage of viral infection of the CD4 T cells and viral reactivation, various epitopes may be exposed that are not necessarily present on the functional Env trimer. Some of the non-neutralizing sites include the CD4-induced epitopes,[66] which can be divided into three portions: cluster A, the gp120 C1 conformational epitope,[67] which is subject to immune escape early in infection[68]; cluster B, a region proximal to the coreceptor-binding site, which involves the V1V2 region; and cluster C, the coreceptor-binding site.[66]

Other non-neutralizing epitopes include the gp120 C5 region and cluster I and II on gp41.[62] Interestingly, emphasis has been placed on particular antibody specificities for both neutralizing and non-neutralizing antibodies related to their (1) conservation across global quasispecies and/or (2) exposure on trimers and linked involvement in key steps of viral attachment/entry.[60] Likewise, the most desirable non-neutralizing mAbs are typically associated with epitopes on highly exposed or induced regions of the virus that result in more effective labeling of the virus or virally infected cells.[62]

Anatomical Sites of the HIV Reservoir

Latently infected cells may reside in multiple compartments, including the blood and tissues,[69–71] where there is suboptimal penetration of antiretroviral drugs and likely variable access by antigen-specific T cells. By contrast, mAbs diffuse more freely and can be modified to gain access to immune privileged sites.[69] Moreover, mAbs can also be engineered to specifically recruit particular populations of innate immune cells, which are differentially distributed in distinct patterns in various tissue compartments (discussed below). For example, while NK cells and neutrophils are abundant in the blood,[72] they represent only a small fraction of tissue-resident innate immune cells. Instead, macrophages are abundantly represented in the gut, brain, and lymphoid tissue.[73] Therefore, HIV-specific mAb-based therapeutics aimed at targeting and killing the reservoir may preferentially aim to recruit macrophage-mediated killing rather than NK cell or neutrophil activity. Moreover, because innate immune cells express different combinations of Fc receptors, complement receptors, and/or lectin-like receptors, specific modifications can be generated to the Fc end of HIV-specific mAbs of interest to tailor a killer monoclonal therapeutic strategy to specifically kill targets as effectively as possible within tissue resident sites; these will be discussed below.

Engineering Monoclonal Function

An antibody can be divided into two relatively artificial functional domains, including the two antibody-binding arms that form the antigen-binding domain (Fab) and the constant domain (Fc).[74] While the Fab is responsible for the antigen specificity of the molecule, and consequently neutralization potential,[60] the Fc domain is responsible for delivering instructions to the innate immune system on how it should destroy anything to which that antibody is bound[75,76] (Fig. 3). Thus, contrary to its name, the constant domain is a highly variable structure that changes both in protein sequence and glycosylation, resulting in more than 120 different states, each of which could theoretically induce disparate antibody effector functions.[77] These functions include antibody-dependent cellular cytotoxicity (ADCC) largely mediated via FcγRIIIa on NK cells, antibody-dependent cellular phagocytosis (ADCP) mainly mediated by FcγRIIa on monocytes, or complement-dependent cytotoxicity (CDC) mediated via circulating C1q or mannose-binding lectin (MBL) as discussed in detail later.

Critically, while great emphasis has been placed on the neutralization of the virus, in the setting of prophylactic vaccine or therapeutic intervention design, these broadly recognizing mAbs can also be harnessed as ''effector'' antibodies to identify and kill infected cells via the recruitment of innate effector cells through their Fc receptors. Likewise, recent animal studies have shown that broadly neutralizing antibodies are indeed effective therapeutically.[78–81] In a human primate study, 3 out of 18 monkeys that exhibited the lowest viral loads prior to treatment exhibited prolonged virological control after the animals were treated with a PGT121-containing mAb cocktail and virus was cleared from the blood. However, while these mAbs cleared systemic virus transiently, the mAbs were unable to eradicate the reservoir in the majority of animals. Moreover, all animals, including the three that exhibited prolonged viral containment, still had detectable proviral DNA in tissue.

