Development of a cell-based AP-1 gene reporter potency assay for anti-anthrax toxin therapeutics

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Abstract and Introduction

Anthrax is a life-threatening disease caused by infection with *Bacillus anthracis*. The pXO1 virulence plasmid of B. anthracis expresses a binary anthrax toxin comprising protective antigen (PA) and two enzymatic moieties, edema factor (EF) and lethal factor (LF). PA binds surface receptors on the host cells, mediating the translocation of the enzymatic moieties into the cytoplasm of the host cells. LF is a zinc-dependent metalloprotease with specific activity to cleave mitogen-activated protein kinase kinases (MKKs) and, in certain mouse and rat strains, NACHT leucine-rich repeat protein 1 (NALP1), a protein involved in cell death pathways. EF is a Ca2+ and calmodulin dependent adenylate cyclase that increases the level of cAMP in the host cell, disrupting water homeostasis, the intracellular signaling pathways and macrophage function. Given the role of PA in mediating entry of the enzymatic moieties into the host cells, anti-PA monoclonal antibodies have been developed as therapeutic for prophylaxis and treatment of inhaled anthrax. The available assays for the potency test of anti-PA antibodies include methods that were developed based on either LT-induced NALP1-mediated cell death or the EF-induced increase in the level of c-AMP. These assays do not fully reflect and/or capture the pathological functions of anthrax toxin in humans, because LT does not cleave human NALP1, and ET may play a less important role than LT in the pathogenesis following infection with *Bacillus anthracis*. Herein, we developed a cell-based gene reporter assay for the potency test of anti-PA antibody based on the rapid LT-dependent reduction of c-Jun protein levels. The c-Jun protein is an important component of the AP-1 transcription factor complex, responsible for regulating numerous physiology and pathology pathways in humans. Our new AP-1 reporter assay has been qualified for specificity, accuracy, repeatability, intermediate precision and robustness. The success in development of this assay may facilitate FDA regulation of anti-PA therapeutic antibodies as well as other products that target the AP-1 signaling pathway.

Materials and Methods

Materials

Cell line: Hepa1C1C7 cells purchased from ATCC (Cata# CRL-2026)

Gene reporter construct: pGreenFire 2.0 AP-1 Reporter purchased from System Biosciences (Cata# TR452VA-P)

Luciferase Assay: One-Glo Luciferase Assay System purchased from Promega (Cata# E6120)

Methods

Establishment of the stable cell line: Hepa1C1C7 cells were transduced with the pGreenFire 2.0 AP-1 Reporter and then cultured in the selection medium containing 5 µg/mL puromycin for one week. The transduced cells were FACS sorted for green fluorescence protein (GFP)-positive cells (Hepa1C1C7-AP1Luc), which were used for single cell cloning by the limited dilution method. The single cell clone 1H3 was selected following testing of the luciferase activity, responsiveness to anthrax toxin and anti-PA antibody.

Assay Qualification: The AP-1 reporter assay was qualitied for specificity, accuracy, repeatability, intermediate precision, robustness.

Results and Discussion

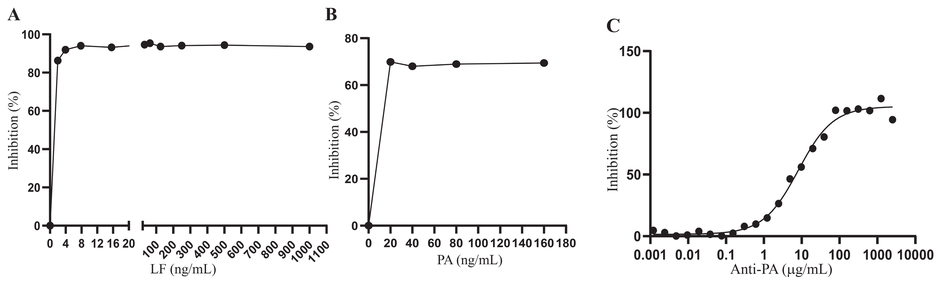

Figure 1. Dose-response of Hepa1C1C7-AP1Luc cells to anthrax lethal factor (LF) (A), protective antigen (PA) (B) and anti-PA antibody (C)

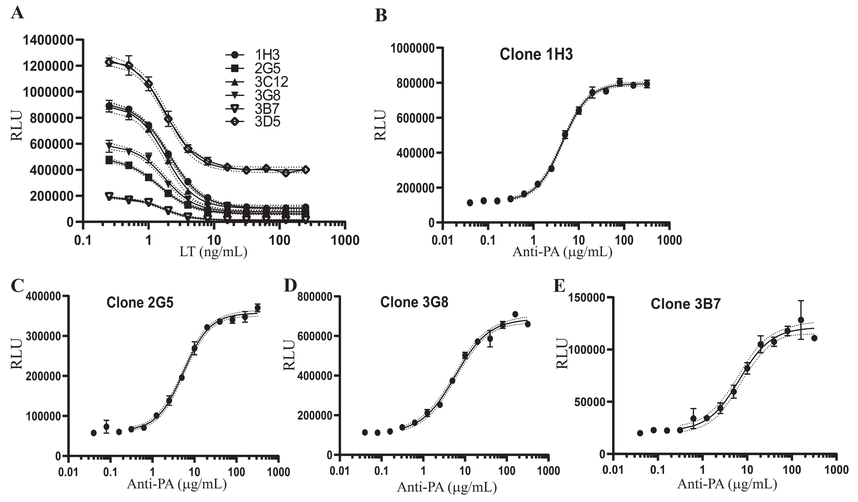

Figure 2. Dose-response of Hepa1C1C7-AP1Luc single clones to anthrax lethal toxin (LT) (A) and anti-PA antibody (B-E)

