Published in final edited form as:
*Vaccine*. 2007 August 29; 25(35): 6458–6473.

# Distinct Gene Expression Profiles in Peripheral Blood Mononuclear Cells from Patients Infected with Vaccinia Virus, Yellow Fever 17D Virus, or Upper Respiratory Infections Running Title: PBMC Expression Response to Viral Agents

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## Abstract

Gene expression in human peripheral blood mononuclear cells was systematically evaluated following smallpox and yellow fever vaccination, and naturally occurring upper respiratory infection (URI). All three infections were characterized by the induction of many interferon stimulated genes, as well as enhanced expression of genes involved in proteolysis and antigen presentation. Vaccinia infection was also characterized by a distinct expression signature composed of up-regulation of monocyte response genes, with repression of genes expressed by B and T-cells. In contrast, the yellow fever host response was characterized by a suppression of ribosomal and translation factors, distinguishing this infection from vaccinia and URI. No significant URI-specific signature was observed, perhaps reflecting greater heterogeneity in the study population and etiological agents. Taken together, these data suggest that specific host gene expression signatures may be identified that distinguish one or a small number of virus agents.

### Keywords

Vaccination; Viral Infections; Gene Regulation

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## Introduction

Expression microarray analysis is a powerful method to determine global profiles of gene expression in cells and tissues under a variety of complex biological conditions. Considerable use has been made of this technology to evaluate the host response to pathogen infection in cells, cell lines and tissues derived from both human and model organisms [1–12]. While much of the original analysis in this field focused on the host response to pathogen infection in tissue culture models, recent methodological improvements in experimental design and data analysis permit the adaptation of this method to more complex biological systems. These new model systems now include a more extensive use of tissue biopsies and complex cell populations from human subjects, as well as the analysis of naturally occurring and experimental infections [13–19].

Existing *in vivo* human studies suggest that specific gene expression signatures are produced during pathogen infection in a variety of tissues (see for example references 15,17, and 19). The present study builds upon existing literature and evaluates the changes in gene expression over time following the onset of both experimental and naturally acquired virus infections in humans. The study further seeks to identify gene expression signatures common to several virus infections and to determine if specific patterns may be defined for individual viruses.

In the present study, peripheral blood mononuclear cells (PBMC) were utilized as easily accessible reporters of the systemic immune response to interrogate the gene response of volunteers immunized with two live-attenuated vaccine strains, the Aventis-Pasteur WetVax® smallpox vaccine and the 17D-derived yellow fever vaccine (Sanofi-Pasteur YF-VAX®). The WetVax vaccine was manufactured between 1956 and 1957 from the New York Board of Public Health vaccinia strain. The analyses were conducted as part of a recent clinical trial involving this material [20]. The yellow fever vaccine was derived from the serial passage of a naturally occurring yellow fever isolate in mouse brain and chick embryo [21]. YF-VAX, while generally safe and effective, can in rare circumstances cause serious illness similar to fulminant yellow fever [22].

The current study describes time-dependent changes in PBMC gene expression following vaccination with these two viruses and compares these observations with the gene expression signatures resulting from naturally occurring upper respiratory infection (URI). Based upon this analysis, we identified specific PBMC gene expression signatures that are characteristic of either vaccinia or yellow fever vaccination. We further identified host gene expression signatures that are more generally associated with virus infection, irrespective of the etiological agent. This study begins to lay the groundwork for the identification of host gene products and gene expression patterns that may be usefully implemented in new tools for the diagnosis of specific virus infections.

## Materials and Methods

### Study Participants

**Smallpox Vaccine Study—**Samples from twenty four healthy subjects participating in a clinical trial of the Aventis Pasteur smallpox vaccine (APSV, Wetvax®) conducted at the University of Iowa [20] were evaluated. The study was sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) and was designed to evaluate the safety and efficacy of the Wetvax vaccine under a variety of dilution conditions. All study procedures were approved by the Institutional Review Board (IRB) of the University of Iowa. Written informed consent was obtained from each subject at enrollment. Healthy adults between the ages of eighteen and thirty-two with no prior history of smallpox vaccination or vaccination scar were eligible for participation in the study. Exclusion criteria included a history of

immunodeficiency, malignancy, transplant, eczema, autoimmune disease, previous vaccination with vaccinia-vectored or other pox-vectored experimental vaccines, or allergies to the vaccine components. Study participants were immunized with one of three dilutions of Wetvax® in a double-blinded manner. Vaccination was carried out by the application of a droplet of liquid containing vaccinia virus to the skin of the deltoid region using a bifurcated needle. Blood collection for the isolation of PBMCs took place at the pre-immune time point (1), two to four days post-vaccination (time point 2), five to seven days post-vaccination (time point 3), and fifty to sixty days post-vaccination (time point 4). At each study visit, vital signs were recorded (temperature, pulse, and blood pressure), and the vaccination site was observed for signs of localized vaccinia infection (vesicle, pustule, or ulcer), diameter of the pock, induration, extent of erythema, and the presence of ancillary nodes or satellite infections. Complete blood counts were not performed on these study subjects.

**Yellow Fever Vaccine Study—**Subjects were recruited from among the population of patients seeking travel related vaccination from the Hall Health Travel Clinic at the University of Washington. Study procedures were approved by the University of Washington Institutional Review Board. Eligible subjects were between the ages of eighteen and forty and in good health. Exclusion criteria included pregnancy, lactation, immune disease, use of immunosuppressive medication, known allergy to a vaccine component, or vaccination within the preceding eight weeks. Blood samples for PBMC isolation and complete blood count with differential (CBD) were collected at the first visit, directly preceding immunization, and at the second visit, four to seven days after immunization. Subjects were immunized with the Sanofi Pasteur 17D-204 yellow fever virus vaccine (YF-VAX®). Vaccine related adverse events were evaluated by a study nurse at the second visit.

**Upper Respiratory Infection (URI) Study—**Subjects were recruited from among the population of patients seeking medical care from the Madrona Medical Group, an integrated primary care and multi-specialty clinic, for symptoms associated with upper respiratory infection. All study procedures were approved by the Western Institutional Review Board (Olympia, WA) and were performed after written informed consent. Eligible subjects were between the ages of eighteen and forty and presented with respiratory symptoms for fewer than ten days with a positive diagnosis of URI by the enrolling physician. Exclusion criteria included a history of asthma, emphysema, chronic obstructive pulmonary disease, cystic fibrosis or other chronic lung condition, immune disease, or the use of an immunosuppressive medication. Blood collection was performed during the first study visit (immune) for PBMC isolation and CBD. Nasopharyngeal washes were collected and evaluated by viral culture with direct fluorescent antibody detection of influenza, parainfluenza, rhinovirus, respiratory syncytial virus, and adenovirus. Return visits for convalescent sample collection were scheduled at least four weeks after the initial visit and included blood collection for PBMC isolation and CBD analysis.

## PBMC Collection and RNA Isolation

For the smallpox vaccine study arm, twenty five mL of blood was collected at each time point into citrate-dextrose containing Vacutainers (Becton Dickinson). PBMCs were prepared by Ficoll-Hypaque centrifugation within two hours of blood collection, washed once with sterile phosphate buffered saline (PBS), resuspended in two mL of TRIZOL® Reagent (Invitrogen Life Technologies), and stored frozen at −80° C. Total RNA was isolated using chloroform extraction and isopropyl alcohol precipitation as per the manufacturer's protocol.

For the yellow fever vaccine and URI study arms, twenty mL blood samples were collected into citrate-containing CPT® Vacutainers and centrifuged according to the manufacturer's instructions to collect PBMCs. Isolated PBMCs were washed once with PBS, resuspended in

RNA*later*® (Ambion), and stored frozen at −20°C. Total RNA was isolated using RNeasy Mini Kits (Qiagen) according to the manufacturer's method.

## RNA Amplification, Labeling, and Microarray Hybridization

The expression array analysis component of this study was conducted using Agilent human 1 cDNA microarrays (catalog # G4100, Vaccinia study) and Human 1A Oligo Microarray Kit (V2) (catalog # G4110B, URI and Yellow Fever studies). The microarray platform was changed midway through the study as the cDNA microarrays were discontinued by Agilent.

