Journal of Microbiological Methods 100 (2014) 27–31

Contents lists available at ScienceDirect

Journal of Microbiological Methods

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

Performance of a multiplexed serological microarray for the detection of antibodies against central nervous system pathogens

Anne J. Jääskeläinen [a,b,*], Sari M. Viitala [c], Satu Kurkela [a,b], Satu Hepojoki [b], Heidi Sillanpää [d], Hannimari Kallio-Kokko [a,b], Tomas Bergström [e], Jukka Suni [a], Ale Närvänen [c], Olli Vapalahti [a,b,f], Antti Vaheri [b]

[a] HUSLAB, Department of Virology and Immunology, Helsinki University Central Hospital, Helsinki, Finland
[b] Haartman Institute, Department of Virology, University of Helsinki, Helsinki, Finland
[c] School of Pharmacy, University of Eastern Finland, Kuopio, Finland
[d] Haartman Institute, Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland
[e] Department of Clinical Virology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.
[f] Faculty of Veterinary Medicine, Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland

ARTICLE INFO

Article history:
Received 12 November 2013
Received in revised form 31 December 2013
Accepted 20 February 2014
Available online 1 March 2014

Keywords:
Microarray
Seroarray
Peptide
Recombinant protein
Glutaraldehyde

ABSTRACT

Central nervous system (CNS) infections have multiple potential causative agents for which simultaneous pathogen screening can provide a useful tool. This study evaluated a multiplexed microarray for the simultaneous detection of antibodies against CNS pathogens. The performance of selected microarray antigens for the detection of IgG antibodies against herpes simplex virus 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), adenovirus, *Mycoplasma pneumoniae* and *Borrelia burgdorferi* sensu lato, was evaluated using serum sample panels tested with reference assays used in a routine diagnostic laboratory. The microarray sensitivity for HSV-1, HSV-2, VZV, adenovirus and *M. pneumonia* ranged from 77% to 100%, and the specificity ranged from 74% to 97%. Very variable sensitivities and specificities were found for borrelial antigens of three different VlsE protein IR(6) peptide variants (IR6p1, IR6p2, IR6p4) and three recombinant decorin binding proteins A (DbpA; DbpAIa, DbpA91, DbpAG40). For single antigens, good specificity was shown for antigens of IR6p4 and DbpAIa (96%), while DbpA91, IR6p1 and IR6p2 were moderately specific (88–92%). The analytical sensitivity of the microarray was dependent on the borrelial IgG concentration of the specimen. The overall performance and technical features of the platform showed that the platform supports both recombinant proteins, whole viruses and peptides as antigens. This study showed diagnostic potential for all six CNS pathogens, including *Borrelia burgdorferi* sensu lato, using glutaraldehyde based microarray, and further highlighted the importance of careful antigen selection and the requirement for the use of multiple borrelial antigens in order to increase specificity without a major lack of sensitivity.

1. Introduction

One of the challenges in the laboratory diagnosis of central nervous system (CNS) infections is the relatively high number of potential causative pathogens, including e.g. herpes simplex virus 1 (HSV-1) and 2 (HSV-2), varicella zoster virus (VZV), *Borrelia burgdorferi* sensu lato, and *Mycoplasma pneumoniae*. Nucleic acid detection from cerebrospinal fluid (CSF) is usually the method of choice for the diagnosis of neuroinvasive virus infections, especially for different herpes viruses. However, virus serology from serum and CSF provides complementary information, and plays an important role particularly in prolonged infections, and in the investigation of long-term sequelae. For neuroinvasive *M. pneumoniae* infections, both nucleic acid detection and serology are generally used, while serology is most sensitive for neuroborreliosis. However, the serodiagnosis of *Borrelia burgdorferi* sensu lato is generally complicated by the very variable antibody responses observed between individuals and those at the different stages of the disease. Furthermore, the borrelial antibody response may not reflect an infection, but merely a contact with a tick carrying the pathogen. Due to the complex nature of borreliosis, several different diagnostic methods, e.g. enzyme immunoassays (EIA) and immunoblotting, are often used in parallel to obtain more specific and reliable results for diagnosis Lyme borreliosis. In addition, for the serodiagnosis of borrelial infection, it is generally suggested that two specific antigens should react with patient's serum to establish the diagnosis.

