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Rapid generation of a human monoclonal antibody to combat Middle East respiratory syndrome

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Summary The last century has witnessed the emergence of several previously unknown viruses as life-threatening human pathogens. Several examples include HIV, Ebola, Lujo, and, most recently, the Middle East respiratory syndrome (MERS) and Ebola. In this study, we describe a method for the swift generation of a human-derived monoclonal antibody, known as LCA60, as a treatment for MERS infections. LCA60 antibody was generated using the Cellclone Technology from the immortalized B cells of a human donor recovering from MERS. Only four months were required from the initial screening of B cells to the development of a stable CHO cell line suitable for the production of clinical grade antibody, thereby delineating a rapid pathway for the development of antiviral therapies against emerging viruses. Currently, the LCA60 antibody is being considered for clinical development, which includes prophylaxis in individuals at risk and a treatment for severe MERS-CoV infections.

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MERS is a highly lethal pulmonary infection [1] caused by a previously unidentified coronavirus (CoV), which is thought to infect Dromedary camels [2]. MERS first surfaced in April 2012, when the virus was rapidly identified by two independent groups. MERS also belongs to a novel subgroup of the C betacoronavirus [3,4] (Fig. 1). MERS-CoV spread to 26 countries, causing episodes of infection over the last three years. As of April 14, 2016, a total of 1741 cases and 675 deaths were reported to WHO, of which were mostly from Saudi Arabia. Most cases of human infection are not transmitted by direct contacts with camels but can occur through direct contact with MERS patients in hospitals [5]. Indeed, healthcare exposure seems to play a key role in the spread of MERS-CoV outbreaks.

Treating viruses is still a challenge in modern medicine. The most effective measure to combat viral diseases is preventive vaccines. However, as observed with the latest Ebola outbreak, vaccines cannot be developed and applied sufficiently rapidly to contain acute outbreaks. Given the danger of newly emerging viruses, novel strategies need to be developed to generate rapidly effective treatments. Interestingly, antibody-based treatments are one of the most promising approaches for the treatment and prevention of viral diseases. Emil V. Behring and Kitasato Shibasaburō pioneered the use of a passive antibody therapy in the early 1890s, when they showed that hyperimmune sera of animal origin could protect against diphtheria and tetanus. Serum therapy for other infections followed and was substituted only when antibiotics were discovered in the 1940s. Unfortunately, the use of polyclonal animal sera is associated with several side effects, including hypersensitivity reactions and serum sickness, that results from the administration of large amounts of animal proteins. These problems led to the replacement of animal sera with human hyperimmune immunoglobulin preparations, such as those used to prevent or treat or prevent cytomegalovirus, varicella zoster virus, hepatitis B virus and respiratory syncytial virus (RSV) infections in high risk populations. Importantly, Respigam, the hyperimmune immunoglobulin product against RSV, was replaced in 1998 by Synagis, the first monoclonal antiviral antibody on the market. Clinical studies showed that in patients with severe pneumonia caused by viral infections, the convalescent sera conferred a therapeutic benefit when administered to patients with severe infections during the Spanish influenza, pneumonia from 1918 to 1919, SARS-CoV in 2003, H5N1 influenza A in 2006, H1N1 influenza A virus in 2009 and, most recently, during the Ebola outbreak [6]. There have been attempts to collect therapeutic antibodies from the serum of recovering MERS patients as well as people living with these patients or health worker contacts. However, it was recently discovered that antibody titers in convalescent plasma are too low to produce a therapeutic effect (communication from Dr. Yaseen Arabi during the MERS-CoV Research Initiative Workshop held on 9—10 September 2015 in Riyadh).

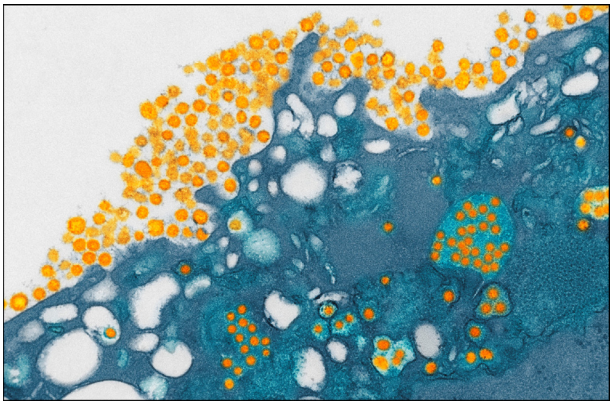

Figure 1 Colored transmission electron micrograph of MERS coronavirus particles (orange) budding from a host cell (blue).
Credit: Public Health England/Science Photo Library.

