ZMapp Reinforces the Airway Mucosal Barrier Against Ebola Virus

Bing Yang,[1] Alison Schaefer,[2] Ying-Ying Wang,[7] Justin McCallen,[2] Phoebe Lee,[2] Jay M. Newby,[3] Harendra Arora,[4] Priya A. Kumar,[4] Larry Zeitlin,[8] Kevin J. Whaley,[8] Scott A. McKinley,[9] William A. Fischer II,[5] Dimple Harit,[1] and Samuel K. Lai[1,2,6]

[1]Division of Pharmacoengineering and Molecular Pharmaceutics, Eshelman School of Pharmacy, [2]University of North Carolina/North Carolina State University Joint Department of Biomedical Engineering, [3]Department of Mathematics and Applied Physical Sciences, [4]Department of Anesthesiology, School of Medicine, [5]Division of Pulmonary and Critical Care Medicine, School of Medicine, and [6]Department of Microbiology & Immunology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; [7]Department of Biophysics, Johns Hopkins University, Baltimore, Maryland; [8]Mapp Biopharmaceutical, Inc, San Diego, California; and [9]Mathematics Department, Tulane University, New Orleans, Louisiana

Filoviruses, including Ebola, have the potential to be transmitted via virus-laden droplets deposited onto mucus membranes. Protecting against such emerging pathogens will require understanding how they may transmit at mucosal surfaces and developing strategies to reinforce the airway mucus barrier. Here, we prepared Ebola pseudovirus (with Zaire strain glycoproteins) and used high-resolution multiple-particle tracking to track the motions of hundreds of individual pseudoviruses in fresh and undiluted human airway mucus isolated from extubated endotracheal tubes. We found that Ebola pseudovirus readily penetrates human airway mucus. Addition of ZMapp, a cocktail of Ebola-binding immunoglobulin G antibodies, effectively reduced mobility of Ebola pseudovirus in the same mucus secretions. Topical delivery of ZMapp to the mouse airways also facilitated rapid elimination of Ebola pseudovirus. Our work demonstrates that antibodies can immobilize virions in airway mucus and reduce access to the airway epithelium, highlighting topical delivery of pathogen-specific antibodies to the lungs as a potential prophylactic or therapeutic approach against emerging viruses or biowarfare agents.

Keywords. immunoglobulin G (IgG); antibody; mucosal immunology; mucins.

Ebola virus readily infects many cell types, including immune cells, fibroblasts, endothelial cells, and epithelial cells [1, 2]. Given this broad tissue tropism, effective treatments of systemic Ebola infection generally require high doses of therapeutic molecules administered systemically. For instance, ZMapp, a cocktail of 3 chimeric monoclonal antibodies (Abs), was evaluated at 50 mg/kg in a randomized, controlled clinical trial in Guinea, Sierra Leone, and Liberia during the 2014–2016 Ebola outbreak [3].

Rather than treating Ebola infections systemically, a potential alternative strategy is to block or treat infections at the portals of entry before virions proliferate and spread throughout the body. In addition to transmission by direct contact with the blood, bodily fluids, or skin of Ebola-positive individuals [4–6], it is possible Ebola may be transmitted via virus-laden droplets generated from a heavily infected individual by coughing, sneezing, vomiting, or medical procedures that are then directly propelled onto the mucus membranes of a nearby person [5, 7]. We use the term *droplet-based aerosol transmission* to differentiate this potential mechanism from strict airborne transmission of individual viruses, which is generally considered an unlikely mechanism of Ebola transmission. Aerosol infection with Ebola delivered directly via inhalation has been demonstrated [8, 9], and multiple studies suggest aerosol transmission between infected and uninfected animals may occur [10–12]. Given the elevated risk of mucosal transmission of Ebola, particularly to healthcare workers [13–15], as well as the potential threat of aerosolized filovirus-based biowarfare agents, we sought to investigate the fate of Ebola deposited at mucosal surfaces.

Mucus membranes are characterized by a layer of mucus secretions that can trap diverse foreign particles and pathogens [16,17], facilitate their elimination through natural mucus clearance mechanisms [18, 19], and consequently reduce the flux of pathogens reaching target cells. Human airway mucus (AM) is likely responsible in part for the relatively modest transmission rates of many respiratory viruses [20–22], but it is also likely that AM can be reinforced to further limit the flux of pathogens reaching the underlying epithelium. We have previously shown that immunoglobulin G (IgG) Abs in cervicovaginal mucus can trap viruses via multiple low-affinity Fc-mucin bonds between IgG accumulated on the virus surface and mucins, akin to a Velcro patch [23]. More recently, we also showed that the immobilization of H1N1 and H3N2 influenza viruses in human AM is correlated with the presence of influenza-binding IgG and immunoglobulin A (IgA) [24].

Received 13 December 2017; editorial decision 27 March 2018; accepted 19 April 2018; published online April 24, 2018.

Correspondence: S. K. Lai, PhD, Division of Molecular Pharmaceutics, University of North Carolina at Chapel Hill, Marsico 4213, 125 Mason Farm Rd, Chapel Hill, NC 27599 (lai@unc.edu).

DOI: 10.1093/infdis/jiy230

Here, we investigate whether topically dosed IgG against Ebola may similarly trap Ebola in AM and facilitate its elimination from the airways.

