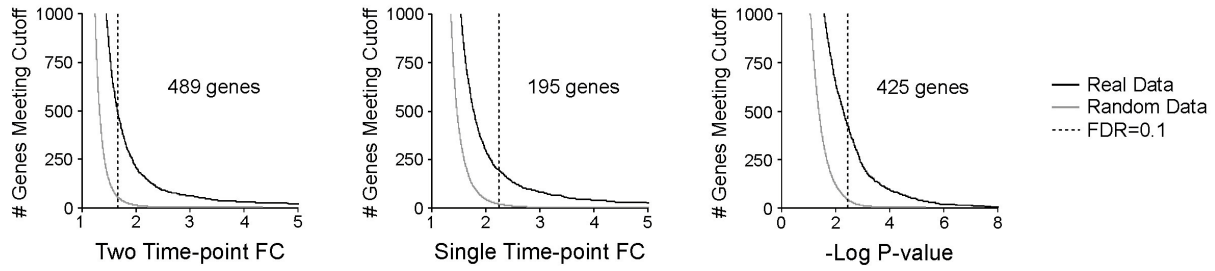
Supplementary Methods

Two-time-point fold-change approach to selecting differentially expressed genes

Common methods of identifying differentially expressed genes in datasets with multiple classes (in our datasets: day 0, day 1, etc.) involve pairwise comparisons with a reference class (e.g. fold-change from day 0) or tests assessing the equivalency of gene expression across classes (e.g. ANOVA) (1). As these approaches do not incorporate the temporal relationship of time-points in our datasets, we considered a modification of the pairwise comparison method. Rather than measuring a gene's fold-change (FC) at each time-point from day 0 and using these values to determine if a gene is differentially expressed, we instead averaged the FC of adjacent time-points. Hence, instead of choosing the greatest of FC1 (FC of day 1 vs. day 0), FC3, FC7, FC12, and FC18, we used the greatest of FC1-3 (average FC of day 1 vs. day 0 and day 3 vs. day 0), FC3-7, FC7-12, and FC12-18. This number was referred to as the two-time-point fold-change (TTFC). We reasoned that true gene expression patterns were likely to involve upregulation or downregulation over at least two time-points and that by averaging adjacent time-points we would dampen false discoveries due to random noise at a single time-point. We compared TTFC to greatest fold-change from day 0 and ANOVA based upon the number of differentially expressed genes that they identified at an estimated false discovery rate (FDR) rate of 0.1. The FDR was estimated by comparing the number of differentially expressed genes identified in an actual dataset to the number in datasets randomly generated from Gaussian distributions. These distributions were created for each gene using the mean expression value across all data points and an aggregate coefficient of variation (i.e. coefficient of variation was calculated for each time-point then averaged across time-points). The results of this comparison are shown below and demonstrate that the TTFC approach yielded more differentially expressed genes than did the greatest single time-point fold change or ANOVA while also providing a measure of the magnitude of the change in gene expression.



The number of genes meeting or exceeding a given test statistic (TTFC, greatest single time-point FC, p-value on a negative logarithmic scale) is plotted for the ankle dataset ("Real Data") and an aggregate of 25 randomly generated datasets ("Random Data"). The number of genes with estimated FDR less than or equal to 0.1 is shown for each test statistic.

Serum-transferred arthritis induction

Arthritis was induced in chemokine and chemokine receptor knockout mice on the B6 background with 150 μl K/BxN serum administered by intraperitoneal injection on days 0 and 2. A higher dose of 200 μl was administered to B6 mice used in the microarray experiments to induce a more synchronous onset of disease, thereby reducing variation in gene expression at each time-point. A lower dose of 75 μl was used for Balb/c mice as this background has enhanced susceptibility to serum-transferred arthritis (2). An early experiment with CXCR2 mice (which are of the Balb/c background) revealed initially increased disease severity in CXCR2-/- mice compared to CXCR2+/- littermates followed by a rapid reduction in disease activity. It was observed that CXCR2-/- mice were frequently smaller in size than their CXCR2+/- littermates and that the smallest mice had the most severe early disease. A weight-based dosing regimen of 2.5 μl/g on days 0 and 2 was adopted and found to equalize disease between the two CXCR2 groups in the early phase. Other strains did not demonstrate a clear variation in weight based on chemokine or chemokine receptor genotype.

References

(1) Grant GR, Manduchi E, Stoeckert CJ Jr. Analysis and management of microarray gene expression data. *Curr Protoc Mol Biol* 2007; Chapter 19:Unit 19.6.

(2) Ji H, Gauguier D, Ohmura K, Gonzalez A, Duchatelle V, Danoy P et al. Genetic influences on the end-stage effector phase of arthritis. *J Exp Med* 2001; 194(3):321-30.