Review

Transcription profiling of rheumatic diseases

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Abstract

Rheumatic diseases are a diverse group of disorders. Most of these diseases are heterogeneous in nature and show varying responsiveness to treatment. Because our understanding of the molecular complexity of rheumatic diseases is incomplete and criteria for categorization are limited, we mainly refer to them in terms of group averages. The advent of DNA microarray technology has provided a powerful tool to gain insight into the molecular complexity of these diseases; this technology facilitates open-ended survey to identify comprehensively the genes and biological pathways that are associated with clinically defined conditions. During the past decade, encouraging results have been generated in the molecular description of complex rheumatic diseases, such as rheumatoid arthritis, systemic lupus erythematosus, Sjögren syndrome and systemic sclerosis. Here, we describe developments in genomics research during the past decade that have contributed to our knowledge of pathogenesis, and to the identification of biomarkers for diagnosis, patient stratification and prognostication.

Introduction

Rheumatic diseases are a diverse group of disorders that involve the musculoskeletal system. Generally, the cause of these disorders is unknown and their pathogenesis poorly understood. Although these diseases involve the synovial joints, they also have many systemic features. For example, rheumatoid arthritis (RA) is a chronic inflammatory disease that - in addition to its systemic manifestations - primarily affects the joints. On the other hand, systemic lupus erythematosus (SLE) is a typical systemic disease with secondary involvement of multiple organs.

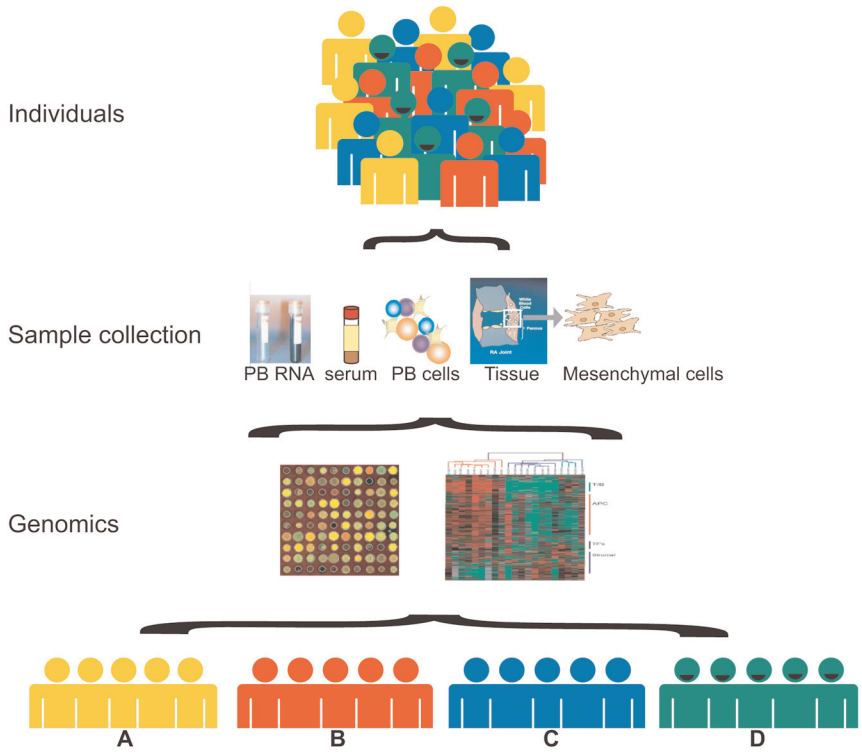
The aetiology of the rheumatic diseases is largely unknown. Clinical and laboratory observations suggest an immune-mediated attack directed against self-antigens in a number of these diseases. This is highlighted by the association between many of these diseases and human leucocyte antigen (HLA) loci, and by the expression of autoantibodies such as antibodies against nuclear components in SLE, Sjögren's syndrome (SS) and systemic sclerosis (SSc), and rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) in RA. That these diseases have an immune-mediated background is corroborated by the ameliorative effect of immunosuppressive therapies.

Most of the rheumatic disorders are heterogeneous diseases with a clinical spectrum that ranges from mild to severe, and variability in secondary organ system involvement (for example, heart failure). The heterogeneous nature is reflected by variation in responsiveness to virtually all treatment modalities. The heterogeneity probably has its origin in the mutifactorial nature of the diseases, in which it is likely that specific combinations of environmental factor(s) and varying polygenic background influence not only susceptibility but also severity and disease outcome. The fact that we generally refer to these diseases in terms of group averages may hamper progress in our understanding of pathogenic mechanisms, genetic background and the efficacy of treatment in subsets of patients. Unfortunately, our understanding of the molecular complexity of these disorders is incomplete, and criteria for subtyping patients (for example, in order to select those patients who will benefit from a specific treatment) are currently lacking.

By definition, nearly every aspect of a disease phenotype should be represented in the pattern of genes and proteins that are expressed in the patient. This molecular signature typically represents the contributions made by and interactions between specific factors and distinct cells that are associated with disease characteristics and subtypes,

ACPA = anti-citrullinated protein antibody; DAS28 = Disease Activity Score using 28 joint counts; DC = dendritic cell; FLS = fibroblast-like synoviocyte; HLA = human leucocyte antigen; IFN = interferon; IL = interleukin; MMP = matrix metalloproteinase; OA = osteoarthritis; PBMC = peripheral blood mononuclear cell; RA = rheumatoid arthritis; RF = rheumatoid factor; SLE = systemic lupus erythematosus; SoJIA = systemic onset juvenile idiopathic arthritis; SS = Sjögren's syndrome; SSc = systemic sclerosis; STAT = signal transducer and activator of transcription; TNF = tumour necrosis factor.

