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Optimized enzyme-linked immunosorbent assay for detecting cytomegalovirus infections during clinical trials of recombinant vaccines

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ABSTRACT

Background: In clinical trials of cytomegalovirus (CMV) glycoprotein B (gB) vaccines, CMV infection is detected by first depleting serum of anti-gB antibodies and then measuring anti-CMV antibodies with a commercially available enzyme-linked immunosorbent assay (ELISA) kit, with confirmation of positive findings by immunoblot.

Objectives: Identification of CMV immunoantigens for the development of an ELISA that detects specifically CMV infection in clinical samples from individuals immunized with gB vaccines.

Study design: Sensitivity and specificity of ELISAs using antigenic regions of CMV proteins UL83/pp65, UL99/pp28, UL44/pp52, UL80a/pp38, UL57, and UL32/pp150 were measured.

Results: An IgG ELISA using a UL32/pp150 [862–1048] capture peptide was the most specific (93.7%) and sensitive (96.4%) for detecting CMV-specific antibodies in sera. The ELISA successfully detected CMV-specific antibodies in 22 of 22 sera of subjects who had been vaccinated with a gB vaccine but who had later been infected with CMV. The ELISA was linear over a wide range of CMV concentrations (57–16,814 ELISA units/mL) and was reproducible as indicated by a 5% intra-day and 7% inter-day coefficients of variation. The signal was specifically competed by UL32/pp150 [862–1048] peptide but not by CMV-gB or herpes simplex virus 2 glycoprotein D. Lipid and hemoglobin matrix did not interfere with the assay.

Conclusion: The UL32/pp150 [862–1048] IgG ELISA can be used for the sensitive and specific detection of CMV infection in gB-vaccinated individuals.

1. Background

Cytomegalovirus (CMV) [1] is a major cause of congenital infection and is currently the most common nongenetic cause of childhood hearing loss [1]. Congenital CMV infection is also an important cause of mental retardation and neuromuscular impairments and was identified as a high priority target in Institute of Medicine vaccine prioritization reports [2]. A vaccine administered to adolescents or adult women could prevent congenital CMV infection by making them immune prior to pregnancy [1]. Since CMV infections in healthy children and adults are rarely clinically apparent, it is necessary to use serodiagnostic tests to detect infections in clinical trial participants as was done in recent phase II clinical trials of a recombinant CMV glycoprotein B (gB) vaccine [3–6]. Most commercially available CMV antibody assays use purified extracts of virus infected cells as capture antigens. Use of an assay with whole virus antigen will make it difficult if not impossible to distinguish antibody response to infection from the antibody response to a recombinant protein vaccine.

To aid in development of a gB-based CMV vaccine, it would be useful to have an ELISA that can differentiate between antibodies induced by natural CMV infection and those induced by the vaccine. Possible capture antigens for such an assay include viral proteins UL44/pp52, UL32/pp150, UL99/pp28, and UL83/pp65, which are major targets for IgM- and IgG-specific antibody responses to CMV [7–11]. In particular, UL32/pp150 can induce a strong, long-lasting serological response that can be detected several years after viral replication has stopped [8]. Moreover, UL80a/pp38, an assembly protein and UL57, major targets of the IgM response during acute CMV infection [7,8,10–12] could also be considered as capture antigens for early CMV-specific antibody detection.

Abbreviations: CMV, cytomegalovirus; ELISA, enzyme-linked immunosorbent assay; gB, glycoprotein B; HSV2 gD, herpes simplex virus 2 glycoprotein D; Ig, immunoglobulin; OD, optical density

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Table 1
Detection of CMV infection by ELISAs using different capture antigens.

Capture antigen		Correctly identified			Discordant		
		Positive (sensitivity)	Negative (specificity)	Overall concordance	False positive	False negative	Overall discordance
		n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)
Single proteins							
UL32/pp150 [463–511]	IgG	69/128 (53.9)	55/57 (96.5)	124/185 (67.0)	2/57 (3.5)	59/128 (46.1)	61/185 (33.0)
UL32/pp150 [495–692]	IgG	115/124 (92.7)	54/56 (96.4)	169/180 (93.9)	2/56 (3.6)	9/124 (7.3)	11/180 (6.1)
UL32/pp150 [762–819]	IgG	112/128 (87.5)	55/57 (96.5)	167/185 (90.3)	2/57 (3.5)	16/128 (12.5)	18/185 (9.7)
UL32/pp150 [862–1048]	IgG	119/127 (93.7)	54/56 (96.4)	173/183 (94.5)	2/56 (3.6)	8/127 (6.3)	10/183 (5.5)
UL32/pp150 [1011–1048]	IgG	107/128 (83.6)	56/58 (96.6)	163/186 (87.6)	2/58 (3.4)	21/128 (16.4)	23/186 (12.4)
UL44/pp52 [202–434]	IgG	77/128 (60.2)	56/58 (96.6)	133/186 (71.5)	2/58 (3.4)	51/128 (39.8)	53/186 (28.5)
UL57 [545–601]	IgM	19/128 (14.8)	56/58 (96.6)	75/186 (40.3)	2/58 (3.4)	109/128 (85.2)	111/186 (59.7)
UL80a/pp38 [117–373]	IgG	0/41 (0.0)	19/19 (100.0)	19/60 (31.7)	0/19 (0.0)	40/41 (97.6)	40/60 (66.7)
UL83/pp65 [297–510]	IgG	5/41 (12.2)	19/19 (100.0)	24/60 (40.0)	0/19 (0.0)	36/41 (87.8)	36/60 (60.0)
UL99/pp28 [130–160]	IgG	28/41 (68.3)	18/19 (94.7)	40/60 (66.7)	1/19 (5.3)	13/41 (31.7)	14/60 (23.3)
Combinations							
UL32/pp150 [762–819] + [1011–1048]	IgG/IgG	118/127 (92.9)	53/57 (93.0)	171/184 (92.9)	4/57 (7.0)	9/127 (7.1)	13/184 (7.1)
UL32/pp150 [463–511] + [1011–1048]	IgG/IgG	109/127 (85.5%)	53/57 (93%)	162/184 (88.%)	4/57 (7%)	18/127(14.5%)	22/184 (12%)
UL32/pp150 [762–819] + [862–1048]	IgG/IgG	120/127 (94.5%)	52/56 (92.6%)	172/183 (94%)	4/56(7.1%)	7/127(5.5%)	11/183(6%)
UL32/pp150 [495–692] + [862–1048]	IgG/IgG	118/124 (95.2)	52/56 (92.9)	170/180 (94.4)	4/56 (7.1)	6/124 (4.8)	10/180 (5.6)
UL32/pp150 [862–1048] + UL57 [545–601]	IgG/IgM[a]	124/126 (98.4)	53/56 (94.6)	177/182 (97.3)	3/56 (5.4)	2/126 (1.6)	5/182 (2.7)
UL32/pp150 [495–692] + UL57 [545–601]	IgG/IgM[a]	118/123 (95.9)	53/56 (94.6)	171/179 (95.5)	3/56 (5.4)	5/123 (4.1)	8/179 (4.4)

