Convalescent transfusion for pandemic influenza: preparing blood banks for a new plasma product?

Jonathon P. Leider, Patricia A.R. Brunker, and Paul M. Ness

Due to the potential of a severe pandemic to limit efficacy or availability of medical countermeasures, some researchers have begun a search for new interventions that could complement the planned antiviral- and vaccine-based response to an influenza pandemic. One such countermeasure—the transfusion of pandemic influenza-specific antibodies from surviving patients to the clinically ill—is the focus of this commentary. Passive immunotherapy, which includes the use of monoclonal antibodies (MoAbs), hyperimmune globulin, or convalescent plasma, had been used before the advent of antibiotics and has recently reentered the limelight due to the accelerating development of MoAb therapies against cancer, a number of microbes, allograft rejection, and a host of other conditions. After the plausible biologic mechanism and somewhat limited data supporting the efficacy for this modality against influenza are reviewed, safety and logistical concerns for utilization of this potential new product (fresh convalescent plasma against influenza [FCP-Flu]) are discussed. FCP-Flu could indeed prove useful in a response to a pandemic, but two necessary items must first be satisfied. Most importantly, more research should be conducted to establish FCP-Flu efficacy against the current and other pandemic strains. Second, and also importantly, blood banks and donor centers should examine whether offering this new product would be feasible in a pandemic and begin planning before a more severe pandemic forces us to respond without adequate preparation.

When preliminary morbidity and mortality data started to appear from Mexico in the spring of 2009,[1] it seemed the next great pandemic was nigh—the government, media, and public health professionals all communicated, sometimes in a frenzy, that the pathogen was virulent, novel, and clinically severe—the major requirements of a severe pandemic germ. The rest of the story is unfolding fortuitously: medical countermeasures (namely oseltamivir and zanamivir) worked quite well; supply lines remained intact during the initial scare; and, most importantly, mortality was lower than feared, although the pathogen spread quite efficiently. Indeed, on June 11, 2009, the WHO declared a worldwide pandemic due to breadth of spread, although only 144 deaths were recorded among the 28,774 reported cases (up to 0.5% fatalities).[2,3] After seeing a tremendous case fatality rate with H5N1 infections previously (up to 60%),[4,5] the public health community had been bracing for much worse. Yet, there are signs that the mild spring-summer pandemic wave may yet beget a larger one come fall or springtime.[6]

As the current global influenza pandemic unfolds, we must not assume that the apparent benignity of the current H1N1 virus thus far is assurance of a "false alarm"

ABBREVIATIONS: ADE = antibody-dependent enhancement; ARDS = adult/acute respiratory distress syndrome; FCP-Flu = fresh convalescent plasma against influenza; HIG = hyperimmune globulin; TRIM = transfusion-related immunomodulation.

From the Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; and the Division of Transfusion Medicine, Department of Pathology, The Johns Hopkins Hospital, Baltimore, Maryland.

Address reprint requests to: Jonathon Leider, Hampton House 352, 624 N. Broadway, Baltimore, MD; e-mail: jleider@jhsph.edu.

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or "near-miss." Such complacency would clearly undermine national and international efforts in emergency preparedness. Even if it is not the current virus, it is simply a matter of time until a catastrophic pandemic capable of overwhelming state health care systems due to lack of resources, likely absenteeism among health care and other key workers, and a growing number of infected individuals occurs. Treatment options will be limited, with public and private health care systems and government agencies employing various forms of rationing.[7-13] An opportunity exists for additional, perhaps experimental, clinical interventions to reduce strain on the system. The Defense Advanced Research Projects Agency (DARPA) of the US Department of Defense has recently issued a broad agency announcement that seeks to operationalize passive immune therapy as a medical countermeasure against any potential dangerous biologic agent.[14] This course of events suggests that the political and social climate may be ripe for clinical research into convalescent plasma therapy for influenza.

The prevailing strategy for treating an influenza pandemic focuses on antiviral medication and supportive care and culminates in the rapid development of an effective vaccine.[15] Some researchers have begun to explore additional possibilities such as monoclonal antibody (MoAb) production, hyperimmune globulin (HIG) generation, and convalescent plasma transfusion as potential treatments for a pandemic influenza. The process of infusing antibodies is broadly known as passive immunotherapy.

Limited but promising reports of success of a passive immunotherapy modality exist for H5N1 highly pathogenic avian influenza (influenza A more broadly), as well as a myriad of other infectious diseases.[16] Here, we review evidence supporting the scientific feasibility of the model, considering the great need for additional interventions in a pandemic. We believe that further investigation of passive immunotherapy as a medical countermeasure during a pandemic is warranted. Beyond their roles in additional clinical research and efficacy trials, blood banks, both functioning as donor collection centers and as product distribution centers, have an opportunity to make a significant impact on both investigational protocols and on any successful pandemic response.

A PRIMER ON PASSIVE IMMUNE THERAPY

Antibody infusions have been used in prophylaxis or treatment of tetanus, hepatitis B, botulism, and several other ailments.[17-20] Until recently, this therapy had occurred mainly in the form of convalescent plasma transfusion, also known as serum therapy (reviewed by Casadevall et al.[18]), where whole blood or plasma from survivors is transfused into the ill. Historically, serum therapy using both animal and human serum sources was widely embraced in the 1890s to 1930s (the preantibiotic era). However, a very significant side effect, known as "serum sickness," can result. This systemic immune reaction (or Type III hypersensitivity reaction) is characterized by the deposition of antigen-antibody immune complexes, which was suspected as early as 1911 by Clemens von Pirquet.[21] After administration of a foreign antigen (such as a horse protein present in horse serum), the patient mounts a humoral response to the antigen. The antibody complexes with its target antigen, deposits in small vessels and joints, activates complement, and instigates inflammatory damage. Clinical disease is characterized by fever, arthralgia, and vasculitis, with dermatologic, renal, and pulmonary manifestations, usually within 6 to 21 days of antigen exposure. The potential severity of this complication, particularly when animal plasma is used, coupled with safer and more predictable drug therapies, foretold the decline of this approach. Modern methods of antibody isolation, purification, and production have heralded the potential resurgence of passive immunotherapy in the form of HIG and therapeutic MoAb.