Figure 1



Schematic outline for genomics in rheumatic diseases. Patients with rheumatic diseases exhibited striking heterogeneity, based on clinical, biological and molecular criteria. Categorization of patients is expected to be of the utmost importance for decision making in clinical practice. Application of high-throughput screening technologies such as genomics allows us to characterize patients based on their molecular profile. The procedure starts with collecting different types of material such as serum, peripheral blood (PB) cells, RNA from blood (using, for example, Paxgene tubes), tissue biopsies and isolated mesenchymal cells from the same patients. Gene expression profiles of this material can be determined using genomics technology. When associated with clinical readouts, we could select the clinically useful molecular markers and apply these in routine clinical practice. In addition, these data may help to elucidate the distinct pathological mechanisms that are at play, potentially explaining the inter-patient variation in clinical presentation, disease progression and treatment response. Ultimately, knowledge of the different pathogenic mechanisms may help us to identify new drug targets for selected patient subgroups.

and thus it defines the samples' unique biology. A very powerful way to gain insight into the molecular complexity of cells and tissues has arisen with the advent of DNA microarray technology, which facilitates open-ended survey to identify comprehensively the fraction of genes that are differentially expressed among patients with clinically defined disease. The differentially expressed gene sets may then be used to determine the involvement of a particular biological pathway in disease, and may serve to identify disease classifiers for diagnosis, prognosis, prediction analysis and patient stratification (Figure 1). Hence, the identification of differentially expressed genes and proteins may provide a comprehensive molecular description of disease heterogeneity that can reveal clinically relevant biomarkers.

Initially, several pitfalls were experienced in the use this multistage and relatively expensive technology, which depends critically on perfectly standardized conditions. First of all, handling of blood and tissue samples may differ considerably between laboratories. Usage of different platforms and the lack of standardized procedures limit consistency of study results. For example, variability in the amount and quality of starting RNA; amplification and labelling strategies employed; and dyes, probe sequences and hybridization conditions may all influence the sensitivity, reproducibility and compatibility of datasets. In addition, lack of standardized approaches to normalization and data analysis can influence the outcome of research. Moreover, the high costs associated with use of this technology can impede ability to conduct well powered studies. Therefore, verification of results became an essential step in microarray studies. In order to establish quality criteria for performing and publishing microarray studies, standards for microarray experiments and data analysis were created [1].

Now, after a decade of technical and analytical improvement, the technology and algorithms for data analysis have been shown to be robust and reproducible across properly

designed and controlled experiments, and different research groups. The Paxgene (PreAnalytix, GmbH, Germany) whole blood isolation system, which directly lyses cells and stabilizes the RNA in the aspiration tube, excludes *ex vivo* processing artifacts and forms a crucial step in the standardization of procedures. Although this approach does not *a priori* account for cell subset differences, the gene expression data generated may provide important information from which extrapolations regarding relative distributions and phenotypic differences can be made. Careful standardization is still required for cell subsets and tissues that are obtained via *ex vivo* manipulation.

Encouraging results have been generated with the use of microarray technology in the identification of predictors for disease outcome and metastasis, and underlying pathways in breast cancer and lymphoma [2,3]. The perceived importance and support for large-scale and well powered gene expression profiling studies in oncology have been considerable, and this may account for the success in this area. However, transcriptomics approaches have lagged behind in the field of rheumatology. We believe that collaborative efforts between groups to increase samples size in order to create high-power studies are of critical importance to move the field forward. Equally important is implementation of standardized sample processing procedures and use of the technology, and data analysis and algorithms between different sites. Moreover, to maximize the usage of information from different laboratories, full and open access to genomics data is essential.

Here, we describe novel developments in genomics research conducted to identify biological pathways that contribute to disease and biomarkers for diagnosis, prognosis and patient stratification in rheumatic diseases. An overview of the genomics studies in rheumatic diseases discussed in this review is provided in Table 1. The findings of these studies will also improve our understanding of the underlying biology of the diseases and refine their clinical management. Ultimately, this information may help clinicians to optimise treatment by identifying subgroups of patients who are most likely to respond.

Gene expression profiling in affected target tissues

One of the first studies of gene expression profiles in rheumatic diseases was conducted in RA biopsy tissues, and used a combination of subtractive hybridization and high-density cDNA arrays [4]. This study identified increased expression of genes involved in chronic inflammation, such as immunoglobulins and HLA-DR, in RA synovium as compared with normal synovium. However, because the investigators used pooled tissues from three patients with RA and three healthy control individuals, it was not possible to consider heterogeneity in RA.

Devauchelle and coworkers [5] studied differences in gene expression profiles between the synovial tissue of patients with RA ($n = 5$) and those with osteoarthritis (OA; $n = 10$). A total of 63 (48 known genes and 15 expressed sequence tags) were differentially regulated between RA and OA samples.

Comparative analysis of synovial biopsy tissue from RA, OA and SLE patients with active disease partly confirmed and extended previous observations that distinct diseases were characterized by distinct molecular signatures [6]. Whereas genes involved in T-cell and B-cell regulation were upregulated in RA tissues, in SLE tissues IFN-induced genes were more highly expressed and genes involved in homeostasis of the extracellular matrix were downregulated. Histological analysis confirmed that in RA the synovium was characterized by greater numbers of infiltrating T cells and B cells as compared with SLE and OA synovium.