[a] Combination of two separate ELISAs.

2. Objectives

The objective of this study was to develop an ELISA that can differentiate between antibodies induced by natural CMV infection and those induced by a gB vaccine. This article describes the selection of the final conditions for the ELISA and its performance in detecting CMV infections based on antibody detection in sera from participants in a CMV vaccine trial.

3. Study design

3.1. Proteins, peptides, and lipid

UL99/pp28 [130–160], UL80a/pp38 [117–373], UL44/pp52 [202–434], UL83/pp65 [297–510], and UL32/pp150 [1011–1048] were from GenWayBio (San Diego, CA, USA). UL57 [545–601], UL32/pp150 [762–819] (KL58 peptide) and UL32/pp150 [463–511] (SD49 peptide) were synthesized from Covalab (Villeurbanne, France). UL32/pp150 [495–692] and UL32/pp150 [862–1048] were generated using vectors from Geneart-Life Technologies (Carlsbad, CA, USA) and were expressed as hexa-histidine-tagged fusion proteins in *Escherichia coli* and purified by Ni^{2+} affinity chromatography by R & D Systems (Minneapolis, MN, USA). CMV gB was produced as described previously [13]. Herpes simplex virus 2 glycoprotein D (HSV2 gD) was from GenScript (Piscataway, NJ, USA). Human hemoglobin and glyceryl trimyristate were from Sigma (St. Louis, MO, USA).

3.2. Sera

CMV-positive and – negative human sera were purchased from SeraCare Life Sciences (Milford, MA, USA), Biomex GmbH (Heidelberg, Germany), and Biomnis (Lyon, France). Serum samples from the phase II clinical trial [4] were seronegative at baseline and were naturally infected with CMV during the trial course.

3.3. ELISA

96-well MaxiSorp ELISA plates (Thermo Fisher Scientific, Waltham, MA) were coated overnight with 4 µg/mL candidate capture antigens, blocked with 1% milk, and incubated with two-fold dilutions (100 µL/well) of samples and CMV-positive standard (Cytotect CP; Biotest Pharma, Dreieich, Germany) for 90 min at 37 °C. Bound antibody was detected by incubating for 90 min with 1:1000 horseradish peroxidase-conjugated goat anti-human IgG or IgM (Sigma-Aldrich, St. Louis, MO), followed by detection with tetramethylbenzidine and measurement of the optical density (OD) at 450 and 650 nm. Concentrations were calculated using SoftmaxPro (Molecular Devices, Sunnyvale, CA) by comparison with the best-fit 4-parameter regression line for the linear portion of the standard curve.

3.4. Statistical analysis

Statistical analyses were performed using SAS® version 9.2 (SAS Institute, Cary, NC). Specificity was calculated as 100% × (OD in the presence of competitor or control/OD for serum alone). The extent of interference by serum components was calculated as the difference in mean antibody concentrations in the presence and absence of the matrix component divided by the mean antibody concentration in its absence. Linearity was assessed by regression analysis of the observed vs. expected antibody concentration. Accuracy was calculated as the 90% confidence interval of the mean recovery percentage. Precision was assessed as the coefficient of variation of the antibody concentrations. Intra- and inter-day coefficients of variance were determined by variance component analysis. The lower limit of quantitation was the lowest concentration of antibody with a coefficient of variance < 30% and within the linear portion of the curve.

4. Results

4.1. Initial screening of capture antigens for an IgG ELISA

For the ELISA development, a panel of 128 CMV positive and 58 CMV negative serum samples (screened by Biomnis using diagnostic kits (CMV_IgG_ELISA PKS Medac, CMV_IgM_ELA Tests PKS Medac) were used. As capture antigens, we considered internal CMV proteins that induce a strong immune response and have been reported to be

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