Passive immune therapy, whether as a "crude" source (such as plasma) or as a "refined" material (such as MoAbs or HIG fractionated from plasma), can help the patient respond to a pathogen. The successful immune response is necessarily multifactorial and has been broadly considered as comprising both innate (e.g., phagocytic processes) and adaptive (subdivided into humoral or cell-mediated) components. The relative balance of these processes is both host and pathogen dependent to a certain extent. Passive immune therapy contributes to the humoral response, which derives its pathogen specificity from the precise binding of an antibody to its cognate antigen. Central to an effective adaptive immune response is an overlapping functionality of both humoral (i.e., antibody-based) and cell-mediated components. However, on initial exposure to a pathogen, it can take days to produce the correct type or types of antibody in an adequate concentration for maximal effect, and this ability is host dependent. In the interim, the disease process continues, causing morbidity and mortality. Providing an exogenous pathogen-specific antibody to the patient in this way provides a bridge of passive immunity that can give the host additional time to mount a more effective active response.[22]

Passive immune therapy is currently available and in use in the form of HIG, which is a biologic therapeutic that has features of both convalescent transfusion and manufactured antibodies. There is a strong track record of success with this therapy for either prophylaxis or treatment in many bacterial and viral diseases, including tetanus, botulism, diphtheria, hepatitis A, hepatitis B, respiratory syncytial virus, cytomegalovirus, varicella-zoster, rabies, measles, and complications of smallpox vaccination (reviewed by Keller and Stiehm).[16] These preparations

are derived from human plasma (like convalescent transfusion), but undergo a rigorous cold ethanol fractionation process and may be subjected to solvent/detergent (S/D) treatment, nanofiltration, or other purification processes.[23] These additional manipulations render the product "sterile" and result in "significant removal and inactivation" of enveloped and nonenveloped viruses, as described in a package insert for rabies immune globulin, for instance.[24] Nonetheless, there is still a risk of infectious disease transmission. The source plasma is typically from patients who have been previously vaccinated (not survivors of natural pathogen challenge) and produce high antibody titers against the target antigen. Extensive donor selection criteria, including questionnaires and infectious disease testing, are performed. While not a recombinant protein or produced in sterile cell culture, the additional processing imparts a stronger safety profile to this product, like manufactured antibodies. However, also in common with biologic therapeutics, the production cycle may require a few weeks to several months.[23]

A ROLE FOR PASSIVE IMMUNE THERAPY IN PANDEMIC INFLUENZA?

Jenner's smallpox vaccine in 1789 was the first effective use of active immunization. Though vaccines may take time to develop and may be in short supply, they will be a critical component of pandemic response as a primary means of prevention. Moreover, depending on the type of vaccine prepared, they can engage more than just the humoral branch of the adaptive immune system, as passive immunotherapy does. For example, DNA vaccines are in development for orthomyxoviruses and "elicit broad-spectrum humoral and cellular immunity against influenza virus."[25] Manufacturing delays hinder vaccine availability in pandemic planning and early pandemic response. The current treatment modality—antivirals such as oseltamivir or zanamivir—may be scarce or ineffective.[26,27] Passive immunization, then, may have a role to play in treatment.

Therapeutic antibody use for pandemic influenza would likely take two forms: MoAb production (with either single administration or "cocktail" formulations) and convalescent transfusion (with the possibility of generating HIG, which is usually manufactured after vaccine development has taken place). A third modality still in developmental stages is the production of recombinant human polyclonal antibodies.[28] MoAb research provides a wealth of support for anti-influenza passive immunization as a proof-of-concept for the scientific plausibility for its use in a pandemic response. This MoAb research in animal models and in vitro human cell systems[29] lays important foundations that can be extended to studying convalescent transfusion as an effective antibody-delivery intervention during a pandemic—justifying human clinical research on this potential, but often neglected, therapeutic modality.

MoAbs AS A MODEL FOR PASSIVE ANTI-INFLUENZA THERAPY—AND ASSOCIATED DIFFICULTIES

MoAbs are antigen-specific proteins commonly mass produced by immortalized B cells known as hybridoma cells or by recombinant protein technology.[30] MoAb technology is being harnessed to create new interventions against cancers, allograft rejection, and a multitude of microbes, among other applications. Dozens are commercially available, with many in development and clinical trials (reviewed by Marasco and Sui[31]). Briefly, cell lines are created by isolating plasma cells producing specific (e.g., anti-H5N1 virus) antibodies from surviving subjects, immortalizing them, selecting the desired clone or clones, expansion into a large-scale cell culture system, and isolating and purifying the secreted antibodies. Several cell lines have been shown to be effective in generating neutralizing and protective antibodies against various types of highly pathogenic influenza.[32-42]

Mechanism of action: efficacious anti-influenza MoAbs?

The importance of humoral immunity in both the prevention and the treatment of influenza challenge is apparent. In fact, the critical role of the humoral response in influenza is the basis of influenza vaccine design.[43] Similarly, MoAb therapy also aims to capitalize on this mechanism. Recently, several laboratories have independently identified MoAbs as efficacious avian influenza countermeasures in mouse models, both as a prophylactic agent and as a therapeutic neutralizing agent and the hunt for a "universal" influenza antibody is intensifying (Table 1). These studies suggest that MoAbs may be highly protective if administered up to 3 or 4 days postinfection, with diminished protection if administered at 5 days postinfection and possibly ineffective at 6 or more days postinfection.[44] While certain isolated lines of antibodies and doses prove more effective than others, these reports independently assert a high success rate, leading some authors to suggest a cocktail of antibodies as a potential treatment for influenza infection, H5N1, or otherwise.[36,37]

The ongoing variation between influenza strains caused by mutations that accumulate over time, primarily at the neuraminidase and hemagglutinin proteins, is responsible for the difficulties producing a durable anti-influenza immune response. This process, known as "antigenic drift," is the result of minor genetic changes and is the reason annual vaccine reformulations are necessary.[45] It appears that the reassortment of viral RNA segments, known as an "antigenic shift," causes major protein