METHODS

Preparation and Characterization of Ebola Pseudovirus

Ebola pseudoviruses were prepared by transfecting 293T cells with plasmids encoding Gag-mCherry and Ebola glycoprotein (GP) generously provided by Dr Suryaram Gummuluru (Department of Microbiology, Boston University School of Medicine) and Dr Ronald N. Harty (Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania), respectively. We also prepared Ebola virus-like particles incorporating VP40 filovirus matrix protein, but the titers were insufficient for our microscopy and in vivo experiments. Incorporation of Ebola GP into the pseudovirus was confirmed by Western blot. Additional details are provided in the Supplementary Materials.

Preparation and Characterization of Nanoparticles

Fluorescent, carboxyl-modified polystyrene beads (PS-COOH) and PEGylated nanoparticles (PS-PEG) sized approximately 100 nm were prepared and characterized [25]. Additional details are provided in the Supplementary Materials.

Collection of Airway Mucus

Fresh human AM was obtained from healthy adult patients intubated for general anesthesia during elective surgery, following a protocol deemed nonhuman subjects research by the University of North Carolina at Chapel Hill Institutional Review Board. Additional details, including characterization of total immunoglobulin and IgG isotype levels, are provided in the Supplementary Materials.

Multiple Particle Tracking Analysis

Dilute particle solutions (~10^8–10^9 particles/mL, 1 μL) and different Abs (2 μL, to a final concentration of 22 μg/mL) were added to 20 μL of fresh, undiluted AM in custom-made chambers, and samples were incubated for 1 hour at 37°C before microscopy. All conditions were tested in aliquots of the same AM samples, allowing direct comparison between conditions. Videos of the fluorescent particles in AM were recorded with MetaMorph software (Molecular Devices, Sunnyvale, CA) at a temporal resolution of 66.7 ms. Particle trajectories were analyzed using Video SpotTracker (University of North Carolina at Chapel Hill). Trajectories of $n \geq 130$ particles per frame were analyzed for each experiment, and 8–9 independent experiments were performed. The coordinates of particle centroids were transformed into time-averaged mean-squared displacements (MSDs), calculated as $\langle \Delta r^2(\tau) \rangle = [x(t+\tau) - x(t)]^2 + [y(t+\tau) - y(t)]^2$, where τ = time scale or time lag, from which distributions of MSDs and effective diffusivity (D_{eff}) were calculated [25, 26]. Additional details are provided in the Supplementary Materials.

Ebola Pseudovirus Distribution in the Lung Airways

ZMapp cocktail (25 μL per mouse) or phosphate-buffered saline, followed by Ebola pseudovirus (25 μL per mouse) after a 15-minute interval, was aerosolized and delivered using a Penn Century Microsprayer to the airways of Balb/c mice (female, 8–10 weeks; n = 3 per condition). Mice were killed 30 minutes after the final microsprayer instillation. Ebola pseudovirus distribution was quantified in transverse cryosections of the upper airways. All experimental protocols were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee and conform to the Declaration of Helsinki conventions for the use and care of animals. Additional details are provided in the Supplementary Materials.

Statistical Analysis

Data averages are presented as means with standard error of the mean (SEM) indicated. Statistical comparisons were limited to 2 groups. A 1-tailed, paired Student's *t* test was used for all comparisons because different conditions were tested in aliquots of the same mucus samples. Differences were deemed significant at an alpha level of .05.

RESULTS

Rapid Penetration of Human Airway Mucus by Ebola Pseudovirus

Wild-type Ebola virus requires Biosafety Level 4 containment, which few laboratories have access to. Therefore, to investigate the fate of Ebola in mucus, we prepared fluorescent, noninfectious Ebola pseudovirus comprised of human immunodeficiency virus type 1 (HIV-1) Gag-mCherry capsid proteins in the core and the Zaire GP, from the same species as in the West Africa epidemic in 2014–2016 [27, 28], on the surface. The same strategy was previously used to prepare HIV and influenza virus-like particles (VLP) [24, 29, 30]. Glycoprotein incorporation into Ebola pseudovirus was confirmed by Western blot (Supplementary Figure 1*A*), and dynamic light scattering showed that the pseudovirus possessed a hydrodynamic diameter of 102 ± 3 nm. All 3 IgGs of the ZMapp cocktail bound specifically to the pseudovirus, confirming the presence of structurally intact GP (Supplementary Figure 1*B*).

To avoid dilution effects caused by the use of hypertonic saline to induce sputum expectoration, we obtained undiluted human AM directly from freshly extubated endotracheal tubes [24]. Ebola pseudoviruses were readily diffusive in all AM secretions tested (Figure 1A; Supplementary Movie 1), exhibiting diffusive motion comparable with that of similarly sized, polyethylene glycol–coated polystyrene (PS-PEG) (Supplementary Movie 2) nanoparticles engineered to evade adhesion to mucins and penetrate various mucus secretions [25, 31, 32]. In the same AM secretions, similarly sized carboxyl-modified polystyrene (PS-COOH) nanoparticles that are muco-adhesive were extensively immobilized (Supplementary Movie 3), confirming that the rapid diffusivity observed for Ebola pseudovirus was not

