Review
Gene profiling for defining targets for new therapeutics in autoimmune diseases
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Abstract

The identification of novel targets for improved diagnosis and pharmaceutical intervention is of critical importance for better treatment of autoimmune diseases in the future. The possibility to measure levels of gene expression for tens of thousands of genes simultaneously and in a quantitative fashion will greatly enhance our knowledge of genes and pathways involved in disease pathogenesis. Initial studies have focused on the gene expression profiling of homogeneous cell populations. Genomic-scale gene expression profiling has also more recently been applied to tissue samples from patients with immunopathologies. The scope of the present review is to discuss recent progress in this field with respect to the identification of novel target molecules.

Keywords: autoimmune diseases, high-throughput EST sequencing, microarrays, target identification, T lymphocytes

Introduction

Genomic-scale gene expression profiling has an increasing impact on immunology and, in particular, on the characterization of immunological diseases. This profiling technology can reveal the physiology of cells and tissues on an unprecedented scale by quantitating, in parallel, the mRNA levels of tens of thousands of genes [1].

Global gene expression studies rely mainly on two technologies: spotted cDNA microarrays, and high-density oligonucleotide microarrays [2,3] (for reviews of the two technologies, see [4,5]). Microarray experiments generate an amount of data that cannot be handled by simple sorting in spreadsheets or plotting on graphs. Microarray data analysis therefore requires dedicated algorithms and tools [6]. Sophisticated computational tools are available, but it is important to note that a basic understanding of these tools is required for meaningful data analysis. Several recent reports demonstrated the power of the combination of gene expression profiling and dedicated computational analysis tools for improved diagnosis and prognosis of cancer.

Alizadeh *et al*. used a specially designed 'lymphochip' to characterize gene expression patterns of diffuse large B-cell lymphoma, the most common subtype of non-Hodgkin's lymphoma [7]. A hierarchical clustering algorithm was used to group genes on the basis of similarity in the pattern with which their expression varied over all samples [8]. This strategy allowed the authors to separate diffuse large B-cell lymphoma into two previously not recognized subtypes, which had marked differences in patient survival [7]. A more recent study demonstrated that molecular profiling can also have a significant impact on the prediction of the clinical outcome of cancer. van't Veer *et al*. showed that gene expression analysis of breast cancer tissue can predict patients that will develop metastases with higher accuracy than currently used clinical parameters [9].

In the following, I will review several studies that attempt to further the understanding of autoimmune diseases using molecular profiling. I will focus on the gene expression analysis of T lymphocytes, the key players in several inflammatory diseases, and on the microarray analysis of brain tissue from patients with multiple sclerosis (MS).

EAE = experimental autoimmune encephalomyelitis; Fc = crystallizabe fragment; IL = interleukin; MS = multiple sclerosis; NF = nuclear factor; OPN = osteopontin; Tc = CD8⁺ T cells; Th = T helper cell.

Transcript imaging of human and mouse T helper cell subsets

T helper lymphocytes are essential to orchestrate appropriate immune responses to pathogens. To achieve effective immunity, T helper cells differentiate into at least two specialized subsets that direct type 1 and type 2 immune responses [10,11]. Cell-mediated (type 1) immunity is necessary for protection against most intracellular pathogens and, when excessive, can mediate organ-specific autoimmune destruction [12]. This indicates that the development of Th1 cells must be tightly controlled. To learn more about the functional properties of human Th1 and Th2 cells and to identify molecules that could be of interest for pharmacological intervention in chronic inflammatory diseases, we decided to analyze gene expression profiles of human Th1 and Th2 cells. Polyclonal human Th1 and Th2 cells were generated *in vitro* from cord blood leukocytes [13]. To monitor changes of gene expression occurring early in the differentiation process, Th1 and Th2 cells were purified 3 days after stimulation. In this initial study, we used high-density oligonucleotide arrays with the capacity to display transcript levels of 6000 human genes [14]. After analyzing gene expression data from Th1 and Th2 cells derived from two independent donors, we realized that it was very difficult to discriminate between subset-specific and donor-specific changes in gene expression. We therefore decided to analyze gene expression in Th1 and Th2 cells generated from three additional donors and to analyze the dataset using a statistical algorithm (paired *t* test).

