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REVIEW

The maturation of antibody technology for the HIV epidemic

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Antibodies are one of our most useful biological tools. Indeed, improvements in antibody-based technologies have ushered in a new era of antibody-based therapeutics, research and diagnostic tools. Although improved technologies have led to the development of therapeutic antibodies for treatment of malignancies and inflammatory conditions, the use of advanced antibody technology in the therapy of viral infections is in its infancy. Non-human primate studies have demonstrated that antibodies against the HIV envelope can both prevent viral infection and control viremia. Despite the obvious potential of antibody therapies against HIV, there remain limitations in production and purification capacity that require further research. Recent advances in recombinant antibody technology have led to the development of a range of novel antibody fragments, such as single-domain nanobodies and bispecific antibodies, that are capable of targeting cancer cells to cytotoxic T cells. Novel antibody production techniques have also been designed, allowing antibodies to be obtained from non-mammalian cells, bovine colostrum and the periplasm and cytoplasm of bacteria. These advances may allow large-scale production of HIV antibodies that are capable of protecting against HIV infection or serving as therapeutics that reduce the need for life-long antiretroviral treatment. This review summarises recent advances in antibody-based technologies and discusses the possibilities and challenges of using these advances to design prophylactics and therapeutics against HIV.
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The vast binding repertoire and high specificity of antibodies have made them one of our most useful laboratory reagents. Antibodies have found their place as successful therapeutics, with over 40 approved by the FDA and with sales in the United States exceeding $24 billion in 2012.[1] Production of conventional monoclonal antibodies (MAbs) as IgGs, however, is relatively expensive, creating a major economic burden for health care.[2] Given the scale of the HIV epidemic, with over 35 million individuals currently infected and two million new infections occurring each year (2013 UNAIDS Global Report), it is unfeasible to use IgGs as preventatives or wide-scale therapeutics with current costs of production. Fortunately, new technologies allow recombinant antibodies to be developed and produced at much lower costs and with a variety of modifications that add specific functions. Concurrent advances in the HIV field have demonstrated that antibodies that neutralise a broad range of HIV subtypes (broadly neutralising antibodies (bNAbs)) are promising candidates for new preventatives and therapeutics.[3–7] This review describes recent advances in antibody technology and their potential application to the HIV epidemic, as well as the challenges that need to be addressed in order to produce useful antibodies to combat HIV on the global scale.

NATURAL AND RECOMBINANT ANTIBODIES

Monoclonal and polyclonal antibodies produced for use in the laboratories or in the clinic are usually IgGs, consisting of two heavy chains and two light chains, and reliant on disulphide interactions for functional conformation (Figure 1a). A number of smaller antibody formats have been developed (Figure 1) that have many advantages over the IgG molecule, particularly in terms of easier and cheaper production techniques. Many of these such as the single-chain variable fragment (scFvs) can be expressed from single genes, unlike IgGs. Bispecific antibody fragments and nanobodies (Nbs) are examples of modified antibody fragments that can be tailored to specific functions and are often easier to produce than IgGs (Figure 1). This section reviews antibody design technologies and recent advances.

Monoclonal and polyclonal IgGs

MAbs in the IgG format have historically been developed by immunising mice or rats with antigens, then establishing clonal plasma cell/myeloma hybrid cell lines (hybridomas) that secrete one specific antibody. 'Humanisation' of rodent MAbs, by converting the rodent amino acid regions of the scaffold to the equivalent residues in the human orthologue, minimises immunogenicity in humans,