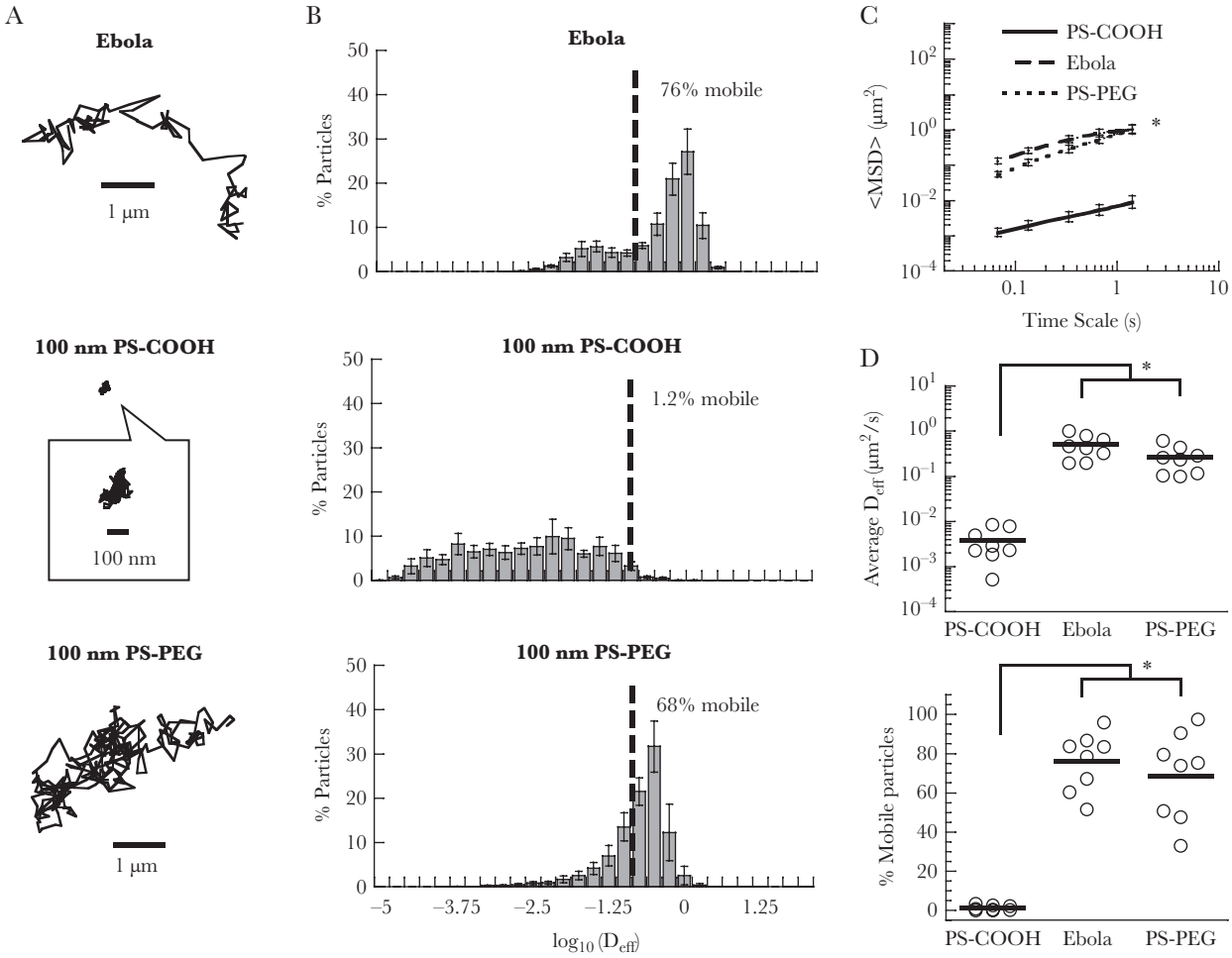

Figure 1. Diffusion in human airway mucus (AM) of Ebola pseudovirus and comparably sized polystyrene nanoparticles that are either carboxylated and mucoadhesive (PS-COOH) or modified with polyethylene glycol and muco-inert (PS-PEG). *A*, Representative trajectories for pseudovirus and particles exhibiting effective diffusivities within 1 standard error of the mean of the ensemble average at a time scale of 0.2667 seconds. *B*, Distributions of the logarithms of individual particle effective diffusivities (D_{eff}) at a time scale of 0.2667 seconds. Log D_{eff} values to the left of the dashed line correspond to particles with displacements of less than approximately 200 nm (ie, roughly twice the particle diameter) within 0.2667 seconds or D_{eff} approximately 20-fold reduced from theoretical diffusivity in water. *C*, Ensemble-averaged geometric mean square displacements (<MSD>) as a function of time scale. *D*, Ensemble geometric D_{eff} at a timescale of 0.2667 seconds, and fraction of mobile particles are plotted for distinct samples with averages indicated by solid lines. Data represent the ensemble average of 8 independent AM specimens. Error bars represent standard error of the mean. * indicates a statistically significant difference compared with PS-COOH ($P < .05$) based on a 1-tailed, paired Student's *t* test. Abbreviations: D_{eff}, effective diffusivity; MSD, ensemble mean squared displacement; PS-COOH, carboxyl-modified polystyrene; PS-PEG, polyethylene glycol–coated polystyrene.

due to a degraded mucin matrix. Nearly all Ebola pseudoviruses (>50% in all samples; on average ~76%) possessed diffusivities in excess of approximately 200 nm, or twice the particle diameter, at a time scale of 0.2667 seconds (Figure 1B). The geometrically averaged ensemble mean squared displacement (<MSD>) of Ebola was only approximately 10-fold reduced compared with their theoretical speeds in buffer (Figure 1C; at a time scale of 0.2667 s), with a slope α of 0.80 for the log <MSD> versus log time scale plot (α = 1 for pure unobstructed Brownian diffusion—eg, particles in water—and α becomes smaller and approaches 0 as obstruction to Brownian diffusion increases). The geometrically averaged effective diffusivity (<D_{eff}>) for Ebola pseudovirus was 0.43 μm²/s, approximately 150-fold higher than that of PS-COOH nanoparticles (Figure 1D).

