Figure 1: Gene expression profiles of circulating leukocytes in response to bacterial endotoxin infusion. Samples from eight healthy volunteers were tested at baseline (0 h) and 2, 4, 6, 9 and 24 h after intravenous administration of endotoxin (four subjects) or vehicle (four subjects). a, Significant (false discovery rate of <0.1%) probe sets (5,093) were subjected to K-means clustering into ten bins (0–9). Probe sets for which the abundance was above the mean are shown in red, below the mean are shown in blue, and equivalent to the mean are in white. b, Principal component plot of the significant probe sets at the indicated times after endotoxin administration.

The observed interactome provides a framework for structuring the existing knowledge regarding mammalian biology, and enables a new analytical approach that objectively examines experimental data in the context of known genome-wide interactions in order to identify significant functional modules. This method is applicable to data of high-throughput platforms such as microarray expression profiling, polymorphism analysis and proteomics. Furthermore, the original literature detailing the genetic interactions can be accessed to further examine and verify the findings.

For a better understanding of the temporal response of gene expression in the innate immune system, we constructed a prototypical inflammatory cell containing 292 representative genes and detailing all direct interactions in our database (Fig. 2). Closer inspection of the temporal response reveals the fine structure of dynamic changes in RNA abundance by highlighting the transient and self-limiting nature of this response. As an example, the apparent expression of several secreted proinflammatory cytokines and chemokines (TNFSF2 (TNF), IL1A, IL1B, CXCL1 (Groα), CXCL2 (Gro-β), CCL2 (MCP-1), CXCL9 (Il-8) and CXCL10) reached a maximum 2–4 h after endotoxin administration, consistent with early activation of innate immunity. Subsequently, the expression of several members of the nuclear factor kappa/relA family of transcription factors (NFKB1, NFKB2, RELA and RELB) reached their zenith.

The time period 4–6 h after endotoxin injection seemed critical, as the expression of a number of transcription factors was increased, including both those that initiate and those that limit the innate immune response. In the former group, these included the signal transducer and activators of transcription (STAT) genes, and the cAMP-response element-binding protein (CREB) and CCAAT/enhancer binding protein (CEBP) gene families. Transcription factors limiting the innate immune response included suppressor of cytokine signalling 3 (SOCS3) and IKBK genes. There was also a delay (4–6 h) in increased mRNA abundance of secreted and membrane-associated proteins that limit the inflammatory response, including IL1RAP, IL1R2, IL10 and TNFRSF1A. Together, these data comprehensively document the temporal modulation of genes controlling the innate immune response in a human model that progresses from an acute proinflammatory phase to unencumbered counter-regulation, concluding with full recovery and a normal phenotype.

To further elucidate the global changes during inflammation and subsequent return to homeostasis, we sought to computationally decipher the principal networks involved. The specificity of connections for each gene was calculated, as defined by the percentage of its direct connections to other genes showing significant transcriptional changes. A network pathway was initiated by the gene with the highest specificity of connections, and was propagated according to the descent of the specificity. Individual significant pathways identified by a statistical likelihood calculation (P < 0.0001) were merged to represent the biological processes.

Our global representation of the inflammatory response to endotoxin, shown in Fig. 3a, comprises a network of 1,556 genes and their interactions. This network consists of a subset of 1,214 genes (78%) responsive to in vivo endotoxin administration, and 342 additional, highly interconnected genes. The gestalt of the temporal response to endotoxin is suggested at the level of interconnecting functional modules, which could not be readily extracted from the experimental data alone (Fig. 1). Simultaneous survey and evaluation of the subnetwork regions enables us to identify new endotoxin-responsive modules in addition to the innate immunity network described above. Examples of the diversity in such modules include (a) increased expression of components of the superoxide-producing phagocyte NADPH-oxidase system, a multicomponent enzyme important for host defence; (b) decreased expression of the major histocompatibility (MHC) II complex, consistent with reduced antigen presentation following endotoxin stimulation; (c) decreased expression of the TCP1 ring complex required for folding of cytoskeletal proteins; (d) increased expression in the family of tubulin-A microtubule genes; (e) suppressed expression of several subunits of the anaphase-promoting complex, which has a key role in cell-cycle regulation; and (f) reduced integrin-α and -β chain expression, affecting cell–cell and cell–matrix adhesion (see Supplementary Methods 2, containing Supplementary Fig. 3a–f).

Further, significant decreases in messenger RNA abundance were