This inability to ''cure'' was likely related to the fact that these mAbs effectively complexed or ''trapped'' virus,

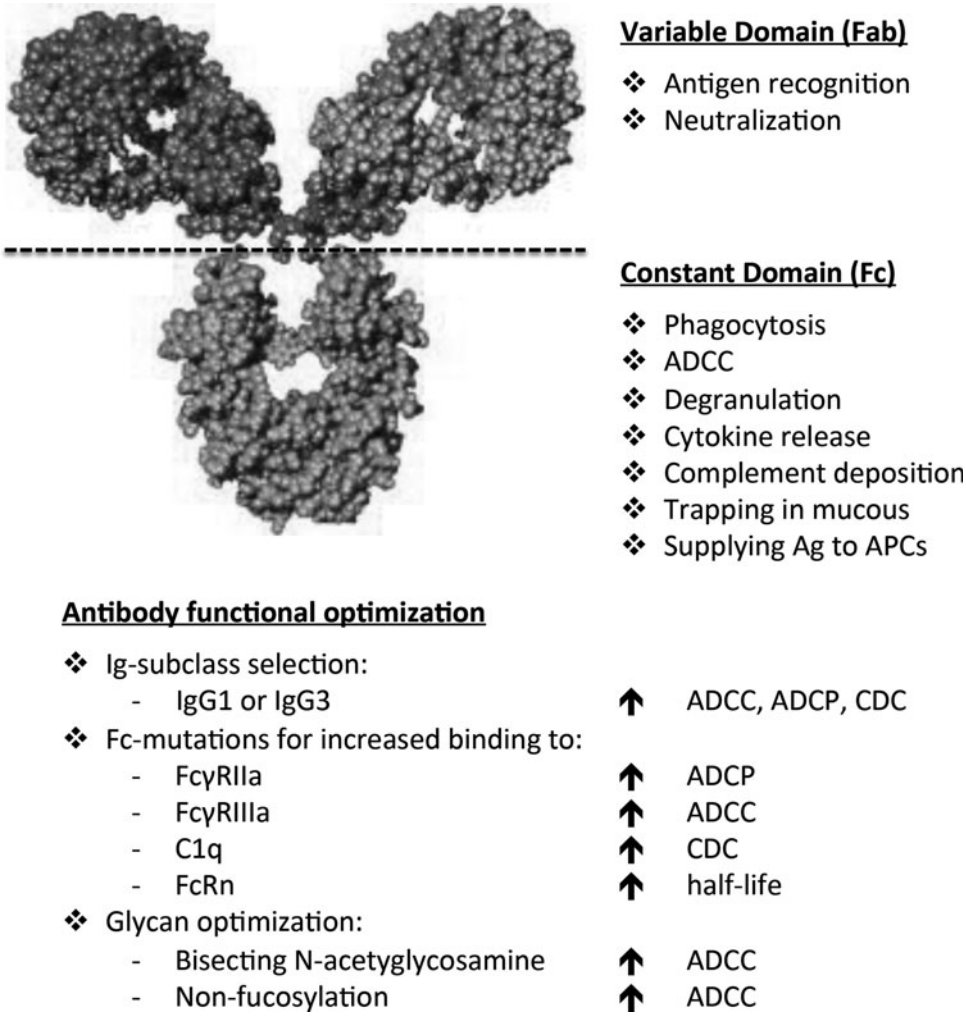

FIG. 3. Antibody functions and optimization related to structure. IgG comprise identical pairs of heavy and light chains. Antibodies have a highly selective variable antigen-binding domain (Fab) and a constant domain (Fc) that mediate various functions as described. Engineering of the IgG constant domain allows modulation of effector functions: antibody-dependent cellular cytotoxicity (ADCC) largely mediated via Fc(RIIIa on NK cells, antibody-dependent cellular phagocytosis (ADCP) mainly mediated by Fc(RIIa on monocytes, or complement-dependent cytotoxicity (CDC) mediated via circulating C1q or mannose-binding lectin (MBL), or increased half-life through increased binding to the neonatal Fc receptors (FcRn).

