Transcriptional response in the peripheral blood of patients infected with *Salmonella enterica* serovar Typhi

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We used microarrays and transcriptional profiling of peripheral blood to investigate the host response of 29 individuals who contracted typhoid fever in the Mekong Delta region of Vietnam. Samples were taken over a nine month period encompassing acute disease, convalescence, and recovery. We found that typhoid fever induced a distinct and highly reproducible signature in the peripheral blood that changed during treatment and convalescence, returning in the majority of cases to the "normal" profile as measured in healthy uninfected controls. Unexpectedly, there was a strong, distinct signature of convalescence present at day 9 after infection that remained virtually unchanged one month after acute infection and in some cases persisted as long as nine months despite a complete clinical recovery in all patients. Patients who retain the convalescent signature may be genetically or temporarily incapable of developing an effective immune response and may be more susceptible to reinfection, relapse, or the establishment of a carrier state.

immune response | microbe–host interaction | transcriptional profiling | typhoid fever

Typhoid fever is a systemic febrile disease caused by *Salmonella enterica* serovar Typhi (*S. typhi*). The disease is endemic in many areas of the world where sanitation and water quality are poor; 22 million cases of the disease are reported each year (1). Typhoid is characterized by a variable incubation period (range 8–14 days) (2, 3), followed by the development of fever >38.5 °C, rash, headache, abdominal complaints, relative bradycardia, enlarged liver, enlarged spleen, and lethargy (1, 3). The definitive diagnosis of typhoid depends on a positive blood culture, although such cultures may be positive in only 40–60% of cases (1, 3).

Typhoid fever must be differentiated from a number of other common febrile diseases including malaria, tuberculosis, and rickettsial disease to facilitate the appropriate clinical management of patients (2). The most common antibiotics used to treat typhoid fever in South Asia are fluoroquinolones, but the increasing microbial resistance is rendering these antibiotics less effective (1, 3). The incidence of typhoid fever in the Mekong Delta region of Vietnam, where our study was based, has been reported as 198 per 100,000 (4).

S. typhi infects humans exclusively. Thus, the majority of research on salmonellosis has been done using a mouse model of the disease using a related bacterium, *Salmonella enterica* serovar Typhimurium (*S. typhimurium*). This animal model system resembles, but does not faithfully reproduce, the enteric fever associated with typhoid; therefore, there is a relative scarcity of knowledge about the host responses in natural human infection.

Several studies investigating typhoid fever have demonstrated that there is a broad humoral- and cell-mediated immune (CMI) response to infection (5–7). The CMI response induced by *S. typhi* is associated with IFNγ production by CD4$^+$ and CD8$^+$ T cells, T-cell proliferation, and the production of specific CD8$^+$ cytotoxic T lymphocytes (CTL) (5, 8, 9). Despite this robust immune response to *S. typhi* infection, 2–3% of infected individuals have recurring infection or exhibit relapse (10). Moreover, about the same number of infected individuals become asymptomatic carriers of the typhoid bacillus and shed bacteria in their stool intermittently for up to 1 year or even a lifetime. These carriers are the primary reservoir of the disease and are a major public health concern (3, 11).

Transcriptional profiling of the peripheral blood using microarray analysis is a recent useful method for monitoring the host response to disease (12–15). We used this technique to investigate the host response to typhoid fever during acute disease, convalescence, and recovery in naturally infected individuals from the Mekong Delta. We show here that blood transcriptional profiling reflects several facets of the host response to infection and during recovery that heretofore have not been appreciated. The most dramatic finding is that most infected individuals continue to exhibit marked changes in their peripheral blood transcription profiles many months postinfection, which suggests a more profound and lengthy impact on the host immune system than was appreciated in previous clinical studies.