**cDNA Microarray Processing (Vaccinia Study Arm)—**RNA samples were analyzed using standard fluorophore-reversal methodologies [23]. 500 ng of total RNA from each sample was linearly amplified using the Low RNA Input Fluorescent Linear Amplification kit (Agilent Technologies). Amplified RNA (500 ng) was then labeled separately with Cyanine 3-dCTP (Cy3) or Cyanine 5-dCTP (Cy5) (Perkin Elmer), purified, and prepared for hybridization according to the manufacturer's protocol. Samples were hybridized twice to replicate arrays on a single glass slide using a reciprocal labeling strategy. RNA isolated from the three post-vaccination time points were all compared to RNA isolated at the pre-immune time point from the same subject. Normal gene expression variation was assessed by comparing RNA isolated from the pre-immune time point of each subject with a control RNA sample composed of pooled RNA from two healthy volunteers.

**Oligonucleotide Microarray Processing (Yellow Fever and URI Studies)—**RNA samples were analyzed using Human 1a 60-mer oligo microarrays purchased from Agilent Technologies. Amplified, fluorescently labeled RNA was generated from 500 ng of total sample RNA using the Agilent Low RNA Input Fluorescent Linear Amplification Kit and Cy 3-CTP or Cy 5-CTP (Perkin Elmer). Amplified cRNA was purified using RNeasy columns (Qiagen) and stored at −80°C until hybridization. Hybridization of samples was performed according to the manufacturer's protocol.

Individual infected and convalescent samples from the URI subjects were each compared to a single pooled, convalescent sample constructed from the entire population. Each comparison was done twice using fluorophore-reversal methodology. Samples collected from the Yellow Fever Vaccine studies were compared in three different ways: (i) individual pre-immune (Cy3) to individual post-immune (Cy5), (ii) individual post-immune (Cy5) to pooled pre-immune (Cy3), and (iii) individual pre-immune (Cy5) to pooled pre-immune (Cy3). As in the URI study, pooled RNA samples were made by combining equal quantities of individually labeled samples from all subjects.

## Data Analysis and Normalization Procedures

All microarrays were scanned using a Perkin Elmer ScanArray Express dual-laser microarray scanner. Scan conditions were manually adjusted so that overall fluorescent intensities of Cy3 and Cy5 were approximately equal and without saturation. Raw and background Cy5 and Cy3 intensities from the scanned TIFF images were collected using the Scan Array Express software, and exported to a custom GENELAMP™ data management and analysis database for further analysis.

For each of the three study arms, data analysis was performed using customized procedures from the Limma package in R [24]. The raw dye intensities and their respective background intensity levels were read into an R object, adjusting weights for control spots. The gene name most associated with each spot was taken from the GAL file (GenePix ArrayList V1.0, supplied by Agilent Technologies). After appropriate normalization within and between arrays, a linear model was used to estimate the average expression for each gene. Significance was estimated

using a moderated empirical Bayes t-statistic and its associated p-value. The false discovery rate was controlled at 5% under the Benjamini Hochberg procedure [25]. Genes were ranked according to the probability that a given gene would be differentially expressed as indicated by the B-statistic (log-odds adjusted at 1%) [26].

Within-array normalization was performed on the background-subtracted data using a LOWESS smoothing procedure [27] across all spots. Normalization between arrays consisted of scaling the log-ratios (M) to the same median absolute deviation. LOWESS normalization of the individual channel intensities was used with the URI arrays.

**Model design—**For the vaccinia study, a linear regression model was implemented as a standard, direct two-color design with one dye-swap pair, comparing the initial and post-vaccination samples. A direct design with no dye-swap was used with the yellow fever arrays. The linear model for the URI study was designed as a single channel analysis comparing individual channel intensities for each patient between the two time points [28].

The overall model for each study resulted in a linear fit for each individual gene. The coefficient for each contrast in the model represents the fitted log-ratio ($M_i$) for a given gene over the set of patients included in the study. The criterion for inclusion in the set of genes with the strongest differential expression was the significance of $M_i$ as defined by $B_i > 0$.

The sample size of populations other than Caucasians were too small in any of the studies to support a statistically meaningful comparison of gene expression among demographic groups based on ethnicity and gender.

**Identification of Genes—**As we had identified inconsistencies in manufacturer-provided probe identities, we re-identified the gene best associated with each probe using updated data from NCBI's July 26, 2006 Unigene build 194 [29]. For both array platforms, we compared manufacturer-provided probe sequences against representative Unigene cluster sequences using the Blast algorithm [30] to identify the best match for each probe. In this way, we identified 9,360 distinct Unigene clusters representing 9,184 distinct genes on the human 1 cDNA platform used in the vaccinia study from an available 13,574 probes. We further identified 16,975 distinct Unigene clusters representing 16,790 distinct genes on the human 1a oligo array platform used in the yellow fever and URI studies from an available 21,073 probes. For purposes of comparing results across the three study arms, we used a common subset of genes found on both platforms that comprised 8,143 unique genes.

**Ontology Analyses—**We utilized the Gene Ontology (GO) cross-references provided in NCBI's Gene database to categorize each gene [31,32]. By constructing a hierarchical lookup table with roots at each parent ontology of interest (e.g. *immune response*), we tabulated the number of genes in each subcategory with descendant ontologies. The results in each subcategory were assessed against the expected distribution of genes in that subcategory present on both the cDNA and oligo arrays.

## Results

### Study Summaries and Clinical Observations

A total of fifty-six subjects divided into three groups were recruited for this study. A summary of the demographics of the three groups is provided in Table 1. The first group consisted of twenty-four subjects recruited from one of three sites conducting a 340 person clinical trial of the Aventis Pasteur WetVax® vaccine (APSV), as previously described [20]. All of the subjects recruited onto the microarray arm of this study developed a "clinical take" (formation of a pock), and were evaluated for reactogenicity, and local and systemic symptoms. No significant

deviations in blood pressure or temperature were observed in the subjects after immunization, and there were no significant differences in the measures of induration and erythema between subjects in the different vaccine dilution groups [20].

The second group consisted of twenty individuals who received immunization against the yellow fever virus (YF-VAX®, Sanofi Pasteur) for planned travel to endemic regions. No severe adverse events were reported, although one individual was hospitalized with apparently unrelated symptoms the day after immunization; this individual also received a tetanus booster while in the hospital, and so was excluded from further analysis. The most common side-effects of the vaccine were erythema and induration at the site of vaccination. Complete blood counts with differentials on study subjects indicated an overall post-vaccination decrease in most cell types, with a small mean decrease in red blood cells (−2.4%, p=0.01), and significant mean decreases in white blood cells (−17.3%, p=0.0002), neutrophils (−19%, p=0.002), lymphocytes (−20%, p=0.003), and eosinophils (−25.9%, p=0.0009).

The third group consisted of twelve normally healthy individuals who presented with symptoms consistent with a diagnosis of viral upper respiratory infection (URI), including fever, cough, chills, myalgia, pharyngitis, and rhinorrhea. Subjects experienced symptoms for an average of 3.7 days before enrollment, with a range of one to seven days. No viral agents were identified in any of the subjects' samples. At the second visit, the health status of each subject was reevaluated and subjects were confirmed to be symptom free before collection of the convalescent blood sample. The average number of days between the two visits was thirty-seven, with a range of twenty-eight to fifty-five days. Complete blood counts with differentials in the URI subjects showed significantly more white blood cells (+26.5%, p=0.02), neutrophils (+60.2%, p=0.002), and monocytes (+26.4%, p=0.04) in the symptomatic blood samples as compared to the convalescent blood samples.

## Identification of Differentially Regulated Genes after Virus Exposure

Differentially regulated genes for each study were identified as described in Materials and Methods. The complete gene lists for all three studies will be made available at http://www.illumigen.com/microarrays. Taqman analysis of expression changes in four genes (IFIT1, STAT1, UBE2L6, and VRK2) was performed on individual RNA samples to confirm the microarray results (Supplementary Figure 1).

