# Bioinformatics for Biodefense: Comparison of Global Transcriptional Host Responses across Non-Human Primates Infected with Anthrax, Poxviruses, and Filoviruses

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## Introduction: Category A Biological Agents of Infection

| | |
|---|---|
| Anthrax | • *Bacillus anthracis* (ANX) |
| Botulism | • *Clostridium botulinum toxin* |
| Plague | • *Yersinia pestis* |
| Smallpox | • *Variola major* (SPX) |
| Tularemia | • *Francisella tularensis* |
| Viral hemorrhagic fevers | • *Ebolavirus (EBOV)*<br>• *Marburgvirus (MARV)* |

These diseases (left) are cause by high priority infectious agents (right), which are known for their relative ease of transmission and high rates of mortality. Highlighted agents represent the ones that were analyzed in this study: To better understand Anthrax, Smallpox, Ebola and Marburg. Monkeypox (MPX), a close relative to Smallpox was also analyzed.

### Questions of Interest

- What are the similarities and differences in the transcriptional host immune response?
- What biological pathways are triggered post-infection?
- Are there any early markers of infection?

## Methods: Data Collection and Experimental Set-Up

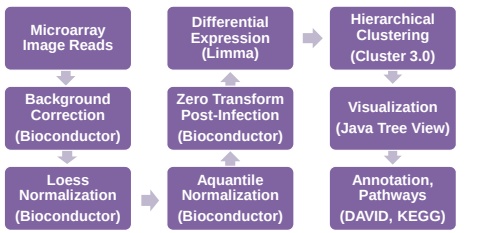
Non-human primates Cynomologous Macaques infected with one of five pathogens

Blood samples collected on subsequent post-infection days until death, mRNA extraction, sample preparation for hybridization

Use of custom two-color microarrays[1] representing ~18,000 genes
experimental sample (cy5) vs. common mRNA reference (cy3)

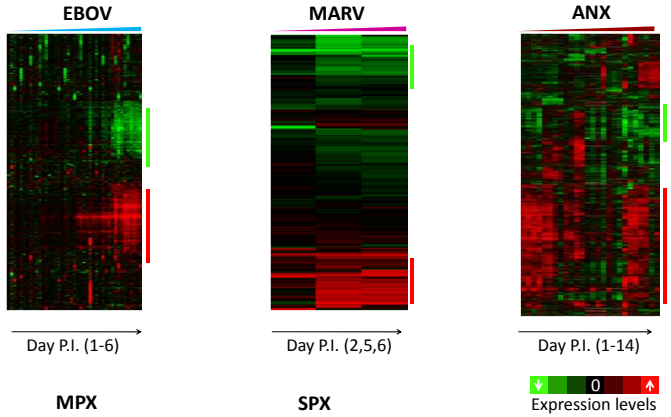
| Pathogen | # Monkeys | Max(Day) Post-Infection | Total Arrays | Cell Type |
|---|---|---|---|---|
| EBOV | 21 | 6 | 85 | PBMC |
| MARV | 1 | 6 | 5 | PBMC |
| ANX | 12 | 14 | 43 | Whole Blood |
| SPX | 22 | 14 | 220 | PBMC |
| MPX | 9 | 12 | 54 | PBMC |

## Methods: Pipeline Developed for Microarray Analysis



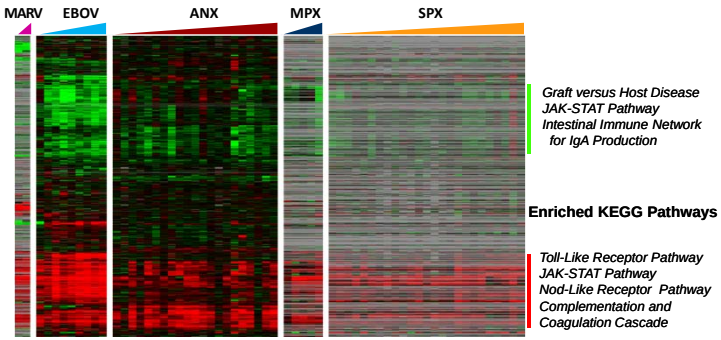

We developed a custom pipeline for the processing of microarray data. Background correction, normalization, and analysis of post-infection versus pre-infection expression levels were first performed. Data filtration, clustering and visualization helped identify temporal expression patterns. DAVID and KEGG were used to investigate biology of specific gene sets.

