Multiplexed Cytokine Expression in Viral Infected Human Cell Lines

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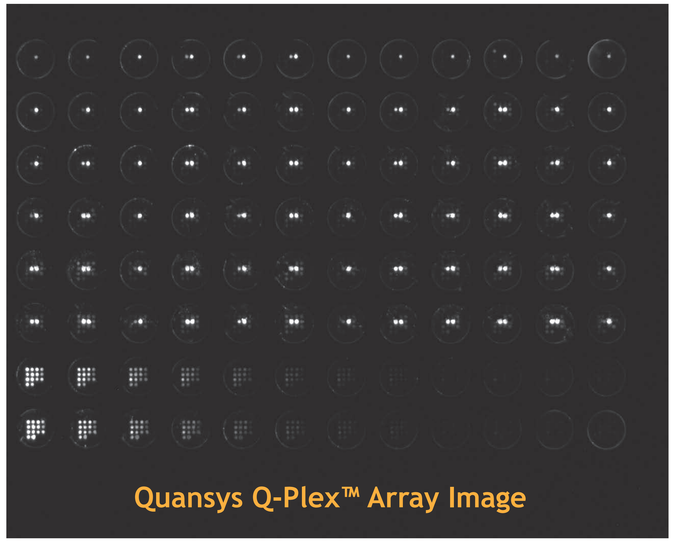
Abstract:

Introduction: Cytokine presence in viral infections has been associated with and is indicative of different disease states as well as treatment options. Multiplexed high throughput applications can aid in the screening of these types of studies. The objective of this study is to screen 3 different human cell lines infected with 10 different virion and characterize the cytokine expression over time.

Materials and Methods: Human cell lines; A498, kidney cells, Hep-G2 liver cells and A549 lung cells were each infected with 10 different viral infections. The virion used were Influenza A H3N2, Influenza A H5N1, Influenza B, Respitory Syncytial Virus (RSV), Parainfluenza 1, Parainfluenza 3, Adenovirus, Echovirus, Herpes Simplex Virus 1 (HSV1), and Cytomegalovirus (CMV).

At 2, 6, 12, 24, and 48 hours, 150 µl of cell culture sample collected. Each sample was then tested using the Quansys Q-Plex™ Human Cytokine Array. This 14 plex array comprises assays for IL-1α, IL-1ß, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, INFγ, TNFα, TNFß, RANTES, and MCP-1. This array is built on a 96 well format and comprises 14 individual ELISA reactions in the bottom of each well. Following the Quansys protocol, the samples are tested following a familiar ELISA based procedure. The image is then processed using Quansys Image Anaglysis software supplied from Quansys.

Results and Conclusion: The Hep-G2 cells showed RANTES production when infected with Influenza A, Influenza B, RSV, and Parainfluenza 3. The A549 lung cells produced significant increases in IL-6 with all of the viral infections except Adnovirus. MCP-1 was modestly present in the uninfected A549 cells though Flu A H3N2, H5N1, RSV, Para 3, Echovirus, HSV1, and CMV infected cells had elevated levels of MCP-1 at all time points. RANTES production was also measured in the A549 cells infected with Flu A H2N2, Flu A H5N1, Flu B, RSV, and Parainfluenza 3. In summary, IL-6, MCP-1 and RANTES play significant roles in viral infection in human cell lines.



Quansys Q-Plex™ Array Image

Introduction:

All human cells have innate defense mechanisms to viral infections. One of the most well known mechanisms involves defenses against dsRNA viruses. The presence of dsRNA is known to activate some cytokine expression pathways. However, viruses are a diverse group of pathogens replicating by many mechanisms. It is our objective to determine cytokine expression profiles in 10 pneumotropic viruses from varied families. It is our hypothesis that cytokine profiles will correlate directly with replication mechanisms, specifically between dsDNA and RNA viruses.

HEP-G2 liver cells, A498 kidney cells, and A549 lung cells were used in this study. These were grown in culture and split into parallel flasks 2 days prior to infection. Each flask was then washed with media and separately infected with the following viruses: Influenza A H3N2, Influenza A H5N1, Influenza B, Respitory Syncytial Virus (RSV), Parainfluenza 1, Parainfluenza 3, Adenovirus, Echovirus, Herpes Simplex Virus 1 (HSV1), and Cytomegalovirus(CMV). The cell culture supernatant was then sampled at 0, 2, 6, 12, 24, and 48 hours post infection, and frozen at -80º C until all the samples were collected.

Cytokine expression was then assessed for each sample using Quansys Biosciences' Q-Plex™ Human Cytokine Array. Using this array we observed expression for the following cytokines: IL-1α, IL-1ß, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, MCP-1, TNF α, TNF ß, and RANTES.

Results:

None of the samples tested expressed measurable levels of IL-1α, IL-1ß, IL-2, IL-4, IL-5, IL-10, IL-13, TNF α, or TNF ß.

HEP-G2 cultures constitutively express IL-8 with no observed difference in expression levels between the different experimental conditions. Unlike the A498 and A549 cell lines the HEP-G2 cells do not express any MCP-1 or IL-6 when virally infected while RANTES production in the HEP-G2 cells is varied depending on the type of viral infection. Flu A H5N1 infected cells had measurable levels of RANTES at 24 hours post infection with high levels at 48 hours. In contrast Flu A H3N2 and Flu B just started expressing measurable levels of RANTES at the 48 hour time point. RSV infected cells expressed measurable levels of RANTES at 12 hours and high levels at 24 and 48 hours. The Parainfluenza 3 infected HEP-G2 cells showed the greatest RANTES response with levels being detected at 6 hours and had high levels for the rest of the time points. Parainfluenza 1, Adnovirus, Echovirus, HSV1, and CMV did not elicit a RANTES response.