Results and Discussion

Time Course of Transcriptional Changes in Response to Typhoid. Twenty-nine residents of the Mekong Delta region of Vietnam with blood culture-documented *S. typhi* were studied. Peripheral blood was drawn from these individuals for total RNA extraction several times during the course of their disease and convalescence. The first sample, T1, was obtained when individuals were initially seen at the clinic, and blood was drawn for blood culture. Sample T3 was obtained day 3 after hospital admission or the report of a positive blood culture for *S. typhi*; T1 and T3 are
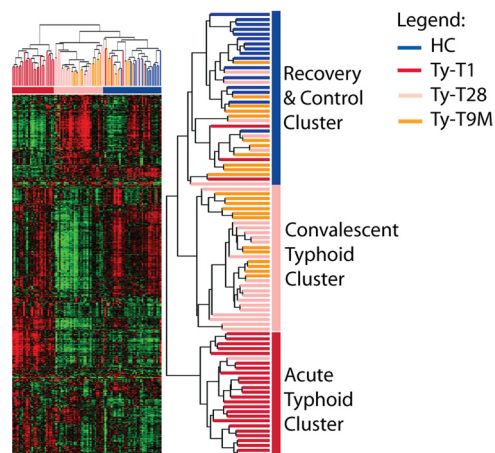
Fig. 1. Unsupervised hierarchical clustering of T1, T28, and T9M samples from typhoid patients and healthy controls using the 1,403 transcripts with greatest variance in abundance. A larger version of the sample dendrogram is shown to the right. Colored bars represent the time point for each sample, and the color key is shown in the legend. The three major branches of the dendrogram are named as shown beside the diagram: recovery and control, convalescent typhoid, and acute typhoid clusters; these contain the majority of the healthy control (HC and Ty-T9M), convalescent (Ty-T28), and acute (Ty-T1) samples, respectively.

collectively termed the "acute" samples. All patients diagnosed with typhoid were treated with an appropriate antibiotic regimen (either a fluroquinolone or azithromycin) for 10 days. Samples T9 and T28 were obtained on days 9 and 28 postdiagnosis, respectively, and termed the early and late "convalescent" samples. Sample T9M was obtained at 9 months postinfection diagnosis and was termed the "recovery" sample. Acute samples also were obtained from 10 individuals who presented with a diagnosis of uncomplicated acute malaria and from 16 healthy Vietnamese individuals (HC). All RNA samples were hybridized to Stanford Human cDNA Microarrays, and the data were initially organized using unsupervised hierarchical clustering to assess the similarity and differences between the samples (16) (Fig. 1). The initial analysis using all five time points from the patients with clinically diagnosed typhoid fever revealed that the expression profiles of T1 and T3 samples were virtually identical; no significant differences in transcript abundance were found using Statistical Analysis of Microarrays (SAM) (17). The T9 time point was intermediate between the acute samples and the T28 sample (Fig. S1). Thus, for simplicity, we used data from the T1 time point to represent acute disease, the T28 time point sample to represent convalescence, and samples taken at nine months after infection to represent recovery.

Notwithstanding the complexity of this host-pathogen interaction, the data fell readily into three general major gene expression clusters as a function of time.

Acute typhoid cluster. As expected, patients with acute typhoid fever have distinct perturbations in their peripheral blood transcriptional profiles as compared to healthy individuals (Fig. 1) and are also largely distinct from patients suffering from acute malaria (Fig. S1).

Convalescent typhoid cluster. Not unexpectedly, virtually all of these patients by day 28 after infection displayed peripheral blood gene transcription patterns that were strikingly different from the pattern they had expressed during the acute phase of the disease. Although all of the patients were clinically well, they displayed a pattern of relative transcript abundance and expression that was dramatically distinct from normal, uninfected subjects. No differences in the transcriptional profile were apparent in patients who received either a fluroquinlone or azithromycin.

Recovery and control cluster. By nine months after diagnosis, the peripheral blood gene transcriptional profiles formed a gradient; most patient samples were clearly distinct from the convalescent samples, and many patients' samples now clustered with the control; however, a subset of patients exhibited transcriptional profiles that remained in the convalescent cluster.

Our data clearly show that the transition of typhoid from the acute phase through recovery is associated with distinct changes in the gene transcriptional profile of peripheral blood cells. Although this is not surprising, the length of time over which changes are observed in the peripheral blood was unexpected. The clustering of the samples taken on day 28 after diagnosis indicates that there seems to be a consistent host response after the acute phase of typhoid. This profile may have been influenced by factors such as antibiotic treatment or preexisting chronic infection with other microorganisms or large parasites. Nevertheless, it is striking that this general peripheral blood transcriptional profile on day 28 differed from the profile shown by the healthy control samples, despite the absence of clinical signs or symptoms of disease in these patients.

