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Antibody-based concepts for multipurpose prevention technologies

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Abstract

Because of the versatility and specificity of monoclonal antibodies, they are candidates for multipurpose prevention technologies when formulated as topical (gels, films, rings) or injectable drugs and as vaccines. This review focuses on antibody-based proof of concept studies for the human immunodeficiency virus, herpes simplex virus and sperm. Opportunities and challenges in antibody evasion/resistance, manufacturing, regulatory, and pharmacoeconomics are discussed. This article is based on a presentation at the "Product Development Workshop 2013: HIV and Multipurpose Prevention Technologies," held in Arlington, Virginia on February 21-22, 2013. It forms part of a special supplement to *Antiviral Research*.

Keywords

Multipurpose prevention technologies; multi-antibody; human immunodeficiency virus; herpes simplex virus; sperm

1. Introduction

Women worldwide frequently confront two concurrent reproductive health challenges: the need for both contraception and protection from sexually transmitted infections (Harrison et al., 2013). Multipurpose prevention technologies (MPTs) are intended to simultaneously address these multiple sexual and reproductive health needs. Conceptually, women could be protected against multiple risks, even if their intention was to address just one perceived health need. MPT products may help alleviate the heavy health and economic toll of unintended pregnancy and sexually transmitted infections (STIs) if women have the option to understand, purchase, store, and use fewer products to maintain sexual and reproductive health.

First generation candidates for MPTs consist primarily of combinations of commercially available hormonal contraceptives and antiretroviral drugs (ARVs). Future generations of MPT candidates are likely to include proteins/peptide-based molecules as drugs (Dereuddre-Bosquet et al, 2012; Kouokam et al, 2011, Lagenaur et al., 2011; Lagenaur et al., 2010), and vaccines (Diekman et al., 1999; Walker and Burton, 2010). Monoclonal antibodies (Abs) are protein-based MPT candidates that are specific for their target, but can be multipurpose when combined to target the array of sexually transmitted pathogens and sperm. *In vivo*

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proof-of-concept studies for the human immunodeficiency virus (HIV), herpes simplex virus (HSV) and sperm are reviewed and serve as the starting point for antibody-based MPTs as topical (gels, films, rings) or injectable drugs, and as vaccines. In addition, challenges in Ab evasion/resistance, manufacturing, regulatory, and pharmacoeconomics are discussed.

2. Topical Antibodies

Antibodies against HIV, HSV, and sperm have demonstrated efficacy *in vivo* when delivered topically. The mechanism(s) by which antibodies afford protection against HIV and HSV have been attributed to both classic neutralization (by steric hindrance) and antibody dependent cellular cytotoxicity (ADCC). Anti-sperm Abs that cause agglutination and mucus trapping may be factors in human infertility (WHO, 1992; Diekman et al., 2000). Antibodies to surface antigens on sperm (and other seminal cells) trap by agglutination and making them “mucophilic”, i.e. the antibodies form adhesive interactions with the mucus gel that stops all forward motility (the “shaking phenomenon”) that appears to be associated with the Fc regions of antibodies (Olmsted 2001). A similar mechanism occurs with mucosal pathogens (Phalipon 2002), i.e. a sufficient number of low-affinity cross-linkages trap the pathogen in the mucus gel, thereby reducing the flux of pathogens that reach target cells.

At present, antibody-based proof-of-concept and mechanisms for active and passive immunization is inconclusive for many other prevalent STIs, e.g. *N. gonorrhoeae* (Cole and Jerse, 2009; Zhu et al., 2011) and *Chlamydia trachomatis* (Rank and Whittum-Hudson, 2010).

2.1. HIV Abs

Many of the new monoclonal antibodies against HIV (PGT121-PGT128) are almost 10-fold more potent than the recently described PG9, PG16 and VRC01, and 100-fold more potent that the original prototype HIV neutralizing antibodies (b12, 2G12, 4E10) (Walker et al., 2011; Hiatt et al., 2013). Analysis of the anti-HIV broadly neutralizing monoclonal antibodies (bnAbs) now available suggests that certain combinations of potent antibodies have superior coverage of the enormous diversity of global circulating viruses and should be sought in active or passive immunization regimes.

Unformulated b12 provides dose-dependent protection when given to macaques vaginally as a single bolus before vaginal challenge with a single high dose of SHIV-162 P4 (Veazey et al., 2003). Similarly, unformulated b12 (5mg) when applied vaginally provided sterilizing immunity in seven of seven animals (Burton et al., 2011); weakly neutralizing or nonneutralizing antibodies showed limited or no protection. Rectal delivery of unformulated HGN194 (dimeric IgA1; 1.25 mg) protected 5 of 6 rhesus macaques against intrarectal challenge with SHIV (Watkins et al., 2013).