**Vaccinia—**Microarray analysis of RNA isolated from PBMCs collected after infection with the smallpox vaccine resulted in a total of 104, 475, and 318 genes exhibiting statistically significant changes in expression at time points 2, 3, and 4, respectively. Analysis of the distribution of $\log_2$ changes in expression indicates that the greatest amplitude changes were observed at T3 (Figure 1), which is coincident with the time of pock formation. This time point represents the acute phase of infection, while time points 2 and 4 represent the early and convalescent phases of infection, respectively. Approximately 50% of the genes significantly modulated at T2 exhibited even greater changes in expression at T3. Box plot analyses of three representative genes that followed this trend are provided in Figure 2. CXCL10 and SERPING1 were both moderately induced at T2 and highly induced at T3, while KLRB1 was moderately repressed at T2 and further repressed at T3. Expression of all three of these genes returned to near baseline at T4.

The complete list of genes that exhibited significant changes in expression at any of the time points is provided in Supplementary Table 1. At T2, many differentially regulated genes are involved in the early or acute phase of the immune response. These include interferon-induced genes that were further induced at T3 (e.g. IRF1, IFIT1, WARS, and CXCL10) as well as TNF-α induced genes (e.g. SOD2 and IER3). Other genes identified as significantly modulated at T2 are involved in the inflammatory response, including ATF3, SOCS3, and BCL6. A number

of ribosomal proteins were moderately repressed at T2 and further repressed at T3. In addition to genes involved in the acute immune and inflammatory response at T3, other genes involved in proteolysis, protein biosynthesis, metabolism, apoptosis, and cell signaling or transcriptional control were identified as significantly modulated. These will be discussed in more detail below. A total of 286 genes (60%) were induced at T3, while 189 genes (40%) were repressed compared to the pre-immune samples. A surprising number of genes exhibited significant changes in expression relative to baseline at T4, the convalescent period. These genes did not display any clear trends in the biological processes represented. Some of the genes, such as TLR8 and OAS1, were induced during the acute phase but then repressed during the convalescent period. Somewhat surprising was the induction of IL8 in the convalescent samples, as this interleukin is generally associated with the inflammatory response and induction of neutrophil chemotaxis.

**Yellow Fever—**Our analysis identified 615 genes exhibiting significant changes in expression in PBMCs four to seven days after vaccination with YF-VAX®. Supplementary Table 2 provides the complete gene list generated from the microarray analysis. Like the vaccinia samples, genes included in this list represent numerous biological processes, largely immune response or protein biosynthesis, but also apoptosis, metabolism, and cell signaling or transcriptional control. Similarly to the vaccinia post-immune samples, 58% of genes (358) were induced in the immune samples compared to pre-immune, while 42% (257) were repressed. Unlike vaccinia, however, a larger proportion of the most significantly changed genes were repressed in the yellow fever samples.

**Upper Respiratory Infections—**127 genes exhibited statistically significant changes in expression in the upper respiratory infection samples. The relatively low number of genes included in this data set is likely due to the small sample size and the variations in time between onset of symptoms and the clinic visit, in contrast to the vaccine studies. The complete gene list is provided in Supplementary Table 3. As in the yellow fever and vaccinia studies, a number of genes that were induced in this study set are involved in the immune response (e.g. IFIT1, IFIT2, IFI30, SERPING1, and CXCL10), along with genes involved in protein processing or antigen presentation (e.g. PSMB6, PSMB9, LAP3, and TAP1). In contrast, only four genes, FCER1A, ZNF521, CDIPT, and C6orf108, were repressed compared to the convalescent sample. No repression of genes involved in protein biosynthesis was observed, in contrast to both the vaccinia and yellow fever studies.

## Comparison and Contrast of Gene Families Modulated by Vaccinia, Yellow Fever, and URIs

In order to compare and contrast the genes identified as modulated in the three different study arms, the data sets were limited to those genes that were represented on both the cDNA and oligo arrays. In addition, the vaccinia data were restricted to observations at time point 3, when the maximal changes in gene expression occurred. These restrictions reduced the total number of genes exhibiting statistically significant expression changes to 475, 355, and 95 for the vaccinia, yellow fever, and URI study arms, respectively. Supplementary Table 4 presents a comprehensive list of the genes included in this analysis with gene expression profiles for each of the three study arms.

A Venn diagram summarizing the overlapping and virus-specific gene expression changes observed across the three studies is shown in Figure 3. Of the 8,143 genes represented on both array platforms, only twenty eight exhibited significant changes in expression in all three study groups. Interestingly, all twenty eight of these genes were induced compared to the pre-immune or convalescent samples. Table 2 lists the commonly induced and repressed genes in the vaccinia and yellow fever study arms. The gene symbols with asterisks were also up-regulated in URI samples (no common genes were down-regulated in the URI samples). The up-regulated

genes include a number of immune or host defense response genes, including known interferon induced genes such as OAS1, OAS2, MX1, MX2, ISG15, and IFIT3. Other genes commonly induced include those involved in proteolysis and antigen presentation, such as CD74, LAP3, PSMA4, PSMB9, and PSME2. Although a significant induction of interferon regulated genes was observed, there was no observable induction of the interferon genes themselves. The commonly down-regulated genes include those involved in protein biosynthesis, specifically ribosomal subunit proteins and translation initiation or elongation factors. None of the down-regulated genes reported in Table 2 were represented in the URI data set.

Virus-specific gene expression responses to vaccinia and yellow fever immunization are listed in Tables 3 and 4, respectively. In order for a gene to be considered virus-specific in this analysis, the gene had to have a 99% or greater probability of being differentially regulated in one group [corresponding to a Bayesian statistic value (B) of ≥ and less than a 1% probability of being differentially regulated in both of the other groups (corresponding to a B value of −4.6 or lower). An interesting feature of the vaccinia-specific gene set was the induction of a number of genes associated with monocytes or B cells, e.g. the HLA family and CD40, with a concomitant decrease in genes associated with T cells, such as TARP, SCAP1, ITPKB, and CD2. Further analysis of B cell or monocyte/macrophage specific markers in the complete vaccinia gene list revealed the repression of B-cell specific markers (e.g. CD19 and CD79B) and the induction of monocyte/macrophage specific markers (e.g. CES, CD36, and CD163). The yellow fever data sets shared a similar trend, with repression of T-cell specific genes (CD3D, KLRB1, and ZAP70) and induction of B-cell or monocyte specific genes (CD74 and CD86). Of note, several genes were on the vaccinia and yellow fever “common” list, including CD74, KLRB1, and ZAP70.

The predominant pattern in the yellow fever-specific gene set (Table 4) was the down-regulation of genes associated with protein biosynthesis, e.g. ribosomal subunit proteins or translation factors (RPS2 and EIF4B). Other genes on the yellow fever specific list are hypothetical or have functions that do not clearly relate to the immune response, such as PRB3, a saliva expressed protein, or SYNGR3, an integral membrane component of the presynaptic vesicle of neuronal cells. Using the same criteria, only one gene was found to be virus specific in the URI study group, FCER1A, the alpha polypeptide of the receptor for the Fc fragment of IgE. This lack of specificity in the URI group may simply be a reflection of the underlying heterogeneity in infectious pathogens sampled.

Analysis of the average $\log_2$ fold change (M) and Bayesian statistic (B) in gene families important for the immune response and protein biosynthetic pathways resulted in the identification of some interesting differences between the three virus studies. For instance, interferon regulatory factors (IRF) 1–8 and major histocompatibility complex, or HLA, gene members were significantly induced only in the vaccinia study samples. Genes in the IRF family are involved in the regulation of the interferon response. The vaccinia study set demonstrated a significant induction of IRF1, 2, and 7; although there was a modest induction of this family in the URI samples, none of the values were statistically significant. HLA genes are required for antigen presentation and are predominantly expressed on B cells, monocytes, and macrophages. Eight of 13 family members present on the Agilent microarrays exhibited significant up-regulation in the vaccinia study set. Small but non-significant increases in expression were also observed in the URI study set, with no change observed in the yellow fever study set.