The expression profile seen in circulating peripheral blood cells at 1 month and in some patients as long as nine months after infection suggests that the host's encounter with the typhoid bacillus, even though interrupted by antimicrobial treatment, has a more profound impact than one might have thought based on the criterion of "clinical recovery." To what extent this may be operative in the long-term host response in endemic regions to infection with other microorganisms, especially *Mycobacterium tuberculosis*, *Helicobacter pylori*, and other persistent microbial infections, needs to be determined. In this context, it may be important to make a distinction between "normal" individuals who reside in regions of the world where there is a high prevalence of unapparent infection by microorganisms and animal parasites as compared to normal individuals from the more developed regions of the globe. Indeed, in a comparison of peripheral blood transcripts seen in normal individuals from the U.S. and from the developing world revealed a subtle, but real increase in the relative abundance of transcripts associated with lymphocytes and lymphocyte activation (see ref. 18 and S. Popper, unpublished observation). This observation requires further investigation and a more rigorous test of this hypothesis.

Patterns of Gene Expression Associated with Temporal Phases of Typhoid. Genes associated with the distinct transcript profiles present at each phase of illness or recovery in the typhoid patients were identified by comparing the relative abundance of transcripts at T1, T28, and T9M to their abundance in the healthy controls using SAM, with a false discovery rate for each comparison of 1%. The resulting set of 1,082 transcripts was then organized using hierarchical clustering before further analysis (Fig. 2). These measurements of transcript abundance were derived from a complex mixture of cell types and may reflect both an altered cellular composition, as well as changes in gene-specific transcriptional activity. In addition, some of these changes may be caused directly by pathogen-specific molecules, while others undoubtedly reflect the host's sequential innate and adaptive response to microbial incursion. We could not completely distinguish between these aspects of variation in gene expression, but we were able to assign individual clusters of genes to particular cellular and physiological categories by identifying enriched gene ontologies (19), by comparing with other studies, and by identifying significant associations between the gene expression patterns and various clinical parameters (CPs) (Fig. 2). As an internal control, we correlated the gender of the patients to the expression pattern of all genes in the set; the result showed a strong correlation for two small specific clusters, one containing Y-linked genes and one containing X-linked genes (clusters 4 and 6; Fig. 2). We also assessed the reproducibility of
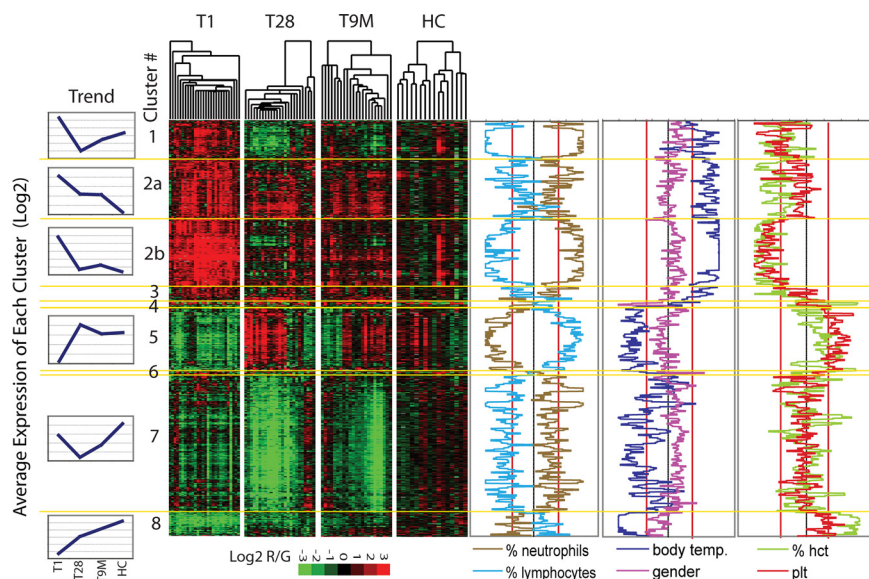
Fig. 2. Temporal changes in gene expression in typhoid patients. Transcripts (1,082) determined by SAM analysis to vary significantly in abundance from the HC samples at T1, T28, or T9M in the typhoid samples were hierarchically clustered; arrays were clustered by time point. Red indicates high expression, and green indicates low expression as shown in the legend; gray indicates missing data. Pearson correlation coefficients and P values were calculated for the expression of every gene and each CP [percent neutrophils, percent lymphocytes, body temperature, gender, percent hematocrit (HCT), and platelets (PLT)] across a selected set of 85 samples that had full clinical data. The plots to the right of the clusters show the negative $\log_{10}$ of the P value signed according to the sign of the calculated correlation coefficient. The P values are plotted as moving averages of three genes (along the vertical axis). The red vertical lines on each plot indicate a P value of 0.05. Gene clusters (1–8) referred to in the text are demarcated by horizontal yellow lines.