TABLE 1. Studies of MoAbs to influenza in animal models

Year	Authors	Source of antibodies	Challenge dose and schedule	Model system	Prophylactic result	Therapeutic result
2006	Lu et al.[34]	Equine vaccinated with inactivated H5N1 virus, anti-H5N1 IgGs purified from HIG sera to F(ab′)2 fragments.	Intraperitoneal injection of 50, 100, or 200 μg F(ab′)2 fragments with normal equine antibodies as control 24 hr after injection with lethal H5N1 dose.	BALB/c mouse	Not tested.	50-μg doses provided 70% protection; 100- and 200-μg doses provided 100% protection. All controls died.
2006	Hanson et al.[39]	Mice injected with attenuated H5N1 virus, MoAbs generated to anti-hemagglutinin 5 of two strains, A/Vietnam/1203/04 and A/Hong Kong/213/03.	Lethal challenge 10 LD50 (50% mouse lethal dose). Prophylaxis tested with MoAb injection 24 hr before challenge in 1, 5, or 10 mg/kg body weight doses. Therapeutic protection tested with MoAb injection 1-3 days postchallenge in 1, 5, or 10 mg/kg body weight doses.	C57BL/6 mice	One MoAb (VN04-2) offered complete protection against death at all doses. One MoAb (VN04-3) offered full protection against death only at 10 mg/kg, with some protection at lower doses.	For injection 1 day postchallenge: 1 mg/kg afforded protection to 80% with major signs of disease, 5 and 10 mg/kg afforded 100% protection with fewer signs of disease. For injection 3 days postinfection, 1 and 5 mg/kg conferred protection to 80% and mice showed signs of disease, with 10 mg/kg affording 100% protection with mice showing few signs of disease.
2007	Sandbulte et al.[85]	Sera taken from mice that were injected with anti-neuraminidase H1N1-derived vaccine and survived lethal H5N1 challenge	Mice received challenge 10 LD50 of H5N1 virus 18 hr after passive immunization with 350 μL sera from positive control (pooled from mice that survived previous H5N1 challenge) or negative control (saline-injected mice), as well as a novel vaccine.	BALB/cJ	All mice that received anti-H5N1 pooled sera survived and were protected from severe disease, 6/13 mice receiving novel vaccine protected, while 12/13 mice that received saline died.	Not tested.
2007	Simmons et al.[36]	Memory B cells immortalized from previously infected H5N1 human survivors. Cell lines were analyzed for neutralizing potential, from which several lines were selected. Also tested was anti-H5N1 sheep sera.	Prophylaxis tested with MoAb injection 24 hr before 10^5 $TCID_{50}$ challenge in 1-mL antibody preparations, with measurements immediately before challenge to determine titer. For therapeutic test, mice injected with 5 LD50 of H5N1, then were injected 1, 2, or 3 days later with 1 mL of MoAb.	BALB/c	Several MoAb strains and sheep sera protected against lethal challenge.	Several MoAb strains and sheep sera protected against lethal challenge up to 72 hr postinfection passive immunization; some offered cross-clade protection.
2008	Yu et al.[42]	Memory B cells from survivors of the 1918 flu were tested for neutralizing ability against reconstituted 1918 virus. From those that were strongly neutralizing, MoAbs were isolated.	Mice challenged with 5 LD50 of the 1918 virus, 24 hr later were injected with 2, 20, or 200 μg of MoAb.	BALB/c	Not tested.	MoAbs in the lowest dose (2 μg) conferred no protection. One MoAb strain at 20 μg conferred no protection, two conferred some protection against lethality, and two conferred complete protection. All MoAbs conferred complete protection against death from strains at highest dose (200 μg).
2008	Throsby et al.[32,44]	Memory B cell libraries generated from seasonal flu vaccines (H1N1). Those lines that showed good neutralization against H5N1 were utilized.	Prophylactic test with 5 mg/kg MoAb (CR6261) 1 day before 10 LD50 H5N1 challenge and 2 mg/kg MoAb for a 25 LD50 H1N1 challenge. Therapeutic test with 15 mg/kg MoAb (CR6261) injection 3, 4, 5, or 6 days post-25 LD50 challenge.	BALB/c	All passively immunized mice survived and showed no sign of disease, while mice that received control died.	Mice immunized at 3 and 4 days postinfection were protected and recovered quickly. Fifty percent of mice immunized at 5 days postinfection survived. No mice immunized at 6 days postinfection survived. Controls did not survive.
2008	Chen et al.[86]	Mice vaccinated with inactivated viruses, hybridomas generated against four H5N1 strains. MoAbs showing strong neutralization were selected.	Mice challenged with 10 LD50 of five H5N1 strains. MoAbs were given at 20 mg/kg body weight 1, 2, 3, or 4 days after challenge. Dose response was determined by administering 1, 5, 10, 20, 40, or 80 mg/kg MoAb preparation against a challenge of 10 LD50 BH Goose/Qh/15C/05.	BALB/c	Not tested.	One MoAb (13D4) offered full protection against all four clades (1, 2.1, 2.2, 2.3) when administered 20 mg/kg body weight 24 and 48 hr postinfection. MoAb preparation conferred complete protection in three of four clades when administered 3 days postinfection. Dose-response experiment indicated minimum dosage to afford full protection after 24 hr was 5 mg/kg, after 48 hr was 10 mg/kg, after 72 hr was 20 and 40 mg/kg, and after 96 hr with 80 mg/kg body weight. Protection was afforded even when virus had advanced beyond lungs.