Both the vaccinia and URI study samples indicated significant changes in expression in the signal tranducer and activator of transcription (STAT) and Caspase families. STAT family members are important for the transcriptional induction of the host anti-viral program. Up-regulation of STAT1 and STAT2 was observed in the vaccinia study samples, while the URI

samples exhibited small but significant induction of STAT1 and STAT3. Induction of various caspase family members, which are involved in host cell apoptosis as well as activation of the cytokine response, was also observed in the vaccinia and URI study arms. Caspase 1, 4, and 5 were induced significantly only in the vaccinia infection system, while caspase 7 was induced in both the vaccinia and URI study samples. No significant changes in expression were observed in either gene family in the yellow fever study samples.

The most striking trend in the yellow fever data is the number of genes involved in protein biosynthesis that were down-regulated after vaccination. These genes include eukaryotic translation initiation and elongation factors and ribosomal subunit proteins. Of the ninety-eight genes represented on the Agilent microarrays, thirty were significantly repressed in the yellow fever study samples, compared to twenty in the vaccinia study set and none in the URI study set. In particular, the translation elongation factors and ribosomal proteins appeared to be targeted for repression in the yellow fever samples. The yellow fever samples also displayed a larger proportion of highly statistically significant gene expression changes in these families, as the Bayesian statistic was greater than 4.6 in seventeen of the listed genes, compared to only two in the vaccinia study set.

## Biased Distribution of Expression Patterns According to Gene Ontology

An analysis of the distribution of gene expression changes by gene ontology during virus infection revealed several biases in the types of genes modulated by each virus. The percentage of differentially regulated genes belonging to each ontological category in the three data sets were analyzed in reference to the composition of genes represented on the entire array to assess over- or under-representation of specific ontologies (Table 5). In this analysis, over-representation of specific gene categories in the immune samples compared to the complete gene set on the microarrays suggests that genes in this category are involved in the host response to the pathogen, while under-representation of specific gene categories suggests that regulation of these gene categories is not affected by infection. In general, the response to vaccinia virus and upper respiratory infection was stronger and more systemic than the response to yellow fever. The response to yellow fever vaccination generally followed the expected distribution of interrogated genes, except for a marginally significant increase in the *response to stimulus* category and slight decreases in the *localization* and *cellular physiological process* categories (Table 5A). This indicates a weak but broadly distributed response to yellow fever vaccination. By contrast, both the vaccinia and URI groups showed significant positive biases in the *response to stimulus* and *organismal physiological process* categories, the latter of which encompasses the immune response. The vaccinia and URI data also showed concomitant decreases in cellular, metabolic, and localization genes. This indicates that the host response to these two infections is generally stronger than that to yellow fever. Vaccinia and URI also have more pronounced effects at the organismal rather than at the host cellular level.

We next evaluated biases in expression patterns related to specific immune response ontologies. While all three viruses exerted a pronounced and significant positive bias in the fraction of *uncategorized immune response* genes (e.g. IFI30, IFI27, IFIT1, IFIT3, IFITM2), they otherwise generally followed the expected distribution of categorized genes undergoing differential regulation with several notable exceptions (Table 5B). During URI infection, there was a significant deficit of *immune cell activation* and *regulation of immune response* genes identified in the microarray analysis. These ontologies contain many cytokine genes and mediators of the cytokine and toll-like receptor responses, indicating poor activation of these pathways in PBMCs during upper respiratory infection. In the vaccinia study, there was a significant deficit in the *humoral immune response* genes with a positive but non-significant bias in favor of *antigen presentation* and *antigen processing*, suggesting that class II HLA responses to vaccinia infection predominate. Finally, the response to yellow fever vaccination,

while generally weaker than expected, was significantly deficient in the fraction of *inflammatory response* genes, an ontology that includes the chemokines, leukotrienes and leukotriene pathway components, interleukin 1 family members, and several acute phase proteins. These data suggest that the yellow fever vaccine is a weak inducer of host inflammation compared to the other viruses sampled (Table 5B and data not shown).

In order to further explore the vaccinia immune response, we separately analyzed the distribution of immune genes according to their direction (up or down) of regulation during virus infection. Decomposition of vaccinia-induced gene expression patterns indicated a significant bias toward down-regulation of *immune cell activation* genes (Table 5C), for example, CD2, CD3D, CD3G, CD8A, CD8B, LAT, and LCK, consistent with suppression of the T-cell response. By contrast there was a significant increase in the number of up-regulated *antigen presentation* and *processing* genes (e.g. CD74, HLAB, HLADOA, HLADPA1, HLADRA1), as well as *uncategorized immune response* genes. These results further confirm that host class II HLA responses are favored during vaccinia infection. At the same time, there are fewer than expected *inflammatory* and *innate immune response* genes that are down-regulated during vaccinia infection, suggesting that the host inflammatory and innate immune responses are not interrupted by the virus.

## Discussion

The analyses performed in these studies provide a view of the peripheral blood gene response to vaccination with attenuated vaccinia and yellow fever virus strains and community acquired upper respiratory infections. The microarray data revealed not only a common immune response gene profile, but also virus-specific gene expression changes that likely reflect the different viral life cycles and host-pathogen interactions.

Exposure to all of the viruses in this study resulted in the induction of acute phase immune response genes, including IF-γ and IF-α inducible genes, as well as genes involved in protein processing or antigen presentation [*e.g*. leucine aminopeptidase 3 (LAP3) and proteasome components]. Notably lacking from this list of genes are the proinflammatory chemokines themselves, including interferons α, β, orγ, interleukin 1 (IL-1), and NFκB. This may be due to generally low expression levels of these cytokines, as the microarray spots with low signal strength produce lower quality data. Although we did not measure cytokine levels in these subjects, Rock *et al*. measured serum levels of a variety of cytokines as part of the WetVax® efficacy study and observed a significant increase in IFN-γ serum levels at time point 3 in all subjects [33].

The vaccinia and yellow fever data shared two interesting features, the apparent down-regulation of B- and T-cell specific genes and the repression of genes in the protein biosynthetic pathway. The reduced expression of lymphocytic genes in the PBMC samples is most likely due to a reduction in the total number of B and T cells in the PBMC population itself. Although complete cell counts with differential were not available for the vaccinia study, the yellow fever vaccine recipients displayed a profound decrease in lymphocytes, neutrophils, and eosinophils. Lymphopenia and thrombocytopenia are common observations in yellow fever patients, indicating that immunization with the 17D yellow fever strain, and likely the vaccinia strain as well, results in an infection that mirrors a wild type, albeit much attenuated, infection. Concomitant with the decrease in B and T-cell specific genes was the induction of genes associated with monocytes or macrophages, including CD74, CD163, and CD36. This induction was more pronounced in the vaccinia study subjects, suggesting a more robust activation of this pathway. Further evidence for a stronger immune response to vaccinia infection is the induction of the Class-II major histocompatibility complex genes, which displayed no changes in the yellow fever study samples.

Down-regulation of protein biosynthesis genes was not observed in the URI samples, and suggests that this may be due to the localized infections in URI patients versus the systemic infections induced by vaccination with live attenuated viruses. As down-regulation of host cell protein biosynthesis is a hallmark of many viral infections, our data are consistent with the hypothesis that circulating PBMCs in the WetVax® and YF-VAX® immunized patients are infected with replicating virus. Viremia in smallpox vaccines is somewhat controversial, as there are conflicting reports regarding the presence of virus in blood [34,35]. Viremia after 17D vaccination, however, is well-described [36,37].

Another interesting difference is the lack of induction of interferon regulatory factors in the yellow fever samples compared to the vaccinia and URI samples. It is possible that the 17D yellow fever strain, like other flaviruses (notably West Nile Virus), is able to disrupt the interferon response by inhibiting the activation of IRF3 and other IRFs [38,39]. The lack of induction of STAT and Caspase family members by the 17D yellow fever strain also suggests viral inhibition of the host defense response.