Molecular tissue markers for heterogeneity within rheumatic diseases

Recently, Lindberg and coworkers [7] studied variability in gene expression levels in synovial tissues within and between RA patients. This study demonstrated that different arthroscopic biopsies taken from one joint yield gene expression signatures that are more similar within the joint of one patient than between patients.

A large-scale gene expression profiling study of synovial tissues from patients with erosive RA revealed considerable heterogeneity between different patients [8,9]. A systematic characterization of the differentially expressed genes highlighted the existence of at least two molecularly distinct forms of RA tissues. One group exhibited abundant expression of clusters of genes indicative of ongoing inflammation and involvement of the adaptive immune response. This subgroup was referred to as the RA high inflammation group. The increased expression of immunoglobulin genes was shown to be one of the main discriminators between high and low inflammatory tissues. Further analyses of the genes involved in the high inflammation tissues provided evidence for a prominent role for genes indicative of an activated IFN/signal transducer and activator of transcription (STAT)-1 pathway. These findings were confirmed at the protein level [10,11]. From the 16 genes that overlapped between the microarray used in this study and the one used by Devauchelle and colleagues [5], seven had comparable gene expression profiles (*TIMP2*, *PDGFRA*, *GBP1*, *Fos*, *CTSL*, *TUBB* and *BHLHB2*). Two of these (*GBP1* and *CTSL*) are known to be regulated by type I IFN.

The expression profiles of the second group of RA tissues were reminiscent of those of tissues from patients with OA. These profiles exhibited a low inflammatory gene expression signature and increased expression of genes involved in tissue remodelling activity, which is associated with fibroblast dedifferentiation. In contrast to the high inflammation tissues, these tissues had increased levels of matrix metalloproteinase (MMP)11 and MMP13 expression, and low expression levels of MMP1 and MMP3 [9].

Table 1

Genomics studies in rheumatic diseases

Disease	Tissue	Number of samples	Approximate number of genes on array	Comparison	Results	Reference
RA	Synovium	13 RA	16,164	Intra- and interindividual patients	Gene expression differences between patients are greater than between biopsies obtained from the same joint	[7]
RA	Synovium	5 RA and 10 OA	5,760	RA versus OA	Genes differentially expressed between RA and OA	[5]
RA	Synovium	21 RA and 9 OA	11,500 and 18,000	Within RA and versus OA	Evidence for the existence of multiple pathways of tissue destruction and repair	[8,9]
RA	Synovium	12 early and 4 late	23,040	Early versus longstanding RA	Early RA fell into two groups based on differences in genes critical for proliferative inflammation	[16]
RA	Synovium	10 RA	30,000 cDNA spots	Before versus after about 9 weeks of infliximab	Genes specifically changed in patients who have a good response to infliximab treatment.	[53]
RA	Synovium	18 RA	18,000	Responders versus nonresponders to infliximab treatment	Patients with high expression levels of genes involved in tissue inflammation before treatment are more likely to benefit from Infliximab therapy.	[54]
RA	Synovium	12 RA	11,500 and 18,000	Within RA	Identification of IL-7 signalling pathway in tissues characterized by lymphoid neogenesis	[15]
RA	FLS	19 RA	18,000	Within RA	Heterogeneity between synovial tissues is reflected in FLSs	[27]
RA	FLS	2 RA	12,600	Resting versus TNF-α or IL-1β 4-hour stimulated cells	Identification of TNF-α and IL-1β regulated genes in RA FLSs	[26]
RA	FLS	5 RA and 5 HC	588	RA versus HC	Over-expression of genes responsible for tumor-like growth in RA FLSs	[24]
RA	Whole blood	35 RA and 15 HC	18,000	Within RA and versus HC	Assignment of a type I IFN signature in a subpopulation of patients	[39,40]
RA	PBMC	19	4,300	Early versus longstanding RA	Gene signature in early disease overlaps with normal response to virus	[38]
RA	PBMC	29 RA and 21 HC	12,626	RA versus HC	Monocyte associated gene signature increased in RA	[36]
RA	PBMC	33	10,000	Before versus 3 months after infliximab	Gene expression profile correlating with treatment response	[51]
RA	PBMC	8 RF^+, 6 RF^- and 7 HC	10,000	RF^+ versus RF^- and versus HC	No genes differentially expressed between RF^+ and RF^- RA patients. Increased expression of immunoinflammatory response genes, especially those related to phagocytic functions, in RA	[35]
RA	PBMC	19	18,500	Before versus 72 hours after etanercept	Gene pairs and triplets predictive for response to treatment at an early stage of treatment	[52]
RA	B-cells	8 RA versus 8 HC	21,329	RA versus HC	Dysregulated B-cell biology in RA is multifaceted	[37]
SSc	Skin biopsies	24 SSc and 6 HC	33,000	Within SSc and versus HC	A 177-gene signature associated with severity of skin disease in diffuse SSc	[14]

Continued overleaf

Table 1 (continued)