TABLE 1. *Continued*

Year	Authors	Source of antibodies	Challenge dose and schedule	Model system	Prophylactic result	Therapeutic result
2009	Sui et al.[37]	Phage library was utilized to identify good H5N1 neutralizing antibodies, aimed at those best inhibiting cell infection.	Mice were challenged with "high" lethal dose of H5-VN04 (Clade 1) or H5-HK97 (Clade 0). Prophylaxis tests occurred 1 hr before infection at 2.5 or 10 mg/kg. Therapeutic tests occurred 1, 2, or 3 days postinfection at 15 mg/kg body weight.	BALB/c	Two MoAbs (F10 and A66) administered at 10 mg/kg conferred complete protection from death against both clades. Others, and at lower doses, afforded some protection.	Various MoAbs afforded effective protection (80%-100%) up to 3 days postinfection.
2009	Sun et al.[87]	Two antibodies were identified after screening a Fab phage library that was derived from a H5N1 survivor.	Mice were challenged with 10 LD50 24 hr after being injected with 0.025, 25, or 2.5 mg/kg purified antibody preparations, with anti-H5N1 rabbit sera as a positive control.	BALB/c	A dose of 2.5 mg/kg purified rhAb preparation conferred protection from death against a lethal Clade 2.3 H5N1 virus.	Not tested.
2009	Yoshida et al.[88]	One anti-hemagglutinin MoAb was identified and test for protective efficacy.	Mice received purified 200 μg of MoAb 1 day before (prophylaxis test) or after (therapeutic test) 10 LD50 challenge of A/Aichi/2/68 (H3N2) or A/WSN/33 (H1N1) virus. Mice were sacrificed on Day 3 to examine virus titer and pathology.	BALB/c	Authors indicate that mice were "almost completely protected" and had lower virus titers compared to controls.	Authors indicate protection among mice, and two of five subjects had no observable virus titer, indicating heterosubtypic protection against H1 and H3 virus.
2009	Prabakaran et al.[89]	Two anti-hemagglutinin MoAbs were identified that could, in combination, neutralize Clades 0, 1, 2.1, 2.2, 2.3, 4, 7, and 8 of H5N1 viruses.	To test prophylaxis, mice were injected intraperitoneally with 0, 1.0, 2.5, or 5 mg/kg MoAb preparation or a negative control, 24 hr before a 10 LD50 lethal challenge of two H5N1 strains. To test therapeutic efficacy, mice were injected with 0, 1, 2.5, or 5 mg/kg MoAb preparation 24 hr after a 10 LD50 challenge. Another iteration offered two injections of MoAb preparation (1 and 3 days postinfection).	BALB/c	Mice prophylaxed with 5 mg/kg body weight of the MoAb preparation were 100% protected from lethal challenges of the two strains, with lower concentrations providing reduced protection in a dose-dependent fashion.	10 mg/kg of one MoAb (ch2D9) afforded complete protection from lethal challenge. Combination therapy of two MoAbs at 5 mg/kg conferred complete protection and limited disease presentation. Mice that received two doses of combination MoAbs were also protected, but recovered weight more quickly than single-dose subjects.
2009	Koudstaal et al.[90]	Human MoAb CR6261, used in an earlier study[44] and an irrelevant antibody were used, as was oseltamivir.	To test prophylaxis, mice were given 15 mg/kg CR6261 1 day before intranasal injection with 25 times median lethal dose of a H5N1 or H1N1 strain. Controls were injected with irrelevant antibody, and another group was given 10 mg/kg oseltamivir for 5 days starting 1 day before challenge. Therapeutic efficacy was tested by first injecting mice intranasally with 25 LD50 of either a H5N1 or H1N1 strain. Then, mice either received 15 mg/kg CR6261 on Day 4 after infection or 10 mg/kg oseltamivir starting on Day 4 onward.	BALB/c	All mice prophylaxed with CR6261 survived challenges from either strain with little weight change, and oseltamivir offered protection for most mice, although subjects experienced more weight loss.	All mice treated with therapeutic CR6261 survived H5N1 challenge, 40% survived H1N1. Oseltamivir protected approximately 20% in H5N1 group and none of the H1N1 infected. Weight recovery was improved in the CR6261 vs. the oseltamivir groups, as was median survival.

reassortments and is responsible for pandemics, since the population is not protected from what are essentially novel antigens.[45,46] Importantly, a bridge of passive immunity in influenza could allow other components of the immune system time to develop an adequate response before the development of irreversible, end-stage disease in very ill patients (e.g., severe pneumonia and the adult/acute respiratory distress syndrome [ARDS]). As the spectrum of clinical disease with H1N1 and H5N1 influenza range from asymptomatic to fatal infection, MoAb therapy may only be necessary for a subset of patients.[47]

Adverse reactions of MoAbs

MoAbs may well play an important role in fighting pandemic influenza; they are highly selective for known strains, have potential to cause fewer adverse side effects than convalescent plasma transfusion, and can be created utilizing standardized methods and technology. However, like all biologic materials, they are not without risk. The most common reaction to these therapeutics is an infusion reaction, consisting of fever, nausea, vomiting, or fatigue, which is usually minor and managed medically.[48] However, severe reactions can occur, including life-threatening cytokine storm.[49] Depending on the degree of chimerism of the MoAb and humanization methods,[50,51] allergic reactions may also ensue, but the severity of the allergic reaction is reduced by increasing use of humanized MoAbs.[52] There are many adverse reactions to existing therapeutic antibodies that are related to a specific antibody performing its intended function on the target ligand (e.g., increased susceptibility to infectious disease with anti-CD20 or anti-tumor necrosis factor therapeutic antibodies). Such an adverse reaction to a MoAb with anti-influenza specificity is unknown, but is unlikely since the target ligand is a foreign pathogen. However, there remains a theoretical risk of an as-yet-unknown cross-reactivity with a host protein.

Therapeutic antibodies that involve laboratory animals in their production are capable of eliciting a specific immune response targeted against constant regions of the immunoglobulin specific to that animal (e.g., anti-mouse or anti-goat). This anti-species activity can result in an important interaction in the clinical laboratory, where many immunoassays use anti-mouse reagents to measure a given analyte. This problem is called heterophile antibody interference in general, or human anti-mouse antibodies (HAMA) in the murine case.[53] By binding an indicator antibody without the presence of the target metabolite, a false-positive result may occur in immunoassays. A recent and remarkable example of this phenomenon was a very common assay for human chorionic gonadotropin, for which positive test results in at least 58 cases resulted in unnecessary invasive procedures and malignant diagnoses.[54] There are many sources of heterophile antibodies in patient specimens, such as other routes of animal exposures, and commercial reagents are available to block these antibodies.[55] Fortunately, with the improved synthesis of chimeric and humanized antibodies, this problem has been reduced.[56]

