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Systemic and Topical Use of Monoclonal Antibodies to Prevent the Sexual Transmission of HIV

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Abstract

Passive immunization, the transfer of antibodies to a nonimmune individual to provide immunological protection, has been used for over 100 years to prevent and treat human infectious diseases. The introduction of techniques to produce human monoclonal antibodies (mAbs) has revolutionized the field, and a large number of human mAbs have been licensed for the treatment of cancer, autoimmune and inflammatory diseases. With the recent discovery and production of highly potent broadly neutralizing and other multifunctional antibodies to HIV, mAbs are now being considered for HIV therapy and prophylaxis. In this review, we briefly review recent advances in the anti-HIV mAb field and outline strategies for the selection, engineering and production of human mAbs, including the modification of their structure for optimized stability and function. We also describe results from nonhuman primate studies and Phase 1 clinical trials that have tested the safety, tolerability, PK and efficacy of mAb-based HIV prevention strategies, and discuss the future of parenteral and topical mAb administration for the prevention of HIV transmission.

Keywords

HIV-1; VRC01; monoclonal antibody; passive immunization; sexual transmission; microbicide; vagina; rectal

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A BRIEF HISTORY OF PASSIVE IMMUNIZATION

In 1890, Emil von Behring and Shibasaburo Kitasato, working at the Institute of Hygiene in Berlin, reported that serum from rabbits that had been immunized with bacterial toxins protected nonimmune rabbits from infection [1]. Their discovery led to the use of immune serum from horses and other animals to treat tetanus and diphtheria infections in humans and marked the start of the age of “serum therapy”. This treatment was hailed as the most important medical breakthrough of the 19th century, and the inventors received the first Nobel Prize in Physiology or Medicine in 1901 [2]. For approximately 40 years, serum therapy was used as front line treatment for a number of major human bacterial and viral infections including tetanus, diphtheria, pneumococcus, meningococcus, influenza, measles and polio. Following the introduction of potent antibacterial drugs, antibody therapy was restricted to a smaller number of selected treatments for snake venoms, bacterial toxins and some viral infections [2]. However, in recent years, passive immunization has experienced a renaissance with the use of monoclonal antibodies to treat a number of cancers, autoimmune and infectious diseases.

The early days of passive immunization with animal immune sera had been hampered by limited availability of quality antibodies, high cost, and frequent adverse reactions to serum components. In the 1940’s, Cohn significantly advanced the field through the introduction of a procedure to purify immunoglobulins from blood, which, with further improvements, led to the use of potent polyclonal “immune globulin (Ig)” formulations for the prophylaxis and treatment of several viral diseases including measles, polio and infectious hepatitis [3], and for the protection of high risk newborns unable to receive colostrum [4]. These products produced fewer side effects, but supplies were limited and expensive. In 1975, the field of passive immunization was revolutionized with the discovery of a technique to make monoclonal antibodies (mAbs) by Kohler and Milstein [5], and in 2003, by transformative technology which introduced the capability of cloning heavy and light chain immunoglobulin genes amplified from single human B cells and their expression in bacteria, and later in other expression systems as described below [6, 7]. This capability accelerated the discovery of new human antibodies, especially when coupled with rapid manufacturing platforms, and made possible their production on a large scale for clinical applications [8]. By the end of 2014, 47 mAb products had been approved for clinical use, and it is projected that 70 mAb products will be on the market by 2020 with combined sales of $125 billion [9].

USE OF ANTI-HIV MABS TO PREVENT THE SEXUAL TRANSMISSION OF HIV

Most HIV transmission events occur across genital or rectal mucosal surfaces following sexual intercourse with an HIV-infected partner [10, 11]. With the introduction of new intervention strategies, such as male circumcision and treatment-as-prevention (TAP), the global HIV incidence has dropped from its peak in 1997 of 3.5 million new infections per year, to 2.1 million/year [12], but this rate is still unacceptably high. A vaccine may be the ultimate goal for HIV prevention, but this approach has remained elusive. MAbs are

currently being explored for HIV therapy and prevention. Approximately one third of HIV-infected individuals make HIV neutralizing antibodies [13], and B cells from these individuals were used to isolate first generation HIV-neutralizing mAbs. These identified conserved epitopes shared between HIV subtypes and isolates; however they had limited breadth and/or potency against global isolates and were only partially effective in SHIV-challenge models. Subsequently, large cohorts of HIV infected individuals were screened for highly effective neutralizing antibodies, and high throughput single cell B-cell receptor amplification techniques and novel soluble trimeric Envs were employed to produce a new generation of extremely potent broadly neutralizing anti-HIV antibodies (bNAbs) [14], and are active across multiple HIV clades. These second generation mAbs are 10–100 fold more potent than the first generation antibodies, and bind to various epitopes on the viral surface (Table 1) which enables the administration of combinations of distinct mAbs to reduce HIV immune escape. Unfortunately, potent HIV neutralization activity is associated with a high degree of somatic hypermutation, making the production of bNAbs through vaccination a challenge [15]. Non-neutralizing HIV mAbs have also been described that may block HIV infection through other antiviral effector functions such as Fc-mediated functional assays (described below). Interest in non-neutralizing anti-HIV antibodies arouse when data from the recent RV144 Thai vaccine trial showed that non-neutralizing antibodies were associated with protection in vaccinated subjects [16]. However, to date non-neutralizing mAbs have been less effective than bNAbs in protecting against SHIV infection in vaginal and rectal transmission models [17–19].