Thus, monoclonal antibodies are an ideal alternative to hyperimmune sera or hyperimmune immunoglobulin preparations. They can be produced by immortalizing memory B cells with Epstein—Barr virus (EBV) or by fusing a B cell with an appropriate partner cell to produce hybridomas. These methods have a very low efficiency, so alternative strategies have been developed. Such alternatives include the humanization of murine monoclonal antibodies through protein engineering, the selection of human antibodies from phage display libraries as well as the immunization of transgenic mice carrying human immunoglobulin loci combined with the production of monoclonal antibodies using the hybridoma technology. Although these methods have led to the development of several therapeutic monoclonal antibodies against cytokines or surface antigens, their impact on infectious disease therapy has been less successful. There are several advantages to using human memory B cells for the production of monoclonal antibodies: (i) antibodies are fully human, (ii) memory B cells are readily accessible in blood and persist for a lifetime, (iii) there is low or no risk of cross-reactivity against self-antigens, (iv) the human immune response is directed against the virulent pathogen, (v) functional assays can be used to isolate antibodies based on their function (''agnostic approach'') with no need to use molecular targets

Table 1 Examples of human monoclonal antibodies isolated using the Cellclone Technology developed at Humabs.

Target	Findings	Reference
SARS	Potent and broadly neutralizing antibodies	*Nat. Med. 2004; J. Virol. 2008*
Diphtheria toxin	Sub-stoichiometric neutralization of the toxin	*Unpublished*
Anthrax protective antigen	Sub-stoichiometric neutralization of the toxin	*Unpublished*
HIV-1	Neutralizing antibodies targeting different sites including heptad repeat 1	*PLoS ONE 2010; PLoS Pathogens 2010*
Influenza A	Broadly neutralizing and pan-influenza A neutralizing antibodies	*J. Clin. Invest. 2010; Science 2011; Nature 2014*
HCMV	Extremely potent neutralizing antibodies targeting the gHgLpUL pentameric complex	*J. Virol. 2010; PNAS 2014*
RSV/MPV/PVM/BRSV	An antibody that neutralizes 4 paramyxoviruses	*Nature 2013*
Plasmodium falciparum	Antibodies to VAR2 CSA, MSP2 and p27. Fc-dependent killing of parasites. Novel antibodies targeting a conserved antigen on the blood stage of malaria	*Mol. Microbiol. 2007; Infect. Immun. 2011; Nature 2016*
Norovirus	Analysis of the GII.4 norovirus evolution	*PLoS Path. 2012; J. Virol. 2014*
Dengue Virus	Fc-engineered antibodies neutralizing all 4 serotypes and effective in an ADE mouse model	*Cell Host Microbe 2010; PLoS Path. 2013*
Rabies Virus	Potent and broadly neutralizing antibodies covering all seven lyssavirus genotypes	*EMBO Mol. Med. 2016*
S. aureus	Anti-MSCRAMMs and other target molecules antibodies with therapeutic potential	*Unpublished*
Avian Flu (H5N1)	Potent and broadly neutralizing antibodies	*PLoS Med. 2007; PNAS 2015*
Ebola	Potent antibody neutralizing Ebola virus as monotherapy in non-human primates	*Science 2016*
HBV	Potent neutralizing antibodies against HBsAg covering all genotypes and drug selected mutants	*Manuscript in preparation*
HCV	Potent and broadly neutralizing antibodies	*Unpublished*
MERS	Potent and broadly neutralizing antibody	*PNAS 2015*

as baits for antibody isolation, and (vi) the process can be very fast.

In 2004, the Lanzavecchia's group at the Institute for Research in Biomedicine (IRB) in Bellinzona described an improved method of EBV transformation of human B cells [7]. This method, called Cellclone Technology (www.humabs.com), was further optimized and used for the isolation of antibodies against a large set of viral and bacterial pathogens (Table 1) [8]. Importantly, antibodies isolated with this approach are being tested in clinical trials through a partnership with pharmaceutical industries.

