Limited Dynamic Range of Immune Response Gene Expression Observed in Healthy Blood Donors Using RT-PCR

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The use of quantitative gene expression analysis for the diagnosis, prognosis, and monitoring of disease requires the ability to distinguish pathophysiological changes from natural variations. To characterize these variations in apparently healthy subjects, quantitative real-time PCR was used to measure various immune response genes in whole blood collected from blood bank donors. In a singletime- point study of 131 donors, of 48 target genes, 43 were consistently expressed and 34 followed approximately log-normal distribution. Most transcripts showed a limited dynamic range of expression across subjects. Specifically, 36 genes had standard deviations (SDs) of 0.44 to 0.79 cycle threshold (C_T) units, corresponding to less than a 3-fold variation in expression. Separately, a longitudinal study of 8 healthy individuals demonstrated a total dynamic range (> 2 standard error units) of 2- to 4-fold in most genes. In contrast, a study of whole blood gene expression in 6 volunteers injected with LPS showed 15 genes changing in expression 10- to 90-fold within 2 to 5 h and returning to within normal range within 21 hours. This work demonstrates that (1) the dynamic range of expression of many immune response genes is limited among healthy subjects; (2) expression levels for most genes analyzed are approximately log-normally distributed; and (3) individuals exposed to an infusion of bacterial endotoxin (lipopolysaccharide), show gene expression profiles that can be readily distinguished from those of a healthy population. These results suggest that normal reference ranges can be established for gene expression assays, providing critical standards for the diagnosis and management of disease.

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