Limitations of MoAbs

The potential benefits of MoAb come with some expense, both time-related and financial. While MoAbs are highly selective for known strains, they take some time to isolate and manufacture.[30] Simmons and colleagues[36] isolated clinically important cell lines in weeks from their research samples, but commercial production may take significantly longer. Exciting new technologies may simplify or otherwise expedite isolation and production; for example, a May 2008 letter in *Nature* demonstrated rapid cloning of influenza-specific human antibodies within 1 month of subject vaccination.[57] However, until such methods are utilized at a broad level, production of influenza-specific MoAbs will not be fast. Mutability of the pandemic strain could also create some difficulty for large-scale implementation of MoAbs as a viable intervention—while broad cross-reactivity is possible,[36,37,44] the epitope-specific nature of MoAbs means that any viral mutations involving the target epitope that develop as a pandemic progresses might render the MoAb ineffective. Creating antibody libraries[33] may thus be a valuable endeavor, although no guarantees exist that the epitopes will remain unchanged during a pandemic. A final consideration is cost. The few MoAb agents on the market are generally quite expensive. Trastuzumab (brand name Herceptin) can retail for more than $3000 a vial. As a point of comparison, intravenous immune globulin (IVIG) is also quite expensive, at approximately $120/g or (for a 70-kg patient), approx. $8400 per day (at a dose of 1 g/kg once a day).[58] However, a federally funded initiative, the Accelerated Manufacturing of Pharmaceuticals, holds the prospect in the not-too-distant future (though not soon enough to be of practical use in the current pandemic) of significantly reducing costs for MoAb production to less than $10 per dose for MoAbs.[59] By soliciting research proposals that specifically target "novel approaches that obviate traditional and rate-limiting steps" of current therapies, DARPA is attempting to spur competition, innovation, and decrease costs. Current high costs and time for isolation and production, as well as uncertainties surrounding production capacity and long-term viability of a MoAb strain dependent on viral mutagenicity all are current impediments to a large-scale intervention during a pandemic.

CONVALESCENT PLASMA TRANSFUSIONS (A.K.A. SERUM THERAPY)

In a convalescent transfusion scenario, survivors will have circulating, virus-specific, polyclonal antibodies

resulting from their successful primary immune response. Moreover, these survivors will have developed memory B cells, which are capable of producing increasingly higher-affinity antibodies upon subsequent infectious challenge. Since survivors are likely to live in an area with other patients, they offer the advantage of proximity to the outbreak that MoAb products (which are mass-produced in a few highly specialized laboratories) will not have. Additionally, the short turnaround time between plasma collection and transfusion (which can be as few as 2-3 days) increases the likelihood that immunoglobulin derived from a convalescent individual will react to the specific viral strain affecting the intended influenza-afflicted recipient. While resources are still available in the early stages of pandemic for testing, donors could be selected based on high anti-influenza titers and possibly on negative assays for influenza viremia, among other criteria.

After donation (either as plasmapheresis or whole blood) and standard plasma processing, the potential new product, which we refer to as "FCP-Flu" (for fresh convalescent plasma against influenza), could be ordered by hospitals caring for infected individuals. At the time of blood or plasma collection, a serum specimen would be sent to a reference lab for influenza antibody titer and possibly influenza viral load determination, along with the standard infectious disease tests currently sent for all whole blood donations. The titer would be used to help dose the product, some evidence-based dosage guidelines would need to be developed, and the plasma can then be frozen at the component laboratory and quarantined until test results are available. The product would require separate labeling and storage space and could be stored in the hospital blood bank or central repository for 1 year, as with standard plasma.

As a special product with unique indications, an order for this product would likely require transfusion or infectious disease physician approval in early phases of an epidemic, when its scarcity makes supplies of FCP-Flu very limited. Possible indications for transfusion could include hospitalized patients who maintain a high influenza viral load or suffer progressive clinical deterioration despite maximal antiviral medication or perhaps as a first-line adjunctive therapy in patients with influenza who were already significantly immunocompromised. To develop solid criteria about specific indications and efficacy, an organized research program to gather such evidence would be absolutely necessary.

Upon an approved request, the product could be thawed, issued, and transfused to the patient. The presumed mechanism of action, as for MoAbs, would be that the transferred antibodies then supplement the patient's own immune response against the pathogen, neutralizing circulating virus.

Mechanism of action: examples of successful convalescent serotherapy

Although well-established models exist for use of fractionated products derived of convalescent plasma, such as pathogen-specific HIG,[18,19,60] evidence of H1N1-specific, H5N1-specific, or other strain-specific passive immunity for influenza is still limited largely to case reports of convalescent serotherapy (Table 2).[61-64] Clinical trials of serotherapy for influenza are limited to historical reports, which have been recently examined in a unique meta-analysis, which could prove useful in response to the current H1N1 pandemic.[65] Recent case reports of its use for H5N1-infected patients are promising, but limited and uncontrolled. The patients are few in number, received other treatments concurrently, and were given various doses of plasma with an unknown anti-influenza antibody titer—400 mL over two infusions,[63] 600 mL over three infusions,[62,64] or 1200 mL over six infusions.[62] Although uncontrolled, these cases each report success under different circumstances, but convalescent transfusion began when other interventions appeared ineffective. The dramatic drop in viral load in one case[64] by an order of magnitude only 8 hours after just 200 mL of convalescent plasma was given (from 1.7×10^5-1.4×10^4 copies/mL) supports the idea that the mechanism of action is clearance of circulating virus. Although these reports are encouraging, it is important to note a potential reporting bias toward patients with favorable outcomes.

Modern examples of success with convalescent serotherapy also exist—reminding us that this treatment modality, while not yet standard of care for influenza, is neither ill-conceived nor impractical (see Table 2). Serum therapy is the standard of care in the treatment of Argentine hemorrhagic fever (caused by the Junin virus), before the neurologic-hemorrhagic phase.[60,66] In terms of other pathogenic respiratory viruses, this model has been used with some success, such as the case reported in 2004 by Cheng and coworkers[67] of a patient with SARS. Similarly, a 2005 case report suggests optimistic results for deteriorating SARS patients after receiving plasma transfusion.[68] With particular relevance for the prospect of an evolving or future influenza pandemic, the historical and thought-provoking literature review by Luke and coworkers[65] in 2006 suggests that individuals treated with convalescent blood products during the 1918 pandemic (H1N1) "may have experienced a clinically important reduction in the risk for death."