Parenteral MAb Administration

Preclinical studies in animal models—Some of the first studies to demonstrate protective effects of HIV mAbs were conducted in the humanized mouse HIV infection model [20–22]. Following the introduction of the chimeric HIV/SIV (SHIV) virus challenge model, nonhuman primates (NHP) have been extensively used to test the ability of anti-HIV bNAbs to prevent SHIV infection via the vaginal or rectal routes (Table 2 [17, 18, 23–37]). The earliest NHP studies used intravenous (IV) infusion of first-generation bNAbs (e.g., 2G12, b12, 2F5); these mAbs required a high dose (≥25mg/kg) to achieve protection against a single high-dose SHIV vaginal challenge, even with Tier 1 (neutralization sensitive) strains [24, 26, 34]. Passive immunization of various combinations of these bNAbs also protected neonatal macaques against high dose IV or oral challenge with SHIVs, providing further proof-of-concept that mAbs can prevent HIV infection [38]. The introduction of more potent cross-clade bNAbs and Tier 2 (neutralization resistant) SHIV strains have provided a more realistic picture of the potential of passive immunization with HIV antibodies. Second-generation bNAbs (particularly the PG, PGT and VRC series) require lower doses (≤1 mg/kg) for protection in passive immunization studies than first-generation bNAbs; furthermore, they have demonstrated efficacy against both vaginal and rectal SHIV challenges with Tier 2 SHIV [32, 35]. Engineering of bNAbs to enhance neutralization efficacy, Fc function and serum half-life has led to increased protection in some studies [29, 36]. This research has paved the way for clinical trials of parenteral bNAb administration in humans to prevent HIV transmission.

Clinical trials: Three Phase 1 clinical trials have been conducted to determine the safety, tolerability, and serum antibody PK in adults receiving IV infusions of the bNAb VRC01 with and without supplemental subcutaneous (SC) injections of antibody. VRC601 and 602 were dose escalation and PK studies of IV vs. SC administration of VRC01 in HIV-infected and uninfected subjects respectively [39, 40]. HVTN104 was a larger Phase 1 trial evaluating the safety of multiple doses of VRC01 administered over 6 months in regimens hypothesized to result in drug levels corresponding to protection against HIV. The study recruited a total of 88 healthy HIV-uninfected volunteers (44 men, 43 women and 1 transgender person). The bNAb infusions and injections were well tolerated and no severe adverse events were reported. PK results showed that: 1) after a 40 mg/kg IV loading dose, VRC01 levels in blood were maintained at >10 μg/ml for 6 months through biweekly 5mg/kg SC injections; 2) 20 mg/kg VRC01 given IV monthly after a 40 mg/kg loading dose maintained blood levels >40 μg/ml; 3) bimonthly VRC01 administered IV at 10, 30 or 40 mg/kg resulted in peak concentrations between 80 and >400 μg/ml and nadirs between 12 and >20 μg/ml. All three of these protocols maintained antibody titers well above the minimum effective HIV neutralization concentration of VRC01 (IC50 = 1 μg/ml). Potentially effective antibody titers were sustained from 53 to 81 days after mAb dosing was terminated [41]. Furthermore, preliminary data indicate that the infused antibody enters vaginal and rectal mucosal tissues at levels conferring protection in *ex vivo* HIV challenge studies [42].

Two large Phase 2B clinical trials, referred to as the Antibody Mediated Prevention (AMP) study (www.ampstudy.org), have been initiated to evaluate the efficacy of the VRC01 bNAbs in reducing acquisition of HIV-1 infection in high risk populations [43]. HVTN704/HPTN085 will test parenteral administration of VRC01 in 2,700 men-that-have-sex-with-men (MSM) and transgender people in the Americas, and HVTN703/HPTN081 will use the same approach in 1,500 heterosexual women at risk for HIV acquisition in sub-Saharan Africa. The mAb will be infused IV at doses of either 10 or 30 mg/kg bimonthly for a total of 8 infusions, and participants will be tested for HIV infection through 80 weeks after initiation of mAb treatment. It is hoped that data from this study will provide additional information on safety, tolerability and efficacy of VRC01 IV infusion, as well as antibody concentrations needed for protection against HIV. Furthermore, if this approach demonstrates protection against HIV acquisition, it may be used to protect vulnerable populations going forward. Other potent bNAbs are under study which could result in combination prophylactic regimens using several bNAbs at the same time to inhibit HIV binding via complementary mechanisms of action [44]. One particularly potent bNAb, 3BNC117, has been shown to suppress HIV rebound after treatment interruption in chronically-infected patients [45]. Other refinements include the development of bispecific antibodies that could enhance the breadth and potency of protection against diverse HIV strains [46].

In the near term, the AMP studies will be pivotal for the assessment of the role of systemic bNAb infusions for HIV immunoprophylaxis, and the findings will inform future efficacy trials of this approach using other bNAbs alone or in combination. If VRC01 infusions are found to protect against HIV acquisition, this will facilitate moving quickly forward to study

other bNAbs that have other favorable characteristics, e.g., longer half-lives, and/or the ability to neutralize a broader array of HIV strains [45]. Analyses of viral isolates from individuals in the AMP trials who received either 30 mg/kg or 10 mg/kg of VRC01 who became HIV-infected will help to inform future dosing regimens of VRC01 and other bNAbs, and provide insights into optimal systemic antibody concentrations needed for protection against HIV acquisition. The pharmacokinetics of the dosing regimens used in the AMP studies has been thoroughly delineated in HVTN 104 [45], so it will be feasible to correlate expected drug concentrations with HIV protection once the AMP data are available. The findings from the AMP studies can be correlated with earlier work with non-human primates, and will allow for enhanced appreciation of the extent to which bNAb protection in the non-human primate model correlates with anti-HIV protection in humans. HVTN 104 and the AMP studies have included mucosal sampling substudies, which will be informative for the determination of bNAb levels necessary for mucosal protection [45], which should facilitate the development of mucosal antibody approaches including preventative HIV vaccines, vectored immunoprophylaxis and topical mAb administration.