Along the major conducting airways, the approximate thickness of mucus lining the bronchial airways is 50 μm, and the entire mucus blanket can be renewed in as little as 15–30 minutes [19]. Therefore, we performed a first passage time estimate, using the measured diffusivities of individual pseudoviruses in AM to determine the time needed for viruses to diffuse across a 50-μm-thick AM layer. We found that upon airway deposition, nearly 10% of Ebola virions can penetrate the luminal AM layer within approximately 5 minutes, and nearly 50% can penetrate the AM layer in as little as 30 minutes. These results suggest limiting rapid Ebola penetration of AM may be an important strategy to reduce Ebola infection by decreasing the flux of virions reaching the underlying airway epithelium.

Immobilization of Ebola Pseudovirus in Human Airway Mucus Treated with ZMapp

Although total immunoglobulin and IgG isotype levels varied substantially across AM samples (Supplementary Table 3), the rapid diffusion of Ebola pseudovirus in AM was remarkably consistent, suggesting endogenous Ab did not impact Ebola pseudovirus mobility. We next evaluated whether Ebola-binding IgG, in the form of the 3 chimeric monoclonal antibody (mAb) cocktail ZMapp, could enhance the diffusional barrier properties of AM against Ebola. In agreement with our prior studies in cervicovaginal mucus [23, 33], addition of modest levels of Ebola-binding IgG into AM (on average ~2% of total IgG in AM) effectively reduced the mobility of Ebola pseudovirus, with the majority of pseudoviruses moving much less than their diameters over at least 20 seconds (Figure 2A; Supplementary Movie 4). Indeed, the $\langle D_{eff} \rangle$ of Ebola pseudovirus in ZMapp-treated AM decreased by approximately 27-fold compared with $\langle D_{eff} \rangle$ in the same native AM secretions without Ab (Figure 3A). Likewise, the fraction of mobile pseudoviruses was reduced from 76% to 13% (Figure 2B), whereas <MSD> was >260-fold lower than theoretical pseudovirus speeds in buffer (Figure 2C; at a time scale of 0.2667 s). The increased hindrance to rapid diffusion is also evident from the log <MSD> versus log time scale slope α of 0.34. Fluorescence of pseudovirus in both