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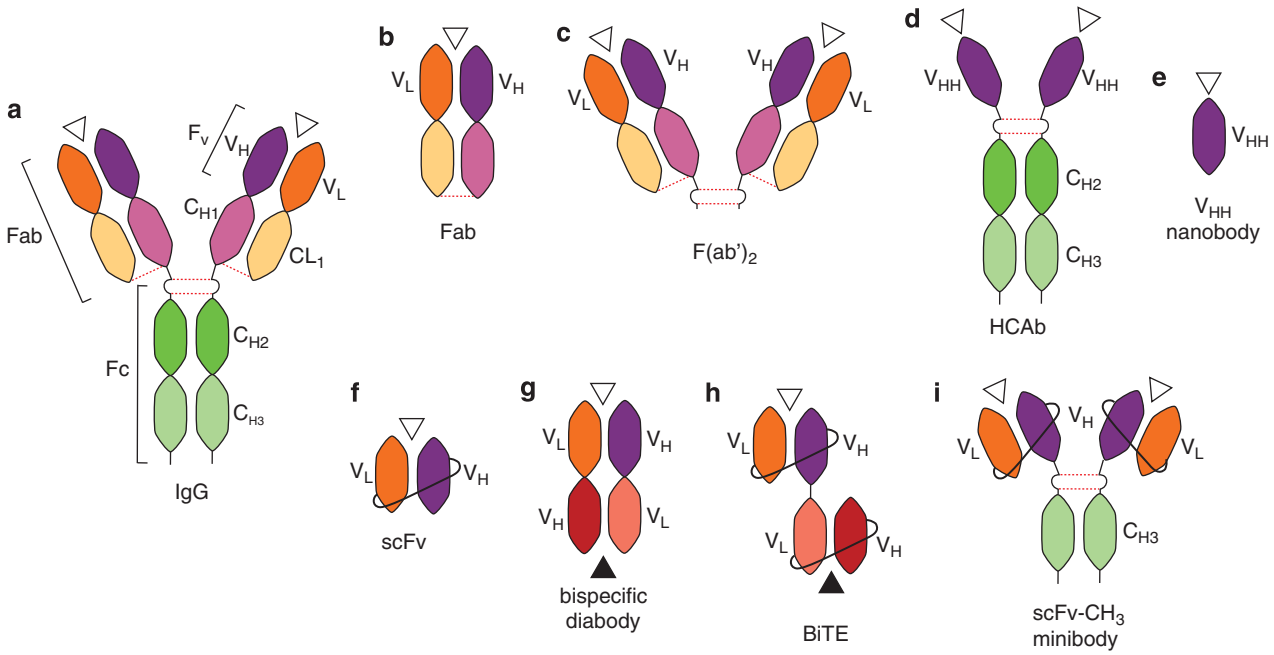

Figure 1 A schematic representation of IgG and various natural or recombinant alternatives. (**a**) IgG (**b**) fragment antigen binding (Fab) (**c**) $F(ab')_2$ (**d**) heavy chain only antibody (HCAb) as found in camelids (**e**) nanobody, based on camelid V_{HH} domain (**f**) single-chain variable fragment (scFv) (**g**) bispecific diabody (**h**) Bispecific T-cell Engager (BiTE) (**i**) minibody. V, variable; C, constant; F, fragment; H, heavy chain; L, light chain. Each oval shape refers to an immunoglobulin domain, antigens are shown as triangles, dotted red lines depict disulphide bonds. It should be noted that disulphide bonds are also present within each immunoglobulin domain that are not depicted in this schematic.

allowing their use in the clinic.[8] MAbs can also be isolated by screening antibody cDNA expression libraries made from human antibody-expressing B cells. Many MAbs are FDA approved for clinical use such as treatment of inflammatory diseases and cancers,[9,10] and as diagnostics, with hundreds more in clinical trials. Large-scale production of clinical MAbs as IgGs uses expression in mammalian cells lines grown in bioreactors. Although well-optimised, this production is relatively expensive (approximately US$3 million for 100 g), prohibiting the potential use of IgGs as a wide-scale preventative or treatment for HIV, given the scale of the epidemic.

There are four subclasses of IgG, (IgG1 to IgG4), which differ in their Fc regions.[11] Production of IgG1 and IgG3 is the dominant response to protein antigens, particularly viruses, with the majority of serum IgGs being IgG1. These forms have the greatest capacity for activation of complement, opsonisation and FcR-mediated effector functions such as antibody-dependent cellular cytotoxicity. IgG2 production is the main response to polysaccharide antigens, whereas IgG4 is only produced after prolonged exposure to antigen.[11] Most therapeutic IgGs are IgG1, with IgG2 and IgG4 sometimes used. The frequent choice of IgG1 is likely because of their potential to elicit FcR-mediated effector functions, and their superior half-life compared with IgG3.[12]

Recombinant antibodies and antibody fragments

Recombinant antibodies are modified versions of natural antibodies, often comprised of smaller fragment/s. Recombinant antibody fragments can be easily produced in a range of cell types, including prokaryotes, at high yields. They can be developed by screening human antibody cDNA libraries or synthetic libraries that harbour greater variation than the human repertoire.[13] One disadvantage of most recombinant antibody formats is the absence of an Fc region, which contributes to inhibiting HIV infection in animals models, discussed below.