The number of genes differentially regulated at the “convalescent” time point (fifty to sixty days after immunization) in the vaccinia study was somewhat surprising, and suggests that the return to baseline gene expression takes longer than two months. It is possible that the continued perturbation of gene expression compared to baseline reflects a change in the composition of PBMCs, but this could not be verified.

The only other *in vivo* study of gene expression changes induced by a pox virus was a study of the gene response in PBMCs from cynomolgus macaques infected with variola virus [40]. The data presented by Rubins *et al* display many similarities to the present study, suggesting that the immune response to vaccinia is similar to that induced by the smallpox virus. Rubins *et al* observed a strong interferon-induced gene response, but a weaker response in NF-κB and TNF-α regulated genes. In addition, they observed lymphopenia in the PBMC samples, which supports the hypothesis that vaccinia infection results in a reduction in circulating B and T cells early in infection. In addition to these similarities, however, variola infection was characterized by an induction of cell proliferation and cell-cycle genes, an effect not observed in the present study. This could reflect either a biological difference between vaccinia and variola infections, or it could be an artifact of the high viral doses administered in the macaque study. During variola infection, a strong correlation between gene expression response and viral dose was observed. We were not able to identify a similar association between gene expression and virus dilution in the present study.

In conclusion, the results presented here show that PBMCs provide a readily accessible source for the interrogation of infection-induced changes in host gene expression. Activation of the immune response is apparent both for localized and systemic infections. While data from all three study arms share common patterns of gene regulation between immune and pre-immune or convalescent samples, distinct differences are noted. These data suggest that there may be narrowly definable gene expression signatures specific to one or a small number of viruses. Such patterns can potentially form the basis for new diagnostic products for evaluating host exposure to, and infection, with a variety of pathogenic agents.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

#### Acknowledgements

We gratefully acknowledge Shannon Leighton for secretarial support and the clinical staff members of the University of Iowa, Hall Health Travel Medicine and Madrona Medical Center for help with subject recruitment and sample processing. This work was supported in part by the Army Research Laboratory’s Chemical and Biological Defense

Program of the Department of Army, Grant #s DAAD19-02-C-0071 and DAAD19-03-C-0053 (Illumigen Biosciences) and NIH Grant # N01 AI65298 (JTS).

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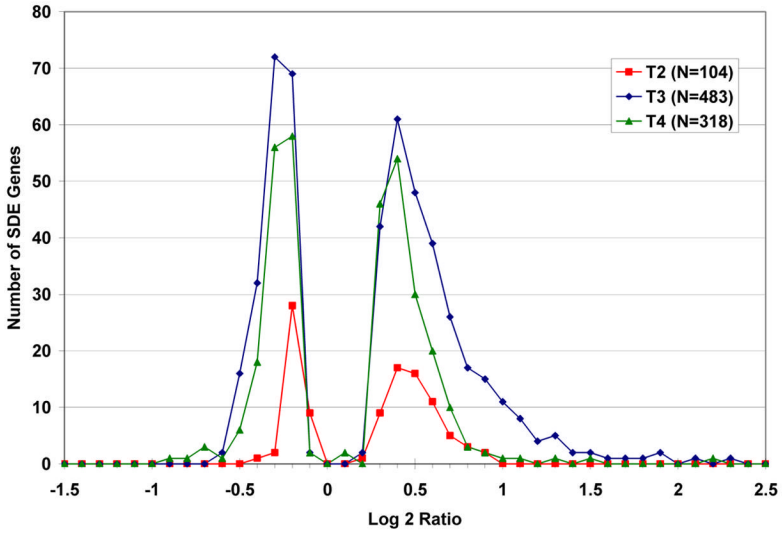


**Figure 1.**

Vaccinia-induced temporal distribution of differential expression. The distribution of the number of genes exhibiting significant differential expression (SDE) and their $\log_2$ ratios is shown for each post-vaccination time point. T2, T3, and T4 were 2–4, 5–7, and 50–60 days after vaccination, respectively. Genes with the greatest amplitude change were observed primarily at T3.

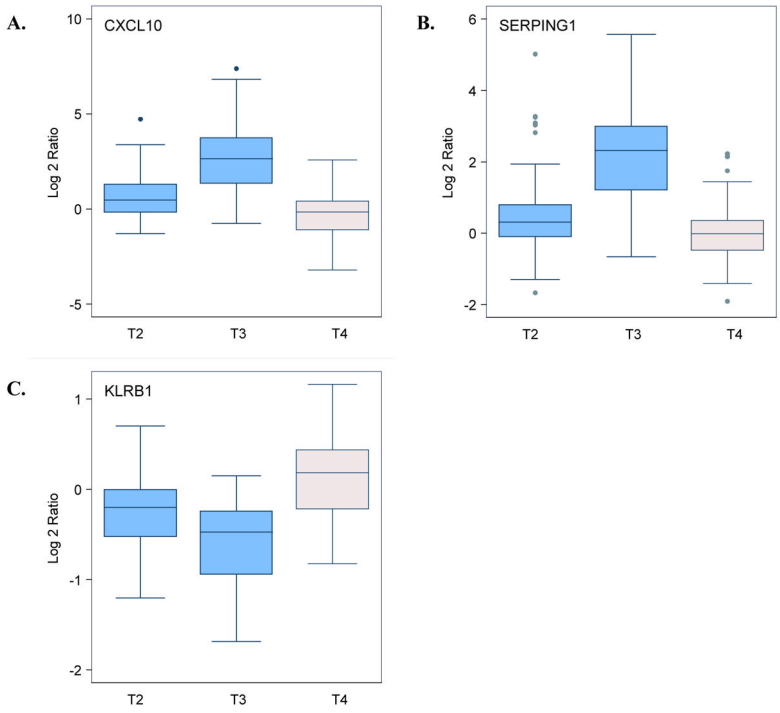


**Figure 2.**

Time variation in subject response to vaccinia vaccination. Summary box plots at three post-vaccination time points of individual subject gene expression changes relative to baseline (T1, not shown) for three representative genes of interest are shown. The midline in each box represents the median subject $\log_2$ ratio, and the box extends from the 25$^{th}$ percentile to the 75$^{th}$ percentile of subject values. Outlier subject values are shown as solid circles. Dark boxes represent time points at which the overall change in gene expression from baseline is significant.

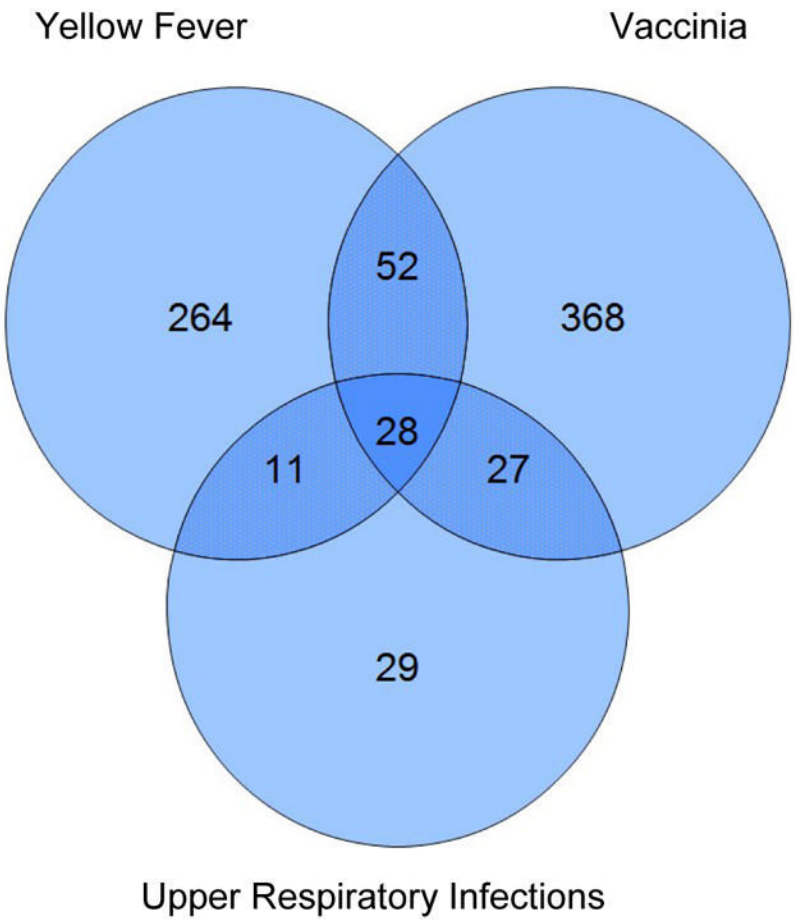


**Figure 3.**
Venn Diagram of Viral-Modulated Gene Expression. Summary of overlapping and specific gene expression patterns resulting from immunization with the yellow fever or smallpox vaccines or infection with pathogens causing upper respiratory symptoms. Overlapping and specific gene numbers are based on the ~8,000 genes that are represented on both the Human 1 cDNA and the Human 1a oligo microarrays.