When formulated as a gel, VRC01 protected seven of nine RAG-hu humanized mice and a multi-Ab gel (b12, 2F5, 4E10, 2G12) provided 100% protection (Veselinovic et al., 2012). MabGel, a multi-Ab gel (4E10, 2F5, 2G12), was shown to be partially protective in a macaque vaginal challenge model (Depo-Provera treated; SHIV162P3; 3-10 AID_{50}) (Moog et al., 2013). In a phase 1 trial of MabGel, the product was shown to be safe (Morris et al., 2010; Charles Lacey 2012, personal communication). Unformulated 2G12 (manufactured in Nicotiana) that was vaginally delivered has completed a phase 1 trial in women and was found to be safe (Julian Ma 2012, personal communication).

2.2. HSV Abs

Unformulated HSV8, a fully human anti-HSV gD Ab which neutralizes a diverse range of low passage clinical isolates of HSV-1 and HSV-2 (De Logu et al, 1998), provided 100% protection at 100 μg/ml in a mouse/HSV model (Zeitlin et al., 1996; Zeitlin et al., 1997). Complete protection against vaginal challenge with an unformulated anti-HSV gB Ab (produced in soy plants and mammalian cells) required approximately 1 mg/ml (Zeitlin et al., 1998). Controlled release of anti-HSV antibodies from EVA-based vaginal rings demonstrated one week of protection in the HSV/mouse model (Sherwood et al., 1996), providing evidence that sustained release of antibodies from an intravaginal device could provide long-term protection.

2.3. Sperm Abs

Agglutination of rabbit sperm with unformulated IgM Ab has been shown to provide contraceptive activity in a rabbit model (Castle et al., 1997); this study mimics the agglutination mechanism that is associated with immune infertility in humans (WHO, 1992). A Nicotiana manufactured IgG_1 against a unique (found only in the human male reproductive tract) glycoform of CD52, i.e. SAGA-1 (Diekman et al., 1999; Diekman et al., 2000), has been shown to co-agglutinate 100% of human sperm and other seminal cells (e.g. white blood cells) in less than thirty seconds at 100 μg/ml (Whaley et al., 2011; Whaley et al., 2012).

3. Injectable Antibody

Systemically delivered Abs have demonstrated efficacy in HIV prevention (Mascola et al., 1999) and therapy (Klein 2012). When 4E10 was delivered intravenously (50 mg/kg on days -1 and +1; day +1 serum concentration = 388-911 ug/ml), the Ab provided complete protection (no viremia) from rectal transmission in macaques (n=6) challenged with SHIV Ba-L (Hessell et al, 2010). Serum concentrations of 25-60 μg/ml of b12 protected against 5 to 28 low dose vaginal SHIV challenges in macaques (Hessell et al., 2009). An injected IgA version of b12 prevented mucosal transmission of HIV in humanized mice (Hur et al., 2012) Systemic delivery of human polyclonal anti-gC1 serum and a murine monoclonal (B1C1) antibody was shown to extend survival time of mice systemically challenged with an HSV-1/Ab mixture (Adamiak et al., 2010). An anti-HSV gB Ab (2c) that was systemically delivered, prevented mucocutaneous disease in a vaginal challenge model; the antibody protected against HSV-1-induced encephalitis independent from complement activation, antibody dependent cellular cytotoxicity, and cellular immunity (Krawczyk et al., 2011).

The half-life of HSV and HIV IgG_1s (∼21 days) may be well matched to the monthly schedule of injectable contraceptives, e.g. *Cyclofem and Lunelle* (25 mg medroxyprogesterone acetate and 5 mg estradiol cypionate), and could be coadministered. Alternatively, since the systemic half-life of Abs can be increased to 3 months by increasing FcRn binding via point mutations to the Fc region (Zalevsky et al., 2010; Dall'Acqua et al., 2006), HSV/HIV Abs could be co-administered with the 3 month injectable contraceptive, Depo-Provera.

Formulating Abs at high concentrations enables delivery by subcutaneous injection which has several benefits, including improved patient convenience, better compliance, reduced pharmacy preparation times, and optimization of medical resources (Ismael et al., 2012). Five highly concentrated Abs (>100 mg/ml) are commercially available; three share very similar lyophilized formulations containing L-histidine as a buffer, sucrose as a cryo-preservative, and a surfactant (Warne, 2010). Self-administration of antibodies and hormonal contraceptives could be achieved by using delivery systems like Uniject (a

prefilled, disposable plastic bubble with needle, administered subcutaneously by squeezing the bubble) for Depo-SubQ Provera 104, or the HumiraPen for anti-TNF Ab.

