Immunological techniques
The expression of the genome during immune responses
Editorial overview

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Just as many paradigms in immunology are beginning to crystallize, genomic approaches to the immune system promise to stir the field up again. The hallmark of experimental approaches that can be deemed 'genomic' is that they aim at a systematic description of a biological system. By definition, these approaches are observational in nature and not necessarily 'hypothesis-driven'. This feature is actually a virtue, in the right hands, in that these new technologies are highly likely to generate a multitude of new hypotheses owing to their systematic nature. Using genomic techniques, the researcher is not limited to looking at a biological system through the lens of existing paradigms but will frequently be led in new directions by the data. However, it is important to emphasize that data collection on a genomic scale should not be considered an end in itself. Critical thinking and biological insight are especially necessary in both the design and interpretation of genomic experiments due to the sometimes overwhelming volume of data that they produce. Scientists with extensive experience in a particular field of biology are likely to 'see' more in a genomic data set than will scientists from other disciplines. Having said this, genomic data from an experiment with immune cells may take the researcher on intellectual excursions outside of immunology into many unfamiliar realms of biology. This can be both a challenge and a pleasure.

The present section of review articles focuses on one mode of genomic research that is aimed at quantitating the expression of thousands of genes in parallel. These techniques have been developed over the past 4–5 years and rely on an ordered microarray of genes on a solid support. The genes can be represented either as oligonucleotides or as cDNA fragments. mRNA from the cells of interest is used to generate total cDNA probes that are then hybridized to a microarray. The hybridization of the probes to each gene on the microarray is quantitated and these measurements reflect the abundance of an mRNA species within the cell. The measurements of relative gene expression obtained by these techniques have been shown to agree well with results obtained using conventional northern blot or quantitative RT-PCR (reverse-transcriptase polymerase chain reaction) techniques.

With current microarray technology, it is quite possible for one researcher to generate millions of gene expression measurements in a month or two. In this issue, Sherlock (pp 201–205) reviews the computational techniques that can be applied to make sense of this torrent of data. Various analytical tools have been adapted to find patterns in gene expression data. These tools can find those genes that are coordinately expressed in, for instance, particular immune cell subsets or during specific immune responses. The same analytical tools can determine whether two cell populations resemble each other in gene expression. For example, gene expression could be compared between blood cells from a patient with an autoimmune disease and various stages of T and B lymphocyte activation or differentiation to gain insight into the pathobiology of the disease. There is currently no consensus as to which of the various analytical algorithms performs best and the most prudent advice is to simply try each one in order to extract the maximum biological insight from a gene expression data set.

The other articles in this section summarize several initial forays into genomic-scale analysis of gene expression in the immune system. Marrack *et al*. (pp 206–209) review studies of gene expression during T cell activation. As expected, a large number of genes that are regulated during the cell cycle are induced during the activation of T cells through the T cell receptor. Less obvious is that roughly the same number of genes are downregulated during T cell activation as are upregulated. Immunologists have generally paid more attention to the induced class of genes but these studies highlight the need to understand the *raison d'être* of genes that are highly and specifically expressed in resting lymphocytes.

Glynne, Ghandour and Goodnow (pp 210–214) summarize experiments that compare gene expression in resting, activated and anergic B cells. These studies demonstrate that the anergic state has a characteristic gene expression signature that is composed of genes that are not highly expressed in activated B cells. In addition, a large number of genes that are normally induced during B cell activation are not expressed in anergic B cells. These studies thus provide a molecular definition of the anergic state. Interestingly, the anergic gene expression phenotype is distinct from the phenotype of B cells activated in the presence of the immunosuppressant FK506. This drug has a number of unwanted side effects and therefore development of new immunosuppressant drugs in the future might use the gene expression phenotype of B cell anergy as a gold standard. As this example suggests, gene expression profiling will undoubtedly become a mainstay of drug

development, allowing target-directed drug effects to be optimized and off-target drug effects to be minimized.

Manger and Relman (pp 215–218) present an overview of gene expression profiling during immune responses to infectious agents. One hoped-for outcome of such studies is that gene expression profiling will define specific gene expression signatures for each pathogen. These could be used for rapid diagnosis of infectious diseases and might be particularly useful when it is technically difficult or impossible to culture the pathogen. Since most infectious processes initiate an ordered series of host responses, gene expression profiles could be used to define the stage of the host response in individual patients as they present for medical evaluation. Physicians might then use this information to select the therapy most appropriate for each patient.

Alizadeh and Staudt (pp 219–225) summarize gene expression surveys in immune cells that have been conducted jointly by the Staudt laboratory and the laboratory of Pat Brown. By creating a large database of observations from various immune cell populations under a variety of activation conditions, gene expression signatures are emerging that are characteristic of particular immune cell types or states of activation. In addition, the authors summarize experiments in which lymphoid malignancies are subdivided on the basis of gene expression patterns. These experiments demonstrate that gene expression profiling can divide an existing diagnostic category of non-Hodgkin's lymphoma, diffuse large-B-cell lymphoma, into two molecularly and clinically distinct diseases. Each newly defined lymphoma subgroup resembles a distinct stage of B cell differentiation and has a significantly different survival following current therapy.

These reviews provide intimations of the impact of gene expression profiling on basic and clinical immunology in the future. It is easy to imagine that the starting point of many immunological investigations will be a visit to a comprehensive gene expression database. Genes that are highly and specifically expressed in a specific immune subset undergoing a response of interest are logical targets for conventional research using the plentiful and powerful techniques of molecular and cellular immunology. In some cases, gene expression profiling alone will provide direct insights and strong conclusions concerning an immune response. In other instances, however, genomic-scale gene expression analysis will generate plentiful and surprising hypotheses, leading immunologists beyond their current paradigms.