Adverse effects

Purified blood components, including plasma-derived antibodies, are generally associated with fewer adverse reactions than unmanipulated donor plasma, due to modern fractionation methods, including viral reduction and nanofiltration procedures.[23] Plasma contains a

TABLE 2. Summary of recent convalescent plasma cases

Pathogen	Year	Case summary (clinical)	Source of plasma	Dose and schedule	Outcome	Reference
Influenza H5N1	2006	Male presented 9 days after flu and pneumonia symptom onset, critically ill with multiorgan failure (lung, heart, renal), toxic hepatitis, upper GI bleeding, DIC, and lung infection with drug-resistant bacteria.	Female survivor of H5N1 infection.	On Day 3 of hospital treatment, patient began receiving 100-mL transfusions every 5-10 hr (500 mL in total).	7-16 days postinfusion, the virus became undetectable and the patient eventually fully recovered and was discharged.	Kong and Zhou[91]*
	2006	31-year-old man presented with 4-day history of fever, chills, and cough with clear sputum. Radiograph showed opacities in a lobe in left lung. Patient was unresponsive to 150 mg oseltamivir twice daily for unknown reasons.	Female survivor from previous H5N1 infection.	Three 200-mL transfusions: 3 days since start of oseltamivir, 3 days 8 hr, and 4 days 8 hr.	More than 8 hr after transfusion, viral load was reduced from 1.68×10^5 to 1.42×10^4 copies/mL and to undetectable levels after 32 hr.	Zhou et al.[64]*
	2007	52-year-old father of index case (who died) with underlying hypertension presented with fever, cough, and chills and took 75 mg of oseltamivir. The next morning, he was hospitalized with mild thrombocytopenia, and bilateral pneumonia. Received levofloxacin, corticosteroids, and additional oseltamivir. Rimantadine treatment commenced on Day 3. Disease progression caused patient to need positive pressure ventilation.	Female participant in an inactivated H5N1 vaccine trial (280 days past final inoculation).	On Day 7, the patient received two 200-mL transfusions 4 hr apart.	Fever resolved after transfusion; a radiograph on Day 10 showed "improvement" in lung. Throat and stool samples showed viral RNA until Day 10. Patient was discharged by Day 22.	Wang et al.[63]
	2005-2008†	44-year-old female with ARDS and a history of bronchiectasis, had received 75 mg orally twice a day for 5 days. Oseltamivir on Days 8-12.	Male H5N1 survivor.	200 mL daily for 3 days, beginning on Day 13.	Viral loads were not determined, but patient recovered and was discharged.	Yu et al.[62]
SARS coronavirus	2005	80 patients that did not respond to ribavirin and prednisolone and experienced severe disease progression were transfused.	Between 600 and 900 mL of plasma was harvested from survivors and stored in approx. 200-mL portions.	Patients were given between 160 and 640 mL plasma (280 on average) at different respective times, range 7 to 30 days (mean, 14 days).	Patients transfused earlier (<14 days) had better outcomes than patients transfused later (6% mortality vs. 22%, respectively, not controlling for other factors). No correlation between volume infused and clinical outcome.	Cheng et al.[67]
	2005	Two health care workers and one lab technician infected with SARS were identified as candidates after ribavirin and methylprednisolone treatments failed.	Three individuals (including an index case) who survived SARS infection. Antibody titers (IgG) were >640.	2 mL/min (500 mL total) over 1 day. One patient also received 400 mg lopinavir and 100 mg ritonavir every 12 hr.	Reduction in fever and pulmonary infiltration after transfusion, viral loads of 495×10^3, 76×10^3, and 650×10^3 copies/mL, respectively (1 hr before transfusion) reduced to undetectable levels 24 hr after transfusion.	Yeh et al.[68]

* It is not clear from these two case reports if they represent the same patient.
† Exact date of this patient's illness is not reported.
DIC = disseminated intravascular coagulation; GI = gastrointestinal.

diverse array of proteins, lipids, and small molecules (such as hormones) and, as a blood component, may still contain some cellular elements to which a patient's immune system might react. The most common adverse reaction reported to plasma-containing products is allergic reaction resulting in urticaria, which is reported in 1% to 3% of blood transfusions.[69] However, this reaction is usually mild and treatable. In contrast, severe allergic reactions to plasma, which have been reported in approximately 1 in 29,000 plasma transfusions,[70] are characterized by rapid and potentially catastrophic bronchospasm and hypotension and are known as anaphylactic (if mediated by immunoglobulin [Ig]E) or anaphylactoid (if clinically indistinguishable but not mediated by IgE). Although the causes of these reactions are not completely known, anti-IgA and anti-haptoglobin appear to be responsible in some patients. More of these severe reactions occur with platelets (PLTs) than with plasma, suggesting a PLT-related factor. Transfusion with IgA-deficient plasma may be considered for deficient patients with anti-IgA, but otherwise, plasma transfusion for patients with a history of anaphylaxis should only be performed sparingly and where emergency medical treatment is immediately available.[71]

Another more important reaction to plasma-containing products, especially in the setting of therapeutic transfusion for pandemic influenza, is transfusion-related acute lung injury (TRALI). Although it is difficult to estimate the incidence of TRALI due to a lack of a standard clinical definition, the move toward male-only plasma was associated with a decrease in the number of TRALI fatalities reported to the FDA from 22 in 2006 to 12 in 2007.[72] The potential for this specific adverse reaction, although relatively uncommon in the general transfused population, is worth noting specifically for a critically ill influenza patient, who is likely already experiencing significant pulmonary injury, including ARDS. As ARDS will likely be one of the main medical complications in an influenza outbreak, new cases or exacerbations of ARDS could occur (caused by TRALI from widespread use of FCP-Flu for this new indication) that may strain the system. TRALI appears to be particularly prevalent in the critically ill (up to 8% of transfused medical intensive care unit patients), and in the subgroup of patients with existing lung injury, 11.6% of patients in one study had worsening of their pulmonary function after transfusion.[73,74] Convalescent plasma therapy given before the onset of ARDS could reduce the total number of ARDS cases if it is effective and thus mitigate the potential impact of FCP-Flu–induced TRALI cases. After the great strides that have been made in the reduction of TRALI incidence by shifting to male-predominant plasma in recent years, it follows that we must start with male-predominant donors for FCP-Flu as well. Pending research quantitating the efficacy of this product, this constraint could be relaxed if a demonstrable benefit eclipses the TRALI risk. Although transfusion of FCP-Flu may result in TRALI in a low percentage of cases, it could prevent many other cases of ARDS from developing, if administered to influenza patients before development of severe lung damage.