Fragment antigen bindings (Fab)s consist of the variable domain (V_H) and first constant domain (C_{H1}) of a heavy chain with the variable and constant domains of a light chain (Figure 1b). A $F(ab')_2$ fragment consists of two Fab fragments linked by disulphide bonds (Figure 1c) that can increase the avidity of antigen binding compared with Fabs. The addition of a flexible peptide linker allows expression of an Fab from one gene, allowing Fab production by eukaryotic cell lines and bacteria.[14] Many Fabs have been approved for clinical use, not only as diagnostics when conjugated to radiolabels, but also as therapeutics such as for Crohn's disease and macular degeneration.[15] They are often used instead of IgGs when solving structures of antibody bound to antigen, as for structures of the HIV Env proteins bound to the Fab domains of various HIV Abs.[16,17] Human and synthetic Fab libraries have been used to develop novel Fabs against Env subunits.[18]

scFvs contain only the variable domains of the light and heavy chain linked by a flexible peptide produced from one gene (Figure 1f). They can be produced by the oxidising periplasm of *Escherichia coli*, which allows disulphide bond formation.[19,20] The scFvs have been extremely useful during phage display, in which scFv from antibody libraries are expressed on the surface of phage then panned by antigen. Diabodies are scFv dimers that are linked with a short (5-amino-acid) peptide that allows dimerisation of the two domains and include single-peptide diabodies that are produced from the one polypeptide (Figure 1g). Higher-order forms of scFvs to form di, tri and even quad-ramers with corresponding higher target avidities can also be produced in bacterial systems by manipulating the length of the linker sequences between the V_L domains.[21] Minibodies have two scFv domains attached to an Fc domain, providing the avidity of two Fv domains with a C_{H3} domain, while being produced from one gene

(Figure 1i). An scFvFc is similar, but contains the C_{H2} domain, thus reconstituting the Fc receptor-mediated functions.

Bispecific antibodies

Bispecific antibodies are engineered to bind to two different epitopes, on either the same, or on different antigens (Figures 1g and h). These antibody formats have been used to bring specific cell types together such as immune effector cells and their targets.[22] Bispecific antibodies can be made in numerous different formats and have been developed by a number of biotechnology companies. The first bispecifics were made as tetravalents based on the IgG form where scFvs were linked to either the Fc, C_{L1}, V_H or V_L domains. Examples are the DVD-Ig and Zybody formats, which have additional domains fused to the IgG-variable or Fc domains.[23,24] However, full Ig bispecific structures are also produced using technologies to pair or separate based on heterodimer formation. Novimmune makes bispecifics that have a lambda/kappa light chain combination, with an identical heavy chain. Genentech produces a full Ig bispecific through modifying the C_{H3} interface to promote heterodimer formation.[25] By expressing only one half of each bispecific in two separate *E. coli* strains, and combining the extract before purification, they combine to produce a full bispecific.

Recombinant antibody technology has allowed the production of a range of smaller and simpler bispecific fragments such as bispecific diabodies (Figure 1g). Bispecific T-cell engagers (Figure 1h) are fused scFvs expressed from one gene that are designed to link cytotoxic T cells, via binding of CD3, to targets such as tumour cells by binding to cell surface markers, leading to killing of the target.[26] The small size and tandem configuration of bispecific T-cell engagersis optimised to enable close association of two cells by binding receptors on opposing membranes.[26] There are currently four bispecific T-cell engagers in clinical trials for the treatment of B-cell malignancies and various solid tumours,[27] but no BiTE candidates have been trialled for HIV yet.

