

Meeting Report

A summary of the workshop on passive immunization using monoclonal antibodies for HIV/AIDS, held at the National Institute of Allergy and Infectious Diseases, Bethesda, 10 March 2006

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Received 5 April 2007; revised 28 June 2007; accepted 28 June 2007

Abstract

Passive immunization with monoclonal antibodies (MAbs) has been shown to prevent a wide variety of diseases. Currently, there are no MAb products that are licensed for use for immunotherapy or immunoprophylaxis against infection by HIV. However, there are several rational arguments that can be advanced for the use of a passive immunization approaches for counteracting HIV much as for other diseases especially with respect to mother-to-child transmission (MTCT) of HIV and immediate post-exposure situations. Several arguments questioning the feasibility of the approach based on availability of effective drugs, high cost of production and distribution of the MAbs among others, also get raised. It seems that the field now is looking at some promising MAbs as well as several alternate ways to manufacture antibodies and which hopefully may positively affect cost-related issues. This summary of a workshop held to assess the role of MAbs in the treatment and prevention of HIV/AIDS provides a fairly comprehensive analysis of the usefulness of MAb technology for future HIV/AIDS research.

Published by Elsevier Ltd on behalf of The International Association for Biologicals.

Keywords: Monoclonal antibodies; Passive immunization; HIV/AIDS prevention; Immunoprophylaxis; Immunotherapeutic; MTCT; MAb

1. Workshop goals

Passive immunization with antibodies has been shown to prevent or treat a wide variety of infectious diseases. In the early use of this technology, antibodies were administered as human or animal plasma. Improvements in technology led to the process of producing the antibodies as a purified immunoglobulin fraction from pooled human plasma for intravenous (IVIG) or intramuscular (IG) use. These immunoglobulin fractions contained specificities against multiple antigens and thus only a fraction of the specificities was directed against any one disease agent. The exquisite specificity of monoclonal antibodies (MAbs) has made it possible to develop antibody products with increased potency and ease of administration. Although several MAbs for infectious diseases are in clinical trials, only one product (against respiratory syncytial virus)

is licensed for use. Currently, no MAb products have been licensed for use of therapy or prophylaxis against HIV infection. However, rational arguments can be advanced for the use of passive immunization to prevent HIV infection, especially to reduce mother-to-child transmission (MTCT) of HIV, and in immediate post-exposure situations. The key arguments questioning the feasibility of this approach relate to the alternate availability and use of effective antiretroviral drugs, and to the high cost of production and distribution of MAb products.

A one-day workshop was held in March 2006 to evaluate the potential role of passive immunization using MAbs for therapeutic or prophylactic outcomes in HIV/AIDS, and to evaluate the feasibility and acceptability of using MAbs in adults, children and infants. An additional goal was to discuss whether there were roles for anti-HIV MAbs and passive immunization studies other than in clinical settings, and if the field needed to pursue them. This workshop had representation from the perspectives of basic science and clinical research, production and manufacturing, cost consideration and

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regulatory issues, among others. The expected output was a better understanding of the importance of MABs for passive immunization against HIV and to evaluate the possibility of identifying protective epitopes, designing appropriate protection models and clarifying regulatory requirements.

At a previous workshop on Immunoprophylaxis for HIV-1 in Pediatrics: Moving Concepts to Reality on Vaccines and Passive Immunity held in 2002, the discussion focused on neonatal immunoprophylaxis strategies and how to incorporate our understanding of the pathogenesis of HIV infection in MTCT into the design of immunoprophylaxis protocols, a review of antibodies and vaccine candidates currently or imminently available, available cohorts of subjects for clinical trials and potential barriers to implementation of neonatal immunoprophylaxis trials. The workshop reviewed both active and passive immunization regimens, as well as ideas for a combination approach [1].

As a result of the 2002 workshop, a few anti-HIV MABs were pursued and a few small studies of safety and effectiveness have been conducted in adults. At that time, a limited number of well-characterized human anti-HIV MABs were available that exhibited potent and broad neutralizing activity. MABs b12, F105 and 2G12 bind to the surface of gp120 envelope glycoprotein. MABs 2F5 and 4E10 recognized linear epitopes near the membrane-spanning region of gp41 (c.f. in Ref. [1]). The currently described March 2006 workshop set out to address a limited number of questions as listed in Table 1. The outcome from the discussions is summarized in this report.