EIA is the most commonly used platform for the serodiagnosis of neuroinvasive infections, sometimes complemented by e.g. immunoblot. Simultaneous screening of antibodies against relevant viral and bacterial targets can provide an advantageous tool for the diagnosis of

* Corresponding author at: Haartman Institute, Department of Virology, P.O.B. 21 (Haartmaninkatu 3), FIN-00014 University of Helsinki, Finland. Tel.: +358 9 19126671, +358 50 5420743 (mobile); fax: +358 9 19126491.
E-mail address: anne.jaaskelainen@helsinki.fi (A.J. Jääskeläinen).

http://dx.doi.org/10.1016/j.mimet.2014.02.011
0167-7012/

CNS infections. Serological platforms with a multiplexed format offer a rapid, cost-effective and clinically comprehensive approach. Such platforms include multiplex bead-based immunoassays and multiple antigen microarrays, which allow for parallel antibody detection. Different multiplex bead-based immunoassays have been developed for e.g. detection of antibodies against influenza virus, *Streptococcus pneumoniae*, Lyme borreliosis, or pneumococcal, meningococcal and haemophilus polysaccharides, and tetanus and diphtheria toxoids (Yan et al., 2005; Yu et al., 2011; Whitelegg et al., 2012; Gerritzen and Brandt, 2012).

Multiplexed serological microarrays have been developed for e.g. simultaneous detection of HIV and its coinfections (Lochhead et al., 2011), as well as for the diagnostics of atypical pneumonia (Gouriet et al., 2008). As the detection of CNS pathogens, previous microarray designs have focused on the viral nucleic acid detection (Jääskeläinen et al., 2006, 2008; Leveque et al., 2011; Mannonen et al., 2012) or detection of herpesvirus, rubella virus and *Toxoplasma gondii* antibodies using nitrocellulose based or amino-silane activated glass microarrays and proteins as antigens (Bacarese-Hamilton et al., 2004; Jääskeläinen et al., 2009). Proteome microarray has been set up to profile immune response to *Borrelia burgdorferi* sensu lato (Xu et al., 2008) but no serological or nucleic acid based microarrays have been described earlier for diagnosis of Lyme borreliosis.

Our aim was to find a suitable microarray platform that supports different kinds of antigens, not just proteins, for the detection of viral and bacterial antibodies, and to set up a microarray for the detection of antibodies against *Borrelia burgdorferi* sensu lato for the diagnosis of Lyme borreliosis. In addition, our aim was to evaluate the feasibility of a multiplexed serological glutaraldehyde based microarray as a platform for comprehensive diagnostics of other CNS infections, especially HSV-1, HSV-2, VZV, adenovirus and *M. pneumoniae*, using serum samples. Glutaraldehyde coated microtiter plate based microarray (Viitala et al., 2013) was set up using different types of antigens, i.e. recombinant proteins, inactivated viruses and peptides, and evaluated for the simultaneous detection of antibodies against HSV-1, HSV-2, VZV, adenovirus, *M. pneumoniae* and *Borrelia burgdorferi* sensu lato from clinical serum specimens, by comparing them to commercial reference assays.

2. Materials and methods

2.1. Reference methods

HerpeSelect® HSV-1 IgG and HSV-2 IgG ELISA kits (Focus Diagnostics Inc, Cypress, CA, USA) were used for HSV antibody detection. *Mycoplasma pneumoniae* IgG EIA (Ani Labsystems Ltd, Vantaa, Finland) was used for *M. pneumoniae* antibody detection. *Borrelia afzelii* and VlsE IgG ELISA kit (Sekisui Virotech GmbH), Liaison® Borrelia IgG (DiaSorin), and Borrelia Europe Plus TpN17 Line IgG Line Immunoblot (Sekisui Virotech GmbH, Rüsselsheim, Germany) were used for the detection of borrelial IgG. The linear measurement ranges were 9–40 AU/ml for the Sekisui Virotech test, and 5–240 VE/ml for the DiaSorin test. A positive reaction in the immunoblot required distinct reactions against at least two of the antigens included in the assay (OspC, VlsE-mix, p39, DbpA, p58 or p83). All tests were performed according to the manufacturers' instructions. A validated and accredited in-house sandwich-EIA (HUSLAB, Finland) with VZV glycoprotein EIA antigen (Institute Virion Ltd, Rüschlikon/Zürich, Switzerland) and in-house adenovirus EIA (HUSLAB, Finland) with inactivated whole adenovirus antigen were used for VZV and adenovirus IgG detection, respectively. In short, the in-house adenovirus IgG EIA was carried out using 96-well microtiter plates coated with attenuated adenovirus 1 (strain 1A891) antigen (HUSLAB, Finland) followed by blocking with 1% bovine serum albumin (BSA; Jackson ImmunoResearch Europe Ltd, Suffolk, UK). Serum samples were diluted 1:100 in phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBST) and incubated for 1 h at +37 °C followed by three washes with PBST. Rabbit anti-human IgG conjugated with horse radish peroxidase (HRP; Dako, Helsinki, Finland) was diluted 1:10,000 and used for a 1 h conjugation at +37 °C. 3,3′,5,5′-tetramethylbenzidine liquid substrate (Sigma-Aldrich) was used for colour reaction after washing three times with PBST. After 30 min incubation at RT, the colour reaction was stopped with 100 μL of 0.5 M H_2SO_4. The absorbance was read at 450 nm (Multiscan RC, Labsystems, Finland).