Nanobodies

Camelids, which include camels and llamas, and some other species such as nurse sharks, express heavy chain only antibodies in addition to conventional antibodies (Figure 1d). Despite having only one variable (V_{HH}) domain per arm, these antibodies are capable of a similar binding diversity to conventional IgGs.[28] The V_{HH} domains of camelid heavy chain only antibodies can be expressed as single-domain antibodies known as Nbs (Figure 1e). The small, highly soluble nature of nanobodies make them easily expressed at high yields in mammalian cell lines, plants and bacteria, with the latter requiring a secretion signal to allow the nanobodies to translocate to the periplasm for disulphide bond formation. A number of Nb features indicate a great potential for the large-scale clinical application. They are very stable, with a long shelf life and remain active at 37 °C for weeks. Nbs can cross the blood–brain barrier.[29] Modified Nbs with a single stabilising disulphide bond are resistant to pepsin and chymotrypsin, raising the possibility that they could be delivered orally as therapeutics.[28] However the small size of standard Nbs allows uptake by the kidneys and rapid clearance from the blood, which will need to be addressed before oral administration becomes feasible.[28,30]

ANTIBODY PRODUCTION BY PROKARYOTES AND EUKARYOTES

The majority of therapeutic antibodies, both monoclonal and recombinant fragments, are currently produced in mammalian cell lines.[21] As of early 2014, at least 40 antibodies are FDA approved for clinical use[1] and many more are undergoing clinical trials.[31] Although most FDA-approved antibodies are full IgG molecules (usually IgG1), a number of Fab, $F(ab')_2$, scFv and bispecific T-cell engagersare also in various phases of clinical trials.[15,27,31] The production of IgGs requires specific protein folding apparatus, the correct oxidising environment for disulphide bond formation, post-translational modifications such as glycosylation and secretion of the antibodies, all of which are all readily accomplished in mammalian cell lines such as CHO cells. The use of specialised bioreactors for MAb production and the optimisation of purification has allowed industrial production of many therapeutic MAbs at concentrations of $>10\,g\,l^{-1}$ of culture.[32] This level of production allows widespread use as therapeutics, but the cost remains a significant challenge, limiting the potential of IgGs as a preventative or therapeutic measure for HIV, given the scale of the epidemic.

Production of antibodies in non-mammalian eukaryotic cells allows folding and secretion (but usually no complex glycosylation) with other advantages that can make costs of production and purification lower. Yeasts combine these features with fast growth and replication kinetics. Some yeast strains, such as *Pichia pastoris*, have been produced with glycosylation pathways that are altered to be more human-like, allowing a kind of 'humanised' glycosylation of antibodies to be produced.[33] Antibodies can also be produced in insect cells transfected with baculoviruses expressing the antibody genes, although complex glycosylation is largely absent. Transgenic plants have great potential for antibody production as they can express very large amounts of IgGs, however, the purification processes are currently extensive and costly.[34]

Recombinant antibodies can also be expressed in *E. coli* and other gram-negative bacteria at high yields.[21] *E. coli* are capable of making a number of antibody formats, such as scFvs, Fabs and full IgGs, but antibodies produced in *E. coli* lack glycosylation. Most *E. coli* expression systems target the protein to be secreted into the periplasmic space, where the oxidising environment allows some disulphide bond formation.[21] Stable forms of scFvs with no cysteines have also been produced in the *E. coli* cytoplasm, allowing very high yields of recombinant antibody.[35]

THE IMPORTANCE OF bNABS TO HIV IMMUNITY

Most successful vaccines elicit neutralising antibodies (NAbs) that correlate with protective immunity. The NAb response to infection, however, is ineffective in controlling existing HIV infection for two main reasons. First, HIV NAb responses are generated only many months after the initial infection when infection is well established. Second, HIV rapidly evolves, owing to the low fidelity of its reverse transcriptase, to escape immune pressures such as neutralising antibodies.[36] Circulating strains of HIV-1 vary greatly, with at least nine different subtypes making up the main group of viruses, and each subtype harbouring much sequence variety itself. Each HIV-infected individual will eventually make NAb against their own autologous strain, but the Env gene of the virus will continue to evolve to escape the NAb response. The NAbs in any individual can therefore only neutralise past strains rather than the current virus.[37] A subset of HIV-infected individuals eventually, often after years of infection, develop NAbs that can neutralise a broad range of HIV strains (bNABs). These bNAbs are of great interest if they could be induced by vaccination. Unfortunately, clinical trials of HIV vaccines have so far failed to elicit bNAbs.

The mutations that HIV-1 undergoes in response to neutralising antibodies cause changes in the Env proteins expressed on the virion surface.[38] Env mediates entry into the cell and is therefore a key target

for prophylactic antibody-based vaccine development. Mature Env 'spikes' consist of three gp120 subunits, and three gp41 subunits that anchor the complex to the membrane, both of which are targets for bNAbs. Many bNAbs targeting Env have now been characterised, some with their structures solved and neutralisation profiles against hundreds of HIV strains determined.[39,40] These antibodies are the subject of numerous recent reviews[40,41] and therefore will not be discussed in detail.