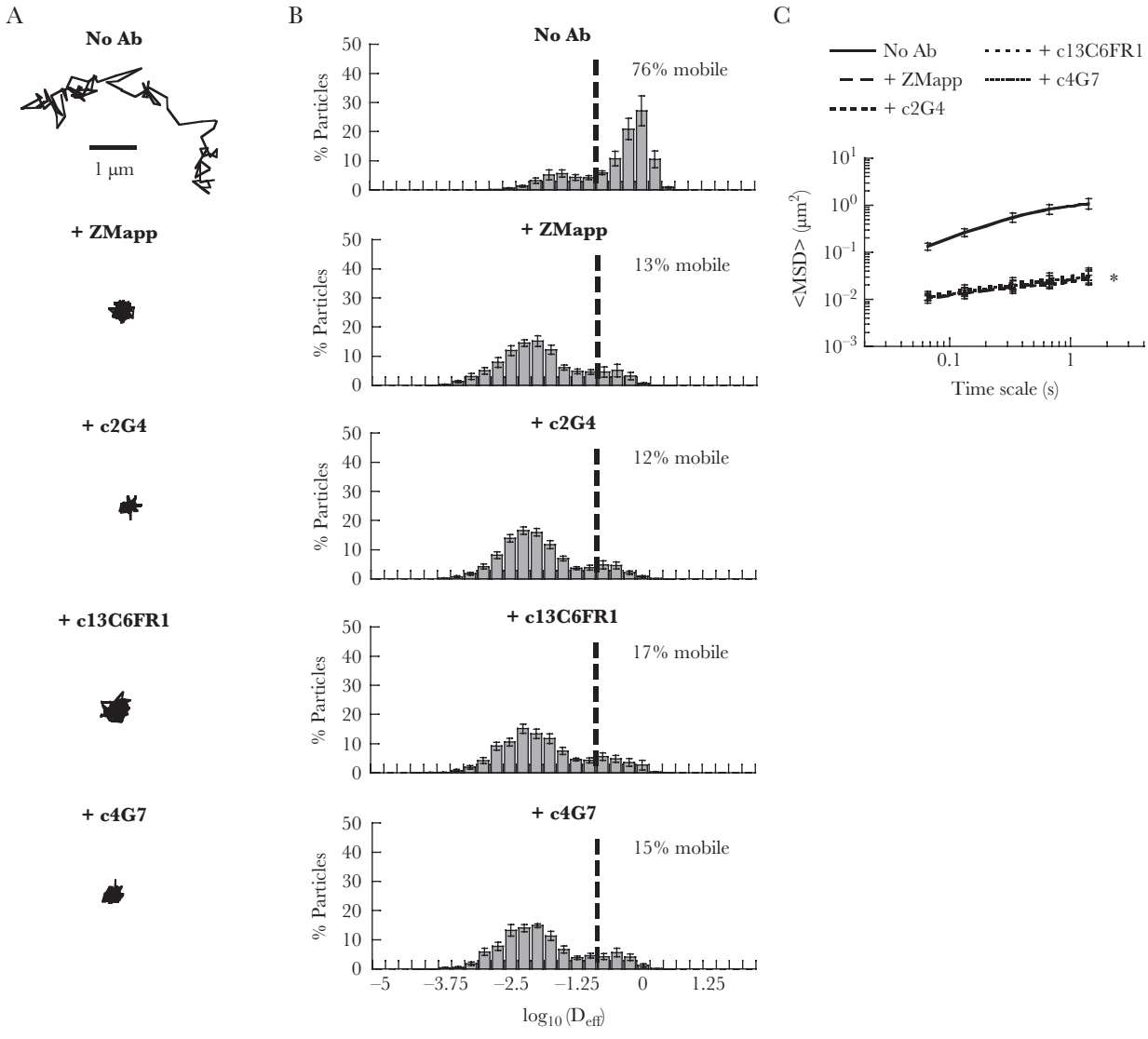

Figure 2. Diffusion of Ebola pseudovirus in human airway mucus (AM) that is untreated (no antibodies) or treated with either ZMapp or individual Ebola-binding immunoglobulin G (IgG; c2G4, c13C6FR1, c4G7). *A*, Representative trajectories for pseudovirus exhibiting effective diffusivities within 1 standard error of the mean of the ensemble average at a time scale of 0.2667 seconds. *B*, Ensemble-averaged geometric mean square displacements (<MSD>) as a function of time scale. *C*, Distributions of the logarithms of individual particle effective diffusivities (D_{eff}) at a time scale of 0.2667 seconds. Log D_{eff} values to the left of the dashed line correspond to particles with displacements of less than approximately 200 nm (ie, roughly twice the particle diameter) within 0.2667 seconds or D_{eff} approximately 20-fold reduced from theoretical diffusivity in water. Data represent the ensemble average of 8–9 independent AM specimens. Error bars represent standard error of the mean. * indicates a statistically significant difference compared with no antibodies ($P < .05$) based on a 1-tailed, paired Student's *t* test. Abbreviations: Ab, antibody; D_{eff}, effective diffusivity.

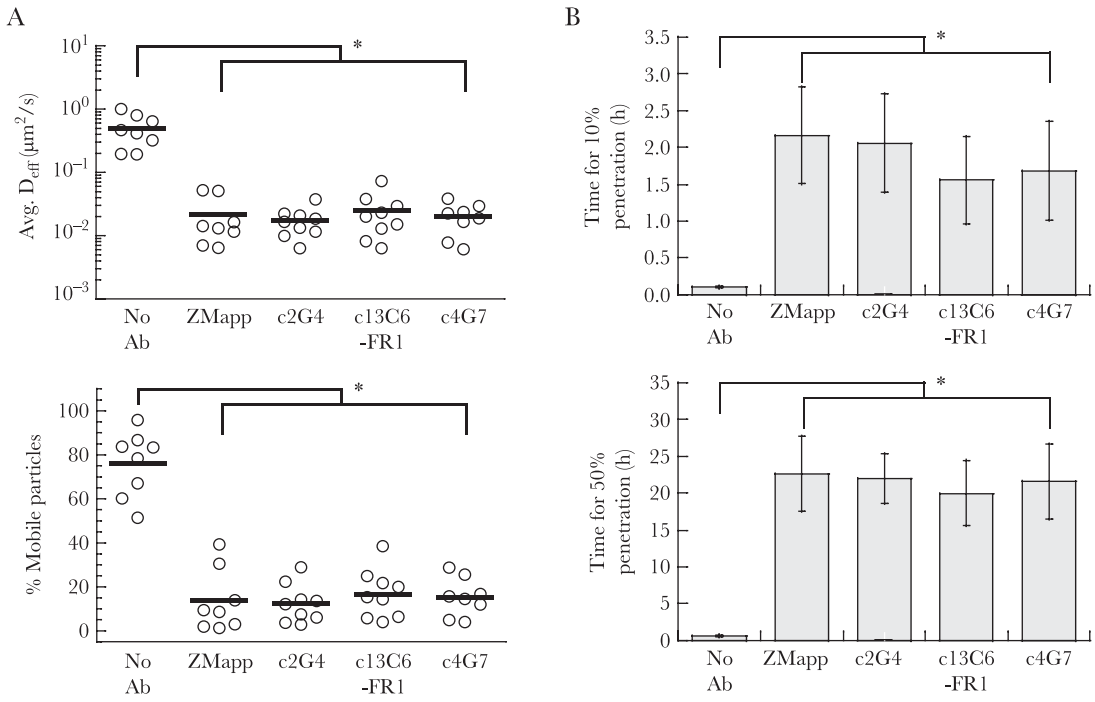

Figure 3. Diffusion and first-passage time of Ebola pseudovirus in human airway mucus (AM) that is untreated (no antibodies) or treated with either ZMapp or individual Ebola-binding immunoglobulin G (IgG; c2G4, c13C6FR1, c4G7). *A*, Ensemble geometric average effective diffusivities (D_{eff}) at a timescale of 0.2667 seconds and fraction of mobile particles. *B*, Estimated time for 10% and 50% of pseudoviruses and particles to diffuse through a 50-μm-thick mucus layer. Data represent the ensemble average of 8–9 independent AM specimens. Error bars represent standard error of the mean. * indicates a statistically significant difference compared with no antibodies ($P < .05$) based on a 1-tailed, paired Student's *t* test. Abbreviations: Ab, antibody ; D_{eff}, effective diffusivity.

native (ie, Ab-free) and ZMapp-treated AM appeared identical in both size and brightness, suggesting ZMapp did not induce agglutination (ie, agglomeration of multiple Ebola pseudoviruses). Thus, the decrease in measured Ebola pseudovirus mobility is most likely attributed to immobilization of individual pseudoviruses due to polyvalent interactions between the array of pseudovirus-bound Abs and mucins. In contrast with ZMapp, control, nonspecific Abs did not alter Ebola pseudovirus mobility.

