Biosensors and Bioelectronics 65 (2015) 211–219

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A novel immunochromatographic system for easy-to-use detection of group 1 avian influenza viruses with acquired human-type receptor binding specificity

Yohei Watanabe [a,*], Tetsuo Ito [b], Madiha S. Ibrahim [c], Yasuha Arai [a], Kozue Hotta [d,e,1], Hoang Vu Mai Phuong [f], Nguyen Le Khanh Hang [f], Le Quynh Mai [f], Kosuke Soda [g], Masaoki Yamaoka [h], Emmanuel Djoko Poetranto [i], Laksmi Wulandari [i], Hiroaki Hiramatsu [j], Tomo Daidoji [k], Ritsuko Kubota-Koketsu [l], Nongluk Sriwilaijaroen [m,j], Takaaki Nakaya [k], Yoshinobu Okuno [l], Tadanobu Takahashi [n], Takashi Suzuki [n], Toshihiro Ito [g], Hak Hotta [h], Tetsu Yamashiro [d,e], Tsukasa Hayashi [b], Kouichi Morita [o], Kazuyoshi Ikuta [a], Yasuo Suzuki [j,**]

[a] Department of Virology, Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan
[b] KAINOS Laboratories, Inc., Tokyo 113-0033, Japan
[c] Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt
[d] Center for Infectious Disease Research in Asia and Africa, Institute of Tropical Medicine, Nagasaki University, Nagasaki 852-8523, Japan
[e] Vietnam Research Station, Nagasaki University, c/o National Institute of Hygiene and Epidemiology, Hanoi, Viet Nam
[f] Department of Virology, National Institute of Hygiene and Epidemiology, Hanoi, Viet Nam
[g] Avian Zoonosis Research Center, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan
[h] Center for Infectious Diseases, Graduate School of Medicine, Kobe University, Hyogo 650-0017, Japan
[i] Indonesia–Japan Collaborative Research Center, Institute of Tropical Disease, Airlangga University, Surabaya 60115, Indonesia
[j] Health Science Hills, College of Life and Health Sciences, Chubu University, Aichi 487-8501, Japan
[k] Department of Infectious Diseases, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan
[l] Kanonji Institute, The Research Foundation for Microbial Diseases of Osaka University, Kagawa 768-0061, Japan
[m] Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, Pathumthani, 12120, Thailand
[n] Department of Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan
[o] Department of Virology, Institute of Tropical Medicine, Nagasaki University, Nagasaki 852-8523, Japan

ARTICLE INFO

Article history:
Received 19 August 2014
Received in revised form
9 October 2014
Accepted 9 October 2014
Available online 22 October 2014

Keywords:
Immunochromatographic strip test
H5N1 avian influenza virus
Group 1 influenza A virus
Detection of receptor binding specificity
Sialylglycopolymer
Pandemic potential

ABSTRACT

A switch of viral hemagglutinin receptor binding specificity from bird-type α2,3- to human-type α2,6-linked sialic acid is necessary for an avian influenza virus to become a pandemic virus. In this study, an easy-to-use strip test to detect receptor binding specificity of influenza virus was developed. A biotinylated anti-hemagglutinin antibody that bound a broad range of group 1 influenza A viruses and latex-conjugated α2,3 (blue) and α2,6 (red) sialylglycopolymers were used in an immunochromatographic strip test, with avidin and lectin immobilized on a nitrocellulose membrane at test and control lines, respectively. Accumulation of a sialylglycopolymer–virus–antibody complex at the test line was visualized by eye. The strip test could be completed in 30 min and did not require special equipment or skills, thereby avoiding some disadvantages of current methods for analyzing receptor binding specificity of influenza virus. The strip test could detect the receptor binding specificity of a wide range of influenza viruses, as well as small increases in the binding affinity of variant H5N1 viruses to α2,6 sialylglycans at viral titers >128 hemagglutination units. The strip test results were in agreement with those of ELISA virus binding assays, with correlations >0.95. In conclusion, the immunochromatographic strip test developed in this study should be useful for monitoring potential changes in the receptor binding specificity of group 1 influenza A viruses in the field.

* Correspondence to: Department of Virology, Research Institute for Microbiology, Osaka University, Osaka 565-0871, Japan.
** Corresponding author.
E-mail addresses: nabe@biken.osaka-u.ac.jp (Y. Watanabe), suzukiy@isc.chubu.ac.jp (Y. Suzuki).
[1] Present address: Laboratory of Veterinary Public Health, Department of Veterinary Medical Science, Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo 113-8657, Japan

http://dx.doi.org/10.1016/j.bios.2014.10.036
0956-5663/

1. Introduction

Emerging infectious diseases, such as severe acute respiratory syndrome (SARS) and avian influenza, have been of increasing public concern in the past few decades. These diseases involve animal-to-human transmission of zoonotic pathogens (Pang and Guindon, 2004). In particular, an influenza pandemic would be devastating and a serious threat to human health and the global economy.

Avian influenza (AI) viruses were the origin of the influenza A viruses and also have been involved in the emergence of all past influenza pandemics (Webster et al., 1992). Therefore, surveillance of AI viruses to assess their evolution in the field is crucial for preparing for an influenza pandemic (Watanabe et al., 2012b).

Influenza viruses are classified into subtypes based on the antigenic properties of their two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA) (Webster et al., 1992). To date, 18 HA subtypes and 11 NA subtypes have been identified. Almost all possible combinations of HA and NA subtypes have been detected in influenza viruses isolated from aquatic birds, poultry and other bird species. The 18 HA subtypes are phylogenetically categorized into group 1 (H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18) and group 2 (H3, H4, H7, H10, H14 and H15) HAs, with HAs in the same group antigenically related, although they can be distinguished by subtype-specific antibodies (Watanabe et al., 2012a).