our findings by examining a second set of acute peripheral blood samples collected from 10 individuals from the same geographical area the following year. Using the list of 783 transcripts that were significantly increased or decreased in abundance in acute typhoid (T1) patients in our primary dataset, the two groups of patients had very similar patterns of expression (Fig. S2).

Gene Expression Associated with Acute Typhoid Fever. The 431 transcripts more abundant in acute typhoid were concentrated in three clusters (clusters 1, 2a, and 2b; Fig. 2). Gene expression in two of these clusters (clusters 1 and 2b; Fig. 2) was strongly correlated with the clinically observed parameters of both temperature and the percentage of neutrophils in the peripheral blood. These findings suggest that a significant feature of the early transcriptional response to *S. typhi* infection is dominated by a neutrophil response. This apparent involvement of neutrophils in the acute typhoid fever response has not previously been appreciated. Mild neutropenia is often noted in patients suffer-

ing from acute typhoid, but the neutropenia is usually attributed to an overall leucopenia (3, 20, 21). However, in the present study, the absolute number of neutrophils stayed constant, while the relative percentage of neutrophils was significantly increased (Table 1).

Cluster 1 contains genes that are increased in abundance in the T1 samples, decreased in the T28 samples, and slowly returned to baseline levels (Fig. 2). Many of these genes are classified as immune response genes including a significant number in the KEGG pathways for JAK-STAT signaling, Toll Receptor signaling, and apoptosis. Cluster 2 contains genes induced during acute typhoid that then decrease toward the normal profile over convalescence and recovery (Fig. 2, Trend graphs). Cluster 2a contains genes coding for intracellular proteins, including transcription factors, secretory granules, and intracellular signaling molecules. A large proportion of the genes in cluster 2b are induced by IFNγ, which is known to be increased during acute typhoid (8, 21). This cluster also includes other genes known to

Table 1. Complete blood counts (CBC) for typhoid patients at T1, T9, and T9M in comparison with those in healthy controls (HC)

	Ty-T1[‡]	Ty-T9	Ty-T9M	HC
Neutrophils, %	71.4 (14.7)***	46.8 (10.0)*	56.0 (9.3)	57.3 (16.7)
Lymphocytes, %	22.3 (10.9)**	41.6 (19.7)*	36.6 (7.2)	32.5 (9.3)
Monocytes, %[†]	5.3 (3.2)	9.3 (9.4)*	6.1 (4.3)	10.2 (6.7)
Hematocrit %	35.1 (4.9)***	34.8 (6.6)***	42.4 (10.7)	40.1 (7.0)
WCC K/μl	5.3 (2.9)**	5.8 (1.0)**	9.7 (2.7)*	7.2 (2.1)
Neutrophils K/μl	3.8 (2.1)	2.7 (1.2)**	5.1 (1.7)	3.9 (1.9)
Lymphocytes K/μl	1.1 (0.8)***	2.4 (1.1)	3.2 (1.3)**	2.4 (0.7)
Monocytes K/μl[†]	0.3 (0.3)***	0.5 (0.6)*	0.6 (0.4)	0.7 (0.6)
Platelets K/μl	129.0 (106.0)***	241.0 (57.0)	254.5 (67.5)	260.5 (96.5)

Mann–Whitney nonparametric t tests showing significant differences from HCs. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[†]Levels consist of combined monocyte, eosinophil, and basophil counts and percentages.
[‡]All values are median (interquartile range).

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be involved in innate immunity (e.g., complement), adaptive immunity, and cell proliferation (22, 23).

As expected, the GO term "immune response" significantly associated with genes expressed at T1, including both innate and adaptive components. The terms inflammatory response, response to virus and bacterium, cell cycle, and cell death categories were also significant. Our annotation of these gene sets was corroborated by identification of other array datasets enriched for the same genes; datasets describing IFNγ (24), IFNα (25), and IL6 (26) responses, as well as gene expression specific to T cells (27) and the cell cycle (28) overlapped significantly with the sets of genes induced in acute typhoid.