resulting in the elimination of the virus from the circulation, but limited elimination of infected cells. Interestingly, in a recent humanized mouse study, a ''shock and kill'' approach was applied in treated, HIV-infected mice. In mice treated with multiple LRAs as well as monoclonal antibodies there was decreased time to viral rebound compared to mice receiving LRAs alone. Furthermore, this study demonstrated that the therapeutic potential of these monoclonal antibodies was heavily dependent on Fc effector function, as the delivery of monoclonal antibodies with nonfunctional Fc domains exhibited limited viral containment.[82] Thus, next generation mAb therapeutic strategies, including neutralizing mAbs or non-neutralizing mAbs that can trap and mop up free virus will likely require some effort in engineering to also recruit innate immune cells to destroy not only the virus, but also the cells to which they are tethered.

These additional functions can be mediated through both soluble and cellular factors, including innate effector cells such as NK cells, monocytes, macrophages, dendritic cells, mast cells, neutrophils, eosinophils, and B cells, which express one or several of a class of Fc receptors (FcRs) as well as complement receptors, and/or innate immune lectin-like receptors that can interact with antibody-opsonized immune complexes.[83,84] These interactions depend on their particular binding affinities and different physical and functional properties, suggesting that all innate immune cell subsets have the potential to be recruited by a monoclonal therapeutic, given the correct modifications. While only a fraction of effector functions have been exploited in the monoclonal therapeutics field, a number are discussed below.

Antibody-dependent cellular cytotoxicity (ADCC)

ADCC occurs when antibody-opsonized antigen-expressing target cells trigger FcγRIIIa activation on an effector cell, resulting in rapid cytokine release, degranulation, and eventual lysis of the target cell.[85–88] FcγRIIIa is mostly expressed on NK cells and neutrophils, but other cell types such as macrophages, and eosinophils also express this receptor at low levels.[89,90] As NK cells are mostly present in the circulatory system, they may play a critical role in killing recently reactivated CD4 T cells in the blood, while tissue-specific killing may rely on other mechanisms. Several neutralizing and non-neutralizing HIV-specific mAbs have been tested in various ADCC assays. This is extensively reviewed by Pollara *et al.*[62] As epitope availability can vary from time of infection to viral release or reactivation (as discussed above), different antibodies may be relevant for prevention as opposed to newly reactivated cells. These antibodies need to be tested in relevant latency assays to determine which

epitopes will be most relevant for killing reactivated cells via ADCC.

Antibody-dependent cellular phagocytosis (ADCP)

Besides direct lysis, target cells can also be phagocytized by monocytes, macrophages, and DCs that express FcγRIIa or FcγRIIb, resulting in the rapid clearance of antibody-opsonized material.[84,91–94] Interestingly, depending on the affinity ratio of FcγRIIa:FcγRIIb with which the immune complex binds on a given innate immune effector cell, phagocytosis may direct immune complexes to vastly different intracellular compartments resulting in either highly inflammatory or attenuated immune responses.[95] Macrophages reside in tissues, including the gut and brain, where reservoirs are likely to reside and therefore represent a critical effector cell that may induce rapid elimination of reactivated cells.

Complement-dependent cytotoxicity (CDC)

Beyond FcRs, the antibody Fc domain interacts with innate proteins including complement proteins C1q (classical complement pathway), mannose-binding lectin (nonclassical), and C3b or C4b, which are further downstream in the complement pathways. Binding of the antibody Fc to these complement pathway components may trigger the rapid recruitment and activation of complement resulting in the formation of the membrane attack complex and lysis or phagocytosis of the antibody-opsonized cell/virus.[96–100] Because of the abundance of complement activating C1q and MBL in tissues and the circulation, where they induce phagocytic clearance or cytotoxicity, respectively, CDC has been exploited broadly by the oncology-targeting monoclonal therapeutic community.[101–103] Thus, CDC activity therapeutics represent a unique and broad acting targeted effector function that may drive rapid clearance of reactivated cells in multiple tissue compartments.