2. Passive immunization studies

Because of the exquisite specificity of antigen recognition by MABs, the ability to characterize them more reproducibly,

Table 1
Points to consider for discussion at the March 2006 workshop

Scientific
(a) Target population (HIV-infected subjects, infants born to HIV-infected mothers, breast-feeding infants, etc.)
(b) Target epitopes for the MABs?
(c) Single MAb or synergistic combination of MABs? How many in the combination?
(d) Synergy between different MAb combinations or synergy between drugs and MABs?
(e) Polyreactivity of antibodies and other properties that may become issues?
(f) Other emerging scientific issues?
Technical
(a) Feasibility of approach (rationale for using MABs; therapeutic or prophylactic)
(b) Production/manufacturing and costs; distribution; ease of administration
(c) Drugs vs MABs (will availability of effective drugs compromise consideration of MABs?)
(d) Other technical/logistical issues?
Clinical trials
(a) Dosage in infants and children
(b) Mode of administration
(c) Formulation
(d) Adverse effects (known or unknown)
(e) Regulatory issues
(f) Other?

to generate quantitative and qualitatively defined amounts consistently and for safety reasons (as compared to harvesting from polyclonal plasma donations), the preference for MAB products for passive protection in clinical settings is clear. MABs can be used to provide evidence for *in vivo* protective effect, for identification of vaccine epitopes as a preventive strategy in newborns or for post-exposure prophylaxis.

In some countries, women of childbearing age are particularly at high risk of HIV infection and primary prevention strategies are desperately needed. While microbicides and vaccines offer the best hope for protecting women against HIV infection, none are currently available. Simian models have been very helpful in understanding the mechanisms and early events in vaginal SIV transmission, and topical or systemic administration of MABs has provided proof of the importance of neutralizing antibodies in protection from infection [2,12]. These studies provide a strong rationale for developing preventive vaccines that elicit strong neutralizing antibodies or the development of microbicides that incorporate MABs to protect women.

Worldwide, MTCT of HIV is the primary mode of pediatric infection. Antiretroviral therapy (ART) can significantly prevent MTCT in the peripartum period; however, the utility of these regimens may be short-lived due to the emergence of ART resistance. In addition, perinatal ART does not prevent MTCT through breast-feeding, which still continues to be a major mode of transmission. The need for preventing MTCT during the breast-feeding period needs special attention especially in financially constrained societies where for several reasons, such as lack of affordable and acceptable options, breast-feeding cannot be discouraged or discontinued [3]. In addition, infants born to HIV+ women do not have a fully developed immune system and may well benefit from passive immunization. More work needs to be done to understand the biology of developing immune responses to HIV in children <6 months of age, such as understanding the neutralization sensitivity of vertically or horizontally transmitted viruses [4].

Studies in juvenile macaques showing that passively introduced neutralizing antibodies resulted in enhanced *de novo* production of neutralizing antibodies in infected animals [5] provide another reason for exploring the value of passive immunization with MABs in a passive–active immunization strategy.

Passive immunization and protection studies in non-human primates (NHP) using the broadly neutralizing MABs 2F5, 2G12, F105 and b12 indicate that a cocktail of two or more MABs is more potent than single MABs, and that passively transferred antibody administered prior to or within a few hours after challenge is more effective at inducing sterilizing immunity [6,7]. It is possible that the administration of neutralizing MABs at the time of birth, and monthly during the neonatal period, in combination with ART, may provide sterilizing protection.

3. Strategies for developing useful MABs for HIV/AIDS

Very few MABs have been used to study vaccine epitopes, and most of these have been generated against Clade B HIV.

Much of the information required for vaccine design or for epitope definition will necessarily need to be generated via induction of neutralizing MAbs and passive protection studies. There is a need for generating MAbs to other Clades in order to define protective epitopes of HIV-1 isolates in endemic regions. The field needs more standardized and cohesive virus panels for screening MAbs; these would also be useful for testing vaccine responses.

The availability of consensus Clades B and C virus panels will be useful to screen for broadly neutralizing MAbs [8,9]. It was also suggested that in view of the differences in the neutralization epitopes between transmitted and parent viruses [10], it may be a good idea to make a consensus panel of newly transmitted viruses, including those passed from mothers to infants during delivery or post-partum through breast milk. Much work is needed in understanding the nature of the transmitted virus. There are a number of questions that need to be addressed with respect to the critical epitopes on the virus that are associated with transmission and which can be targets of neutralizing MAbs. However, it is also unclear if it will be possible to find a conserved epitope that will be present in all transmitted viruses, such as between different mother–infant pairs or hetero-sexual donor–recipient pairs. To add another level of complexity, it is unclear as to whether critical epitopes involved in virus transmission are different as virus is transmitted across different tissues, and whether transmission occurs by cell-associated virus or cell-free virus. Thus, for all these reasons, from the discussions in the context of the utility of compiling a newly transmitted virus panel, it was clear that because there was not sufficient information available regarding the epitopes seen in different transmission settings, whether the search for breadth of reactivity of neutralizing MAbs against HIV would now be further stretched to include not just regional Clades but different transmitted isolates.