To determine whether any particular mAb (c2G4, c13C6FR1, c4G7) within the ZMapp cocktail may confer superior "muco-trapping" potency, we measured the mobility of Ebola pseudovirus in different aliquots of the same AM specimens treated with the individual mAb. Interestingly, all 3 mAbs, including 1 with poor neutralizing activity against Ebola, were similarly effective in reducing the mobility of Ebola pseudovirus in AM (Figure 2A; Supplementary Movies 5–7). Relative to the control with no Ab and similar to ZMapp, the $<D_{eff}>$ of Ebola pseudovirus was reduced by approximately 28-, 22-, and 25-fold in AM treated with c2G4, c13C6FR1, and c4G7, respectively. Similarly, the fraction of mobile Ebola pseudoviruses was reduced to 12%, 17%, and 15%, respectively.

To better illustrate how changes in mobility might alter the flux of virions reaching target cells, we again performed first-passage time analysis. The predicted time for 10% of Ebola pseudoviruses to diffuse across a 50-μm-thick mucus layer increased from approximately 5 minutes for native AM to 2.2, 2.1, 1.6, and 1.7 hours for ZMapp-, c2G4-, c13C6FR1-, and c4G7-treated AM, respectively (Figure 3B). Similarly, the estimated time for 50% of pseudoviruses to cross the mucus layer increased from 0.5 hours for native AM to 23, 22, 20, and 22 hours, respectively. Because mucociliary clearance occurs on the order of 15–30 minutes [19], these results suggest that the vast majority of Ebola virions would be quickly trapped and eliminated from ZMapp-treated airways before they could penetrate AM.

Rapid Elimination of Ebola Pseudovirus from the Lung Airways Treated with ZMapp

Lastly, we sought to evaluate whether ZMapp-induced trapping of viruses in mucus secretions ex vivo would translate to an improved AM barrier against Ebola and consequently altered distribution in the lung airways in vivo. Using a PennCentury microsprayer, we administered ZMapp or phosphate-buffered saline to the mouse lung, followed by addition of fluorescent Ebola pseudovirus 15 minutes later. In control mice, we observed substantial red fluorescence associated with Ebola pseudovirus throughout the lung airways, including fluorescence indicative of mucosal penetration and accumulation in the underlying airway epithelium (Figure 4A and 4B). In contrast, far fewer

