

Multiplexed Analysis of Cytokine Production in Vaccinated and SIV-Infected Animals



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INTRODUCTION

Typically, antigen (Ag)-specific T-cell responses are recognized by production of only one cytokine (IFN γ).

However, many Ag-responsive cells do not make IFN γ .

- Therefore, most assays cannot capture all Ag-responsive cells or report the full breadth of a response.
- Even ICS assays for IFN γ , IL2, TNF α may mark only a subset of Ag-responsive cells.

This problem may be avoided by testing additional cytokines.

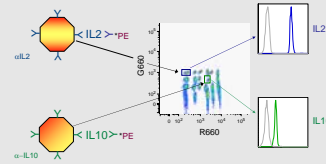
- However, often it's not clear which cytokines should be measured.

Recently, a flow cytometry-based assay (BD Biosciences) has been developed for measurement of 20+ cytokines.

Here, we test the primate cross-reactivity of kit's antibodies.

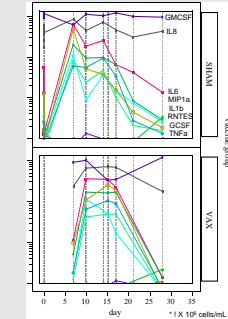
- and determine the cytokines associated with a vaccine's ability to protect animals from SIV challenge.

PRINCIPLE OF THE ASSAY



- 25 vials of beads, one for each cytokine.
- Each bead population is coated with antibodies to one cytokine.
- Each bead population contains a particular ratio of fluorescent dyes, measured in G660 (green laser, 660nm) and R660 (red laser, 660 nm) channels of our cytometer.
- Beads incubated with culture supernatant.
- PE-conjugated anti-cytokine antibodies are added.
- Like a sandwich ELISA.
- More cytokine = more PE signal.
- Compare relative fluorescence or quantify with standards.

APPLICATION CYTOKINES IN ACUTE SIV

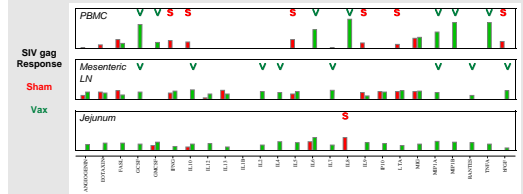


- PBMC (cultured overnight without stimulation) release certain cytokines over acute infection.
- Cytokine kinetics mirror viral load, suggesting these cytokines are released as a consequence of *in vivo* cell stimulation.
- Analysis of purified human T-cells (data not shown) demonstrates that many of these cytokines can be T-cell derived.
- Thus, using a multiplexed screen for 25 cytokines, we found six that may be important in acute infection.
- Five of these cytokines are typically not measured by ICS.

ANTIGEN-SPECIFIC CYTOKINE PRODUCTION AT PEAK VIREMIA (DAY 10 P.I.) BY TISSUE

Next, we examined cytokine production upon stimulation with peptide pools from SIV gag and SIV pol proteins.

The cytokine signals contributed by unstimulated cells were subtracted; thus, data shows only the SIV gag- and pol-specific cytokine responses.



PBMC from vaccinated animals make a number of cytokines (indicated by the symbol V) that are not made by PBMC from sham-injected animals.

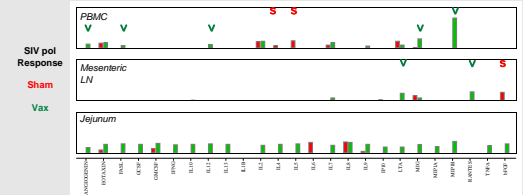
These cytokines may reflect effective anti-SIV responses induced in the vaccine-protected animals.

PBMC from sham-injected animals make a number of cytokines (indicated by the symbol S) that are not made by the vaccinated animals.

These cytokines may correlate with the ineffective anti-SIV responses occurring in natural SIV infection.

Cells from the mesenteric lymph nodes and jejunum of vaccinated animals make cytokines not found in sham-injected animals.

These cytokines may reflect protective immunity. Some of these cytokines were not observed in the peripheral blood.



APPLICATION TO VACCINE DEVELOPMENT

Recent studies of acute SIV infection

60-80% of memory CD4+ T-cells lost from peripheral blood and mucosa within two weeks. Extent of loss predicts survival time.

Prophylactic vaccination

Reduced memory CD4+ T-cell loss, lessened disease severity.

It remains unclear which immune responses mediate protection.

Cytokine responses to vaccination

Typically assessed by measuring IFN γ , IL2, and/or TNF α , but these may not be sensitive markers for vaccine-induced immunity.

Do other cytokines correlate better with protective immunity? We used a multiplexed cytokine assay to address this.