Many (352) transcripts were less abundant in the samples from patients with acute typhoid as compared to healthy controls. These transcripts were concentrated in two clusters (clusters 5 and 8; Fig. 2). The reduced abundance of transcripts in cluster 5 was correlated with the clinical parameters of hematocrit and platelet levels, and with a reduction in both absolute lymphocyte numbers and the percentage of lymphocytes, presumably reflecting the well characterized transient anemia, thrombocytopenia, and lymphopenia associated with acute typhoid. Cluster 5 was highly enriched for genes associated with erythroid differentiation (29) and with high expression in early stage reticulocytes and bone marrow-derived erythroid cells expressing the transferrin receptor (CD71) (30, 31).

Transcript abundance in cluster 8 was strongly correlated with hematocrit, as well as the overall white cell count and to a lesser degree with platelet levels; annotation of the genes in this cluster indicated an association with neutrophils and myeloid differentiation. Genes in cluster 8 were associated with the GO term "cell differentiation" and were expressed at high levels during myeloid differentiation (32). They were also expressed at higher levels in neutrophils than other leukocytes (33). Higher levels of bacteria in the bone marrow of typhoid patients are associated with decreased white count and platelet counts (34), and transient anemia is a classic feature of typhoid fever (3, 35); the patterns of gene expression in clusters 5 and 8 suggest a profound impact on cells of the myeloid cell lineage (i.e., megakaryocytes, erythrocyte precursors, mononuclear phagocytes, and all of the polymorphonuclear granulocytes).

Gene Expression During Convalescence. Cluster 7 contains the majority of the 469 gene transcripts that were significantly less abundant in the convalescent (T28) patient samples as compared with HC (Dataset S1). Gene ontology analysis showed that this cluster does not contain transcripts involved in the immune and inflammatory responses. Rather, the transcripts comprising this cluster fell into ontology categories, such as "membrane-bounded organelle, in particular, endoplasmic reticulum and Golgi stack," as well as "secretory pathway," "cellular metabolism," "ubiquitin cycle," and "lipid modification and transcription." We note that the *Salmonella*-containing vacuole (SCV) has been shown to associate with the Golgi stack and to exploit both endocytic and secretory pathways of cells to survive intracellularly (36–38). Perhaps the relative under representation of ER, golgi and secretory gene transcripts during convalescence reflects a residual effect of pathogen-directed suppression that reflects the formation and integrity of the SCV. Alternatively, the relative paucity of the gene transcripts of this class might reflect host defense response that is mobilized in an effort to thwart intracellular bacterial replication. In this context, we note that the patients who are recovering from typhoid fever in the present study may still be shedding the bacillus at T28 despite having received antibiotic therapy as has been noted in previous studies (6). Unfortunately, we could not specifically assess typhoid shedding in the present study due to the difficulty of locating patients in the setting of the Mekong Delta.

There were only 48 gene transcripts that were significantly

more abundant during convalescent typhoid (T28) as compared with HC (Dataset S1). These were located primarily in cluster 2a (Fig. 2) and included some associated with a response to IFNγ (TNCRNA, LGALS3BP), IGF-1R pathway (SOS1 and IGF1R), and those associated with T-cell receptor signaling (TARP, SOS1, CBLB, and PPP3CA). We note that these gene transcripts might be expected to be found during an adaptive immune response to infection.

Divergent Patterns of Gene Expression 9 Months After Typhoid. Nine months after acute disease, all patients without exception appeared well when they were seen at the time of sampling. Despite their apparent similarity in appearance to the healthy control group, we found 422 transcripts that differed in abundance at T9M as compared to the HC; 359 (85%) of these transcripts were also differentially expressed at 1 month postinfection. This is consistent with the view that no unique gene expression programs were apparent at the 9-month time point (Fig. 2 and Dataset S1).