**Table 1**

Demographics of Study Subjects

| Study: | Total Number: | Caucasian: | Hispanic/Latino: | Asian: |
|---|---|---|---|---|
| Vaccinia | 24 | | | |
| Female | 16 | 15 | 1 | 0 |
| Male | 8 | 8 | 0 | 0 |
| Yellow Fever | 20 | | | |
| Female | 11 | 7 | 1 | 3 |
| Male | 9 | 8 | 0 | 1 |
| URI | 12 | | | |
| Female | 10 | 9 | 1 | 0 |
| Male | 2 | 1 | 1 | 0 |

**Table 2**

Significantly Modulated Genes Common to Vaccinia and Yellow Fever Vaccination[a].

| Gene Symbol | Vacc. M | Vacc. B | Y.F. M | Y.F. B | Gene Name |
|---|---|---|---|---|---|
| ANXA2 | 0.21 | 1.61 | 0.45 | 2.38 | Annexin A2 |
| APOBEC3B* | 0.70 | 9.28 | 1.99 | 10.89 | Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B |
| ARL4C | −0.37 | 0.04 | −0.62 | 7.06 | ADP-ribosylation factor-like 4C |
| B4GALT3 | −0.25 | 0.12 | −0.37 | 5.59 | UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 3 |
| BIRC4BP | 0.52 | 0.83 | 0.86 | 8.27 | XIAP associated factor-1 |
| BLVRA* | 0.63 | 15.92 | 1.00 | 7.55 | Biliverdin reductase A |
| C12orf57 | −0.57 | 5.32 | −0.58 | 5.44 | Chromosome 12 open reading frame 57 |
| CBX7 | −0.38 | 0.33 | −0.41 | 2.26 | Chromobox homolog 7 |
| CD3D | −0.42 | 2.54 | −0.44 | 3.44 | CD3d molecule, delta (CD3-TCR complex) |
| CD74 | 0.45 | 5.06 | 0.55 | 1.82 | CD74 molecule, major histocompatibility complex, class II invariant chain |
| CEBPB* | 0.54 | 3.84 | 0.49 | 0.31 | CCAAT/enhancer binding protein (C/EBP), beta |
| CREG1 | 0.43 | 6.63 | 0.42 | 1.87 | Cellular repressor of E1A-stimulated genes 1 |
| EEF1B2 | −0.27 | 0.05 | −0.60 | 4.53 | Eukaryotic translation elongation factor 1 beta 2 |
| EIF4A1 | 0.36 | 0.31 | 0.42 | 1.80 | Eukaryotic translation initiation factor 4A, isoform 1 |
| FCGR3A | 0.58 | 0.30 | 0.55 | 0.66 | Fc fragment of IgG, low affinity IIIa, receptor (CD16a) |
| FPRL1 | 0.62 | 6.72 | 0.39 | 4.75 | Formyl peptide receptor-like 1 |
| GLRX | 0.29 | 1.22 | 0.52 | 3.75 | Glutaredoxin (thioltransferase) |
| HERC5 | 1.08 | 19.35 | 0.89 | 7.57 | Hect domain and RLD 5 |
| HSBP1 | 0.50 | 0.54 | 0.57 | 4.03 | Heat shock factor binding protein 1 |
| ICAM3 | −0.22 | 1.59 | −0.54 | 5.63 | Intercellular adhesion molecule 3 |
| IFI27* | 1.08 | 8.36 | 1.81 | 6.60 | Interferon, alpha-inducible protein 27 |
| IFI30* | 0.57 | 15.24 | 0.91 | 2.99 | Interferon, gamma-inducible protein 30 |
| IFI44 | 1.33 | 18.34 | 2.08 | 17.26 | Interferon-induced protein 44 |
| IFI44L | 2.00 | 19.79 | 0.98 | 9.57 | Interferon-induced protein 44-like |
| IFI6 | 0.91 | 10.74 | 1.32 | 9.94 | Interferon, alpha-inducible protein 6 |
| IFIT1* | 1.75 | 14.90 | 2.14 | 7.98 | Interferon-induced protein with tetratricopeptide repeats 1 |
| IFIT2* | 1.02 | 5.00 | 1.92 | 10.80 | Interferon-induced protein with tetratricopeptide repeats 2 |
| IFIT3* | 1.23 | 11.06 | 1.67 | 10.41 | Interferon-induced protein with tetratricopeptide repeats 3 |
| IFIT5 | 0.70 | 6.38 | 0.70 | 4.82 | Interferon-induced protein with tetratricopeptide repeats 5 |
| IFITM1* | 0.32 | 2.23 | 1.33 | 13.72 | Interferon induced transmembrane protein 1 (9–27) |
| IFITM2* | 0.58 | 3.97 | 1.28 | 13.59 | Interferon induced transmembrane protein 2 (1–8D) |
| IFITM3 | 1.02 | 11.52 | 0.64 | 3.88 | Interferon induced transmembrane protein 3 (1–8U) |
| IL1RN* | 0.58 | 7.53 | 0.65 | 0.85 | Interleukin 1 receptor antagonist |
| ISG15* | 1.08 | 11.45 | 2.35 | 15.69 | ISG 15 ubiquitin-like modifier |
| ISGF3G* | 0.47 | 4.10 | 0.55 | 3.27 | Interferon-stimulated transcription factor 3, gamma 48kDa |
| KIF22 | −0.22 | 0.12 | −0.38 | 1.51 | Kinesin family member 22 |
| KLRB1 | −0.62 | 16.75 | −0.60 | 2.20 | Killer cell lectin-like receptor subfamily B, member 1 |
| LAP3* | 1.57 | 18.00 | 0.99 | 4.53 | Leucine aminopeptidase 3 |
| LDHB | −0.26 | 0.15 | −0.38 | 0.38 | Lactate dehydrogenase B |
| LGALS3BP | 0.83 | 13.43 | 0.58 | 2.04 | Lectin, galactoside-binding, soluble, 3 binding protein |
| LMO2* | 0.56 | 11.50 | 0.56 | 6.77 | LIM domain only 2 (rhombotin-like 1) |
| LTB | −0.38 | 9.00 | −0.46 | 3.19 | Lymphotoxin beta (TNF superfamily, member 3) |
| LY6E* | 0.79 | 9.70 | 1.07 | 7.25 | Lymphocyte antigen 6 complex, locus E |
| MARCKS* | 0.67 | 9.39 | 1.28 | 7.87 | Myristoylated alanine-rich protein kinase C substrate |
| MT1M* | 0.40 | 1.99 | 0.42 | 1.36 | Metallothionein 1M (functional) |
| MX1* | 1.24 | 16.27 | 2.17 | 17.18 | Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse) |
| MX2* | 0.65 | 12.10 | 1.44 | 14.19 | Myxovirus (influenza virus) resistance 2 (mouse) |
| OAS1 | 0.95 | 15.90 | 1.08 | 7.18 | 2′,5′-oligoadenylate synthetase 1, 40/46kDa |
| OAS2 | 0.88 | 8.82 | 0.36 | 3.55 | 2′-5′-oligoadenylate synthetase 2, 69/71kDa |
| PLSCR1* | 0.64 | 1.02 | 0.74 | 3.40 | Phospholipid scramblase 1 |