The immunomodulatory role of blood component transfusion has been debated and under scrutiny for many years.[75] Known as "TRIM," transfusion-related immunomodulation refers to both immunosuppressive effects (such as increased postoperative infection, enhanced renal allograft survival, or increased reactivation of latent cytomegalovirus or human immunodeficiency virus) and proinflammatory changes (hypothesized to manifest as an increased short-term mortality in transfused patients). Although these effects have not been clearly defined, there are three postulated mechanisms by which TRIM occurs: 1) immunologically active allogeneic leukocyte transfer, 2) the presence of soluble white blood cell–derived mediators (e.g., histamine or soluble Fas ligand), and 3) the presence of soluble HLA molecules. Some of these mechanisms have been researched by evaluating the impact of prestorage leukoreduction on TRIM, with an emphasis on demonstrating this effect in cellular blood components. Frozen plasma could contribute to TRIM if soluble immunoreactive molecules underlie this phenomenon, but this effect has not been clearly demonstrated. If the immunosuppressive effects of convalescent plasma transfusion are significant, then the issue becomes determining the relative benefits of viral neutralization against further suppression of the patient's inadequate anti-influenza immune response. Although the limited data available are promising, knowing whether transfusion will be beneficial may well be difficult to ascertain before a pandemic strikes and such a possibility cannot be easily dismissed.

It is also important to establish if pathogen antibodies in active infection could actually be detrimental. Since the humoral response is a normal and essential part of the natural immune system, it seems plausible that it is always helpful for the patient to have these pathogen antibodies. Interestingly, this is not always the case. An important phenomenon, called antibody-dependent enhancement (ADE), occurs at subneutralizing concentrations when an antibody serves to actually facilitate viral particle cell entry, rather than result in neutralization.[76] The best-characterized human example of this process is Dengue shock syndrome, found in some patients with Dengue virus infection.[77] ADE is thought to occur in most cases by the binding of the Fc portion of the antibody to an Fc-receptor on an immune cell (usually a macrophage) or by a complement-mediated mechanism. Importantly, for influenza A, ADE has been demonstrated in vitro in mice[78] and in humans.[79] However, a clinical syndrome due to enhancement by influenza antibodies has not been characterized in vivo.

The existence of ADE in influenza may at first appear to be a potential adverse reaction of serotherapy, but it could actually reveal a new mechanism for potential patient benefit by enhancing cell-mediated immunity. ADE facilitates viral entry into Fc-receptor–bearing cells (such as the antigen-presenting cells that are central to cell-mediated immunity). Since macrophages are not permissive for influenza infection,[80] increased viral uptake is unlikely to compromise cell function, but instead may increase viral antigen processing and presentation to T cells. As a result, the presence of cross-reactive, nonneutralizing antibodies may be beneficial in influenza to augment cell-mediated immunity.[78] This area should be researched as part of a convalescent plasma program for pandemic influenza, since the impact of transfusing antibodies against influenza on a large scale in human populations has not been established.

Limitations: focus on product safety

Specifically recruiting blood donors who have recently been ill seems counterintuitive to the current dogma of the "healthy blood donor." How long after illness is it necessary to wait for collection to assure maximal neutralizing antibody titers? What is the risk of cotransfusion of infectious virus as a result of persistent donor influenza viremia? It is controversial whether influenza viremia routinely occurs during natural infection,[81] and although patients with influenza viremia have been reported, the question has not been extensively studied. It is thought that seasonal influenza "would not pose a large risk to the safety of the blood supply,"[82] but since viral load and viremia rates can differ by strain (e.g., as high as 56% with H5N1 [9 of 16 patients]),[83] a more clinically severe virus could. A conservative approach might be deferring donors at least 2 weeks after their last fever, but evidence on this question is lacking. Since influenza is an enveloped orthomyxovirus, deriving its lipid membrane from the host cell upon the completion of virion formation, it is susceptible to the process of S/D treatment and other pathogen reduction processes. However, these processes take time and are not readily available in most blood centers. Another option would be nanofiltration of individual donations;[84] however, this process is too specialized for widespread application in the field. A critical advantage of convalescent serotherapy is its community-based, local application (i.e., using plasma from donors in close proximity to patients who may be suffering from infection by the same viral strain) and this benefit could be lost if a centralized manufacturing step were necessary.

An additional product safety hurdle to overcome would be potential shortages of testing reagents in a time of widespread infection. Reagents for testing donors for high antibody titers may be rapidly depleted and eventually exhausted. Regardless of the issue of convalescent plasma use, anticipating reagent limitation for routine donor infectious disease testing and blood component preparation testing is already a component of planning for a severe pandemic at blood donor centers.

A third way to protect against virus transmission would be to transition passive immunotherapy treatments from convalescent plasma to HIG as a pandemic progresses. The development of more rapid manufacturing platforms within the plasma fractionation sector should be encouraged. The safety benefits of commercial manufacturing processes of HIG could produce a safer product, but the tradeoffs between preparation time and wide availability may favor convalescent plasma in the early stages.

LOGISTICS OF SEROTHERAPY

As with most pandemic-related resources, rationing would almost assuredly be necessary under present "severe" pandemic planning scenarios.[8] Questions of differential allocation—i.e., whether states ought to create a separate rationing scheme for a convalescent transfusion intervention—will require consideration. However, these questions, along with ones pertaining to the notion of "compelled donation" (forcing a survivor to give plasma products for the sake of the ill), what to do about individuals prioritized to vaccines and antivirals, directed donation, and other difficult ethical questions are beyond the scope of this commentary.