Disease	Tissue	Number of samples	Approximate number of genes on array	Comparison	Results	Reference
SSc	Dermal fibroblasts	15 SSc twins and 5 HC	16,659	Lesional versus nonlesional and versus twin pair and versus HC	At the molecular level, concordance for the SSc fibroblast phenotype is high in MZ twins and greatly exceeds that in DZ twins	[31]
SSc	Dermal non-lesional fibroblasts	21 SSc and 18 HC	16,659	Lesional versus nonlesional and versus HC	Fibroblasts from nonlesional sites in SSc have detectable abnormalities in a variety of cellular processes, including ECM formation, fibrillogenesis, angiogenesis and complement activation	[30]
SSc	PBMC	18 SSc and 18 HC	16,659	SSc versus HC	Differentially regulated expression of genes involved in IFN and vasculopathy	[42]
SSc	PBMC	9 early diffuse SSc and 4 HC	38,500	SSc versus HC	Type I IFN induced Siglec-1 is increased on circulating SSc $CD14^+$ monocytes	[43]
SS	Minor salivary glands	10 SS and 10 HC	6,803	SS versus HC	Increased expression of genes involved in chronic inflammation and type I IFN	[13]
SS	Minor salivary glands	7 SS and 7 HC	7,261	SS versus HC	Activation of IFN pathways in SS	[17]
SS	Whole saliva	10 SS and 8 HC	38,500	SS versus HC	Activation of IFN pathway in SS	[18]
SLE	Synovium	6 SLE, 7 RA and 6 OA	38,500	SLE versus RA versus OA	The different diseases were characterized by distinct molecular signatures. Upregulation of IFN-induced genes and downregulation of genes involved in ECM homeostasis in SLE	[6]
SLE	Glomeruli	12 SLE and 4 HC	3,602 and 4,030	SLE versus HC and within SLE	Characterization of heterogeneity in the molecular pathogenesis of lupus nephritis	[12]
Paediatric SLE	PBMC	30 SLE, 12 JCA and 9 controls	12,626	SLE versus JCA versus controls	IFN signature in the majority of SLE patients and upregulation of granulocyte specific transcripts	[32]
SLE	PBMC	48 SLE and 42 HC	10,260	Within SLE and versus HC	About half of the patients studied exhibited dysregulated expression of genes in the IFN pathway associated with more severe disease	[33]
SLE	Whole blood	269 patients	256	Within SLE	Categorization of SLE patients into two groups based on a high or low IFN signature. Disease activity correlates with the high IFN signature	[34]
Paediatric SoJIA	PBMC	44 SoJIA, 94 infected patients, 38 SLE, 6 PAPA and 39 healthy controls	17,454	SoJIA versus controls	A SoJIA-specific gene signature containing 88 genes. Blood transcriptional patterns in the systemic phase of SoJIA are more similar to those of patients with infections than to those of SoJIA patients in a later arthritic stage of disease	[45]
Paediatric SoJIA	PBMC	8 untreated and 5 infliximab treated SoJIA	17,454	Treated versus untreated patients	Increased expression of type I IFN regulated genes in the anti-TNF treated SoJIA patients, suggesting cross-regulation between TNF and type I IFN	[55]
Autoimmune diseases	PBMC	20 RA, 24 SLE, 5 type I diabetes, 4 MS and 9 HC	4,329	Between autoimmune disease	Overlapping gene expression profiles in RA, SLE, type I diabetes and MS, which is distinct from a normal immune response profile	[48,58,59]
RA, SLE	Whole blood	6 HC, 4 RA, 4 SLE and 5 family members	4,000	RA versus SLE versus HC versus family	Shared autoimmune gene expression signature in patients and unaffected first-degree relatives	[47]

DZ, dizygotic twin; ECM, extracellular matrix; FLS, fibroblast-like synoviocyte; HC, healthy control individuals; OA, osteoarthritis; IFN, interferon; IL, interleukin; JCA, juvenile chronic arthritis; MS, multiple sclerosis; MZ, monozygotic; PAPA syndrome, a familial autoinflammatory disease that causes pyogenic sterile arthritis, pyoderma gangrenosum and acne; PBMC, peripheral blood mononuclear cell; RA, rheumatoid arthritis; RF, rheumatoid factor; SLE, systemic lupus erythematosus; SoJIA, systemic onset juvenile idiopathic arthritis; SS, Sjögren's syndrome; SSc, scleroderma; TNF, tumour necrosis factor.

Histological analyses revealed that the differences observed in global gene expression between the different groups of patients are related to differences in cell distribution. Tissues that contain germinal centre-like structures were selectively found among the high inflammation tissues. The increased immunoglobulin transcript expression is in accordance with the presence of B cells and/or plasma cells, and may reflect local production of antibodies. Increased immunoglobulin transcripts were also found in target tissues of other rheumatic diseases such as SLE [12], SS [13] and SSc [14]. Germinal centre-containing tissues in RA also exhibited enhanced expression of the chemokines C-X-C chemokine ligand-12 and C-C chemokine ligand-19 and the associated receptors C-X-C chemokine receptor-4 and C-X-C chemokine receptor-5, which are important for the attraction of T cells, B cells and dendritic cells. Pathway analysis revealed increased expression of genes involved in Janus kinase/STAT signalling, T-cell and B-cell specific pathways, Fc receptor type I signalling in mast cells, and IL-7 signal transduction in the tissues with ectopic lymphoid follicles, accompanied by increased expression of IL-7 receptor α, IL-2 receptor γ chains and IL-7. Protein expression of IL-7 in RA tissues was localized within fibroblast-like synoviocytes, macrophages and blood vessels, and was co-localized with extracellular matrix structures around the B-cell follicles. These findings indicate that activation of the IL-7 pathway may play an important role in lymphoid neogenesis, analogous to its role in the development of normal lymphoid tissue [15]. Tissues with a diffuse type of infiltrate exhibited a profile that indicated repression of angiogenesis and increased extracellular matrix remodelling.