The importance of replicate microarray experiments has recently been emphasized in a study addressing the natural differences in mouse gene expression [15]. The authors used a 5406-clone spotted cDNA microarray to quantitate transcript levels in the kidney, the liver, and the testis from each of six normal male C57BL6 mice. Analysis of variance was used to compare the variance across the six mice with the variance among four replicate experiments performed for each tissue. The striking finding was that statistically significant variable gene expression was detected for 3.3%, 1.9%, and 0.8% of the genes in the kidney, the testis and the liver, respectively [15]. Importantly, many of the transcripts that were found most variable were immune-modulated genes, stress-induced genes, and hormonally regulated genes. This finding may raise some doubt about the validity of the data reported in several published microarray studies performed with only one or two replicate experiments. Pritchard *et al.* further point out that genetically diverse populations such as humans are very likely to show an even greater variability in gene expression than inbred mice [15]. This suggests that a meaningful interpretation of global gene expression in humans will require many replicate experiments and/or an extensive characterization of normal variability.

To exert their functions, type 1 and type 2 T lymphocytes have to home into different sites. We reported an increased expression of mRNA for fucosyltransferase VII, which codes for an enzyme that mediates the fucosylation of selectin ligands on the surface of T cells [14]. This fucosylation is required for the first step of lymphocyte adhesion to endothelial cells ('rolling'). Recent *in vivo* observations have validated the biological relevance of this finding. Fucosyltransferase VII was in fact found to be upregulated on T cells infiltrating the inflamed joints of patients affected by either rheumatoid arthritis [14] or juvenile idiopathic arthritis [16]. In both diseases, the T cells infiltrating the synovium have a clear Th1 phenotype.

In a subsequent study, Chtanova *et al.* used high-density oligonucleotide microarrays to analyze gene expression in murine CD4$^+$ Th1 and Th2 cells, as well as CD8$^+$ type 1 and type 2 T cells (Tc1 and Tc2) [17]. In contrast to our study where Th1-overexpressed genes predominated [14], Chtanova *et al.* identified more type 2-biased genes [17]. It is important to note that different protocols were used to generate polarized T-cell subsets in the two studies. Chtanova *et al.* stimulated purified naïve mouse CD4$^+$ and CD8$^+$ T cells with anti-CD3/CD28 antibodies, IL-2 and IL-6 plus the polarizing cytokine cocktail. Cells were cultured for 7 days and then restimulated for 24 hours with anti-CD3 before extracting RNA. A previous report has demonstrated that IL-6 is able to polarize naïve CD4$^+$ T cells into Th2 cells by inducing the initial production of IL-4 in CD4$^+$ T cells [18]. In addition, it has been shown that IL-6 inhibits Th1 differentiation in an IL-4-independent manner through the induction of SOCS1 [19]. The addition of IL-6 to the cultures could therefore be a possible explanation for the Th2-bias observed in that study [17]. As expected, CD4$^+$ and CD8$^+$ T-cell subsets expressed their signature cytokines. In addition, Chtanova *et al.* found two members of the tumor necrosis factor receptor-associated factor family to be differentially expressed in type 1 and type 2 cells. TRAF4 was expressed at a higher level in type 1 cells while TRAF5 was preferentially expressed in type 2 cells. Members of this family serve as adapter proteins that mediate cytokine signaling; in particular, they seem to play a role in tumor necrosis factor and Toll/IL-1 signaling, resulting in activation of transcription factors NF-kB and activator protein 1. Much work clearly remains to be done to address the biological relevance of these findings.