3. Antibody-based Multipurpose Vaccines

Neutralizing antibodies serve as a correlate of protection for most successful antiviral vaccines, and broadly neutralizing antibodies are the basis of rational HIV vaccine design (Walker and Burton, 2010). The parallel paths of HIV Abs as drugs and Ab-based vaccines stem from the relatively recent discovery of many potent bnAbs in serum samples from HIV-positive individuals. Passive immunization trials are expected to provide proof-of-concept that purified forms of these potent bnAbs protect against HIV in sero-negative individuals. Ab-based vaccines, e.g. anti-idiotype Abs (Mader and Kunert, 2012) and recombinant immune complexes (Chargelegue et al., 2005), can be designed to stimulate systemic and mucosal antibody production. Immunization with fusion proteins like HIVgp120-FcRn (Lu et al., 2011) and HSVgD-FcRn (Ye 2011) have been shown to protect mice against vaginal challenge; these antibody-based vaccinogens can utilize FcRn (neonatal receptor) binding to enhance serum residence time and mucosal uptake. Ab-based subunit vaccines may provide additional advantages by utilizing Ab platforms in manufacturing, purification and formulations.

A hybrid vaccine/Ab strategy has been developed with the use of systemic Adeno-associated virus (AAV)-vectored antibodies (Balazs 2011). In this study, a single intramuscular injection of an AAV-vector containing an anti-HIV antibody gene resulted in long-lasting and high expression of the antibody, and protected humanized mice against intravenous HIV challenge. Using similar technology, anti-HIV antibody fragments were produced in cervico-vaginal epithelial stem cells and were protective *in vitro* (Abdel-Motal, 2011).

4. Challenges and Opportunities for Antibody-based MPTs

4.1. Antibody Evasion and Resistance

Infectious agents can circumvent B and T cell immune responses by a variety of means, including accumulation of point mutations on immunodominant regions of surface proteins, glycosylation of functionally pivotal residues (the glycan shield), association with host serum components (e.g., lipoproteins) in order to mask them from the immune system, cell-to-cell transmission, molecular mimicry between viral proteins and host self-antigens, and interference by non-neutralizing Abs. The pressure for selection of escape mutants is likely higher in a therapeutic context -- where viremic conditions may exist -- than in prevention; Ab-based MPTs that use antibodies against two or more conserved regions of each pathogen are likely to minimize emergence of resistance.

4.1.1. HIV—Founder HIV and antibody gene sequencing reveal concomitant virus evolution and antibody maturation (Liao et al., 2013), suggesting that antibody evasion is transitory. The antibody response to transmitted/ founder virus drives viral escape, such that virus mutants become resistant to neutralization by autologous plasma. This antibody–virus race leads to evolved variants of the transmitted/founder virus that induce antibodies with considerable neutralization breadth.

Although HIV-1 escapes from antibody monotherapy, multi-Ab combinations of potent and broadly neutralizing antibodies can effectively control HIV-1 infection and suppress viral load to levels below detection in mice (Klein et al., 2012). Antibodies differ from other therapeutic modalities for HIV in several important respects: (1) they can neutralize the pathogen directly; (2) they have the potential to clear the virus and infected cells through engagement of innate effector responses; (3) immune complexes produced by the passively

transferred antibodies may stimulate enhanced immunity to HIV-1; and (4) antibodies have far longer half-lives (IgG_1 = 21 days) than currently used antiretroviral drugs. The prolonged control of infection in mice with a penta-mix (3BC176, PG15 45-46^{G54W}, PGT128, 10-1074) was primarily attributed to the long serum half-life of the injected antibodies. The efficacy of antibody-based therapy may be further enhanced with modifications that extend half-life several fold (Hinton et al., 2006).

Mounting evidence from in vitro, animal, and clinical studies indicates that infected cells ('Trojan Horse' leukocytes) may be important vectors of HIV-1 mucosal transmission (Anderson 2010; Anderson et al., 2010). One of the broadly neutralizing HIV mAbs, 4E10, has been shown to have activity against cell-associated HIV in vitro (Sagar 2012). Now that a macaque model for cell-associated SIV/HIV vaginal transmission has been developed (Anderson 2010; Salle et al., 2010), 10E8 (Huang et al., 2012), 4E10, and HC4 (Ab to CD52 glycoform; male reproductive tract unique that co-agglutinates seminal cells) can be evaluated for efficacy in NHP studies. In addition, Abs to antigens found on both cell vectors and free virus (e.g.CD 25, CD26, CD36, CD44, HLA-Class I and II, HLA-RD, ICAM-1) could be evaluated.