Topical MAb Administration

Topical administration of mAbs directly to sites of mucosal HIV transmission in the genital tract or rectum is another promising approach for HIV prevention. Topical mAb-based microbicides potentially offer several advantages including: use of synergistic mAb combinations, ease of application, delivery of concentrated product directly to site where needed, reversibility, few if any side effects, and cost effectiveness.

Preclinical studies in animal models—Several studies have demonstrated that topical administration of anti-HIV mAbs can protect macaques from SHIV mucosal challenge (Table 3 [17–19, 47–50]). MAbs have been administered vaginally [18, 19, 48, 50] or rectally [17, 49], followed by high dose or repeated low dose SHIV challenges to the site shortly after mAb delivery. The half-life of mAbs in mucosal secretions after topical delivery of 5–20 mg mAb was about 4 hours; animals were protected to varying degrees against the SHIV challenges, and no side effects were noted. Efficacy of topically administered mAbs has also been demonstrated in the humanized mouse model [51].

Clinical trials—MAb-based vaginal microbicides have been evaluated for safety in at least 3 Phase 1 clinical trials. A vaginal microbicide containing a combination of 4E10, 2F5 and 2G12 (MABGEL), developed by the European Microbicides Programme, was recently tested for safety and pharmacokinetics (PK) [52]. The mAbs were produced in Chinese hamster ovary (CHO) cells as human IgG1, were formulated at 20 mg/ml (high dose) or 10 mg/ml (low dose) in gel, and 2.5 ml of gel was administered vaginally to women daily for 12 days. None of the women reported serious adverse events, and effective concentrations of mAbs could be observed in cervicovaginal secretions up to 8 hours post treatment. Furthermore, there was no evidence of systemic absorption of the mAbs. Another European consortium recently conducted a first-in-human, double-blind, placebo-controlled Phase 1 clinical trial with a plant-derived human HIV bNAb, 2G12, documenting the safety of a single vaginal administration of this mAb [53]. Our team of U.S. scientists is developing mAb-based vaginal microbicides using a transient expression system in *Nicotiana* (tobacco

plants) [54]. A prototype vaginal microbicide, called MB66, containing the HIV bNAb VRC01 and the anti-HSV mAb HSV-8 [55], has been formulated into gels and films and tested for efficacy and stability *in vitro* and *in vivo*. MB66 film is currently undergoing evaluation in a Phase 1 clinical trial. Segment A of the study, a single dose of MB66 film in 8 women, was recently completed with no serious adverse effects; Segment B, 7 daily vaginal doses of MB66 or placebo film in 15 women/group, is currently underway. The next generation MB66 microbicide may contain additional mAbs, such as more potent bNAbs and mAbs that block cell-associated HIV transmission, to improve efficacy. In addition, mAbs that agglutinate sperm may be added to provide contraceptive protection [56]. A prototype MB66 vaginal ring has been developed and tested in macaques [57]. Sustained release of mAbs from a vaginal ring could improve user compliance, overcoming the significant disadvantage of poor adherence to study protocols that has plagued other vaginal microbicide trials [58].

PRODUCTION AND ENGINEERING OF ANTI-HIV MABs

Engineering mAbs for enhanced performance in the mucosal environment

Recent research indicates that passive immunization with anti-HIV mAbs may be a viable approach for HIV prevention and therapy. Non-traditional antibodies such as s-IgA, and mAbs engineered with Fab or Fc modifications to improve function may in the future be significantly more efficacious and cost effective, further enhancing the feasibility of this approach.

Engineering mAb isotype—Most monoclonal antibodies are manufactured as IgG1, which is the most abundant Ig subclass in blood [59]. However, polymeric secretory IgA (s-IgA) is the predominant immunoglobulin type in most mucosal secretions [60], and has structural characteristics that enhance its presence, stability and function at mucosal sites [61].

It is possible to manufacture IgA, dimeric (d) IgA and s-IgA mAbs, but this is currently an industrialization challenge [62]. However, several studies provide evidence that IgA mAbs may provide superior protection in the mucosal environment, indicating that it may be worthwhile to produce IgA mAbs for passive mucosal protection.

i. Dimeric and secretory forms of IgA have double the number of available antigen-binding sites as IgG, and multiple antigen-binding sites are crucial for the formation of viral aggregates [63]. Furthermore, the flexibility of diametrically opposed F(ab′) portions of dIgA with a higher extension span overcomes steric hindrance when accessing vulnerable epitopes on the viral envelope [64].

ii. IgA1 subclass antibodies have a more flexible hinge region and a broader reach than IgG and IgA2 subclass antibodies (16.3 nm vs 10.2 nm). In a recent study the dIgA1 form of HCGN194 captured approximately twice as many virions as dIgA2 in a virion capture assay, and inhibited the transcytosis of cell-free SHIV across an epithelial monolayer *in vitro* whereas IgA2 and IgG mAbs did not [49].

iii. s-IgA is particularly suited to function in the mucosal environment due to resistance to protease digestion and its ability to anchor onto the apical side of epithelial tissues by interacting with mucins [49, 65].

iv. Association of the polymeric Ig receptor (pIgR) with HIV-bound IgA mAbs mediates HIV virion excretion from polarized epithelial cells suggesting a possible mechanism of viral efflux and subsequent reduction in viral load from mucosal epithelia [66].

v. Evidence for superior protection by IgA anti-HIV antibodies has been recently reported: a neutralizing monoclonal dIgA1 provided better protection than IgG in a SHIV rectal challenge study [49], *in vitro* studies showed intracellular abrogation of HIV replication/transcytosis by HIV-specific IgA mAbs [67], and vaginal transmission of HIV-1 in humanized mice was inhibited by polymeric recombinant forms of IgA but not monomeric IgG mAbs [68].

