Diagnosing Lassa Virus Infection by Tracking the Antiviral Response

Ignacio Sánchez Caballero[1], Gracia Bonilla[1], Judy Y. Yen[2], John H. Connor[2]

Departments of Bioinformatics[1] and Microbiology[2], Boston University, MA. Correspondence: jhconnor@bu.edu

Motivation

Lassa fever is a severe hemorrhagic fever caused by the Lassa virus. It is estimated that 300,000 West Africans are infected with it every year resulting in a mortality rate of 15% in hospitalized patients (1,2). Its symptoms (fever, sore throat, cough, headache and abdominal pain) are easily confused with those of other hemorrhagic fevers, or with the common flu.

With the goal of helping diagnose the disease, we set out to identify a subset of genes whose levels of expression changed in a distinctive manner soon after a host was infected with the Lassa virus. This pattern of expression would have to be strong enough to be detected in a low-throughput setting at an early stage of infection, and distinctive enough to distinguish between Lassa, Influenza A, and other viral infections.

Modeling Lassa Infection

Total RNA was extracted from the peripheral blood mononuclear cells (PBMCs) of Cynomolgus macaques before they were infected with the Lassa virus, and at different timepoints after infection.

The use of an animal model is justified because most clinical symptoms are shared among primates. It also provided us with a greater ability to control and track the spread of infection.

Data Sets

Samples from individual monkeys, corresponding to specific timepoints (see Figure 2), were hybridized to two-color Agilent microarrays to determine the rate of transcription of each gene at different stages of the disease.

A similar type of experiment was previously carried out by Huang and colleagues (3), infecting patients with Influenza and performing transcriptional analysis at increasing times along the disease period. We used their data to compare the transcriptional patterns of individual genes in both Lassa and Influenza.

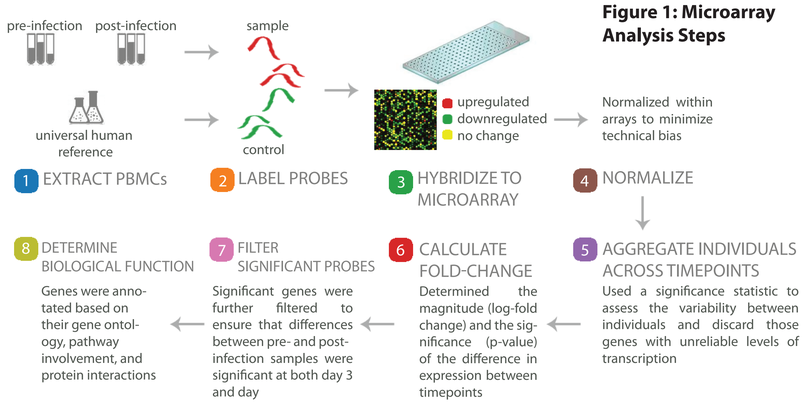

Figure 1: Microarray Analysis Steps

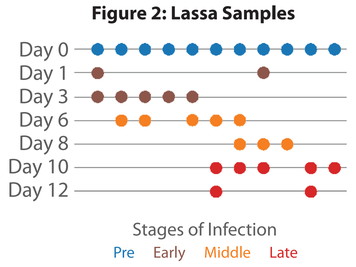

Figure 2: Lassa Samples

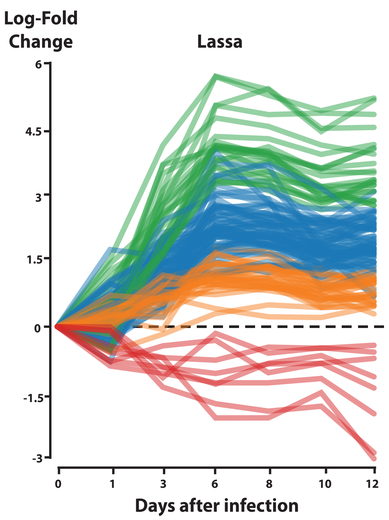

Figure 3
Expression patterns of 150 significant genes throughout Lassa infection, using the pre-infection samples as baseline. The genes are clustered into four groups based on the magnitude of their log-fold change. Most genes show moderate levels of expression, while others show very high levels of up- or down-regulation.