2.2. Clinical specimens

The study material included serum samples from individual patients tested for IgG antibodies against the following antigens at the routine diagnostics of the Helsinki University Central Hospital Laboratory (HUSLAB) in Finland in 2009–2012: HSV-1 ($n = 81$), HSV-2 ($n = 82$), VZV ($n = 77$), adenovirus ($n = 77$), *M. pneumoniae* ($n = 70$), and *Borrelia burgdorferi* sensu lato ($n = 53$). The sera tested for HSV-1, HSV-2, VZV, adenovirus and *M. pneumoniae* IgG antibodies were deemed either positive or negative by using the respective reference methods. The sera deemed positive for *Borrelia* IgG ($n = 35$) were reactive both in two different EIA tests (Sekisui Virotech and DiaSorin) and in IgG immunoblot. The sera deemed negative for *Borrelia* IgG ($n = 18$) were non-reactive in one EIA test (Sekisui Virotech). In order to better evaluate the analytical sensitivity of the microarray, the reactive borrelial specimens were further divided into three subcategories according to their IgG antibody concentrations as follows: low IgG concentration ($n = 9$), intermediate IgG concentration ($n = 13$), and high IgG concentration ($n = 13$). The sum of numeric values from the two EIA tests (Sekisui Virotech and DiaSorin) was used for this categorization (low IgG concentration 25–59; intermediate 60–179; high >180).

2.3. Microarray design

Single antigens for HSV-1, HSV-2, VZV, adenovirus and *M. pneumoniae* were used for simultaneous screening of specific IgG antibodies. The antigens used for the microarray were as follows: recombinant HSV-1 glycoprotein G (gG, amino acids 84–175; Acris Antibodies GmbH, Herford, Germany), recombinant HSV-2 glycoprotein G [gG, amino acids Leu343-Asp649 fused with human superoxide dismutase (SOD); Acris Antibodies GmbH, Herford, Germany], purified whole virus antigen of attenuated adenovirus 1 (strain 1A891) (HUSLAB, Finland), *M. pneumoniae* 1 AG (4MP67; Ani Biotech Oy, Vantaa, Finland), and recombinant VZV gE (University of Gothenburg, Gothenburg, Sweden; 11). This recombinant VZV gE antigen is produced in mammalian cells in large scale and is known to be specific for VZV showing no cross-reaction with HSV (Thomsson et al., 2011; Grahn et al., 2011).

The complex nature of the immune response to *Borrelia burgdorferi* sensu lato infection requires the use of multiple serological assays to increase diagnostic sensitivity and specificity (Bacon et al., 2003; Porwancher, 2003; Porwancher et al., 2011). With this in mind, six antigens for *Borrelia* were selected for the microarray: three different VlsE protein IR6 peptide variants (Core Facility of Protein Chemistry, Haartman Institute, University of Helsinki, Helsinki, Finland; Sillanpää et al., 2007) of *Borrelia burgdoferi sensu stricto* B31 (IR6p1), *B. garinii* IP90 (IR6p2), and *B. afzelii* ACAI (IR6p4), and three recombinant decorin binding proteins A (DbpA) (University of Helsinki, Helsinki, Finland; Panelius et al., 2007) derived from *B. burgdorferi sensu stricto* IA (DbpAIa), *B. garinii* 40 (DbpAG40), and *B. afzelii* A91 (DbpA91).

Prior to spotting, HSV-1, HSV-2, *M. pneumoniae*, and all six borrelial antigens were diluted in spotting buffer [10 mmol/L $NaHCO_3$ (pH 9.5)] in a final concentration of 0.1 mg/ml. VZV and adenovirus antigens were diluted in a final concentration of 1:10.

The 96-well polystyrene plates were activated with pre-polymerized glutaraldehyde, and each well was spotted with the 11 viral and bacterial antigens in triplicates by using the BioRobotics MicroGrid II microarray printer (BioRobotics, Cambridge, UK) as previously described (Viitala et al., 2013). Human IgG (~95% HPLC purified; Sigma-Aldrich, Helsinki, Finland) was spotted in triplicates and used as a positive control on the microarray. The spotting buffer and rabbit myosin (Sigma–Aldrich)