Studies showing that passive transfer of bNAbs protected macaques from infection with SHIV (Simian Immunodeficiency Virus with human Env)[42,43] as well as the correlation of neutralising antibody titre with immunity in a macaque vaccination model[44] support the notion that induction of bNAbs in humans could be protective. bNAbs are therefore likely to be an essential component of a successful vaccine. Designing vaccines that elicit bNAbs has been difficult for numerous reasons. Many bNAbs have unusual characteristics, such as very long CDR3 domains, tethered V_H domains that form the paratope, interactions with the plasma membrane, autoreactivity or polyreactivity.[45–47] This suggests that they could be rarely produced in the host. The extensive Env glycosylation that shields epitopes also hampers the generation of bNAbs by vaccines. HIV bNAbs often have a high degree of somatic mutation that take over a year to be generated in infected individuals[48–50] and mirroring this by vaccination is a particularly difficult aspect of bNAb induction. The emerging vaccination strategy aims to elicit bNAbs by making use of studies that predict the unmutated ancestors of specific bNAbs as well as maturation intermediates, and therefore use a series of epitopes predicted to act as 'guidelines' to promote this affinity maturation pathway.[17,51]

The isolation of bNAbs from HIV-infected patients required advanced screening technologies. Indeed, the first discovered bNAb, named b12, came from panning a phage display Fab library made from an HIV-1-positive patient's bone marrow cDNA with gp120.[52–54] Since the discovery of b12 using phage display, many bNAbs have been isolated from HIV-1-infected individuals using an impressive array of specialised techniques. The formation of clonal cultures from antibody-producing memory B cells from patients with broadly neutralising sera is the basis for these techniques. This culture technique has been adapted for high-throughput screening of tens of thousands of individual B cells followed by IgG heavy and light chain gene cloning from these cells.[55–57] A more-targeted screening approach uses recombinant Env proteins aimed at directing immune responses to specific epitopes and regions. The resurfaced stabilized core 3 form of gp120 has exposed surfaces substituted with SIV homologues, but maintains the HIV CD4-binding site in a stabilised 'CD4 binding' conformation.[49] By using this molecule to screen the binding of memory B cells from broadly neutralising sera, many CD4-binding site bNAbs have been discovered.[17,49,58] Screening B-cell clones with a different recombinant gp120, which has a stabilised 'CD4 binding' conformation and variable loops removed, has also allowed numerous bNAbs to be identified.[50]

HIV ANTIBODIES AS PREVENTATIVES AND THERAPEUTICS

HIV antibodies have the potential to be used as preventative measures such as topical microbicides and pre- or post-exposure prophylaxis. As therapies, antibodies that block HIV-1 transmission could be useful in patients with multi-drug resistance and those with low tolerance of existing therapies. It is also feasible to use antibodies in combination with future latency reactivation therapies to target reactivated cells for eradication.

Early studies indicated the potential for antibody-mediated protection by showing that passive immunisation with IgG purified from infected chimpanzees or a mix of HIV antibodies could protect macaques from SHIV.[42,59] Oral SHIV infection could also be prevented by antibody treatment of infant macaques.[60] In humans, HIV antibodies were well tolerated and capable of transiently delaying rebound after cessation of antiretroviral therapy.[7,61] Recently, a number of studies in macaques and humanised mice have demonstrated the potential for HIV bNAbs as therapeutics. bNAb treatment resulted in an impressively delayed viral rebound, reduced viral load and reduced cell-associated viral DNA in these animal models.[3–6]