Despite considerable evidence favoring IgA for mucosal protection, contrary evidence also exists. A recent study that compared the protective effects of a number of neutralizing and nonneutralizing mAbs of IgG vs. IgA2 isotypes in both *in vitro* and *in vivo* NHP studies did not find an advantage to the use of IgA mAbs for protection against HIV and SHIV infection [17]. Furthermore, the RV144 HIV vaccine trial showed an in inverse relationship between the presence of IgA HIV antibodies in serum and protection in vaccine recipients [69]; *in vitro* experiments using serum from vaccinated subjects indicated that HIV Env-specific IgA antibodies competed for Env binding sites with vaccine-elicited IgG antibodies and diminished their ADCC antiviral effector function [70]. In light of this evidence, Ruprecht and colleages administered both IgG and IgA isotype HIV-specific mAbs to NHP to determine if the dIgA2 mAbs compete with IgG mAbs to affect protection in a rectal SHIV challenge experiment; they found that instead of competing, IgG and dIgA2 mAbs worked synergistically to completely protect macaques from rectal SHIV challenge [47]. Clearly, this is an important research area that calls for further study.

Engineering Fab—A variety of approaches for enhancing the function of anti-HIV mAbs have been devised through engineering of the Fab region. Passive immunization has demonstrated a strong correlation between anti-HIV mAb neutralizing potency, a property of the Fab region, and protection against SHIV mucosal challenge *in vivo* [18]. VRC07-523, a clonal relative of VRC01 engineered to have increased neutralization potency, was 5X more effective in a SHIV mucosal challenge study [36]. Antibody valence is another important feature. Additional F(ab′) fragments provide increased possible orientations for maximal ligation and crosslinking of HIV-1 envelope spikes [63]. In addition, bispecific antibodies have been engineered that demonstrate extremely potent and broadly neutralizing activity due to their ability to bind to more than one HIV Env epitope [71], and chimeric molecules such as one consisting of a PG16 antibody heavy chain and CCR5-like peptide have also been shown to be highly effective at neutralizing HIV [72].

Engineering Fc—Engineering of the immunoglobulin Fc region provides another approach to potentially enhance mAb protective functions. There are at least four Fc-mediated antibody activities that are important in regulating HIV infection (Figure 1). The

Fc region can activate complement, leading to the enzymatic destruction of virions or infected cells [73]. Antibody-dependent cellular cytotoxicity (ADCC) is another principal Fc-mediated protective immune effector mechanism. Classic experiments illustrated killing of HIV antigen-coated or infected cells by immune cells that had captured antibodies from immune serum from HIV-infected individuals [74, 75], and this ADCC mechanism has been recapitulated in a number of studies using mAbs instead of immune serum [76–78]. Antibody-dependent cellular phagocytosis (ADCP), another Fc-mediated antibody function, similar to ADCC but involving phagocytosis of infected cells and immune complexes, has also been demonstrated for HIV antigen-coated beads *in vitro* [79], and is associated with improved clinical outcomes in chronically infected HIV patients [80]. Antibody-dependent cell-mediated viral inhibition (ADCVI) is an Fc receptor-mediated effector function that mediates viral reduction not only through cytotoxicity but also by inhibition with antiviral factors such as cytokines [81].

Passive immunization studies have implicated the Fc region in protection against HIV/SHIV challenge *in vivo*. Fab fragments of the 2F5 anti-HIV mAb were not protective against vaginal SHIV challenge whereas whole mAbs (with Fc region) were protective [28]. Furthermore, b12 mAbs with diminished Fc function due to the LALA mutation were less protective than unmodified mAbs [24, 25]. On the other hand, HIV mAbs engineered for enhanced Fc function have been more protective in SHIV mucosal challenge studies [29, 33]. Various engineering approaches have been used to modify the Fc region of mAbs for enhanced function:

i. There is a direct link between Fc glycosylation patterns and mAb effector function [79, 82]. IgG molecules with Fc glycans lacking the core fucose residue display an increased affinity for Fcγ RIIIa and enhanced ADCC [82, 83]. Nonfucosylated glycan forms on antibodies from elite controllers were shown to minimize viral load during chronic HIV-1 infection through ADCVI [84], and nonfucosylated HIV bNAbs have been made that demonstrate higher affinity for Fcγ RIIIa receptors and enhanced ADCC activity [33]. Furthermore, Fc glycosylation also affects antibody interactions with mucins; antibodies with shorter glycan profiles preferentially associated with MUC16 and captured more virions [85]. These data suggest that modification of Fc glycosylation could improve antibody function in the mucosal environment. However, one *in vivo* SHIV vaginal challenge experiment was conducted comparing nonfucosylated b12 mAb to normally glycosylated b12, and the results were inconclusive [33].

ii. Another approach has been to modify the protein structure of the Fc region. GASDALIE is an Fc domain variant that enhances FcRγ binding and function [86]. GASDALIE-modified anti-HIV bNAbs demonstrated enhanced protection against HIV infection in the humanized mouse model [87].

iii. The neonatal Fc receptor (FcRn) is another receptor of interest in designing mAbs with enhanced circulation time in blood and improved function at the mucosal surface. This receptor is expressed by mucosal epithelial cells, and is especially abundant in the placenta and newborn intestine where it directs the polarized uptake of maternal IgG antibodies for fetal immune protection [88].