4. Safety of *in vivo* administered MAbs

While the *in vivo* studies in NHP models and Phase I studies in humans have been encouraging for contemplating the use of these broadly reacting anti-HIV MAbs in clinical settings (especially for breast-feeding infants), the chance discovery that these antibodies exhibit cross-reactive binding to self-antigens has begun a whole new process of inquiry into the safety issues associated with the *in vivo* use of MAbs [11]. MAb 4E10 has not been studied by itself in NHP, and there are limited data from three juvenile macaques studied with 2F5 alone in NHP [12]. In light of the “anti-phospholipid” reactivity of these antibodies *in vitro*, a study to assess the long-term *in vivo* adverse effects of administering such antibodies in NHP may be worthwhile and could provide valuable information.

With respect to the adverse events noted in the NHP studies [7] it seems that the one infant rhesus (out of 45 studied), which had neurological symptoms including hind limb paralysis, may have been predisposed to possible intracranial bleeding. Observations such as these are disturbing and could

potentially be an obstruction for further development; however, when such incidents are rare occurrences, a more careful and perceptive analysis needs to be done before discontinuing such approaches. Regulatory agencies such as the FDA can help or guide in interpreting such data, to enable sound decisions regarding pros and cons of pursuing potential candidates.

The polyspecificity and/or autoantigen reactivity of some of the current MAbs (such as 4E10 and 2F5) need to be critically looked at. There does not appear to be a consensus that the anti-cardiolipin and other autoantigen reactivities of the currently studied MAbs are associated with pathogenicity [13,17]. Anti-phospholipid antibodies have been reported in infectious disorders, such as AIDS and syphilis, without the clinical features of anti-phospholipid syndrome. Despite the prolonged clotting times, bleeding is not a typical feature associated with administration of these antibodies. Thrombocytopenia, if present, is usually mild. Patients may have either a lupus anticoagulant or an anti-cardiolipin antibody or they may have both antibodies. A high percentage of patients with systemic lupus erythematosus (SLE) or related autoimmune diseases have these antibodies, which may also develop in patients without an underlying disorder and transiently in association with certain medications (e.g., hydralazine, phenytoin) or infections. The human immunodeficiency virus (HIV) is commonly associated with positive tests for anti-phospholipid antibodies [14]. Infection-associated antibodies may not be associated with clinical symptoms of anti-phospholipid antibody syndrome, and they tend to recognize phospholipid rather than the phospholipid–protein complexes described [15]. In fact, it is entirely possible that broadly cross-reactive potent neutralizing MAbs will intrinsically be polyreactive, in which case the biological and biochemical properties of these antibodies need to be better studied. As a start to collecting such information, partial thromboplastin time (PTT, a measure of blood clotting time) measurements were made on four human subjects being treated with a cocktail of 2G12, 4E10 and 2F5. It was observed that PTT was prolonged; however, it was considered to be mild (symptoms causing no or minimal discomfort). One patient showed a low level of lupus anticoagulant activity. No other overt/significant clinical adverse event was reported [16].

5. Therapeutic considerations for *in vivo* administration of MAbs

In adult therapeutic trials, resistance to MAbs has been shown to develop *in vivo* in HIV-infected patients who discontinue ART. In studies performed to assess the *in vivo* efficacy of passively administered pool of 2F5, 4E10 and 2G12, viral rebound upon cessation of antibody infusion was noted [17]. In general the antibody infusions were well tolerated and there were no significant adverse effects. The conclusion from clinical trials with anti-env MAbs was that resistance to 2G12 developed early, and also that 2G12 had a longer elimination half-life than 2F5 and 4E10. In general viral fitness was significantly decreased in the presence of 2F5 and 4E10 [18]. Passive immunization using broadly neutralizing antibodies as

an adjunct to HAART needs further evaluation, using combinations of antibodies with a more favorable half-life or tissue distribution profile, as well as doses that would provide neutralization responses above that achieved with autologous plasma.

An anti-CD4 MAb, TNX-355 (Tanox, Inc.), is currently in Phase II trials and shows some promise of efficacy; however, resistant viruses using CD4 are still generated *in vivo*. Further work with these resistant viruses is ongoing to understand the underlying mechanisms of resistance development. MAb dB4 (United Biomedical, Inc.) is another anti-CD4 MAb that has shown encouraging results in NHP challenge studies and is currently being contemplated for clinical trials. Anti-cell surface antibodies may have promise as therapeutic MAbs; there are plans to use them in combinations to improve neutralizing responses. It was suggested that antibodies targeting CCR5 may have clinical utility.