Among the 76 gene transcripts more abundant in the T9M samples than in the healthy controls, there were many associated with GO terms and cellular processes that indicate an active on-going immune response: "T-cell receptor signaling pathway," "complement activation," the IGF-1R pathway, and transcripts generally associated with cytotoxic T lymphocytes and/or natural killer cell-mediated cytotoxicity. These findings are consistent with our clinical observation of a relative increase in lymphocytes still evident in patients even 9 months postinfection (Table 1). CD8^{+} CTL are induced after vaccination of volunteers with attenuated *S. typhi* strains, and these cells can specifically lyse *S. typhi* infected cells (8, 9). Thus, the data from the present study suggests that a similar class of CTL may also be induced after natural infection in the convalescent/recovery period.

Inspection of both the overall transcription profiles (Fig. 1) and the subset of transcripts associated with earlier time points in infection (Fig. 2) indicated that differences from gene expression levels in healthy controls were present in some but not all patients. To define this subset of patients, we used the set of transcripts that differed in abundance at T28 and in the healthy controls to cluster samples from T28, T9M, and the healthy controls. There were seven T9M patient samples that clustered with and were indistinguishable from the healthy controls (normal). Another group of seven patient samples displayed a transcription profile that was indistinguishable from the profile seen at T28; these patients were called "unusual." The remaining nine patient samples taken at T9M had an intermediate profile but clearly were much closer to normal healthy controls than were the unusual patient group (Fig. S3). The distinctive nature of the unusual patients can be appreciated by noting that when we examined the entire set of 1,403 transcripts assessed in this study, there were only nine transcripts that differed significantly in abundance in healthy controls and in those patients who had normal T9M expression profiles. In contrast, 499 transcripts differed when the unusual samples were compared to the controls. A direct comparison of the T9M samples from the unusual patients and the patients whose profile had returned to normal, revealed 38 gene transcripts that were more abundant and 430 gene transcripts that were less abundant in the unusual patients (Dataset S1).

In an effort to identify any unique trait of the unusual patients that may have resulted in such a striking retention of a convalescent signature nine months after acute disease, we directly compared patient samples from the same individuals (normal and unusual) at earlier time points. We found no significant differences in expression during the acute phase of the disease (T1, T3, or T9), but at T28 there were 11 transcripts more abundant in the unusual patient samples (Dataset S1). This small gene set was significantly enriched for genes associated with "ion

transmembrane transporter activity" ($P < 0.010$); the three genes associated with this term were SLC1A6, GLRB, and CNGB3. Both GLRB (a glycine receptor) and SLC1A6 (a glutamate receptor) encode neurotransmitter receptors. There is growing recognition that neurotransmitters may play a role in the immune response (39). There is evidence that neurotransmitters play a role in the activation and function of macrophages, the primary cellular target of *S. typhi*: Ion-dependent glutamate transport occurs in activated macrophages (40), GLRB is expressed in hepatic Kupffer cells (41), a known site of *S. typhi* replication, and it is documented that neurotransmitters affect the ability of activated macrophages to respond to bacterial pathogens and LPS (42, 43).

The altered gene expression patterns in the unusual patient group may indicate that these individuals were genetically or temporarily incapable of developing an effective immune response. None of these patients were found to be persistent carriers of *S. typhi* as assessed by a single stool culture at nine months, but it is also possible that these patients still retained a significant reservoir of actively replicating microbes. Further investigation is required to determine if their persistent convalescent transcription profile is indicative of a persistent carrier state, relapse, or genotype. Based on the findings in these patients, we have followed the peripheral blood transcripts in the mouse model of persistent *Salmonella* infection and found a similar pattern of persistent transcripts in animals that are asymptomatic but still retain a reservoir of actively replicating bacteria that need not be associated with active bacterial shedding (44).

Summary and Conclusions. Typhoid fever has a complex pathogenesis. It presents as an acute febrile disease, but this follows a relatively long incubation period that involves the transmigration of the microorganism through the Peyer's patch, localized multiplication in the mesenteric lymph nodes, and subsequent spread to the liver and spleen before there are clinical symptoms (45).

Thus, in the patients who were studied here, the organism had intimately engaged both the innate and adaptive immune system at the time their symptoms brought them to the clinic and their peripheral blood was sampled. The peripheral blood contains components of both the innate immune system and the adaptive immune system including neutrophils, CD4$^+$ and CD8$^+$ T lymphocytes, B lymphocytes, natural killer cells, monocytes, and dendritic cells each expressing a unique combination of genes. Blood represents both a pool and a migration compartment for these cell types. We find that typhoid fever induces a distinct and highly reproducible signature in the peripheral blood that changed during treatment and convalescence, returning in the majority of cases to the normal profile as measured in healthy uninfected controls. Unexpectedly, a strong signature of convalescence present as early as day 9 remained at one month after acute infection and in some cases persisted as long as nine months.