MAbs engineered with point mutations that alter the binding of IgG to FcRn for greater stability have been shown to have a prolonged half-life in blood [89, 90]; this activity could extend to the mucosa as Fc-engineered mAbs with enhanced FcRn function showed enhanced protection against SHIV vaginal challenge in animal studies [29].

Overview of mAb production platforms

Mammalian cell platforms and alternative systems are rapidly being developed and optimized for more efficient mAb production, and promise to deliver clinical grade mAbs at reduced cost in the future (Figure 2). Evaluation of various technologies and production options is best accomplished early in product development. Ideally this occurs after product feasibility is established but well before product-related process development is initiated. Cost analysis for production and processing needs to be applicable to the complete platform process from initiation to harvest and downstream processing. The employed analytical methods are applicable to a variety of users and organizations and contain standardized and relevant cost data [91].

Understanding the cost implications of new technologies that may include continuous processes and modular facilities can be difficult. This evaluation at different production scales adds to the complexity. One example is mAb expression in mammalian cells. The production levels have increased dramatically in the last two decades and now titers of 3–5 g/L are not uncommon [92]. During the past 10 years, an industry-wide emphasis on upstream process optimization has resulted in up to 100- fold increases in productivity. Based on current rates of optimization, it would not be surprising to witness further increases in the range of 10–15 g/L. Due to the success of upstream process optimization, attention has shifted toward new or improved technologies that will augment the productivity of the downstream purification process. In particular, single use systems that have high throughput and minimize expensive chromatography resin turnover are being developed. Examples include continuous, multicolumn fed streams using disposable columns (such as simulated moving-bed chromatography (SMB) [93] as well as novel membrane chromatography technologies [94]. Single use technologies offer the advantages of low capital costs. This can ultimately reduce the initial investment as well as distribute cost expenditures across the entire lifetime of the product [95]. In this scenario, the increased cost burden of consumables results in an enhanced response rate when market demand changes. This flexibility is now increasingly important as drug pipelines transition from traditional mAbs with average demands of 250 kg/yr to more heterogeneous product capabilities such as fusion proteins, nanobodies, bispecifics and other emerging antibody structures, with production rates ranging up to 500 kg/yr.

Mammalian Cell MAb Production Systems—The prevalent stably transformed mammalian cell lines for recombinant mAb production are Chinese Hamster Ovary (CHO), NS0, Sp2/0, HEK293, and PER.C6 [96]. The majority (~70%) of mAbs approved for human therapy are produced in CHO, although NS0 and Sp2/0 have also been used for clinical mAb production [97]. The primary importance of mammalian cells compared to microbial or plant production systems lies in the mammalian post-translational modifications such as

glycosylation [98]. Despite an excellent safety and efficacy record, the use of mammalian cells for recombinant mAb expression does have some drawbacks including a relatively slow growth rate, potential risk of viral contamination, and complex manufacturing process. Furthermore, the current standard production process is expensive, cumbersome and time-consuming [99].

Alternative MAb Production Systems—In the interest of cost-savings and potential increased scale of production, numerous transgenic technologies have been explored during the last two decades. These transgenic systems have involved yeast, bacteria, insects, animals, and plants [100]. Challenges for these transgenic platforms include immunogenic glycosylation, a susceptibility to viral pathogen co-propagation, developmental instability, complex genetics, environmental containment concerns, and most significantly, prolonged development times. One or more of these deficiencies is associated with every transgenic system, including current mammalian cell-based techniques. In addition, major GMP challenges for large-scale production can also contribute to significantly longer development times and unfavorable economics. For global health indications, the existing mammalian manufacturing platform for antibodies is viable, but emerging platforms like yeast/fungus, plants, and transient mammalian transfection could potentially play a complementary role when cost, scale and speed are critical factors.

GAPS, CHALLENGES AND CONCLUSIONS

1. Recent mAb HIV protection studies have measured antibody levels in female genital tract and rectal tissue to correlate antibody titers with protection. The male genital tract has not been studied but also requires protection from female-to-male HIV transmission. It is unknown whether systemically-administered mAbs reach transmission sites in the male genital tract (foreskin and urethra), and whether mAbs can effectively be administered topically to the male genital tract.
2. Infectious virus was detected in draining lymph nodes and gastrointestinal tissues from macaques that had been infused with protective levels of bNAbs and then challenged vaginally with SHIV [101]. This indicates that protective mAb titers must be sustained in tissues distal from the site of viral entry. MAb titers have not been monitored at these sites, and implications of this finding for topical mAb-based microbicides are unknown.
3. More studies are needed to identify synergistic combinations of bNAbs to maximize efficacy against a wide range of HIV strains and to minimize viral immune escape.
4. There is a growing interest in the use of mAbs to block cell-associated HIV transmission [102]. HIV-infected cells are present in genital secretions of HIV-infected men and women, and could play a role in the sexual transmission of HIV [103]. Cell-cell HIV transmission is 10 to >1,000-fold more efficient than cell-free transmission *in vitro*, and since cell-cell viral transmission entails transfer of HIV though specialized intercellular synapses, the virions are partially

protected from environmental agents including antibodies [104, 105]. A number of studies have ranked existing bNAbs for their ability to block cell-cell HIV transmission [102]; the outcome of these studies has varied depending on the timing and concentration of antibodies and the model systems used. More research is needed with improved mucosal transmission model systems to identify anti-HIV mAbs that effectively block cell-associated HIV transmission for addition to HIV-prevention mAb cocktails. Such antibodies could prevent early events in HIV acquisition such as spreading of virus from HIV-infected cells in genital secretions, and between cells in the genital mucosal epithelium [103, 106].