Tsubaki and colleagues [16] demonstrated that tissue heterogeneity within RA can already be observed in the early phase of RA. In this study, gene expression profiles were analyzed from synovial lining tissues from 12 patients with early RA (duration <1 year after diagnosis) and four with longstanding RA (duration >3 years after diagnosis). As seen in the previous study using biopsies from longstanding RA patients, the early RA patients could be divided into at least two different groups based on their gene expression profiles.

A study conducted in minor salivary gland tissue from 10 patients with primary SS and 10 healthy control individuals identified 200 genes that were differentially expressed [13]. Clear upregulation of IFN-inducible genes (*ISGF3G*, *IFIT3*, *G1P2* and *IRF1*) was identified, besides increased expression of genes related to lymphocyte development and activation, and antigen processing and signal transduction. Other studies confirmed that genes in the IFN pathway were upregulated in salivary glands of SS patients [17,18].

Upregulated IFN-induced gene expression has also been reported in affected skin of SSc patients [19]. In addition, Milano and coworkers [14] described distinct patterns of gene expression profiles in skin tissues when patients were grouped into those with diffuse SSc and those with limited SSc. Moreover, these data provided evidence for the existence of three different subgroups of patients with SSc: one in those with diffuse SSc and two among those with limited SSc.

Two main subgroups of lupus nephritis biopsies were identified based on clustering of genes with the highest interbiopsy variance [12]. One patient subgroup was characterized by high expression of fibrosis-related genes in the absence of an IFN signature. The other subgroup had high expression of IFN signature genes but low expression of the fibrosis cluster. The clinical features of the patients were not significantly different, although the fibrosis subgroup tended to have higher indices of activity (acute, reversible damage) and chronicity (irreversible damage), whereas the IFN subgroup generally had lower activity/chronicity indices. These results hint at a molecular and biological explanation for severity of renal injury.

Overall, tissue profiling in rheumatic diseases has led to an increase in our understanding of disease pathogenesis. In particular, an IFN signature was observed in target tissues of subsets of patients with RA, SLE, SS and SSc. This provides insights that will facilitate assessment of disease activity and identification of therapeutic targets. Moreover, this information will provide a basis for categorization of patients with rheumatic diseases.

Gene expression in mesenchymal cells derived from affected target tissues

Fibroblasts are ubiquitous mesenchymal cells that play important roles in organ development, inflammation, wound healing, fibrosis and pathology [20]. In chronic inflammation, fibroblasts are considered sentinel cells that contribute to leucocyte migration and local immune response through the production of various immune modulators [21]. These observations suggest that these fibroblasts may acquire the capacity to modulate the immune response [22,23].

Fibroblast-like synoviocytes (FLSs) are major players in joint destruction in RA. One of the first gene expression profile analyses of FLSs revealed over-expression of genes responsible for tumour-like growth of rheumatoid synovium [24]. In this study a cDNA array membrane containing 588 cDNA fragments of known cancer-related genes was used to compare the gene expression profiles of FLSs from five patients with RA with those of five traumatic control patients. Increased expression levels were found for *PDGFR*α, *PAI-1* and *SDF1A* in FLSs derived from rheumatoid synovium when compared with normal FLSs. Because the sample size was very small in this study, heterogeneity between FLSs derived from different RA patients was not considered. Other investigators studied the influence of tumour necrosis factor (TNF) on FLSs [25,26]. TNF has been shown to be of primary importance in the pathogenesis of chronic inflammatory

diseases. These studies are instrumental in defining TNF-α response signatures for application in pharmacology studies to monitor the effects of TNF blockade.

We recently profiled FLSs derived from 19 RA patients using microarrays with a complexity of 24,000 cDNA elements. Correlation studies of paired synovial tissue and FLS clustering revealed that heterogeneity at the synovial tissue level is associated with a specific phenotypic characteristic of the cultured resident FLSs [27]. The high inflammation tissues were associated with an FLS subtype that exhibits similarity with so-called myofibroblasts. The myofibroblast is a specialized fibroblast that has acquired the capacity to express α-smooth muscle actin, an actin isoform that is typical of vascular smooth muscle cells. It is now well accepted that the myofibroblast is a key cell for connective tissue remodelling and contributes to cell infiltration. These cells are characterized by a markedly increased expression of genes that represent the transforming growth factor (TGF)-β response programme. Among these response genes were *SMA*, *SERPINE1*, *COL4A1* (type IV collagen-α chain), *IER3* (immediate early response 3), *TAGLN* (transgelin) and the gene encoding activin A, which is a potential agonist for the induction of the TGF-β response programme. Similar cells were recently identified in the human $TNF^{+/-}$ transgenic mouse model of arthritis [28]. Studies in the field of oncology indicate that myofibroblasts present in tumours play a crucial role in angiogenesis through the production of extracellular matrix proteins, chemokines and growth factors. Hence, it is hypothesized that myofibroblast-like synoviocytes in RA synovial tissue contribute to angiogenesis.

These data support the notion that cellular variation between target tissues is reflected in the stromal cells, and provide evidence for a link between an increased myofibroblast-like phenotype and high inflammation in the target tissue.