| Gene Symbol | Vacc. M | Vacc. B | Y.F. M | Y.F. B | Gene Name |
|---|---|---|---|---|---|
| PSMA4* | 0.62 | 10.63 | 0.45 | 0.02 | Proteasome (prosome, macropain) subunit, alpha type, 4 |
| PSMA6 | 0.28 | 2.94 | 0.52 | 4.66 | Proteasome (prosome, macropain) subunit, alpha type, 6 |
| PSMB9* | 0.65 | 11.66 | 0.52 | 0.76 | Proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2) |
| PSME1* | 0.40 | 5.63 | 0.39 | 0.95 | Proteasome (prosome, macropain) activator subunit 1 (PA28 alpha) |
| PSME2* | 0.75 | 16.41 | 0.90 | 7.16 | Proteasome (prosome, macropain) activator subunit 2 (PA28 beta) |
| PYHIN1 | 0.51 | 5.81 | 0.53 | 5.08 | Pyrin and HIN domain family, member 1 |
| RPL10A | −0.22 | 0.54 | −0.72 | 8.72 | Ribosomal protein L10a |
| RPL13 | −0.26 | 0.17 | −0.45 | 7.17 | Ribosomal protein L13 |
| RPL23A | −0.26 | 1.29 | −0.39 | 2.38 | Ribosomal protein L23a |
| RPL9 | −0.27 | 0.21 | −0.48 | 4.92 | Ribosomal protein L9 |
| RPS10 | −0.26 | 0.87 | −0.37 | 1.30 | Ribosomal protein S10 |
| RPS15A | −0.26 | 2.40 | −0.58 | 7.15 | Ribosomal protein S15a |
| RPS21 | −0.36 | 3.42 | −0.57 | 5.77 | Ribosomal protein S21 |
| RPS3A | −0.28 | 2.02 | −0.78 | 10.04 | Ribosomal protein S3A |
| SERPINA1 | 0.24 | 6.79 | 0.49 | 3.44 | Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 |
| SERPING1* | 2.29 | 20.88 | 1.38 | 6.65 | Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1, (angioedema,hereditary) |
| SP110 | 0.47 | 12.32 | 0.63 | 4.32 | SP110 nuclear body protein |
| TAP1* | 0.67 | 7.60 | 0.72 | 5.04 | Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) |
| TCF3 | −0.30 | 2.06 | −0.26 | 0.79 | Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47) |
| TMC6 | −0.29 | 0.33 | −0.58 | 9.63 | Transmembrane channel-like 6 |
| TNFSF10 | 1.15 | 14.84 | 0.79 | 3.28 | Tumor necrosis factor (ligand) superfamily, member 10 |
| TRIM21 | 0.50 | 1.21 | 0.48 | 2.10 | Tripartite motif-containing 21 |
| TRIM22* | 0.63 | 15.37 | 0.81 | 6.78 | Tripartite motif-containing 22 |
| TSPAN4 | −0.30 | 1.29 | −0.34 | 0.27 | Tetraspanin 4 |
| UBE2D3 | 0.26 | 0.22 | 0.43 | 1.43 | Ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast) |
| USP18 | 0.82 | 4.81 | 0.53 | 3.58 | Ubiquitin specific peptidase 18 |
| VRK2 | 0.63 | 11.68 | 0.38 | 4.03 | Vaccinia related kinase 2 |
| ZAP70 | −0.38 | 0.72 | −0.40 | 1.65 | Zeta-chain (TCR) associated protein kinase 70kDa |

[a]Significant Modulated Genes Common to Vaccinia and Yellow Fever Vaccination. Analysis was limited to genes that are represented on both the cDNA and oligonucleotide microarrays. Genes exhibiting statistically significant induction (red type) or repression (green type) after immunization with Vaccinia virus (Vacc.) and the live attenuated yellow fever vaccine (Y.F.) are presented. Genes that were also significantly induced in the URI "immune" study samples are indicated with an asterisk (*) after the gene symbol. M represents the average $\log_2$ fold change across spots at the given gene, while B represents the Bayesian statistic on expression change (>0 is significant).

**Table 3**

Genes Altered Specifically by Vaccinia Virus Exposure[a].

| Gene Symbol | M | B | Gene Name |
|---|---|---|---|
| IRF1 | 0.90 | 16.47 | Interferon regulatory factor 1 |
| APOL1 | 0.82 | 14.27 | Apolipoprotein L, 1 |
| HLA-DRA | 0.54 | 13.94 | Major histocompatibility complex, class II, DR alpha |
| NFX1 | 1.17 | 12.68 | Nuclear transcription factor, X-box binding 1 |
| CD40 | 0.69 | 12.53 | CD40 molecule, TNF receptor superfamily member 5 |
| TJP2 | 0.59 | 11.84 | Tight junction protein 2 (zona occludens 2) |
| C3AR1 | 0.70 | 11.82 | Complement component 3a receptor 1 |
| ZNF671 | 0.55 | 11.59 | Zinc finger protein 671 |
| LILRA2 | 0.42 | 10.83 | Leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2 |
| SLC8A1 | 0.84 | 9.22 | Solute carrier family 8 (sodium/calcium exchanger), member 1 |
| SPIB | −0.48 | 9.20 | Spi-B transcription factor (Spi-1/PU.1 related) |
| LLGL2 | −0.46 | 9.20 | Lethal giant larvae homolog 2 (Drosophila) |
| ALDH1A1 | 0.68 | 9.06 | Aldehyde dehydrogenase 1 family, member A1 |
| IRF7 | 0.71 | 9.04 | Interferon regulatory factor 7 |
| HLA-DPB1 | 0.41 | 8.62 | Major histocompatibility complex, class II, DP beta 1 |
| SLC38A1 | −0.44 | 8.19 | Solute carrier family 38, member 1 |
| IL24 | −0.39 | 8.17 | Interleukin 24 |
| KSP37 | −0.52 | 8.06 | Ksp37 protein |
| IRF2 | 0.45 | 8.01 | Interferon regulatory factor 2 |
| CAMK2D | 0.50 | 7.97 | Calcium/calmodulin-dependent protein kinase (CaM kinase) II delta |
| C1QB | 1.29 | 7.87 | Complement component 1, q subcomponent, B chain |
| TNFRSF1B | 0.56 | 7.83 | Tumor necrosis factor receptor superfamily, member 1B |
| SPTLC2 | 0.51 | 7.72 | Serine palmitoyltransferase, long chain base subunit 2 |
| SPOCK2 | −0.41 | 7.37 | Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 2 |
| GLS | 0.53 | 6.78 | Glutaminase |
| GRAP | −0.38 | 6.62 | GRB2-related adaptor protein |
| MS4A1 | −0.51 | 6.49 | Membrane-spanning 4-domains, subfamily A, member 1 |
| RPL12 | −0.30 | 6.06 | Ribosomal protein L12 |
| HYAL2 | −0.51 | 5.95 | Hyaluronoglucosaminidase 2 |
| FN1 | 0.53 | 5.84 | Fibronectin 1 |
| ATOX1 | 0.40 | 5.56 | ATX1 antioxidant protein 1 homolog (yeast) |
| TARP | −0.54 | 5.51 | TCR gamma alternate reading frame protein |
| NAP1L4 | −0.31 | 5.44 | Nucleosome assembly protein 1-like 4 |
| SCAP1 | −0.37 | 5.42 | Src family associated phosphoprotein 1 |
| CBLB | −0.39 | 5.32 | Cas-Br-M (murine) ecotropic retroviral transforming sequence b |
| ITPKB | −0.36 | 5.32 | Inositol 1,4,5-trisphosphate 3-kinase B |
| FLNB | −0.35 | 5.25 | Filamin B, beta (actin binding protein 278) |
| CES1 | 0.33 | 5.06 | Carboxylesterase 1 (monocyte/macrophage serine esterase 1) |
| P2RX5 | −0.41 | 5.05 | Purinergic receptor P2X, ligand-gated ion channel, 5 |
| CD79B | −0.44 | 5.00 | CD79b molecule, immunoglobulin-associated beta |
| C6orf48 | −0.24 | 4.77 | Chromosome 6 open reading frame 48 |
| C20orf111 | −0.39 | 4.71 | Chromosome 20 open reading frame 111 |
| LIMK2 | 0.53 | 4.71 | LIM domain kinase 2 |
| SORT1 | 0.47 | 4.62 | Sortilin 1 |
| CD2 | −0.36 | 4.62 | CD2 molecule |

[a]Genes Altered Specifically by Vaccinia Virus Exposure. Genes that are altered specifically in response to vaccinia virus at the 5–7 day post-immunization time point are listed. M represents the average $\log_2$ fold-change in expression, with increases in expression designated by red type and decreases in expression designated by green type. B represents the Bayesian statistic on expression change (>0 is significant).