5. MAb-based prevention methods may never enter the main stream due to cost considerations.

Existing mammalian cell manufacturing platforms are very costly for clinical grade mAb production. Emerging platforms such as yeast/fungus, plants, and transient mammalian transfection may prove advantageous when cost, scale and speed are critical factors. Use of potent mAbs engineered for enhanced function and half-life could also reduce costs.

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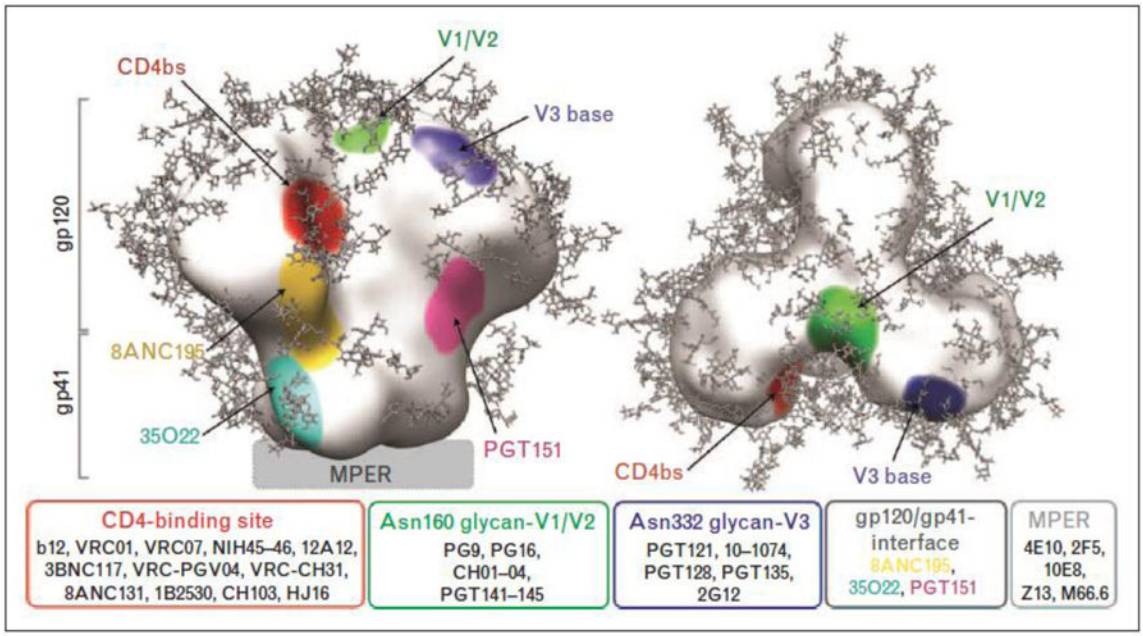

Figure 1. HIV broadly neutralizing monoclonal antibodies and their binding sites
From: Sievers, Schaarf, West, Bjorkman; Curr Opin HIV AIDS 2015.

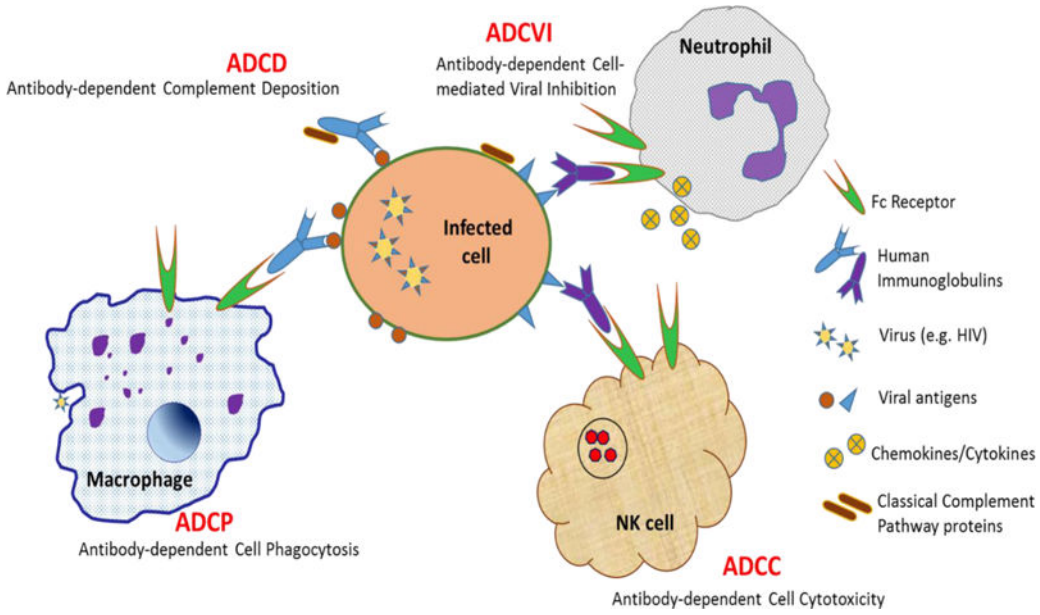

Figure 2.