Genes characteristically expressed in fibroblasts are differentially expressed between SSc and normal tissue biopsies [29]. Detectable abnormalities in the expression of genes involved extracellular matrix formation, fibrillogenesis, complement activation and angiogenesis are also present in dermal fibroblasts cultured from nonlesional skin of SSc patients [30]. No significant differences in gene expression levels were observed between lesional and nonlesional fibroblasts [31]. The finding that fibroblasts from discordant monozygotic SSc twin pairs were not significantly different indicates that there is a strong genetic predisposition to the SSc phenotype [31].

Gene expression in peripheral blood cells

Although the gene expression analysis of tissue samples of affected organs offers insights into the genes that are instrumental in patient stratification and primarily involved in disease activity and pathogenesis, it is not feasible to use this approach to study large cohorts of patients. Because of the systemic nature of a number of rheumatic diseases and the communication between the systemic and organ-specific compartments, we and others also have studied whole blood and/or peripheral blood mononuclear cells (PBMCs) to obtain disease-related gene expression profiles. The peripheral blood may not have direct implications for our understanding of disease pathogenesis, but it is especially suitable for analyzing gene expression profiles that can be used as biomarkers to permit improved diagnosis and individualized therapy.

Gene expression profiling in the peripheral blood of patients with SLE revealed the presence of an IFN signature in approximately half of the patients studied [32-34]. This signature included well known IFN-regulated genes (for example, the anti-viral *MX1* [myxovirus {influenza virus} resistance 1, interferon-inducible protein p78 {mouse}]) as well as additional IFN response genes. The group of patients carrying the IFN signature had a significant higher frequency of certain severe manifestations of disease (renal, central nervous system and haematological involvement) as compared with those who did not. Furthermore, the expression of these genes was significantly correlated with the number of American College of Rheumatology criteria for SLE. Pascual and colleagues [32] also noted that IFN genes were among those most highly correlated with the Systemic Lupus Erythematosus Disease Activity Index. The same molecular signature is found in SLE synovial tissue [6]. The imbalance between IFN molecules and other molecules in SLE synovial tissue might be of interest pathophysiologically during the course of SLE arthritis.

RA has systemic manifestations, and a number of investigators have studied gene expression levels in peripheral blood cells to address the issue of whether disease characteristics correlate with gene expression levels in peripheral blood cells. Bovin and colleagues [35] studied the gene expression profiles of PBMCs in RA patients ($n = 14$; seven RF positive and seven RF negative) and healthy control individuals ($n = 7$) using DNA microarrays. Using two independent mathematical methods, 25 genes were selected that discriminated between RA patients and healthy control individuals. These genes reflected changes in the immune/inflammatory responses in RA patients, and among these were the genes encoding the calcium-binding proteins S100A8 and S100A12. No significant differences between RF-positive and RF-negative RA were observed.

Batliwalla and colleagues [36] studied gene expression differences between PBMCs from RA patients ($n = 29$) and those from healthy control individuals ($n = 21$). They identified 81 differentially expressed genes, including those encoding glutaminyl cyclase, IL-1 receptor antagonist, S100A12 and Grb2-associated binding protein, as the main discriminators. This profile was associated with increased monocyte count in RA. Szodoray and colleagues [37] studied gene expression differences in peripheral blood B cells from eight RA patients

and eight healthy control individuals. A total of 305 genes were upregulated, whereas 231 genes were downregulated in RA B cells. However, the investigators did not address heterogeneity in peripheral blood gene expression profiles among patients with RA.

Olsen and colleagues [38] studied gene expression levels in PBMCs in order to identify differentially expressed genes between early (disease duration <2 years) and established RA (with an average disease duration of 10 years). Out of 4,300 genes analyzed, nine were expressed at threefold higher levels in the early RA group, including the genes encoding colony stimulating factor 3 receptor, cleavage stimulation factor, and TGF-β receptor II, which affect B-cell function. A total of 44 genes were expressed at threefold lower levels. These genes were involved in immunity and cell cycle regulation. The observation that a quarter of the early arthritis genes overlapped with an influenza-induced gene set led the authors to suggest that the early arthritis signature may partly reflect the response to an unknown infectious agent.

We examined the gene expression profiles of whole blood cells and also identified clear and significant differences between RA patients ($n = 35$) and healthy individuals ($n = 15$) [39]. The microarray data confirmed previous observations of increased expression of, for instance, the calcium-binding proteins S100A8 and S100A12. Application of pathway analysis algorithms revealed increased expression of immune defence genes, including type I IFN response genes, which indicates that this pathway is also activated systemically in RA. This type I IFN signature may be a direct reflection of increased activity of type I IFN. However, it cannot be excluded that another ligand known to activate the IFN/STAT-1 pathway is involved. The increased expression of the type I IFN response genes was characteristic of not all but approximately half of the patients. Moreover, the immune defence gene programme that was activated in a subgroup of RA patients was reminiscent of that of poxvirus-infected macaques [40]. This subgroup of RA patients expressed significantly increased titres of anti-cyclic citrullinated peptide antibodies (anti-CCP/ACPA). Based on these findings, we conclude that activation of an immune response, with a type I IFN signature among the gene sets, defines a subgroup of RA patients characterized by increased autoreactivity against citrullinated proteins.