Therapeutic Monoclonal Antibodies and Mode of Action

The advantages of using killer mAbs in a cure strategy are many fold. The potential of mAbs is most evident in the cancer, autoimmunity, or inflammation field, where as of May 2014, 43 monoclonals have been approved or are in review for human therapy[104] (www.landesbioscience.com/journals/mabs/about/) in the United States and Europe. While some of these antibodies work by antagonizing specific receptors, for instance inflammatory targets, such as tumor necrosis factor (TNF) in rheumatoid arthritis, several function through the recruitment of the innate immune system to destroy the cells to which they are tethered through Fc-mediated effector functions. The best known of these is the anti-CD20 drug (rituximab) for chronic lymphocytic leukemia, whose proposed mechanism of action is through ADCC and CDC.[43,103,105,106] Interestingly, it was shown that patients treated with rituximab exhibited differential therapeutic efficacies depending on the presence of a specific polymorphism in FcγRIIIa[106,107] (necessary for ADCC) indicating the crucial role of Fc-mediated functions in tumor clearance/control. These same polymorphisms have been shown to also play a role in antibody-mediated therapies to other cancers such as colorectal and breast cancer.[108–110] Moreover, antibody engineering efforts have overcome these differential outcomes via the generation of mAbs that bind with high affinity to both FcγRIIIa variants.[111–116] However, next generation antibody engineering approaches have significantly broadened the landscape of potential antibody effector mechanisms that may be harnessed with these potent, antigen-specific therapeutics.

Next Generation Monoclonal Antibodies

Many natural as well as artificial Fc engineering strategies have been utilized to tune and promote more effective Fc effector function. These include differential subclass selection, altered Fc glycosylation, and/or generation of subclass Fc mutations with differential affinity for particular Fc receptors (Fig. 3).

Subclass

Although all FcRs can bind immune complexes, there is a bias among activating and inhibitory receptors for particular IgG isotypes/subclasses.[117] Moreover, antibody affinity is directly related to the potency of biological effector activity. For instance, ADCC, which is largely recruited through FcγRIIIa, is driven most effectively by IgG3 > IgG1 > IgG4 > IgG2, which coincides with the hierarchy of subclasses affinity for FcγRIIIa.[75,118–120] Similarly, phagocytosis, largely mediated through FcγRIIa on monocytes, is driven by IgG3 > IgG1 > IgG2 > IgG4.[121] Although IgG3 exhibits the highest functional activity, it also has the shortest half-life at 1 week, compared to 3 weeks for other subclasses.[122] Thus, most therapeutic antibodies are generated as IgG1s. Conversely, for the treatment of some autoimmune conditions, therapeutic antibodies with limited effector function are desired, and therefore generated as IgG4s.[123] Other subtypes include IgA, which binds to the FcαRI on myeloid cells and is especially good at inducing neutrophil-mediated tumor cell killing.[124–126]

IgG subclass-associated Fc-mediated effector functions have been recently linked to reduced risk of HIV infection following vaccination.[127] Interestingly, reduced risk of infection was associated with the selective induction of HIV-specific IgG3 antibodies that exhibit a polyfunctional profile, able to simultaneously recruit ADCC, ADCP, and NK degranulation, potentially providing enhanced protection from diverse modes of HIV acquisition through a broader capacity to recruit a variety of innate immune effector cells. Similarly, spontaneous control of HIV infection is associated with the selective induction of gp120-specific IgG3 antibodies,[54] which normally decline following acute infection in non-controllers.[128] These data strongly argue that IgG3 antibodies, despite their short serum half-life, represent a potent Fc modification able to rapidly control and clear HIV-infected cells. However, subclass alone does not mediate FcγR binding and function.

Glycosylation

Beyond subclass selection, all antibodies are glycosylated at a single N-linked glycosylation site at asparagine 297 of the CH2 domain.[75,120] Moreover, this glycan changes rapidly in the setting of inflammatory conditions, with age, in pregnancy, following vaccination, and following infection.[127,129–132]

However, the critical nature of the antibody glycan is most clearly illustrated following antibody deglycosylation, which abrogates all FcR and complement binding.[75] Glycosylation is therefore essential not only for antibody folding/secretion, but also for effector functions.