6. Future considerations for research and development of anti-HIV MAbs

Immunoglobulin class (heavy chain) is an important factor to consider in that virus neutralization can be brought about by functions such as antibody dependent cellular cytotoxicity (ADCC) and cell-mediated lysis (CML) in an *in vivo* setting. It was noted that 2F5 and 4E10 were initially isolated as IgG3 isotype and it was also shown that F105, when isotype-switched from an IgG1 to an IgG3, showed enhanced neutralizing activity. Because of the abundance of non-neutralizing antibodies present in HIV-positive individuals, studies were encouraged for investigating whether such non-

Table 2
Action items from the March 2006 workshop

1. Better understanding of the mechanism/early viral replication following mucosal transmission (including MTCT).
2. Better understanding of the genetics and biological properties of transmitted viruses (including susceptibility to entry inhibitors or MAb).
3. Better understanding of how to elicit highly neutralizing Ab using vaccination.
4. Better understanding of the clinical relevance of anti-phospholipid Abs.
5. Better understanding of the bases and clinical significance of differences in readouts from different neutralizing Ab studies.
6. Continue to develop high-throughput assays that can speed basic and clinical research; including the development of transmitted virus panels and standardized biologically relevant assay methods.
7. Continue to drive clinical evaluation of promising, available products, particularly in infants and children. There is a need for generating MAbs to other Clades to define protective epitopes in those endemic regions.
8. Further study synergy between non-neutralizing antibodies in mixtures with either non-neutralizing or neutralizing antibodies as well as cell-surface directed antibodies and anti-HIV antibodies.
9. Investigate the role of antibodies as microbicidal agents, and microbicides in general (with specific focus on antibody-based microbicides) as an intervention for perinatal transmission.
10. Further evaluate passive immunization using broadly neutralizing antibodies as an adjunct to HAART using combinations of antibodies with a more favorable Pk profile, as well as doses that would provide neutralization responses more than what can be achieved with autologous plasma.

Table 3
Participating panel members at the March 2006 workshop

Dr. Cavacini, Lisa	Beth Israel Deaconess Medical Center
Dr. Clouse, Kathleen	FDA/CBER/UPS/DMA
Dr. Finstad, Connie	United Biomedical Inc.
Dr. Fung, Michael	Tanox, Inc.
Dr. Hanson, Carl	California Department of Health Services
Dr. Haynes, Barton	Duke University
Dr. John-Stewart, Grace	University of Washington
Dr. Katinger, Hermann	Polymun Scientific, Austria
Dr. Koenig, Scott	MacroGenics, Inc.
Dr. Lewis, Stanley	Tanox, Inc.
Dr. Lockshin, Michael	Cornell University
Dr. Luzuriaga, Katherine	University of Massachusetts Medical School
Dr. Markowitz, Martin	Aaron Diamond AIDS Research Center
Dr. Markham, Richard	Johns Hopkins University
Dr. Mascola, John	Vaccine Research Center/NIH
Dr. Meade, Harry	GTC Biotherapeutics
Dr. Mynahan, Richard	Leerink Swann/MEDA Corp.
Dr. Ruprecht, Ruth	Dana-Farber Cancer Institute
Dr. Safrit, Jeffrey	Elizabeth Glaser Pediatric AIDS Foundation
Dr. Trkola, Alexandra	University Hospital, Zurich

neutralizing antibodies may exhibit synergistic action in mixtures with other less studied non-neutralizing or neutralizing MAbs.

Although a long way off from a potential product stage, the possible use of antibodies as microbicidal agents was discussed. *In vitro* experiments looking at antibody release from vaginal rings are very promising. The use of vectors such as lactobacilli to express antibodies may be worth contemplating since they (antibodies and lactobacilli) may provide some benefits with respect to safety or decreased toxicity compared to some of the current formulations under study, and can be contemplated for counteracting perinatal transmission of some infections.

Finally, the development of an MAb-based product should include an early recognition of marketability, production logistics and cost-related issues, as well as regulatory and safety considerations. Discussion with the FDA (www.fda.gov/cder/ode4/preind/default.htm) and/or industry would be worthwhile if begun early at the time product oriented research is contemplated.

Table 2 lists some of the gaps and action items that were identified for consideration while revisiting issues related to the use of MAbs in passive immunization studies for HIV/AIDS by the panel members who participated in the workshop, as shown in Table 3. From this brief summary of the workshop presentations and discussions, it appears that priorities should include more work on identifying the nature of the transmitted viral epitopes, isolating neutralizing MAbs with breadth of reactivity, investigating the autoimmune reactivity and its relevance, and in developing consensus assays for the screening of relevant and useful MAbs.

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