Together, these results demonstrate the impact of large-scale gene expression profiling on the analysis of distinct T lymphocyte populations. The analyses of the expression of 6000 genes in human Th1 and Th2 cells and of 11,000 genes in mouse Th1, Tc1, Th2 and Tc2 cells were first attempts to understand the molecular mechanisms underlying the functional diversity of distinct T-cell subsets. The finding that genes regulating key steps in the process of

leukocyte extravasation into inflamed tissues are coregulated in human T-cell subsets sheds light on the importance of the correct positioning of T cells within tissues to eliminate pathogens. Moreover, autoimmune diseases are associated with the presence of specialized subsets of T helper cells at the site of inflammation. Knowledge of the genetic program that controls the differentiation and functional properties of Th1 cells versus Th2 cells may therefore increase the understanding of inflammatory diseases.

Gene expression analysis of MS lesions

MS is characterized by the infiltration of T cells and other immune cells into the white matter of the central nervous system. The resulting inflammation and subsequent destruction of myelin cause progressive paralysis and a variety of other neurological symptoms [20,21]. The diversity of symptoms and of the disease course complicates diagnosis and the understanding of the pathogenesis of MS. Much of our current knowledge of MS stems from the analysis of a mouse model of MS. Experimental autoimmune encephalomyelitis (EAE) is a T-cell-mediated autoimmune disease with striking clinical and histopathological similarities to MS. Unfortunately, EAE has failed several times in predicting the efficacy of new therapeutics [22].

In an attempt to identify genes that contribute to lesion pathology, several groups have analyzed gene expression in brain tissue obtained postmortem from MS patients and compared it with tissue samples from individuals without MS [23–26]. Given the heterogeneity of the disease, and also the small numbers of patients that have so far been analyzed, it is not surprising that the results are only partially overlapping. Yet the results are encouraging since they provide the first hints of novel targets for anti-inflammatory treatment of MS. Whitney *et al*. screened cDNA microarrays with ^{33}P-dCTP-labeled cDNAs generated from MS lesions and normal white matter [24]. Among other genes, they identified arachidonate 5-lipoxygenase, a key enzyme in the biosynthetic pathway of leukotrienes, to be overexpressed in MS. Immunohistochemistry confirmed this finding, and 5-lipoxygenase staining was mainly detected in cells resembling macrophages and monocytes. The authors point out that 5-lipoxygenase upregulation is not unique to MS, but is also found in other central nervous system diseases where macrophages and monocytes are activated, such as cerebral infarction and meningitis.

Using large-scale sequence analysis of cDNA libraries generated from brain tissue of MS patients, Chabas *et al*. identified a number of cDNAs that were over-represented in the MS libraries when compared with libraries constructed from control brain tissue [25]. Among these was osteopontin (OPN), a cytokine with pleiotropic functions including roles in inflammation and immunity to infectious diseases. Previous work had attributed a key role to OPN in the regulation of Th1-mediated immune responses by its effects on IL-12 and IL-10 production [27]. Immunohistochemistry revealed increased expression of OPN adjacent to lesions observed in the brain tissue of MS patients, as well as in rodents that develop an experimental form of the disease [25]. The induction and severity of EAE and the expression of inflammatory cytokines by T cells were greatly reduced in mice lacking the OPN gene [25]. Increased expression of OPN has also been found in inflamed joints of rheumatoid arthritis patients [28]. These observations make OPN an attractive target for anti-inflammatory therapy of MS, and possibly rheumatoid arthritis.

Lock *et al*. more recently compared gene expression in two distinct types of neuronal lesions: acute active lesions with inflammation, and chronic silent lesions without inflammation [26]. Granulocyte colony-stimulating factor was found highly expressed in acute lesions, but not in silent lesions. In contrast, transcripts encoding the IgG Fc receptor I were found overexpressed in silent lesions. The importance of these two molecules in the pathogenesis of MS was assessed in the EAE model. Treatment with granulocyte colony-stimulating factor before the onset of EAE decreased the severity of the acute phase but had no effect on the late stages of the disease. The role of Fc receptors in EAE was studied by comparing the severity of disease in Fc receptor-deficient and wild-type mice. Acute disease was less severe and chronic disease was absent in Fc receptor knockout mice [26]. This study by Lock *et al*. is the first example demonstrating the value of microarray technology for the analysis of distinct disease states of MS. Together with additional studies that are sure to follow, it may pave the way for an improved diagnosis and tailored treatment.

Competing interests

None declared.

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