The A498 kidney cells constituently expressed IL-8, IL-6, RANTES, and MCP-1. There were no observable differences in cytokine expression between all the experimental conditions.

The A549 lung cells had varied IL-6 production, in response to infection, with all viruses inducing measurable levels by the 2 hour time point except Adnovirus. Flu A H5N1 had markedly higher levels of IL-6 production than Flu A H3N2, and both H5N1 and H3N2 had higher levels than Flu B. Additionally RSV, Parainfluenza 3, Echovirus and CMV had very high levels of IL-6 production at the 4 hour time point. The A549 cells also express MCP-1 in response to viral infection. MCP-1 was modestly present in the uninfected cells though Flu A H3N2, H5N1, RSV, Para 3, Echovirus, HSV1, and CMV infected cells had elevated levels of MCP-1 at all time points. The cells infected with FluB, Parainfluenza 1, and Adnovirus expressed MCP-1 at levels comparable to uninfected cells. Flu A H2N2, Flu A H5N1, Flu B, RSV, and Parainfluenza 3 infected cells expressed RANTES that was measurable at the 6 hour time point. RANTES was never measured in the Parainfluenza 1, Adnovirus, Echovirus, HSV1, and CMV infected cells. Like the HEP-G2 and A498 cell lines the A549 cells constitutively expressed IL-8.

A549 Cells

	Influenza A H3N2			Influenza A H5N1			Influenza B			RSV			Parainfluenza 1		
	IL-6	RANTES	MCP-1	IL-6	RANTES	MCP-1	IL-6	RANTES	MCP-1	IL-6	RANTES	MCP-1	IL-6	RANTES	MCP-1
0 Hour	-	-	-	-	-	-	-	-	-	++	-	-	++	-	-
2 Hour	+	-	+	++	-	+	-	-	+	++++	-	++	+++	-	+
6 Hour	++	-	++	+++	++	++	+	-	++	++++	-	+++	+++	-	++
12 Hour	+++	++	+++	++++	+++	+++	+	+	++	++++	+	++++	+++	-	+++
24 Hour	++++	+++	++++	++++	+++	++++	++	+	+++	++++	+++	++++	+++	-	++++
48 Hour	++++	++++	++++	++++	++++	++++	+++	++	++++	++++	++++	++++	+++	-	++++

	Parainfluenza 3			Adenovirus			Echovirus			Herpes Simplex Virus 1			Cytomegalovirus		
	IL-6	RANTES	MCP-1	IL-6	RANTES	MCP-1	IL-6	RANTES	MCP-1	IL-6	RANTES	MCP-1	IL-6	RANTES	MCP-1
0 Hour	+++	-	+	-	-	+	-	-	-	-	-	-	-	-	-
2 Hour	++++	+	++	+	-	+	+++	-	++	++	-	++	++++	-	+++
6 Hour	++++	++	+++	+	-	++	+++	-	+++	++	-	+++	++++	-	++++
12 Hour	++++	+++	++++	+	-	++	++++	-	++++	+++	-	++++	++++	-	++++
24 Hour	++++	++++	++++	+	-	++	++++	-	++++	++++	-	++++	++++	-	++++
48 Hour	++++	++++	++++	+	-	+++	++++	-	++++	++++	-	++++	++++	-	++++

HEP-G2 Cells

	Influenza A H3N2	Influenza A H5N1	Influenza B	RSV	Parainfluenza 1
	RANTES	RANTES	RANTES	RANTES	RANTES
0 Hour	-	-	-	-	-
2 Hour	-	-	-	-	-
6 Hour	-	-	-	-	-
12 Hour	-	-	-	+	-
24 Hour	-	++	+	+++	-
48 Hour	++	++++	++	++++	-

	Influenza A H3N2	Influenza A H5N1	Influenza B	RSV	Parainfluenza 1
	RANTES	RANTES	RANTES	RANTES	RANTES
0 Hour	++	-	-	-	-
2 Hour	-	-	-	-	-
6 Hour	++	-	-	-	-
12 Hour	+++	-	-	-	-
24 Hour	++++	-	-	-	-
48 Hour	++++	-	-	-	-

Conclusion:

We demonstrated that HEP-G2 and A549 cell lines present a rapid cytokine response when viral infected. The amount and type of cytokine expressed varied between the cell types and infecting virus. In the A549 cell line RANTES expression was only present in the minus sense RNA viruses whereas it was not present in the DNA viruses. Conversely MCP-1 coupled with IL-6 may prove to be an accurate indicator of DNA viral infection. These facts could be used in viral screens to determine the type of infecting virus. Additionally, the rapid IL-6 response, within 2 hours, presented by the A549 cells when infected with Flu A, Flu B, RSV, Parainfluenza 3, Echovirus and CMV virions could be used to rapidly diagnose the early stages of a viral infection. The early diagnosis and subsequent identification of viral type would aid clinicians in more rapid and effective treatment of viral infections.