Fc Receptor-Mediated Antibody Dependent Functions that Boost the Immune Response to HIV and HIV-infected Cells

	Mammalian	Plant (transient)	Yeast/Fungus
Target Cost	$10/gram (TEM =$20–$200/g)	$10/gram (TEM=$100-$1000/g)	$10/gram
Productivity	1 – 5 g/L	0.1 – 1 gram/kg biomass	1 – 5 g/L
Scale	up to 20,000L	3,500 kg biomass/ week	up to 300,000L
Yield	20 – 100 kg	0.3 – 3.0 kg	300 – 1500 kg
Production Cycle (Start to DS fill)	30 – 40 days	10-12 days	12 days
Annual Production	0.45 – 2.2 tonne/20K L bioreactor	20-100 kg/ 3.5K kg biomass	13.5 – 67.5 tonne/ 300K L fermenter

Figure 3. Manufacturing Estimates for mAb Drug Substance
TEM = Techno-economic models

Table 1

Broadly neutralizing HIV monoclonal antibodies [107]

Monoclonal Antibody	Epitope
1° Generation	
2F5	gp41 membrane-proximal external region (MPER)
2G12	gp120 glycan
b12	Gp120 CD4 binding site (bs)
2° Generation	
PGT121	V3 glycan
10-1074	V3 glycan
2G12	gp120 glycan
VRC34	fusion peptide
PGT151	fusion peptide
VRC01	gp120 CD4 bs
3BNC117	gp120 CD4 bs
4E10	MPER
10E8	MPER
C5022	gp120/41 extended glycan
PGT151	gp120/41 extended glycan
3° Generation	
VRC07-523	CD4 bs
3BNC117	CD4 bs
10-1074	V3 glycan

Table 2

Protection against SHIV mucosal challenge following intravenous parenteral administration of HIV-MAbs

Reference	MAbs	MAb Dosage	SHIV Challenge	Protection	Notes
Mascola [30]	2G12 2G12 + 2F5	15 mg/kg 15 mg/kg @	Vaginal, sHD[a] $SHIV_{89.bPD}$ 〃	2/4 2/5	First demonstration of modest protection against mucosal SHIV challenge w/mAbs.
Parren [34]	b12 〃 〃	1 mg/kg 5 mg/kg 25 mg/kg	Vaginal, sHD $SHIV_{162.P4}$ 〃 〃	0/4 2/4 4/4	MAb protection was dose-dependent.
Hessell [24]	b12 $b12_{KA}$ $b12_{LALA}$	25mg/kg 〃 〃	Vaginal, sHD $SHIV_{SF162P3}$	8/9 8/9 5/9	Decreased protection without Fc function.
Hessell [25]	b12 $b12_{LALA}$	1 mg/kg (weekly) 〃	Vaginal, rLD $SHIV_{162.P4}$ 〃	20X RR[b] 10X RR	First repeated low dose SHIV challenge study: less mAb required for protection. Fc important.
Hessell [26]	2G12	40 mg/kg	Vaginal, sHD, $SHIV_{SF16273}$	3/5	Half-life of 2G12 in serum: 7.7-12.2 days. Half-life in vaginal secretions: <24 hrs.
Hessell [27]	2F5 4E10	50 mg/kg 50 mg/kg	Rectal, sHD $SHIV_{Ba-L}$ 〃	5/6 5/6	MPER mAbs are also protective.
Burton [18]	b12 b6	25 mg/kg 25 mg/kg	Vaginal, sHD $SHIV_{SF162P2}$ 〃	3/4 0/4	Strong neutralizing mAb (b12) more protective than weakly neutralizing mAb (b6).
Moldt [32]	PGT121 〃 〃	0.2 mg/kg 1 mg/kg 5 mg/kg	Vaginal, sHD, $SHIV_{SF162P3}$ 〃 〃	3/5 5/5 5/5	Potently neutralizing mAb (PGT121) achieved protection at low dose
Moldt [33]	b12 NFb12	1 mg/kg (weekly)	Vaginal, rLD[c], $SHIV_{SF162}$ 〃	4.5X RR 3.0X RR	Nonfucosylated b12 (higher affinity for Fcγ RIIIa and ↑ADCC) did not show enhanced protection.
Klein [28]	2F5 〃 〃 2F5 (Fab)	5 mg/kg 25 mg/kg 50 mg/kg 25 mg/kg	Vaginal, sHD $SHIV_{BaL}$ 〃 〃 〃	3/5 5/5 5/5 0/4	Serum and vaginal PK and PD: serum mAb levels were higher and more predictive of protection than vaginal mAbs. Fab mAb not protective.
Ko [29]	VRC01 VRC01-LS	0.3 mg/kg 〃	Rectal, sHD $SHIV_{BaLP45}$ 〃	2/12 7/12	VRC01-LS, engineered for enhanced FcRn binding, had 3X longer serum half-life and was more protective than VRC01.
Pegu [35]	VRC01 〃 〃 10E8 〃 〃 PG9 〃 〃	0.3 mg/kg 5 mg/kg 20 mg/kg 0.3 mg/kg 5 mg/kg 20 mg/kg 0.3 mg/kg 5 mg/kg 20 mg/kg	Rectal, sHD $SHIV_{BaLP4}$ 〃 〃 Rectal, sHD $SHIV_{BaLP4}$ 〃 〃 Rectal, sHD $SHIV_{BaLP4}$ 〃 〃	4/10 6/6 6/6 3/6 6/6 6/6 0/6 3/6 4/6	Various bNAbs directed against different epitopes on HIV Env were protective.