There is evidence of the synergistic activity of multiple bNAbs in prevention of HIV transmission. Using the macaque SHIV infection model, Mascola *et al.*[42] showed that sterile protection could only be achieved by a triple combination of antibodies, with a double combination more effective than single MAbs. Other studies suggest single bNAbs can protect against infection.[62] However, *in vitro* neutralisation studies have also shown the synergistic or additive effects of antibody combinations on infection.[63–66] This synergism was also apparent in therapeutic studies; combinations of bNAbs were more effective than single bNAbs as therapeutics in the HIV-infected humanised mouse model.[5] A cocktail of three antibodies was able to delay viral rebound in acutely-infected people who had ceased antiretroviral therapy.[7] In this study, viral load resurged in most of the subjects and escape mutations were found for only one of the three antibodies, indicating that this was the dominant of the three in the cocktail. A recent study of the therapeutic potential of newer bNAbs in macaques showed the very high-affinity PGT121 bNAb having the same, or possibly a stronger effect on viral load compared with combinations of antibodies.[3] Obviously, more experiments in humans are needed before an optimal mix of antibodies can be determined. The mechanism behind the ability of antibody combinations to synergise is not well understood but predicted to entail more than simply increasing the density of antibodies on the surface of the antigen.[67]

There are no antibodies targeting HIV proteins that are currently approved by the FDA. In fact, the most advanced investigational antibodies for the HIV epidemic target HIV's target-cell receptor (CD4) or co-receptors (CCR5 or CXCR4). PRO140 is a humanised IgG4 that binds the extracellular domain of the HIV co-receptor CCR5, acting as an entry and fusion inhibitor. It is currently in phase IIb clinical trials and has been awarded 'fast track' candidate status by the FDA. Previous trials demonstrated antiviral effects with no serious adverse effects.[68] Ibalizumab is a humanised anti-CD4 with viral entry inhibitory activity, of which the safety and efficacy have been demonstrated in phase I and II clinical trials (www.taimedbiologics.com/en/info/TMB355.aspx).

DEVELOPMENT AND PRODUCTION OF HIV ANTIBODIES *IN VITRO*

The discovery and characterisation of bNAbs derived from HIV-infected patients has led to a significant progress in our understanding of how to prevent infection over a broad range of HIV-1 subtypes and therefore new vaccination strategies. However, antibodies made *in vitro* for the prevention or treatment of HIV need not be restricted by the repertoire of the human immune system. It is this field that should benefit most from recent advances in antibody technology.

Numerous bispecific antibodies have been developed with the aim to diagnose, prevent or treat HIV. Early approaches produced modified antibodies that, although are not bispecifics, exploit a

similar principle. Examples are 'immunoadhesins' and 'janusins', which bind gp120 via recombinant CD4 domains attached to an anti-CD3 that targets $CD3^+$ cytotoxic cells to kill HIV-infected cells with gp120 on their surface.[69–71] No HIV immunoadhesins or janusins have been approved for use in the clinic to date. A rCD4-IgG2 named PRO 542 entered phase II clinical trials in the year 2000[72] but was unsuccessful, although more recently an immunoadhesin based on the HIV bNAb PG9 has entered phase I trials (http://clinicaltrials.gov/ct2/show/NCT01937455). Early bispecifics used similar principles such as targeting gp41 using a Fab attached to anti-FcγRI to elicit both antibody-dependent cellular cytotoxicity and neutralisation,[73] or anti-gp120 attached to anti-FcγRs leading to phagocytosis of infected cells.[74] To avoid the need to bind gp120 or gp41 from a broad range of HIV subtypes, the HIV co-receptor CCR5 has been used as a target of bispecific antibodies; Schanzer *et al.*[75] developed IgG-scFv antibodies that bind the two alternative docking sites on CCR5 and Bruhl[76] depleted $CCR5^+$ leukocytes from the circulation by targeting them to cytotoxic $CD3^+$ cells using a bispecific single-chain antibody to both CD3 and CCR5. After the characterisation of many bNAbs, their Fv regions were incorporated into a new generation of bispecifics. Attaching an scFv containing the Fv of HIV bNAbs PG9 or PG16 to the V_H domain of Ibalizumab (the anti-CD4 MAb in current clinical trials) created a bispecific antibody with very good potency and breadth that the study authors propose could be used in passive immunisation to prevent infection.[77] Bispecific anti-gp41 or anti-gp120 antibodies with broad-recognising activity were also used to target infected cells for neutrophil- or complement-mediated lysis, respectively.[78,79]

Anti-Env Nbs have been made by immunising llamas with Env proteins followed by cloning the V_{HH} domains to make a phage library and panning this against Env targets.[80–83] Numerous V_{HH} with broad neutralisation of HIV-1 subtypes have been made, in some cases identifying novel sites of vulnerability on Env.[84] Broadly neutralising Env V_{HH} can be produced in large amounts in *Saccharomyces cerevisiae* and are being developed as microbicides. Initial data on formulation of V_{HH} in a vaginal ring study showed favourable release and tissue penetration properties in pre-clinical trials.[85]