4.1.2. HSV—HSV antibodies play a role in mother to child transmission as the severity of HSV infection in the fetus and newborn are greatly reduced when antibodies pass transplacentally (FcRn mediated). A possible explanation for the difficulty in developing an effective HSV-2 vaccine is that the virus has evolved mechanisms to escape immunity. Many herpes viruses encode antibody evasion molecules that interfere with activities mediated by antibody and complement, suggesting their importance in host defense against herpes infections (Hook and Friedman, 2007). HSV evasion of neutralizing Ab by altering complement and ADCC functions may not be relevant to protection by cervicovaginal antibody (acting by either neutralization or mucus trapping) in a mucosal environment with little complement present. However, for injectable HSV Ab it could play a role, i.e. HSV expressed Fc receptors could create a coat of outward facing host IgG that block neutralizing gD Abs. Active (Awasthi et al., 2011) and passive (Adamiak et al., 2010) immunization studies suggest that HSV gC Abs may mitigate this evasion strategy.

4.1.3. Sperm—Sperm are "non-self" to both the male and female immune systems, and it is not surprising that sperm, like STI pathogens, use antibody evasion mechanisms (Cone and Whaley, 1994). Semen contains factors that inhibit cell-mediated immunity, natural killer cell and macrophage function. The female reproductive tract also secretes factors that inhibit complement-mediated damage. Targeting surface coating antigens, e.g. the surface glycolipid CD52 added to sperm in the epididymis, is one potential approach for immunocontraception (Cone and Whaley, 1994; Diekman et al., 1999; Diekman et al., 2000).

4.2. Antibody Dependent Enhancement

The use of antibodies as therapeutic or prophylactic agents for viruses raises the potential for exacerbation of disease by increasing the cellular uptake of viruses resulting in higher viremia, a phenomenon termed antibody-dependent enhancement (ADE). The neonatal Fc receptor (FcRn) enhances transcytosis of IgG-bound HIV across intact epithelial monolayers. Appropriate selection (e.g. dimeric IgA) and dosing of Abs is a strategy likely to avoid the potential for ADE on mucosal surfaces. Additionally, modifications to IgG Fc regions that disrupt antibody interaction with Fcγ receptors have been shown to be effective strategies in preventing ADE-mediated lethal disease in a mouse model (Beltramello et al, 2010).

4.3. Manufacturing and Pharmacoeconomics

Antibodies have become a commercial blockbuster drug platform, with the biggest portion of sales growth in the pharmaceutical industry, but most have indications for oncology and immunological diseases, such as rheumatoid arthritis (RA). There is one commonly used licensed product for prevention of respiratory syncytial virus (RSV) in premature babies, another recently FDA approved for inhalational anthrax disease, and a handful of Ab products undergoing clinical evaluation for infectious disease indications, including methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile* (CB-UPMC, 2013). In spite of the lack of commercial attention to infectious disease Abs, there are a number of reasons to believe they may be more desirable in the future: (a) declining clinical effectiveness of antibiotics; (b) a large number of immunocompromised people; (c) microbiome disruption by antibiotics; and (d) an increased availability of diagnostic tests that may make mAbs more feasible to administer (CB-UPMC 2013).

Cost can be a major determinant of access and acceptability for drugs and vaccines, e.g. the cost of the HPV vaccine is considered a factor in U.S. acceptability (Stupiansky et al., 2010; Liau et al., 2012). As a biologic, Abs cost more to manufacture than small-molecule drugs; FDA-licensed Abs are currently among the most expensive drugs. Many factors contribute to the cost of a particular Ab, but the most important factor influencing their price appears to be the market, i.e. the therapeutic market will bear a high cost for Abs, so they carry a big price tag (CB-UPMC, 2013). With the incredible opportunities of Ab-based drugs and vaccines in global health, there is now significant pressure to dramatically lower the costs.

Antibody manufacturing in mammalian cells has made tremendous strides over the last three decades in lowering the cost of antibody manufacturing and increasing the scale. For example, using existing and conventional unit operations for very large scale Ab manufacturing and purification costs are frequently reported to be < $300/g Ab (Kelly 2007). However, the shear size of the unmet need for Ab-based products in global health may be beyond the current worldwide manufacturing capability of animal cell based production (Farid, 2007).