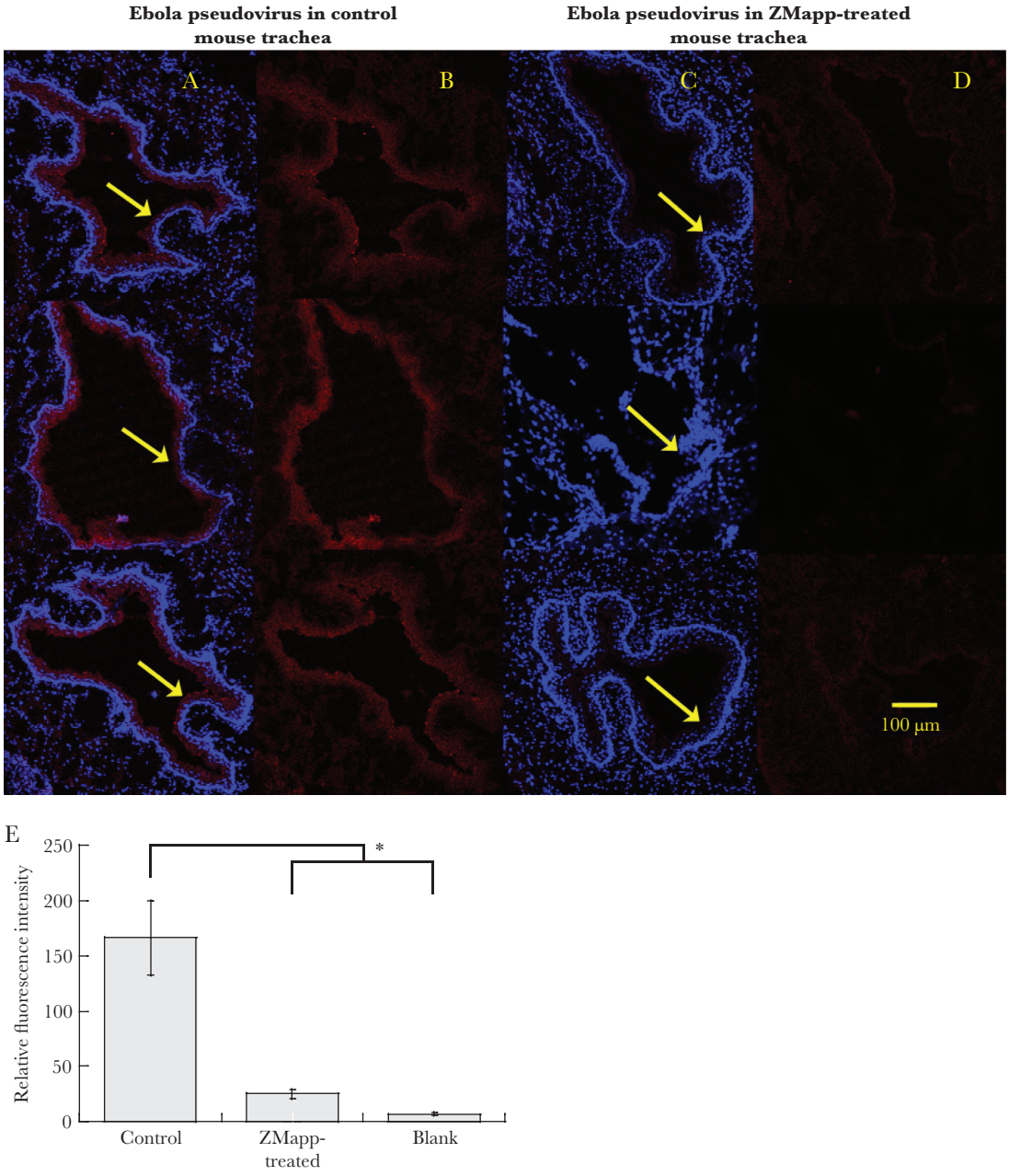

Figure 4. Ebola pseudovirus distribution in the mouse lung airways. *A–D*, Representative transverse 50-μm-thick frozen tissue sections showing the distribution of Ebola pseudovirus in the mouse trachea treated with phosphate-buffered saline (PBS) (*A*, *B*) or ZMapp (*C*, *D*). Red corresponds to Ebola pseudovirus, and blue corresponds to 4',6-diamidino-2-phenylindole (DAPI)-stained cell nuclei. Arrows indicate the inner lining of the trachea. *E*, Quantification of Ebola pseudovirus signal in mouse trachea treated with PBS (control) or ZMapp compared with blank tissue. Data represent n = 3 mice per group with, on average, 10 tissue sections quantified per mouse. Error bars represent standard error of the mean. * indicates a statistically significant difference ($P < .05$) based on a 2-tailed Student's *t* test assuming unequal variance.

Ebola pseudoviruses were present in the airways of mice treated with ZMapp (Figure 4C and 4D), presumably because pseudoviruses were trapped in the luminal mucus and rapidly cleared. Fluorescence levels in lung tissues of ZMapp-treated mice were almost 7-fold lower than those in control mice and only 3.5-fold above background. These results are consistent with previous observations that nanoparticles that bind to mucin mesh fibers are unable to penetrate the mucus layer and reach the underlying epithelium.

DISCUSSION

We showed here that Ebola pseudoviruses readily penetrate fresh human AM secretions but can be effectively immobilized in AM by antigen-specific IgG (ZMapp). Trapping in turn facilitated rapid elimination of Ebola pseudovirus from the mouse airways. Although Ebola is generally not considered an airborne pathogen, aerosol transmission of Ebola virus is biologically plausible. Ebola virus is present in saliva, feces, blood, and other body fluids that can be aerosolized through Ebola symptoms (eg,

coughing, vomiting, diarrhea) and through healthcare delivery (intubation, suctioning, delivery of nebulized medications) [5, 6]. Large droplets from a human sneeze can travel up to 1–2 m, whereas smaller droplets can travel up to 6–8 m away within seconds to a few minutes [34]. Studies have also found Ebola to survive in aerosol form for tens of minutes if not hours [35]. Ebola can initiate infection in cells present in the respiratory tract, and fatal respiratory infection has been observed in guinea pigs and nonhuman primates following intranasal and aerosol exposure [8, 9, 12]. Furthermore, Ebola transmission has been shown between infected and healthy macaques and between infected pigs and macaques without direct physical contact, likely through aerosol or droplet transmission [10, 11], although potential cross-contamination during animal husbandry practices could not be discounted entirely. A study frequently cited in arguments against airborne transmission found no detectable Ebola transmission when monkeys inoculated intramuscularly were housed in neighboring open-barred cages separated by a Plexiglas divider that prevented direct contact [36]; nevertheless, high viral titers were only detected in the blood, suggesting virus titers in the lungs may not have achieved the critical titers necessary for respiratory transmission before the animals succumbed to systemic effects of infection. In contrast, recent studies in humans have reported substantial quantities of Ebola in respiratory secretions [37, 38], and pathology studies have also found viral antigen in lung tissue [1, 39]. Finally, aerosol dissemination of weaponized forms of filoviruses, a version of which has reportedly already been developed for Marburg virus [40], presents a substantial threat to both the military and potentially the general public. Altogether, these reports substantiate continued concern over aerosol transmission of Ebola virus and the need to explore strategies to prevent and treat Ebola transmission at mucosal membranes.

Although the secretion of mucus can increase in response to infection, mucus is generally viewed as a passive rather than adaptive barrier against pathogens and is consequently overlooked in most studies of mucosal infection. The notion that Abs can work in tandem with mucus to reinforce the diffusional barrier properties of mucus has remained largely unexplored, despite the fact that large quantities of Abs, including both IgG and IgA, are secreted into AM [41, 42]. Here, in good agreement with our recent discovery that IgG can trap herpes simplex virus type 1 in human cervicovaginal mucus [23], we showed that Ebola-binding mAbs, in the form of ZMapp, were able to facilitate effective trapping of the majority of Ebola pseudoviruses in AM. Antibody-mediated trapping of Ebola pseudovirus markedly reduced the fraction of virions predicted to penetrate the mucus layer over the first few minutes of exposure. Because trapped viruses are quickly eliminated by natural mucociliary clearance in the airways, ZMapp-mediated trapping of Ebola would likely reduce the total flux of viruses arriving at the airway epithelium and thus the likelihood and/or severity of infection, rather than simply delaying the onset of infection. Consistent with this hypothesis, we observed topical delivery of ZMapp into the mouse lung greatly reduced the amount of fluorescent Ebola pseudovirus retained in the conducting airways within 30 minutes. Coupled with our earlier studies with genital mucus secretions [23, 33] and unpublished observations with gastrointestinal mucus, IgG-mediated trapping of viruses in mucus appears to be a universal protective immune function across different mucosal surfaces that enables protection directly at the portals of entry for viral transmission. Importantly, adhesive interactions between the array of pathogen-bound Abs and mucus gel would explain how the otherwise relatively nonadaptive and nonspecific biochemistry and microstructure of mucus secretions—the first line of defense against most infections—can be fortified with adaptive Abs to fend off a diverse and ever-changing spectrum of pathogens.