Repeatability		Test Results										
Theoretical RP	Exp.1	Exp.2	Exp.3	Exp.4	Exp.5	Exp.6	Exp.7	Average	SD	RB%	CV	
50	46.68	51.42	45.33	49.27	49.83	49.94	54.27	49.53	2.95	-0.93107	5.951151	
75	71.39	86.74	74.05	79.53	73.44	70.47	76.26	75.98	5.63	1.310525	7.407261	
100	95.49	93.57	101.21	102.26	97.41	95.58	88.98	96.36	4.53	-3.64253	4.69914	
125	123.09	125.32	129.06	126.69	126.74	138.00	134.28	129.03	5.29	3.220964	4.099003	
150	152.88	149.39	155.5	130.85	136.83	149.77	138.91	144.88	9.29	-3.41595	6.415266	
Intermediate Precision		**Test Results**										
Theoretical RP	**Analyst**	**Exp.1**	**Exp.2**	**Exp.3**	**Exp.4**	**Exp.5**	**Exp.6**	**Exp.7**	**Average**	**SD**	**RB%**	**CV**
100	1	95.49	93.57	101.21	102.26	97.41	95.58	88.98				
100	2	99.83	103.02	116.27	108.98	94.97	101.30	101.83	100.05	6.80	0.05	6.79

Table 1. Repeatability and intermediate precision of the AP-1 gene reporter assay

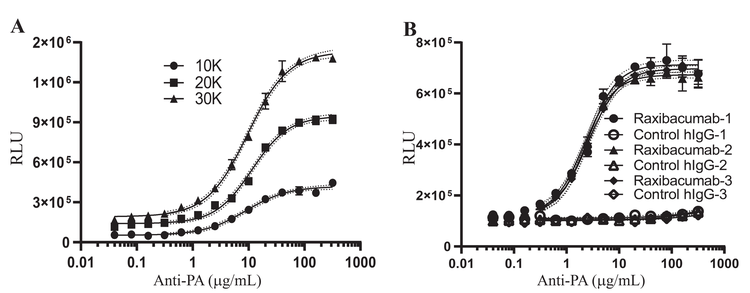

Figure 3. Optimization of the cell number for the AP-1 gene reporter assay (A) and the specificity of the assay (B)

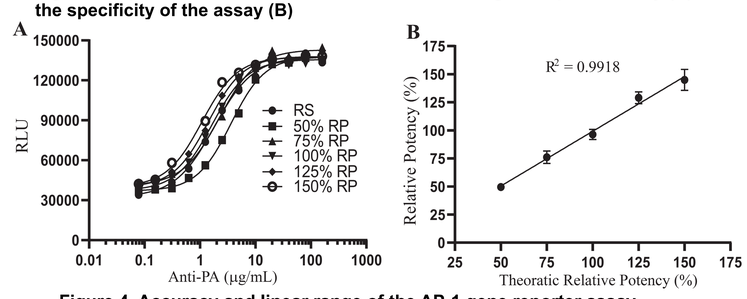

Figure 4. Accuracy and linear range of the AP-1 gene reporter assay

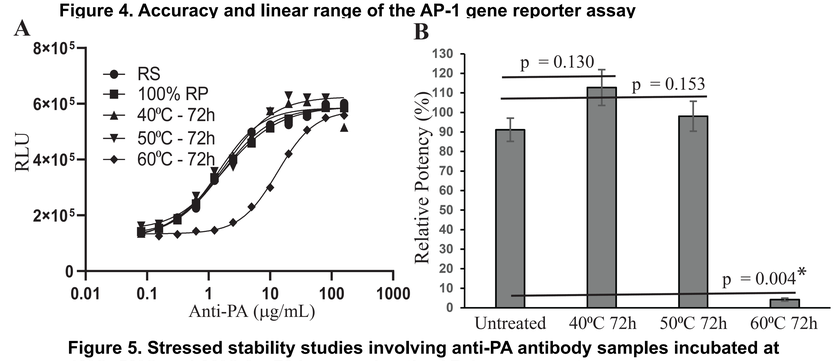

Figure 5. Stressed stability studies involving anti-PA antibody samples incubated at 40°C, 50°C and 60°C for 72 hours using the AP-1 gene reporter assay.

Conclusion

We developed a cell-based gene reporter assay for the potency test of anti-PA antibody based on the rapid LT-dependent reduction of c-Jun protein levels. This new AP-1 reporter assay has been qualified for specificity, accuracy, repeatability, intermediate precision and robustness. Using this assay, we detected the reduction in the potency of the stressed anti-PA antibody sample, suggesting that it may serve as a stability-indicating assay. The success in development of this assay may facilitate FDA regulation of anti-PA therapeutic antibodies as well as other products that target the AP-1 signaling pathway.