The Cellclone Technology was also used for the isolation of a potent monoclonal antibody that was able to neutralize a MERS-CoV infection [9]. The second patient ever reported with MERS, a 49-year-old man from Qatar, was hospitalized with severe respiratory failure in London. Public Health England isolated the MERS virus from this patient (A/England/1/2012) and determined the presence of neutralizing antibodies during recovery from his initial illness, which was suitable for the Cellclone approach. Memory B cells were obtained from this patient eight months after the onset of the MERS infection in May 2013 (when neutralizing antibodies were still detectable in peripheral blood), and supernatants from single antibody-producing B cell clones were tested for virus neutralization using a pseudotyped neutralization assay. This assay was rapidly developed once the sequence of the homologous MERS-CoV spike protein was available. Despite a weak neutralizing serum titer, we identified one B cell clone among the 4600 B cell

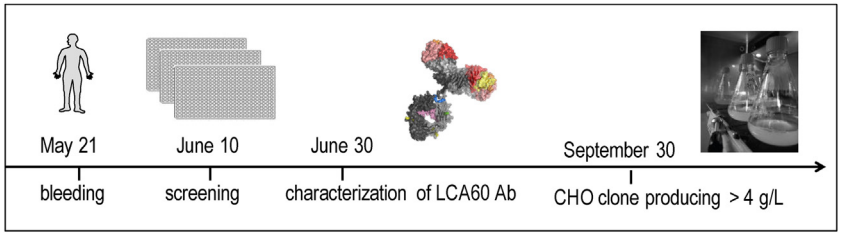

Figure 2 Rapid isolation of MERS-CoV human neutralizing antibodies from a survivor in 2013.

clones tested, that produced a monoclonal antibody (dubbed LCA60) with potent neutralizing activity against the MERS-CoV pseudovirus. A stable CHO cell line producing the neutralizing LCA60 antibody was established, allowing the production of high yields of LCA60. Of note, the entire project took only four months from drawing the blood samples to establishing the stable CHO cell line that produced LCA60 (Fig. 2). LCA60 was efficiently neutralized the infectious MERS-CoV from the London patient, as well as additional isolates obtained from Saudi Arabia and Jordan (IC50 values, 279 ng/mL for England/1/2012, 150 ng/mL for EMC/2012, and 110 ng/mL for the Jordan-N3/2012 isolate). Furthermore, LCA60 efficiently neutralized a panel of monoclonal antibody-resistant viral mutants (MARMs) that were previously selected using phage-derived antibodies that were mapped to 3 different regions in the receptor-binding domain (RBD) of the spike protein. LCA60 was found to target the receptor-binding domain (RBD) of the spike protein of the virus. The spike protein mediates binding to CD26 (also known as dipeptidyl peptidase 4), which serves as the host cell receptor of MERS-CoV and facilities cell entry of the virus by membrane fusion. LCA60 showed subnanomolar affinity for the spike protein (K_D 0.12 nM), which was ~500-fold higher when compared to that of the human receptor CD26.

The epitope recognized by LCA60 was identified using a combination of computational predictions, experimental validation and the generation of escape variants. According to a predicted model, the LCA60 footprint on the RBD was shown to partially overlap with that of the CD26 receptor. This model was confirmed by the results of cross-competition experiments showing that LCA60 prevented the binding of CD26 to RBD, thus demonstrating that LCA60 neutralizes the MERS-CoV infection by blocking the binding to its cellular receptor.

The rapid isolation of LCA60 was also paralleled by the rapid development of an animal model for MERS-CoV based on transient expression of the human CD26 receptor in wild-type or immune-deficient mice. In this model, LCA60 showed high efficacy both in prophylaxis and therapy by reducing lung viral titers of 100—1000-fold, as well as by reducing disease signs and pathology.

This research has demonstrated several findings applicable to all viral emerging diseases for which no vaccines or medications are currently available, but it also raises important considerations for future research:

(1) In an emergency, the isolation of memory B cells from convalescent patients allows the swift generation of effective therapeutic antibodies [10].
(2) A rapid development path for testing new therapeutics in the clinic should be implemented to combat emerging virus outbreaks.
(3) It is important that regulatory agencies and global public health authorities continue implementing and supporting adaptive clinical trials design aimed at testing and comparing multiple candidate drugs during outbreaks [11].
(4) LCA60 represents a promising approach for both prevention and therapy of MERS-CoV infections.

The LCA60 anti-MERS monoclonal antibody in brief

LCA60 is the only antibody isolated from a recovered MERS patient. It is naturally affinity-matured fully human IgG1 monoclonal antibody and potently neutralizes MERS-CoV infection of multiple isolates in vitro and in vivo by preventing the interaction of the viral spike protein with its cellular receptor. The product has finished preclinical development and a GMP-approved cell line expressing the purified and highly potent antibody in high concentrations is established. The non-profit development path will reduce end-user costs considerably.

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Competing interests

None declared.

Ethical approval

Not required.

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