The concept of mucosal Ab prophylaxis and/or therapy based on Abs designed to work together with mucus to trap pathogens represents a unique and complementary approach in the arsenal of protective methods against infectious disease. First, the concept radically shifts the first line of defense against respiratory viruses to extracellular mucus gels instead of cellular targets, which is especially important against viruses that are either exceptionally virulent (eg, Ebola) and/or without a cure (eg, HIV, Ebola). Second, Abs that trap viruses in mucus need not bind to neutralizing epitopes; this greatly broadens the potential antigen targets that can be exploited to achieve protection. Indeed, one of the mAbs in the ZMapp cocktail is actually a poor neutralizer. Third, because the viral load during the transmission episode at mucus membranes is likely low, the overall dose of mAbs needed at mucosal surfaces, either before or immediately following a high-risk exposure event, may be substantially less than the mAb dose needed to treat a proliferating systemic infection. Thus, ZMapp delivered topically may be a particularly useful preventative measure or emergency intervention for populations at the highest risks of acquiring Ebola infections, such as healthcare workers, for reducing both the odds of becoming sick as well as the viral load entering the circulation following an exposure event.

Because of the continuous secretion and elimination of mucus coating the airways, the half-life of topically dosed Abs in the airways is relatively limited, likely necessitating at least once a day delivery to ensure adequate concentrations of mucosal Abs. Given the prolonged circulation times of IgG systemically, an attractive strategy could theoretically involve long-circulating IgG that can also effectively extravasate and enter the AM. However, the extent to which systemically dosed IgG can enter the AM remains poorly understood. In guinea pig studies, intramuscular administration of a potent neutralizing IgG did not protect against influenza transmission, whereas intranasal administration of the IgG did [43]. In humans, although the distribution of Ab isotypes appears to be similar in genital secretions compared with systemic Abs [23], total IgG and IgA

in cervical mucus secretions correlate poorly with systemic levels in normally cycling women [44, 45], reflecting substantial variations in Ab transudation across individuals. Thus, systemic delivery of IgG intended for mucosal protection may be challenging. In comparison, improvements in pulmonary delivery methods, including nebulizers, may enable uniform delivery of mAbs to the lung, while minimizing protein degradation and aggregation [46–48]. Combined with rapidly falling costs of Ab production, nebulizers may enable inhaled mAbs for prophylaxis to become a practical reality.

One limitation to our work is that we did not have sufficient AM sample volume to test control virus or control Abs in the same samples. Nevertheless, we validated in pilot studies that control Abs do not alter the mobility of Ebola pseudovirus compared with no treatment control. We have also shown that wild-type influenza viruses and influenza VLP, but not HIV VLP, are trapped in the same AM containing influenza-specific Abs [24]. Finally, we have previously demonstrated using multiple viruses/particles in different mucus types that control, nonspecific Abs do not trap the viruses/particles, whereas specific Abs do so potently [23, 33]. We were also not able to test wild-type Ebola virus in either particle tracking or in vivo experiments. Although our pseudovirus has the same surface GPs as wild-type virus, it is possible that differences in morphology or GP density can lead to varying trapping efficiency by Ebola-binding IgG. Because of Biosafety Level 4 requirements, we were unable to directly demonstrate protective efficacy against Ebola in vivo, although we have shown that trapping in mucus and rapid clearance of viral particles from the mouse vagina correlate to protection against herpes [23]. Lastly, because of major differences in lung physiology, rodent studies are ill suited to inform the local pharmacokinetics of inhaled Abs in the human lung. Although prior work suggests the half-life of inhaled Abs in humans is approximately 1 day [49, 50], studies exploring the effectiveness and potential duration of this strategy in larger animal models will be needed for translation into humans.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. Plasmids encoding Gag-mCherry and Ebola glycoprotein were generously provided by Dr Suryaram Gummuluru (Department of Microbiology, Boston University School of Medicine) and Dr Ronald N. Harty (Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania), respectively. We thank the developers of Video Spot Tracker: Center for Computer Integrated Systems for Microscopy and Manipulation (CISMM) at University of North Carolina at Chapel Hill, supported by the National Institutes of Health (NIH) National Institute of Biomedical Imaging and Bioengineering (NIBIB) (NIH 5-P41-RR02170).

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Financial support. This work was supported by North Carolina Translational & Clinical Sciences Institute (NC TraCS) (NCATS; 1UL1TR001111 to W. A. F. and S. K. L.), the National Institute of Allergy and Infectious Diseases (1K23AI121516 to W.A.F.), the David and Lucile Packard Foundation (2013–39274 to S. K. L.), and the Eshelman Institute for Innovation (to S. K. L.).

Potential conflicts of interest. The findings described in this publication are related to technology being developed by Mucommune. S. K. L. is cofounder and member of the Board of Directors of Mucommune. S. K. L. owns company stock, which is subject to certain restrictions under university policy. The terms of this arrangement are being managed by the University of North Carolina in accordance with its conflict of interest policy. S. K. L. and Y.-Y. W. have a patent WO 2014/070786 A1 licensed to Mucommune, LLC. ZMapp was developed by Mapp Biopharmaceutical. K. J. W. and L. Z. are co-founders as well as CEO and President, respectively, of Mapp Biopharmaceutical. K. J. W. and L. Z. own company stock, and have pending U.S. patent applications related to antibody affinity and antibody cocktails. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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