Moreover, significant advances have been achieved in our understanding of the role of monoclonal therapeutics in tuning antibody functionality through changes in antibody glycosylation. For example, removal of a fucose at the base of the biantennary structure results in enhanced binding to FcγRIIIa and therefore in potentiated cytotoxic activity,[111–116] resulting in improved clinical efficacy of a large number of monoclonal therapeutics.[43] More recently, a second modification, the addition of a bisecting *N*-acetyl-D-glucosamine (GlcNAc), was introduced in an anti-CD20 antibody, resulting in extraordinary clinical improvement of CLL treatment and remission rates, likely attributed to enhanced ADCC as well as other antibody effector functions.[44,45]

Interestingly, mutations abrogating Fc effector functions in a neutralizing mAb, b12, resulted in the abrogation of the sterilizing protection from SIV acquisition in nonhuman primates.[133] These data stress the importance of Fc effector function in protection from HIV.[133] In a follow-up study, using an afucosylated form of b12, which should drive enhanced ADCC, demonstrated limited improvement in antibody protective efficacy in a vaginal challenge model.[134] However, FcγRIIIa+ NK cells are necessary for ADCC, which are limited at mucosal barriers,[135] including the vaginal walls. This most likely resulted in the limited protection of these antibodies *in vivo*. Conversely, because other innate immune cells, such as macrophages, are highly abundant at the sites at which HIV is transmitted, it is plausible that delivery of an HIV-specific mAb with engineered modifications aimed at recruiting phagocytic cells may provide enhanced protection from infection. Thus, tuning antibody glycosylation to harness relevant innate immune effector cells represents an untapped opportunity in HIV-specific monoclonal therapeutic design.[136]

Fc mutation optimization

Beyond natural changes in subclass and glycosylation, the monoclonal therapeutics community has identified a large array of point mutations in the Fc domain of an antibody that results in differential affinity for distinct innate immune receptors, including Fc receptors[137,138] and complement.[139] These single or multiple Fc mutations have been tailored to specifically alter binding to single, or combinations of Fc receptors to drive specific functional profiles. For example, the G236A mutation results in the selective binding of an antibody to the activating FcγRIIa versus the inhibitory FcγRIIb, both expressed on monocytes and macrophages, resulting in enhanced phagocytosis.[137]

In contrast, the S239D:E330L:I332E mutation specifically drives preferential binding to FcγRIIIa on NK cells,[137] aimed at increasing ADCC potency up to $\sim$100-fold.[138] Moreover, several mutations have been identified that result in enhanced binding and recruitment of complement, boosting complement activity by $\sim$600-fold, which have been widely exploited on several anti-CD40 and anti-CD19 variants.[139] Similarly engineered Fc variants have been generated in the CD4-binding site-specific mAb, b12,[140] resulting in increased ADCP and ADCC activities in an HIV-specific manner, and can be easily extended more broadly to panels of HIV-specific monoclonal therapeutics aimed at selectively harnessing specific antiviral functions of the innate immune system to kill HIV-infected cells.

Pharmacokinetic half-life extension

Interestingly, outside of their role in driving changes in antibody interactions with Fc receptors, several point mutations have been developed that alter the half-life and homeostasis of IgG through enhanced antibody interactions with the recycling neonatal Fc receptor, FcRn.[141] The half-life of circulating IgG1 in humans is $\sim$21 days[122] while engineered chimeric, humanized, and human antibody drugs range between 3 and 27 days.[142] Increasing the half-life and stability of these engineered antibodies lower the cost, dose, and tissue access of particular monoclonal therapeutics.[46,143] Similarly, modifications have been made to IgG3 to enhance its half-life.

Specifically, a natural H435A substitution increases the IgG3 7-day half-life to mirror the 21-day IgG1 half-life,[144] potentially offering an opportunity to exploit the polyfunctional nature of this potent antibody subclass. Moreover, the serum-extended IgG3 antibody was shown to have enhanced protection against a pneumococcal challenge in mice.[144] Importantly, because the point mutations involved in altering FcRn binding site at a distance from the CH2 domain mutations that affect Fc receptor and complement binding, these half-life modifications can be co-engineered to further enhance monoclonal therapeutic strategies for cure and beyond.