HA is the main determinant of viral infectivity and consists of a head region and a stalk region (Imai and Kawaoka, 2012; Sriwilaijaroen and Suzuki, 2012). Influenza viruses attach to host cells by specific binding between the HA head region and sialylglycan that is expressed on the host cell surface. Influenza viruses also recognize terminal sialic acid (Sia) and galactose linkage patterns on sialylglycans (Imai and Kawaoka, 2012). Human influenza viruses preferentially bind to α2,6-linked Sia (α2,6 Sia), whereas most avian viruses preferentially bind to a sugar chain ending in α2,3-linked Sia (α2,3 Sia). This plays a key role of the interspecies barrier that prevents AI viruses from easily infecting humans. Therefore, it is believed that a switch of HA receptor specificity from α2,3 Sia to α2,6 Sia is essential for the emergence of a pandemic influenza virus (Watanabe et al., 2012b).

The highly pathogenic AI virus subtype H5N1 (H5N1 virus) that emerged in China around 1997 has become endemic in birds in some areas, including China, Viet Nam, Indonesia and Egypt (OIE, 2014). H5N1 virus can be directly transmitted from birds to humans and cause a severe respiratory disease with high morbidity (60%) (WHO, 2014). Fortunately, all human H5N1 infections have been restricted to people with close contact with infected poultry and there has been no sustained human-to-human transmission. However, repeated bird-to-human transmission may allow H5N1 viruses to acquire HA mutations that change their receptor specificity from α2,3 Sia (bird-type) to α2,6 Sia (human-type), thereby generating a pandemic virus. H5N1 virus has now diverged genetically to form 10 phylogenetically and phenotypically distinct clades (designated clades 0–9) in different geographic areas (Watanabe et al., 2013). Such complex ecology and diversification in the field increase the pandemic potential of H5N1 virus (Peiris et al., 2007). In addition, other subtype AI viruses, such as H9N2 and H7N9, have also been directly transmitted to humans (Garcia-Sastre and Schmolke, 2014).

Thus far, human-adaptive changes in AI viruses had been thought to occur during AI virus infections in humans and/or pigs. However, recent studies showed that AI virus could acquire increased human-type receptor binding affinity during viral transmission and infection in birds (Watanabe et al., 2011; Sriwilaijaroen and Suzuki, 2014). These results highlight the importance of monitoring possible changes in AI receptor binding specificity in the field to enable rapid response to the emergence of a pandemic influenza virus.

Several assay techniques have been developed to analyze receptor binding affinity of influenza virus (Smith and Cummings, 2014; Stevens et al., 2006; Watanabe et al., 2011; Yamada et al., 2006). The solid-phase virus binding assay is a quantitative system that can detect small changes in receptor binding affinity of influenza virus (Watanabe et al., 2011; Yamada et al., 2006). Glycan microarrays enable multiplex analysis using a panel of glycans with different topologies (Smith and Cummings, 2014; Stevens et al., 2006). A new assay system has been developed combining a virus binding assay and real-time RT-PCR (Takahashi et al., 2013). However, these very sensitive assay systems require a high level of technical expertise, expensive reagents and specialized equipment.

These limitations indicate the need for a system that is easier to use for detecting changes in influenza virus binding specificity. Immunochromatographic assays based on specific antigen–antibody reactions are very useful diagnostic tools and do not require specialized equipment or complicated handling procedures (Gopinath et al., 2014; Sakurai and Shibasaki, 2012). Despite their relatively moderate sensitivity, immunochromatographic assays have been used in various rapid diagnostic kits to detect virus infections for clinical diagnosis and surveillance, since they are fairly simple and rapid. In this study, we developed a new easy-to-use immunochoromatographic strip test to detect the emergence of AI viruses with increased human-type receptor specificity and confirmed the applicability of this test using AI viruses isolated in several different geographic areas.

2. Materials and methods

2.1. Virus isolation and preparation

Avian and human influenza viruses were grown in 10-day-old embryonated chicken eggs and MDCK cells, respectively. The allantoic fluids and culture supernatants were then harvested, precleared by centrifugation at 3000 rpm for 20 min, passed through 0.45 μm filters, and stored as seed viruses at −80 °C. Viral titers were assayed as hemagglutination units (HAU) by hemagglutination assays as described below. All experiments with live H5N1 viruses were performed in Biosafety Level 3 (BSL3) conditions at Osaka University (Japan), Tottori University (Japan), National Institute of Hygiene and Epidemiology (Viet Nam) and Airlangga University (Indonesia).

2.2. Generation of viruses by reverse genetics

Recombinant viruses were generated with a plasmid-based reverse genetics system in the genetic background of influenza virus A/duck/Egypt/D1Br/2007 (EG/D1), which is one of the parental Egyptian H5N1 strains, as previously described (Watanabe et al., 2011). Mutant HA genes were generated by PCR-based site-directed mutagenesis. Recombinant EG/D1 virus and an EG/D1 variant carrying an HA mutation were denoted here as rEG/D1 and $rEG/D1_{mutation}$, respectively. Recombinant viruses were propagated by single passage in eggs. The HA genes of the virus stocks were sequenced to detect the possible emergence of revertants during amplification. All studies with recombinant DNAs were conducted at Osaka University under the applicable laws and approved by the Biological Safety Committee of the Research Institute for Microbial Diseases, Osaka University (approval number 3439).

2.3. Hemagglutination titration

Stocks of avian and human influenza viruses were serially diluted with phosphate-buffered saline (PBS) and mixed with 0.5%