HIV antibodies have been produced by a range of different organisms, such as an anti-capsid Fab produced by *E. coli*,[86] anti-gp41 antibodies produced by various insect cells lines[87] and an scFvFc produced by mammalian CHO cells.[88] Numerous HIV antibodies have been produced in transgenic plants such as maize, *Arabidopsis* and tobacco, but few have been progressed to clinical trials.[34] MAPP66 (a mix of antibodies that includes an anti-CCR5 to inhibit HIV infection) produced by *Nicotinia Benthamiana* crops is currently being trialled for the prevention of HIV and Herpes infection as a topical microbicide (www.bumc.bu.edu/ipcp/projects/). One innovative and inexpensive example of HIV antibody production uses bovine colostrum. Cows vaccinated with recombinant gp140 Env trimers produced IgG in their colostrum that had broad neutralising activity, with specificity for the CD4-binding site.[89] Anti-gp41 antibodies could also be produced by goat colostrum.[90] The use of bacterially-produced antibodies as therapeutics requires extensive purification to remove all toxic bacterial products. This is a particularly important consideration for HIV, as activated T cells are most likely to be infected, therefore any low level of bacteria product that could either directly or indirectly activate T cells will likely negate any benefit of the antibody. Additionally, antibodies produced in bacteria will not be glycosylated, meaning that many known bNAbs could not be produced in bacteria as they rely on glycosylation for function. The benefits of production in bacteria are very high yields, which could be a great advantage for preventatives that will need to be made at very high levels for a low price.

PROTECTION FROM HIV TRANSMISSION IS ENHANCED BY FC-MEDIATED EFFECTOR FUNCTIONS

Despite their high costs of production, the IgG1 format has the advantage of maintaining an Fc region, compared with the most smaller antibody fragments. The Fc of IgG1 (and IgG3) confer upon antibodies the ability to trigger innate immune cells to mediate antibody-dependent cellular cytotoxicity and antibody-dependent phagocytosis. These effector functions may allow for cytolysis-mediated elimination of antibody-coated HIV-infected autologous lymphocytes, or infected allogeneic cells within the genital secretions of the transmitting partner, by NK cells and/or monocytes. Opsonisation of infected lymphocytes or viral particles with antibodies also allows innate immune cells to phagocytose and degrade the coated particle. These effector functions could be essential to prevent HIV transmission in situations where neutralisation may not be sufficient, for instance, cell-to-cell transmission of HIV through virological synapses formed between infected T cells and uninfected cells.[91] If HIV-infected autologous or allogeneic cells are present during early HIV exposure, it might be necessary for certain neutralising antibodies to use non-neutralising effector functions to prevent systemic infection from taking hold. However, some antibodies, such as the very high-affinity PGT121, have been shown to enter the virological synapse and prevent cell-to-cell transmission *in vitro*.[92]

The importance of the Fc receptor for prevention of HIV transmission has been indicated by animal studies. Macaques receiving an infusion of the Fab version of the bNAb 2F5 showed no significant protection from vaginal challenge with SHIV, whereas animals receiving the IgG1 version were protected.[93] Macaques treated with intravenous bNAb b12 IgG or a b12 IgG-based vaginal microbicide were protected from vaginal challenge with SHIV.[94,95] However, protection against SHIV by the b12 Nab decreased when Fc receptor and complement-binding activities were engineered out of the antibody, but not by elimination of complement binding alone.[96] Fc-mediated effector functions, but not complement, are therefore important for protection in animal models of SHIV infection. Whether this Fc domain of PGT121, the most potent bNAb known, is required for protection is currently unknown.

PRODUCTION OF ANTIBODIES WITH FC-DIRECTED FUNCTIONS FOR WIDE-SCALE USE: CHALLENGES AND POSSIBILITIES

Aside from issues of efficacy, there are many challenges that need to be addressed before the wide-scale use of antibodies that can be employed to combat HIV. In particular are the twin issues of the necessity for the Fc-directed functions for full inhibition of HIV transmission and the prohibitive costs of IgG production for wide-scale use.