The gene expression analyses in peripheral blood of individuals at high risk for developing RA (RF and/or ACPA positive arthralgia patients) that we performed provide a framework for the identification of predictive biomarkers that may permit identification of individuals who will develop arthritis within 2 years [41].

Tan and coworkers reported increased IFN-response gene expression in SSc [42]. Similar observations were made by York and coworkers [43], who described increased expression of *Siglec-1*, an IFN-response gene, in both the diffuse and the limited cutaneous type of disease as compared with healthy individuals. Recent findings from our group indicate an association between the IFN response signature and anti-centromer autoantibodies and digital ulcers in SSc [44].

An analysis of significance across several febrile inflammatory disease (44 paediatric systemic onset juvenile idiopathic arthritis [SoJIA], 94 paediatric infections, 38 paediatric SLE, six PAPA [a familial autoinflammatory disease that causes pyogenic sterile arthritis, pyoderma gangrenosum and acne] and 39 healthy children) revealed a SoJIA-specific signature composed of 88 genes in peripheral blood [45].

Common denominators

Upregulation of IFN-response genes has now been observed in peripheral blood cells and/or target tissues of (a subset of) patients with autoimmune diseases such as RA, SLE, SSc, SS, multiple sclerosis and type 1 diabetes. These findings suggest that an activated IFN response gene expression programme is a common denominator in rheumatic diseases, and autoimmune diseases in general.

Type I IFNs, which are the early mediators of the innate immune response that influences the adaptive immune response through direct and indirect actions on dendritic cells (DCs), T and B cells, and natural killer cells, could affect the initiation or amplification of autoimmunity and tissue damage through their diverse and broad actions on almost every cell type and promotion of T-helper-1 responses. It is speculated that the IFN response programme could be associated with activation of immature monocyte-derived DCs, which regulate deletion of autoreactive lymphocytes. Subsequently, IFN-matured DCs may activate autoreactive T cells, leading to autoreactive B-cell development, representing the first level of autoimmunity [46]. Loss of tolerance may lead to autoantibody production. In the case of SLE, autoantigen/autoantibody complexes may trigger pathogen recognition receptors (such as Toll-like receptors) that induce IFN-α production and thereby perpetuate the IFN response programme.

Apart from a role for the IFN response programme as a common denominator in autoimmune diseases, other gene profiles have been identified that are shared by autoimmune diseases. In particular, Maas and colleagues [47] studied the overlap of gene expression profiles between different diseases. They identified 95 genes that were increased and 117 genes that were decreased in the PBMCs of all patients with RA, SLE, type 1 diabetes and multiple sclerosis. These genes were involved in, for example, inflammation, signalling, apoptosis, ubiquitin/proteasome function and cell cycle. Hierarchical cluster analysis on the basis of gene signatures in PBMCs revealed that RA and SLE patients were intermixed with one another. Moreover, they reported that from the genes that were differentially expressed between PBMCs from patients and those from unrelated unaffected individuals,

the gene expression profile of 127 genes was shared between patients with autoimmune diseases and unaffected first-degree relatives. This commonality between affected and unaffected first-degree relatives suggests a genetic basis for these shared gene expression profiles. Accordingly, the investigators showed that these genes are clustered in chromosomal domains, supporting the hypothesis that there is some genetic logic to this commonality [48].

Pharmacogenomics in rheumatic diseases

Given the destructive nature of most rheumatic diseases, it would be highly desirable to predict at an early stage the most beneficial treatment for those patients at risk. If we rely solely on clinical or radiographic manifestations, we will probably be responding too late and failing to maximize protection. Ideally, it would be desirable to make predictions on success before the start of therapy. Ultimately, this may lead to a personalized form of medicine, whereby a specific therapy will be applied that is best suited to an individual patient.

TNF antagonists are approved worldwide for the treatment of various rheumatic diseases. Clinical experience indicates that there are 'responders' as well as 'nonresponders', but clear criteria for such classification are still lacking. For RA, treatment is only effective for approximately two-thirds of patients [49], which has attracted interest in the pharmacology and mechanisms of action of the available therapies. We present the results of studies assessing progress in exploiting pharmacogenomics (in particular transcriptomics for disease profiling) and pharmacodynamics to predict response to therapy. The term 'pharmacogenomics' emerged in the late 1990s and pertains to the application of genomics in drug development. 'Pharmacogenomics' is defined as, 'The investigation of variations of DNA and RNA characteristics as related to drug response'. Here, we focus on transcriptomics studies.

Until now a few pharmacogenomics studies have been conducted to gain insight into pharmacodynamics and to identify genes predictive of responsiveness to TNF blockers.

The pharmacogenomics of RA patients ($n = 15$) before and 1 month after the start of infliximab treatment revealed a similar change in the expression of a pharmacogenomic response gene set in the peripheral blood compartment of all patients treated, irrespective of clinical response. This result indicates that all RA patients exhibit an active TNF response programme that contributes to disease pathogenesis [50].