Reference	MAbs	MAb Dosage	SHIV Challenge	Protection	Notes
Rudicell [36]	VRC01 〃 VRC07-523	0.05 mg/kg 0.2 mg/kg 0.3 mg/kg	Rectal, sHD $SHIV_{BaLP4}$ 〃 〃	0/4 3/4 5/12	VRC07-523, a clonal relative of VRC01, engineered to have increased neutralization potency, was more protective than VRC01. EC_{50}: 0.47 μg/ml vs. 2.5 μg/ml.
Shingai [37]	VRC01 〃 〃 PGT 121 〃 〃 10-1074 〃 〃	20 mg/kg 30 mg/kg 50 mg/kg 1 mg/kg 5 mg/kg 20 mg/kg 1 mg/kg 5 mg/kg 20 mg/kg	Rectal, sHD $SHIV_{DH12-V3AD8}$ or $SHIV_{AD8E0}$ 〃 〃	0/2 1/2 1/2 3/4 1/2 6/6 1/4 4/4 4/4	Plasma protective neutralization titer (50% animals) ~ 1:100. [3BNC117 and 45-46m2 mAbs showed little to no protection in same concentration range.]
Gautam [23]	VRC01 VRC01-LS 3BNC 117 10-1074	20 mg/kg 〃 〃 〃	Rectal, rLD $SHIV_{AD8-EO}$ 〃 〃 〃	RR=2.6 RR=4.8 RR=4.3 RR=4.2	A single mAb injection provided protection against several weeks of low dose SHIV rectal challenge (n=6/group).
Moldt [31]	PGT126 〃 〃 PGT126 〃 〃	0.4 mg/kg 2 mg/kg 10 mg/kg 0.4 mg/kg 2 mg/kg 10 mg/kg	Vaginal, sHD $SHIV_{SF163P3}$ 〃 〃 Rectal, sHD $SHIV_{SF163P3}$ 〃 〃	1/5 2/5 5/5 0/4 2/4 3/4	bNAbs protect against both vaginal and rectal challenge routes.
Astronomo [17]	CH54 IgG CH38 $mIgA_2$	50 mg/kg 〃	Rectal, sHD $SHIV_{BaLP4}$	0/8 1/6	These non-neutralizing mAbs were not protective against rectal SHIV challenge.

[a] sHD: Single high dose SHIV challenge;

[b] RR: Relative risk, Cox Proportional Hazard Model;

[c] rLD: Repeated low dose SHIV challenge

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Table 3

Protection against SHIV mucosal challenge following topical administration of HIV-MAbs

Reference	MAb(s)	MAb Dosage	Route	SHIV Challenge	Protection	Notes
Veazey [48]	b12	5 mg	Vaginal	Vaginal, sHD[a] $SHIV_{162P4}$	9/12	First topical mAb efficacy study.
Burton [18]	b12 b6 F240 b12 b6 F240	5 mg 〃 〃 5 mg 〃 〃	Vaginal 〃 〃 Vaginal 〃 〃	Vaginal, sHD $SHIV_{162P4}$ 〃 〃 Vaginal, sHD $SHIV_{162P3}$ 〃 〃	5/5 0/5 2/5 1/4 1/4 1/4	Strong neutralizing mAb (b12) more protective than weakly neutralizing mAbs (b6, F240). MAbs less effective against Tier 2 virus ($SHIV_{162P3}$)
Watkins [49]	HGN 194 IgG1 HGN 194 dIgA1 HGN 194 dIgA2	1.25 mg 〃 〃	Rectal 〃 〃	Rectal, sHD $SHIV_{1157ipEL-p}$ 〃 〃	2/6 5/6 1/6	dIgA1 was more protective than IgG1 or dIgA2 mAbs.
Moog [19]	2G12 + 2F5 + 4E10 246-D + 4B3	20 mg @ mAb 30 mg @ mAb	Vaginal Vaginal	Vaginal, sHD $SHIV_{162P3}$ 〃	10/15 0/15	Neutralizing mAbs (2G12, 2F5, 4E10) were more effective than non-neutralizing mAbs (246-D and 4B3). Tier 2 SHIV was used.
Sholukh [47]	HGN 194 IgG1 HGN 194 IgG1 + dIgA2	1.45 mg/kg 1.45 mg/kg + 1.25 mg	IV IV + Rectal	Rectal, sHD $SHIV_{1157ipEL-p}$ 〃	0/6 6/6	Rectal administration of dIgA2 mAb enhanced protection against rectal SHIV challenge.
Zhao [50]	4E10-N[b] 〃 〃 VRC01-N 〃 〃	1.25 mg 5 mg 20 mg 1.25 mg 5 mg 20 mg	Vaginal 〃 〃 Vaginal 〃 〃	Vaginal, rLD[c] $SHIV_{162P3}$ 〃 〃 Vaginal, rLD $SHIV_{162P3}$ 〃 〃	0/5 0/5 0/5 3/5 4/5 5/5	First study to use “plantibodies”, repeated low dose challenge with Tier 2 SHIV.
Astronomo [17]	CH31 IgG_1 CH31 m-IgA_2 CH31 d-IgA_2 CH31 s-IgA_2	5 mg 〃 〃 〃	Rectal 〃 〃 〃	Rectal, sHD $SHIV_{SF162P3}$ 〃 〃 〃	6/6 3/6 5/6 4/6	IgG_1 was more protective than IgA_2 forms.

[a] sHD: Single high dose SHIV challenge;

[b] -N, mab made in *Nicotiana benthamiana*;

[c] rLD: Repeated low dose SHIV challenge