Manufacturing of whole antibodies in *Nicotiana benthamiana* may meet the demands of large, cost-sensitive markets (Whaley et al., 2012; Whaley et al., 2011). The transient expression system relies on the co-infection and co-replication of two different, non-competing plant viral vectors, tobacco mosaic virus (TMV) and potato X virus (PVX) (Giritch 2006). *Agrobacterium tumefaciens*-mediated transfer-DNA (T-DNA) is used as the delivery system to introduce the components necessary to assemble the TMV and PVX-based plant viral vector expression systems *in planta*. With the development of transgenic strains of *N. benthamiana* with fucosyl- and xylosyl-transferase knocked out by RNAi (Strasser 2008; Strasser 2009), Abs are produced with a highly homogenous mammalian glycoform (GnGn). An important aspect of this versatile and adaptable manufacturing platform is that it has been shown to be a linearly scalable system. In addition, the Nicotiana-based technology is portable (i.e. minimal capital cost requirements) and could be used to manufacture in countries with unmet need, assuming a local biopharmaceutical industry. Production of Ab-expression in transgenic Nicotiana may further lower costs. The quality of cost estimates are likely to improve over time now that GMP manufacturing of Nicotiana-based Abs is becoming routine (e.g. Kentucky BioProcessing LLC, Owensboro KY; Icon Genetics, Halle, Germany).

Several types of antibody fragments can be produced in microbial cells (mainly bacteria or yeast).

Manufacturing of antibody fragments has been conducted with *Lactobacillus*, *Bacillus*, *Streptomyces*, and *Staphylococcus*. The use of Lactobacilli as vectors for antibody fragments is being pursued (Lagenaur et al., 2010), as is AAV-vectored antibodies delivered systemically (Balazs 2011) or to cervico-vaginal epithelial cells (Abdel-Motal, 2011). Cost estimates for vectored antibodies are not currently available.

4.4. Regulatory Strategy for Ab-based MPTs

Most of the current pediatric vaccines are multipurpose vaccines (MMR, DTaP), including recent approvals that provide simultaneous prevention of multiple diseases (diphtheria, pertussis, typhoid, polio, hepatitis B, Hemopholius influenza, mumps, measles, rubella). All of these vaccines were approved as single vaccines and then evaluated as multipurpose vaccines. The FDA has provided Guidance for Industry for the evaluation of combination vaccines for preventable diseases (FDA 1997) including a vaccine that prevents multiple diseases. Regulatory considerations for developers of combination vaccines have been reviewed (Vose, 1999).

Today, there are over 30 FDA-approved monoclonal antibody products, with >200 in clinical development. In some instances, Abs do not carry as much regulatory risk as other drugs because the FDA has recent and historical experience with evaluating mAb products and Abs are essentially naturally occurring human molecules (CB-UPMC, 2013). Because many infectious disease indications require administration of multiple Abs, the FDA has allowed multi-Ab drugs to be clinically tested as a single product. A Phase 1 clinical trial has been performed with a three Ab cocktail for botulinum toxin being developed by Xoma (Nyak et al., 2013) and Phase 2 trials have been performed by Symphogen involving a 25 mAb and a two mAb cocktail (Stasi, 2010), and by Crucell with a two Ab cocktail for rabies (Bakker et al., 2008). New and cost efficient cell banking and manufacturing concepts for multi-mAb products have been described (Frandsen et al., 2011) and it has been demonstrated that a complex mAb composition containing 25 antibodies can be manufactured in a highly consistent manner in a scaled-up production process. The FDA has provided draft Guidance for Industry on the co-development of two or more unmarketed investigational drugs for use in combination, i.e. multidisease products (FDA, 2010).

5. Summary and Conclusions

There is significant safety and efficacy data on antibodies to support continued development of antibody-based concepts for MPTs. Formulation of injectable Abs is well established, but formulations for MPT films and rings is now emerging. Regulators are familiar with reviewing antibody based products, but are less familiar with multi-Ab products for simultaneous protection from STIs, but they have extensive experience with combination vaccines administered to children. Acceptability and access of Ab-based MPTs are dependent in part on pharmacoeconomics that are currently undetermined, but the cost of traditional cell culture manufacturing continues to drop, and the intent of Nicotiana-based and other novel manufacturing technologies is to significantly lower the cost of antibody-based products for prevention.

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Highlights

- Antibodies are specific and versatile candidates for multipurpose prevention technologies.
- Low cost and scaleable manufacturing is required to complement Ab versatility.
- Antibodies can be formulated as topicals (gel, film, device), injectables, and vaccines