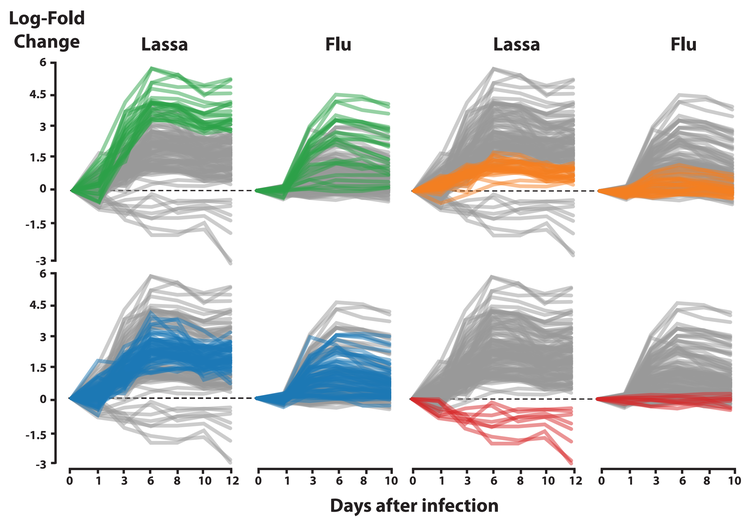

Figure 4
Expression patterns of 150 significant genes throughout Lassa and flu infection, using their pre-infection samples as baseline. Genes are colored based on the Lassa cluster they belong to. The majority of genes follow a similar pattern in both types of infection, with the exception of those in the green and red clusters.

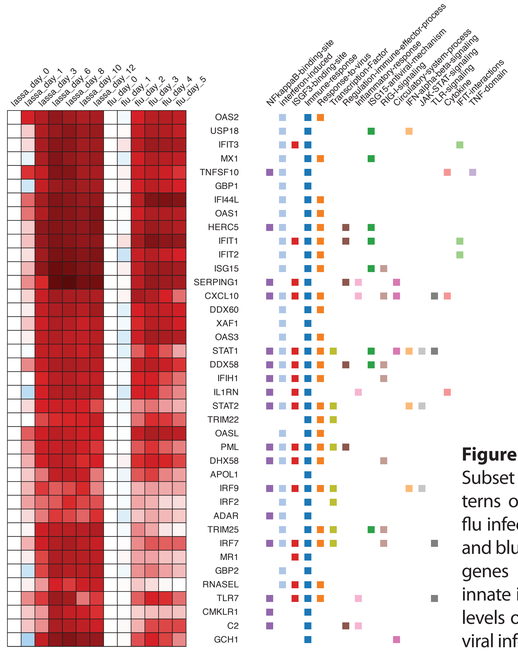

Figure 5
Subset of genes that show common patterns of expression between Lassa and flu infection. Red indicates upregulation, and blue, downregulation. Most of these genes play an important role in the innate immune response, and their high levels of upregulation are indicative of a viral infection.

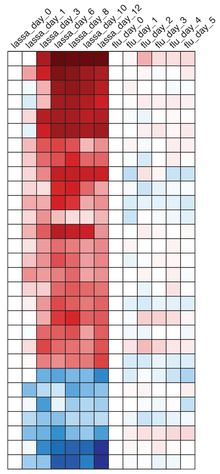

Figure 6
Subset of genes that show distinctive patterns of expression between Lassa and flu infection. Red indicates upregulation, and blue, downregulation. Most of these genes have not been previously characterized, or their main function wasn't thought to be affected by the host immune response.

Results

We clustered the set of candidate genes with respect to the magnitude of their log-fold change between the post- and the pre-infection samples (see Figure 4). In Lassa, we observed that gene transcription is very strongly regulated for a subset of genes, while it is more moderate for others. Most upregulated genes are involved in the innate immune response (pattern recognition receptors, alpha/beta interferon signaling pathways). The role that the remaining genes play in the antiviral reponse remains unclear.

By comparing the transcriptional patterns of both Lassa and Influenza we noticed a high overlap between the genes that make up the innate immune response and those that behaved similarly in both diseases (see Figure 5). There are also a number of genes that follow a unique transcriptional trajectory in each disease (see Figure 6). These are the most promising candidate biomarkers, and for most of them, little is known about their immune activity.

Conclusion

We performed a transcriptomics analysis over the course of Lassa virus infection looking for genes whose changes in expression could be used as diagnostic biomarkers. We identified a set of candidate genes with unique transcriptional patterns whose role in the immune response remains poorly characterized, which indicates a potentially interesting area of investigation. We have plans of performing RT-PCR validation of the candidate genes using clinical samples of influenza- and Lassa-infected patients.

References

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2. Drosten, C., Göttig, S., Schilling, S., Asper, M., Panning, M., Schmitz, H., and Günther, S. (2002). Journal of Clinical Microbiology, 40(7):2323–2330
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Acknowledgements

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