**Table 4**

Genes Altered Specifically by Yellow Fever Virus Exposure[a]

| Gene Symbol | M | B | Gene Name |
|---|---|---|---|
| PRB3 | −0.73 | 16.05 | Proline-rich protein BstNI subfamily 3 |
| PYCR2 | 0.66 | 15.02 | Pyrroline-5-carboxylate reductase family, member 2 |
| SYNGR3 | 0.80 | 11.25 | Synaptogyrin 3 |
| DPP7 | −0.51 | 10.34 | Dipeptidyl-peptidase 7 |
| LIMD2 | 0.49 | 9.97 | LIM domain containing 2 |
| NHLH2 | 0.64 | 9.16 | Nescient helix loop helix 2 |
| FBXW5 | −0.56 | 8.78 | F-box and WD-40 domain protein 5 |
| VAV1 | −0.62 | 8.66 | Vav 1 oncogene |
| LOC44059 | −0.61 | 8.60 | Similar to eukaryotic translation elongation factor 1 alpha 2 |
| RPS2 | −0.77 | 8.58 | Ribosomal protein S2 |
| NDUFV1 | −0.53 | 7.94 | NADH dehydrogenase (ubiquinone) flavoprotein 1, 51kDa |
| LIF | −0.80 | 7.68 | Leukemia inhibitory factor (cholinergic differentiation factor) |
| RPS7 | −0.68 | 7.57 | Ribosomal protein S7 |
| HAND1 | 0.52 | 6.89 | Heart and neural crest derivatives expressed 1 |
| RPS13 | −0.50 | 6.73 | Ribosomal protein S13 |
| NRBP2 | 0.50 | 6.63 | Nuclear receptor binding protein 2 |
| CCNI | −0.39 | 6.50 | Cyclin I |
| EIF4B | −0.62 | 6.49 | Eukaryotic translation initiation factor 4B |
| THRB | 0.90 | 6.23 | Thyroid hormone receptor, beta (erythroblastic leukemia viral (v-erb-a) oncogene homolog 2,avian) |
| APRT | −0.41 | 6.19 | Adenine phosphoribosyltransferase |
| APOD | 0.67 | 5.83 | Apolipoprotein D |
| CACNA2D2 | 0.40 | 5.77 | Calcium channel, voltage-dependent, alpha 2/delta subunit 2 |
| TAGLN3 | 0.70 | 5.65 | Transgelin 3 |
| C14orf2 | −0.44 | 5.61 | Chromosome 14 open reading frame 2 |
| C7orf27 | −0.45 | 5.26 | Chromosome 7 open reading frame 27 |
| SLC25A6 | −0.48 | 4.91 | Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6 |
| COL6A2 | −0.35 | 4.90 | Collagen, type VI, alpha 2 |
| CORO1A | −0.43 | 4.74 | Coronin, actin binding protein, 1A |
| EGLN2 | −0.43 | 4.71 | Egl nine homolog 2 (C. elegans) |
| C19orf6 | −0.47 | 4.70 | Chromosome 19 open reading frame 6 |
| DKFZp762 | 0.43 | 4.65 | Hypothetical protein DKFZp762E1312 |

[a]Genes that are altered specifically in response to yellow fever are listed. M represents the average $\log_2$ fold-change in expression, with increases in expression designated by red type and decreases in expression designated by green type. B represents the Bayesian statistic on expression change (>0 is significant).

## Table 5

Gene Ontology Bias in Differential Response to Study Infections[a]

| Category: | Array: | Vaccinia: | Yellow Fever: | URI: |
|---|---|---|---|---|
| **A. Percent Representation in Physiological Process Subcategory** | | | | |
| Cellular Physiological Process | 90.4 | 78.7 | 87.3 | 73.2 |
| Metabolism | 68.1 | 59.8 | 69.4 | 53.7 |
| Regulation of Physiological Processes | 31.7 | 30.2 | 29.5 | 26.8 |
| Localization | 20.6 | 15.1 | 13.8 | 14.6 |
| Response to Stimulus | 20.4 | **43.9*** | **27.2*** | **54.9*** |
| Organismal Physiological Process | 19.2 | **41.0*** | 21.6 | **47.6*** |
| Death | 6.6 | 10.8 | 5.6 | 9.8 |
| **B. Percent Representation in Immune Response Subcategory** | | | | |
| Uncategorized I.R. | 37.5 | **55.3*** | **60.4*** | **61.1*** |
| Inflammatory Response | 27.9 | 23.5 | 16.7 | 16.7 |
| Humoral I.R. | 21.6 | 12.9 | 16.7 | 11.1 |
| Immune Cell Activation | 13.9 | 13.6 | 10.4 | 0.0 |
| Cellular Defense Response | 13.0 | 12.1 | 10.4 | 11.1 |
| Regulation of I.R. | 11.5 | 8.3 | 6.3 | 0.0 |
| Innate I.R. | 7.8 | 5.3 | 2.1 | 5.6 |
| Cytokine Production | 6.1 | 3.0 | 2.1 | 0.0 |
| Cytokine Metabolism | 5.4 | 2.3 | 2.1 | 0.0 |
| Antigen Presentation | 5.0 | 8.3 | 2.1 | 0.0 |
| Antigen Processing | 4.3 | 6.8 | 0.0 | 0.0 |
| Acute-phase Response | 3.3 | 3.0 | 4.2 | 5.6 |
| **C. Percent Representation in Immune Response Subcategory (Vaccinia only)** | | | | |
| **Category:** | **Array** | **Vaccinia Upregulated** | **Vaccinia Downregulated** | |
| Uncategorized I.R. | 37.5 | **58.7*** | **47.5*** | |
| Inflammatory Response | 27.9 | 27.2 | 15.0 | |
| Humoral I.R. | 21.6 | **12.0*** | 15.0 | |
| Immune Cell Activation | 13.9 | **6.5*** | **30.0*** | |
| Cellular Defense Response | 13.0 | 8.7 | **20.0*** | |
| Regulation of I.R. | 11.5 | 6.5 | 12.5 | |
| Innate I.R. | 7.8 | 7.6 | 0.0 | |
| Cytokine Production | 6.1 | 3.3 | 2.5 | |
| Cytokine Metabolism | 5.4 | 2.2 | 2.5 | |
| Antigen Presentation | 5.0 | **10.9*** | 2.5 | |
| Antigen Processing | 4.3 | **9.8*** | 0.0 | |
| Acute-phase Response | 3.3 | 4.3 | 0.0 | |

[a] For each study infection, the table shows the percent of genes that are annotated for one or more of the following Gene Ontology categories: (A) “Physiological Process” (GO:0007582), (B) “Immune Response” (GO:0006955), or (C) “Immune Response” (GO:0006955) for vaccinia only, sorted according to either up- or down-regulation. Bold-type numbers with an asterisk (*) indicate significant biases ($p<0.05$) in category representation for each of the study infections relative to the expected reference distribution.