For all medical interventions, supply and supply chains are expected to quickly become an issue during a severe pandemic,[22] but several complications would be specific to transfusion-related ancillary resources. The case of convalescent plasma transfusion pushes this difficulty further, as supply will largely depend on public willingness to donate. This critical component of supply—a necessary reliance on the altruism of individuals under stress—complicates any allocation rationale aimed at objectivity and fairness.

While some local and national blood bank associations may not be planning specifically for large-scale plasma collection if antibody transfusion proves a worthwhile intervention, there is still potential for success. A typical whole blood donation of approximately 450 to 500 mL of blood would yield 250 to 300 mL of plasma. Plasmapheresis harvesting typically yields between 250 and 600 mL, but does require additional equipment and specialist training that may not be widely available. Although blood supplies are expected to diminish during a pandemic, there is the possibility of using source or recovered plasma without irreparably thinning reserves for other transfusion purposes. However, this proposition would need to be weighed against the everyday

demand for the fractionated products of plasma, such as IVIG.

A NEED FOR RISK-BENEFIT ANALYSES

In considering the risks associated with passive immunotherapy as a large-scale intervention, one of the most important problems is that there exists relatively little research on influenza-specific human models at present. Given the onset of the 2009 H1N1 pandemic and potential for increased severity in the winter and following spring, it is unclear whether enough data now exist to unequivocally support a role for antibody transfusion in influenza treatment.

Although passive immune therapy holds the potential for a treatment in a future pandemic, there are several difficulties and risks that could arise. Many concerns are regulatory in nature. However, the circumstances surrounding a pandemic—scarcity, fear in the ill population, broad levels of absenteeism on the job—would compound these sorts of problems. As such, risk-benefit analyses should be conducted to evaluate the appropriateness of incorporating this type of novel clinical intervention at a broad level. Policy makers should decide what level of certainty is needed about product quality or efficacy given scarcity of other resources. For example, unexpected shortfalls in routine blood screening test kits or blood containers could make safety and availability of the general blood supply very difficult. Any convalescent plasma-based intervention would suffer alongside regular blood transfusions in times of such shortage. Weighing blood supply safety against need for convalescent plasma therapy will be required in either case.

A final set of analyses regard cost. The state or federal government would need to support passive immunotherapy to be viable. Money would need to be taken from some other pot, one likely tagged to tend to costs related to pandemic response or research into other, more conventional influenza treatments (such as vaccine or antiviral drug development). Given the expectation of scarcity and rationing, this probability should not be understated. Where passive immunotherapy could serve as an intervention to influenza-affected individuals when treatment options are scarce, it would also need financial backing, which could detract from other aspects of pandemic response. State governments would likely need to assume liability for this type of treatment, as well as develop response and allocation protocols for local health systems or departments. Additionally, the FDA may need to issue an emergency use authorization for such a product.

IN CONCLUSION: WHY DO IT?

As hospitals, blood centers, and others focus on the very difficult task of responding to the current pandemic, the question should arise why anyone should attempt to plan for yet another "unknown." Models for passive immunotherapy are generally quite positive, but human data are limited for influenza specifically. The data we have reviewed here demonstrate effectiveness in several animal models, document efficacy in human case reports of similar diseases, and raise important questions that can only be addressed by engaging in active research projects during pandemic periods. Moreover, potential antiviral resistance, possible geographic variation, and tremendous scarcity of resources and alternatives support the pursuit of convalescent plasma therapy as a countermeasure and an important focus of preparedness.

As with any biologic therapeutic, there are important safety concerns that mitigate the appeal to move forward on convalescent plasma treatment and research programs (Table 3). On balance, available data on passive immunotherapy, influenza-specific and otherwise, suggest that research on this potential new modality should be prioritized. Concurrently, substantial planning in the blood banking community should occur to make this modality widely available if the evidence emerges that FCP-Flu is an effective therapy. Pointing to a perceived deficit of unequivocal scientific proof is not sufficient to put logistical analyses of convalescent transfusion on the back burner. We need well-designed clinical trials to help answer the important lingering questions about clinical efficacy. Such trials could demonstrate that large-scale convalescent transfusion is

TABLE 3. Pros and cons of convalescent transfusion

Pro	Con
Proximity of donors increases likelihood of transfusing strain-specific antibodies	Greater risk for adverse side effects than MoAb or HIG mix
Does not require an existing vaccine	Safety concerns (e.g., allergic and anaphylactic reactions, TRALI, TRIM)
May be only effective treatment modality if oseltamivir or zanamivir resistance develops	Could distract from pandemic response if not effective
Less costly than equivalent current MoAb technologies	Influenza-specific case reports are few and many confounding factors are present
Treatment modality used successfully in other infectious diseases: promising case reports for H5N1 patients	No controlled human research
Murine data suggest significant benefit in influenza-specific cases	Regulatory concerns could delay product availability
Allows public to contribute personally to pandemic response efforts	

imprudent, impractical, or even unsafe—but this will only come from extensive testing and modeling. As the safety of the product is of ultimate concern, trials should be conducted now.

MoAbs or HIG could be directed in a similar manner to other standard pharmaceutical interventions during a pandemic. The logistics of convalescent transfusion, however, merge the traditional blood product system with that of a biologic therapeutic. The type of interdisciplinary response needed to collect, test, and distribute FCP-Flu on a large scale is considerable. When so many sectors—government, public health, blood banks, and hospitals—are focusing on continuity of operations and other respective challenges in pandemic planning and response, adding a promising-though-unproven intervention into the mix may seem imprudent to some. In addition, policy issues, including questions of an equitable distribution of FCP-Flu, the ethics of directed transfusion, and of compensating or compelling donors to "donate" will similarly arise. When picturing a more severe pandemic, one must wonder how convalescent transfusion could ever be tenable on a large scale if some planning has not occurred. Even with the great deal of unknowns associated with a pandemic, planning is still a priority. During the height of a pandemic, when times are difficult, it is probably too late to attempt to generate new protocols. Consequently, lines of communication need to be opened and well established now, and test exercises should be performed or designed. In other facets of pandemic planning, a guiding philosophy is that it is better to be caught prepared than not; so too is it better to have planned for a model of FCP-Flu collection and distribution and not use it than to realize one could have an effective intervention, but no practical way to collect and distribute a much-needed product.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

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