Non-naked antibodies

While antibodies can be used as ''naked'' monoclonal therapeutics that aim to direct the killing activity of the innate immune system, monoclonal antibodies have also been used as vehicles that drive the delivery of killing ''cargo'' to target cells of interest, also known as antibody-drug conjugates. Thus antibodies can be tethered to toxic and/or radioactive agents to kill target cells.

In a recent study by Denton *et al*. HIV-infected BLT mice under ART therapy were treated with an HIV-specific antibody (3B3) coupled to a toxin and administered to mice to target persistently infected cells in the presence of ART.[145] The toxin-conjugated antibody depleted infected cells to a greater degree than ART alone, suggesting that a monoclonal therapeutic Trojan horse approach may successfully lead to HIV eradication. Importantly, because this toxic delivery strategy does not rely on the presence of immune-competent innate immune cells, it is likely that this type of mAb approach could provide a broader application in subjects that may have suffered irreparable immune damage due to persistent progressive infection prior to the initiation of HAART. However, more exciting, the Denton *et al*.[145] study also strongly suggested that viral antigens can persist despite HAART, and that a shock and kill strategy including a monoclonal therapeutic can have a meaningful clinical impact in the setting of viral eradication.

Bi-specific antibodies

Bispecific antibodies were first discussed more than 50 years ago,[146] and then developed in 1985[147] to redirect

cytotoxic T cells to kill specific tumor cells by combining specific antibodies to CD19 and CD3, thereby bringing cytotoxic T cells in close proximity to the target cell for more efficient killing. In 2009, the first bispecific IgG, catumaxomab (anti-CD3 and the antitumor-associated antigen, epithelial cell adhesion molecule), was approved in Europe for the intraperitoneal treatment of patients with malignant ascites, which results in longer puncture-free survival rates.[148] This same application has been suggested for mAbs against HIV antigens and CD3. Moreover, because of the emerging understanding of the role of PD1 as a marker of latently infected cells,[149] it is plausible that a bispecific PD-1+ anti-Env antibody could provide enhanced specificity for the detection and rapid destruction of reactivated cells in the setting of an LRA-based therapeutic eradication strategy.

Conclusions

Tremendous advances have been made in the potentiation of mAb therapeutics in the fields of oncology and autoimmunity over the past 3 decades. These breakthroughs have come through the realization that antibodies not only label target cells, but can also be engineered to function as effective killers in conjunction with innate effector cells, aimed at rapidly and specifically targeting and eliminating tumor/autoimmune cells.

These discoveries have laid the groundwork for the fastest growing class of drugs, the monoclonal therapeutics, that can be customized for defined effector functions through the selection of specific subclasses, glycans, and/or point mutations that drive enhanced clinical efficacy. Moreover, because antibody effector function correlates with decreased infection risk and slower disease progression it is likely that functional mAbs can control and clear HIV-infected cells, offering a unique opportunity within a shock and kill strategy to rapidly destroy cells following reactivation. Further characterization of unique humoral functional profiles that track with post-treatment control (PTC) and/or viral reservoir elimination in clinical trials following LRA treatment may point to the functional mechanism(s) by which antibodies may naturally contribute to reservoir eradication. Yet animal studies that rapidly test ''Fc signatures'' of reservoir killing are desperately needed to define the most potent ''shock and kill'' strategy.

Thus, with the discovery of multiple broadly reactive neutralizing and non-neutralizing monoclonal antibodies, as well as emerging next generation sequencing technologies that allow for the rapid identification of thousands of novel monoclonals, we believe that the targeted development of one or a cocktail of monoclonal therapeutics with robust killing activity is an attainable reality for a kill strategy. The success of a mAb-based kill strategy can be further enhanced through the exploitation of mAb engineering approaches aimed at manipulating the most broadly reactive HIV-specific mAbs, customized for function at the correct anatomic site. Such a mAb approach may work independently of LRAs, or in a synergistic manner with LRAs and other cure strategies.

Author Disclosure Statement

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