*Vaccine regimen

DNA plasmids encoding SIVmac239 env, gag-pol, administered IM thrice, followed by rAd boost. Animals challenged with SIV (Mattapalli, et al. J. Exp Med. 2006; J. Med. Primatol. 2006)

CYTOKINES MEASURED

CYTOKINE	SOURCE	TARGET	NOTABLE
IL1	Monocyte, Dendritic	IL1R1	First Cytokine
IL2		T, B, NK	T Cell Growth Factor
IL3	T, B, Myeloid	IL3R1, IL3R2	Stim. B Cell, Myeloid Proliferation in Allergic
IL4	Many Mononuclear Hemopoietic	Many	
IL5	Several Hemopoietic	T, B	Hemopoietic T Cell Signaling
IL6	Many	Neutrophils	Hemopoietic, Hemopoiesis, Myelopoiesis, IL6R1, IL6ST
IL8	Activated T	Unknown	IL8R1, IL8R2, IL8R3, IL8R4, IL8R5, IL8R6, IL8R7, IL8R8, IL8R9, IL8R10, IL8R11, IL8R12, IL8R13, IL8R14, IL8R15, IL8R16, IL8R17, IL8R18, IL8R19, IL8R20, IL8R21, IL8R22, IL8R23, IL8R24, IL8R25, IL8R26, IL8R27, IL8R28, IL8R29, IL8R30, IL8R31, IL8R32, IL8R33, IL8R34, IL8R35, IL8R36, IL8R37, IL8R38, IL8R39, IL8R40, IL8R41, IL8R42, IL8R43, IL8R44, IL8R45, IL8R46, IL8R47, IL8R48, IL8R49, IL8R50, IL8R51, IL8R52, IL8R53, IL8R54, IL8R55, IL8R56, IL8R57, IL8R58, IL8R59, IL8R60, IL8R61, IL8R62, IL8R63, IL8R64, IL8R65, IL8R66, IL8R67, IL8R68, IL8R69, IL8R70, IL8R71, IL8R72, IL8R73, IL8R74, IL8R75, IL8R76, IL8R77, IL8R78, IL8R79, IL8R80, IL8R81, IL8R82, IL8R83, IL8R84, IL8R85, IL8R86, IL8R87, IL8R88, IL8R89, IL8R90, IL8R91, IL8R92, IL8R93, IL8R94, IL8R95, IL8R96, IL8R97, IL8R98, IL8R99, IL8R100
IL10	T, NK, B	Many	Regulates Immune Response in Multiple Ways
IL12	APCs	T, NK	
IL15	Activated T	T, Myelopoietic	Hemopoietic
IL16	Many	Many	Inflammatory
MIP1a	Many	Many	Inflammatory, CC Chemokine, Binds CXCR1
MIP1b	Many	Many	Inflammatory, CC Chemokine, Binds CXCR2
IP10	Many	T, NK	CC Chemokine, Binds CXCR3
LTA	T, B, NK		Receptor for Lipoteichoic Acid
MIP	Many	T	Macrophage Chemoattractant (MIP-1)
MIP1a	Many	T	Macrophage Chemoattractant (MIP-1a)
MIP1b	Many	T	Macrophage Chemoattractant (MIP-1b)
MIP2	Many	T	Macrophage Chemoattractant (MIP-2)
MIP3	Many	T	Macrophage Chemoattractant (MIP-3)
MIP4	Many	T	Macrophage Chemoattractant (MIP-4)
MIP5	Many	T	Macrophage Chemoattractant (MIP-5)
MIP6	Many	T	Macrophage Chemoattractant (MIP-6)
MIP7	Many	T	Macrophage Chemoattractant (MIP-7)
MIP8	Many	T	Macrophage Chemoattractant (MIP-8)
MIP9	Many	T	Macrophage Chemoattractant (MIP-9)
MIP10	Many	T	Macrophage Chemoattractant (MIP-10)
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MIP13	Many	T	Macrophage Chemoattractant (MIP-13)
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MIP98	Many	T	Macrophage Chemoattractant (MIP-98)
MIP99	Many	T	Macrophage Chemoattractant (MIP-99)
MIP100	Many	T	Macrophage Chemoattractant (MIP-100)

NEW CYTOKINES TO MEASURE?

Cytokine	Tissues	May Reflect Immunity or Progression?
GM-CSF	PBMC, ME, SN	Immunity
IP10	PBMC	Immunity
IL10	PBMC	Progression
IL2	MESN	Immunity
IL4	MESN	Immunity
IL5	PBMC	Progression
IL6	PBMC	Immunity
IL7	MESN	Immunity
IL8	Jejunum	Progression
IL9	PBMC	Immunity
IL12	PBMC	Immunity
IL15	PBMC	Immunity
IL17	PBMC	Progression
IL18	PBMC	Immunity
IL21	PBMC	Immunity
IL22	PBMC	Immunity
IL23	PBMC	Immunity
IL27	PBMC	Immunity
IL28	PBMC	Immunity
IL29	PBMC	Immunity
IL30	PBMC	Immunity
IL31	PBMC	Immunity
IL32	PBMC	Immunity
IL33	PBMC	Immunity
IL34	PBMC	Immunity
IL35	PBMC	Immunity
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