As detailed above, studies using *in vitro* infection experiments and animal models indicate that the Fc portion of the many bNAbs is necessary for their full protective capacity when used as infusions or vaginal microbicides. The Fc region can also extend the serum half-life of antibodies, from hours to days, however this can also be achieved by a number of alternative methods including PEGylation, glycosylation or fusion to albumin (or albumin-binding proteins).[29] One alternative to making IgG antibodies is to make recombinant antibodies that contain an Fc receptor. Minibodies are an example in

which two scFvs are connected to a C_{H3} domain (Figure 1i). Minibodies could be produced in more cost-effective manner than IgGs, as they can be made from a single gene and can be expressed in bacteria.[97] Minibodies, however, do not contain C_{H2} domains that are necessary for effector functions such as antibody-dependent cellular cytotoxicity.[98,99] The similar scFvFc format antibodies have C_{H2} domains. The scFvFcs based on b12 successfully prevented HIV transfer in an *in vitro* model using epithelial cells from the female genital tract, raising hope that this approach could protect humans.[100]

Whereas large-scale production of IgGs using mammalian cells remains expensive, other methods of production may be scaled up with lower costs. As discussed above, the greatest and cheapest yields of IgGs come from expression in plants; however, the current costs of purification as well as the perceived dangers of genetically modified crops are challenges that need to be overcome to make this a viable option. Although production of recombinant antibodies in yeasts and bacteria is very cost efficient, contaminating host products may lead to activation of T cells, dendritic cells or macrophages. As HIV preferentially infects activated cells, any cell activation could increase the incidence of infection. One way to address this concern could be to use a bacterial strain that produces less-harmful impurities, such as species of *Lactobacillus* that are commensal bacteria of the human vagina, to express a scFv as a vaginal probiotic. Preliminary studies have shown that *Lactobacilli* expressing a scFv against ICAM-1 could block cell-mediated HIV-1 transmission in cervical epithelial transwell cultures[101] and *Lactobacilli* expressing cyanovyrin-N, an HIV-1 entry inhibitor, could reduce vaginal SHIV transmission in macaques.[102] The gut might also be protected by similar mechanisms using appropriate commensal bacteria. Although impurities from *Lactobacilli* may be well tolerated in the vagina, they would not be well tolerated in the blood, so other production methods would be needed for intravenous treatments.

An exciting approach to HIV prevention is the administration of antibodies using vectored immunoprophylaxis (gene therapy). The vector adeno-associated virus, a ubiquitous commensal human virus with no known pathology, has been used to deliver antibody genes in animals models using intramuscular injection to induce antibody gene expression.[103,104] Balazs *et al.*[104] demonstrated protection from HIV infection of humanised mice after vectored immunoprophylaxis delivering IgG genes for bNAbs b12 or VRC01. Johnson *et al.*[103] used this approach to induce expression of immunoadhesins against SIV that produced long-lasting serum neutralising activity and complete protection against intravenous SIV challenge in macaques. The immunoadhesins used had an IgG2 Fc fragment attached to Fvs that recognised SIV gp120. An immunoadhesin based on the HIV bNAb PG9 has recently entered phase I clinical trials (www.clinicaltrials.gov NCT01937455). Using this approach avoids the necessity for costly IgG production, while maintaining Fc fragments and avoiding contaminants from yeasts or bacteria that may activate HIV target cells.

SUMMARY

Although no FDA-approved antibody-based prophylactics or therapeutics for HIV are currently available, promising candidates are currently entering clinical trials. These trials will build upon several elegant animal studies that have reignited hopes for antibody-based preventative and therapeutic medicines. For these hopes to reach fruition, much research is needed to identify the antibody-dependent effector functions that are necessary to achieve protective outcomes, as well as to produce antibodies that elicit Fc-mediated functions at low cost. Recent advances in recombinant antibody technology should facilitate the design of HIV-antibody-based prophylactic and therapeutic medicines. Similarly, lessons learned from previous trials of antibody-based drugs should expedite the transition of these medicines to the clinic.

CONFLICT OF INTEREST

Potential conflicts of interest may arise from the industry affiliations of some of the authors. Specifically, Matthew Parsons is on the Scientific Advisory Board of Network Immunology Inc. Rob Center is a cofounder of Reef Pharmaceuticals Pty Ltd. Ben Kiefel and Matthew Beasley are the co-founders of Affinity Biosciences Pty Ltd.

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