## Results: Detection of Time-Dependent and Independent Trends




To help visualize the transcriptional changes in the host response to infection, we created five separate heatmaps. Rows represent probes with a greater than or equal to 3.0 expression fold change relative to pre-infection levels in at least two arrays. Columns are arrays sampled from individual monkeys at different days post infection (P.I.), with the samples taken closest to the date of infection on the far left. The intensity of red and green denotes the magnitude of the respective increase or decrease in expression compared to pre-infection values. Bars of red and green denote clusters of dense up or down-regulation.

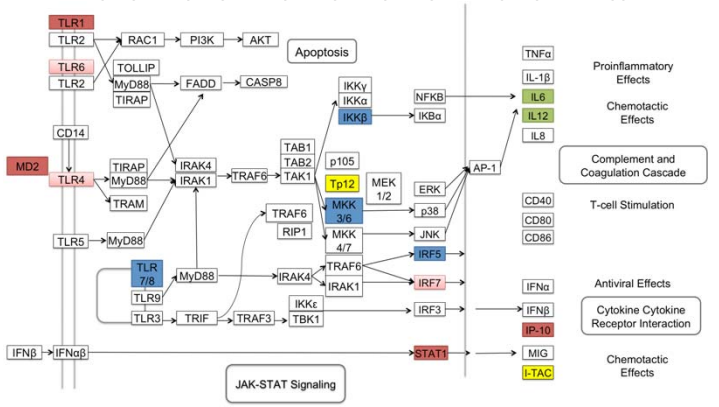
## Results: Analysis of Top Differentially Expressed Probes




To identify commonly regulated genes and pathways, a meta-heatmap was created to incorporate the top differentially expressed probes that were up-regulated and down-regulated within each infection group. The following days were selected for this analysis: MARV (Day 5, 6), EBOV (Day 4, 5), ANX (Day 3, 5), MPX (Day 2, 3), SPX (Day 2, 3, 5). A total of 1,235 unique probes (rows) are displayed. Grey rows represent probes that did not map across datasets. The Toll-Like Receptor (TLR) pathway is an example of a highly enriched pathway.

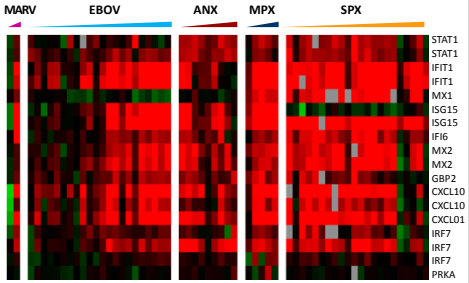
## Results: Toll-Like Receptor Pathway Activated Across All Infections

**Transcripts were identified as activated in at least :**
**Five (Red), Four (Pink), Three (Yellow), Two (Green), or One (Blue) Infection(s)**




This KEGG diagram maps the molecular interactions of the TLR Pathway, helping us visualize the specific transcripts that are uniquely and/or commonly activated across the five infections.

## Results: Early Activation of STAT-1 and STAT-1 Regulated Transcripts




STAT1, a signal transducer and activator of transcription, was activated across all five types of infection. If phosphorylated, STAT1 can form homo- or heterodimers that translocate to the cell nucleus and mediate the expression of a variety of genes. To determine whether or not STAT1 activation may have triggered a causal response in activating other downstream transcripts, we examined arrays from early days (2,3,5) post-infection, and extracted the probes corresponding to IFN-responsive genes that have been experimentally shown to be up-regulated by STAT1.

## Summary and Conclusions

- These studies help us to better understand the pathogenic mechanisms of high priority agents
- Host responses to the two hemorrhagic fevers (EBOV, MARV) exhibited greatest degree of differential expression during the latter days of infection, whereas ANX, SPX and MPX agents display more heterogeneous transcriptional host responses throughout the course of infection
- Activation of Toll-Like Receptor Pathway and STAT1 gene may be used to help identify early onset or pathogenesis of these diseases
- Identification of unique temporal signatures may serve as diagnostic markers or potential therapeutic targets

## References and Acknowledgements

[1]Rubins KH *et al.* "The Host response to smallpox: analysis of the gene expression program in peripheral blood cells in a nonhuman primate model". Pro*c Natl Acad Sci USA 2004,* 101:15190-15195.

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