About 20 years ago, the phenomenon of *Salmonella*-induced immune suppression in mice was described in ref. 46, and suppression of T-cell responses has been found to occur in vivo in *Salmonella*-infected mice (47–49). This effect is not antigen-specific and is consistent with the potential for *Salmonella*-induced suppression of lymphocyte responses during murine infection (49) and, we believe, may be the reason for our observations of a slow and inefficient development of immunity in a subset of patients suffering from proven human typhoid.

Thus, the process of returning to homeostasis in individuals who have experienced an acute *S. typhi* infection takes much longer than expected and may affect immune competence during this time. Our data may provide clues as to why individuals sometimes relapse from *S. typhi* infection, become reinfected, and/or become persistent carriers. The mRNA profiles of peripheral blood cells provide a clear view of differences of gene expression in the diseased and the recovered state of the same subject. It is likely that one can use this kind of information to obtain insight into the immune response to natural infection, as well as immunization with vaccines.

Materials and Methods

Patient Selection, Treatment, and Sample Collection. Three hospitals were involved in the study: Dong Thap Provincial and An Giang Provincial hospitals, both located in the Mekong Delta region of Vietnam where most of the typhoid patients were admitted, and the Hospital for Tropical Diseases, Ho Chi Minh City, where the malarial patients were admitted. Infection status of each patient was determined by blood culture for the typhoid patients and by microscopic examination of stained thick and thin peripheral-blood slides for the patients suspected of having malaria. Patients with blood cultures positive for *S. typhi* or *S. enterica* serovar Paratyphi A (*S. paratyphi* A) or peripheral blood smears positive for *Plasmodium falciparum* or *Plasmodium vivax* were consented and enrolled in the study. A total of 28 *S. typhi* positive patients and one *S. paratyphi* A positive patient were enrolled in the first phase of the study, and a further nine *S. typhi* and one *S. paratyphi* A positive patient were enrolled in the second phase. Nine patients with uncomplicated malaria caused by *P. falciparum* and one caused by *P. vivax* were enrolled. Sixteen uninfected healthy individuals were also enrolled in the study (one sample each). The typhoid patients were randomly assigned to receive either azithromycin (20 mg/kg/day) or the fluoroquinolone gatifloxacin (10 mg/kg/day) for 7 days as a once oral dose. Uncomplicated malaria patients were treated for three days with Artekin (Holleykin Pharmaceutical Co Ltd). Venous blood (2.5 mL) was collected for total RNA extraction into PaxGene RNA collection tubes (Qiagen) at various times. The PaxGene tubes were stored at 4 °C until RNA was extracted as per the manufacturer's instructions. Purified RNA was shipped on dry ice to Stanford University. CBCs were also taken at various times during treatment and analyzed using an automated Coulter counter in Vietnam.

cDNA Microarrays and Hybridization. Experimental and reference samples (Human Universal RNA reference; Stratagene) were amplified using the MessageAmp II aRNA kit (Ambion) as per the manufacturer's instructions. Four micrograms amplified RNA were labeled through indirect incorporation of Cy5 (experimental) and Cy3 (reference) dyes. The labeled probes were then mixed and hybridized to Stanford Human 42K cDNA arrays manufactured by the Stanford Functional Genomics Facility. Arrays were scanned using a Genepix 4000A laser scanner, and data were extracted with Genepix Pro Software version 5.1 (Axon Instruments). The data were uploaded into the Stanford Microarray Database (SMD) and are freely available (http://genome-www5.stanford.edu/). The data have also been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE7000.

Data Filtering and Scaling. Array features with a regression correlation of <0.6 and signal to background ratio of <2, and <80% good data were excluded. Using the arrayed samples (93/173) for which full clinical parameters were available, genes that varied by at least a standard deviation of 1.2 across the arrays were retrieved, leaving 1,403 genes (unique cDNA features). Data for replicate arrays were averaged using the arithmetic mean leaving a total of 162 arrays for further analysis. This dataset was used for all presented analyses (Dataset S2). Additional details are available in *SI Text*.

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