Lequerre and colleagues [51] studied 13 patients (six responders and seven nonresponders) who began treatment with an infliximab/methotrexate combination. Treatment response, determined after 3 months, was based on a difference in Disease Activity Score using 28 joint counts (DAS28) of 1.2 or more. Gene expression analysis of the PBMCs identified a preselected set of 2,239 transcripts out of 10,000 transcripts screened, which exhibited abnormal expression in at least one out of the 13 patients. Subsequent statistical (t-test and serial analysis of microarrays) analysis identified a total of 41 transcripts, covering a diverse set of proteins and functions, which discriminated between responders and nonresponders. In a validation study conducted in 20 patients (10 responders and 10 nonresponders) and with a set of 20 transcripts, correct classification of 16 out of the 20 patients was found (90% sensitivity and 70% specificity). Koczan and colleagues [52] determined pharmacogenomic differences after 72 hours in 19 RA patients (12 responders and seven nonresponders) using a microarray with a complexity of about 18,400 genuine transcripts after administration of etanercept. They identified an informative set of genes, including *NFKBIA*, *CCLA4*, *IL8*, *IL1B*, *TNFAIP3*, *PDE4B*, *PP1R15* and *ADM*, which are involved in nuclear factor-κB and cAMP signalling, whose expression changes after 72 hours was associated with good clinical responses (DAS28 >1.2). Comparative analysis did not reveal an overlap between the two gene sets.

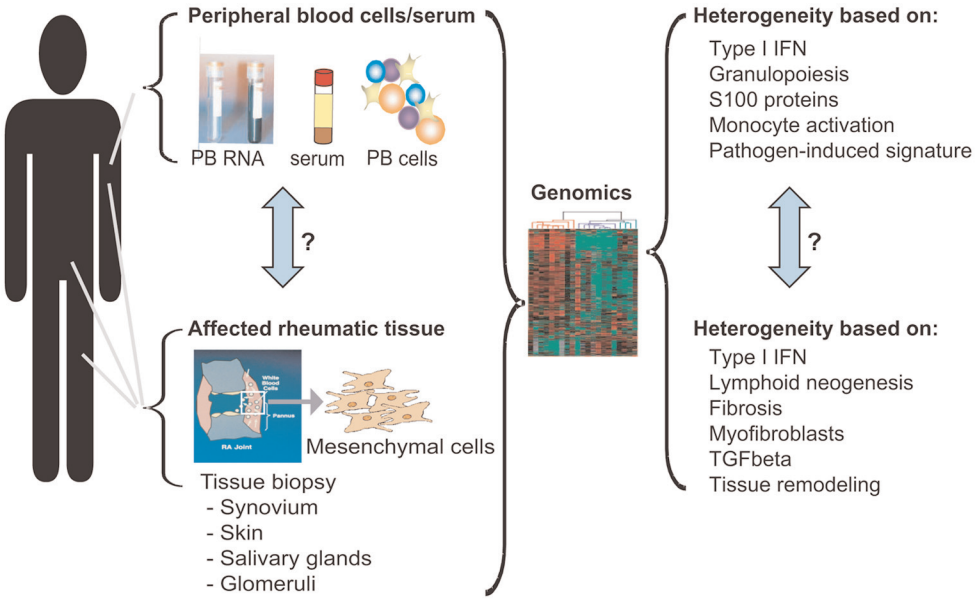
Lindberg and colleagues [53] studied synovial tissue gene expression profiles in 10 infliximab-treated patients (three responders, five with moderate response and two non-responders). The data revealed 279 genes that were significantly differentially expressed between the good responding and nonresponding patients (false discovery rate <0.025). Among the identified genes was that encoding MMP3. Moreover, their data revealed that TNF-α could be an important biomarker for successful infliximab treatment.

We conducted a gene expression profiling study in synovial biopsies from 18 patients (12 responders and six non-responders, based on DAS28 ≥ 1.2 after 16 weeks). Several biological processes related to inflammation that were upregulated in patients who responded to therapy, as compared with those who did not show clinical improvement, were identified. These findings indicate that patients with a high level of tissue inflammation are more likely to benefit from anti-TNF-α treatment [54].

Overall, identification of biomarkers before treatment to predict response to anti-TNF treatment in RA has not yet yielded consistent results. Therefore, additional studies using large cohorts of patients and more stringent response criteria are necessary.

A comparative microarray analysis of PBMCs from eight SoJIA patients without anti-TNF therapy and five SoJIA patients undergoing therapy with infliximab [55] revealed over-expression of IFN-α-regulated genes after TNF blockade. Conversely, the addition of IFN to stimulated human PBMCs inhibits the production of both IL-1 and TNF, and induces the production of IL-1 receptor antagonist [56]. These findings indicate that cross-regulation of type I IFNs

Figure 2



Discovery of molecular rheumatic disease subtypes. Schematic overview of the discovery of rheumatic disease subtypes in peripheral blood cells and affected target tissues. Heterogeneity in rheumatic diseases have been demonstrated at peripheral blood as well as tissue level using high-throughput genomics technology. Several studies have described the presence of at least two subgroups of patients based on the presence or absence of an activated type I interferon (IFN) induced gene expression profile in peripheral blood as well as in affected tissues. In addition, peripheral blood cells of rheumatic patients exhibit heterogeneous expression levels for genes involved in granulopoiesis and monocyte activation, as well as for genes encoding the inflammatory S100 proteins. Moreover, subsets of patients exhibit gene expression profiles similar to pathogen-induced profiles. Apart from type I IFN, tissue heterogeneity is reflected at the level of lymphoid neogenesis, fibrosis, myofibroblasts, tissue remodelling and transforming growth factor (TGF)-β signalling. The exact relationship between the peripheral blood profile and tissue profile needs to be further investigated.

and TNF plays an important role in the regulation of pathological inflammatory responses. Because TNF plays a critical role in the pathogenesis of certain rheumatic diseases (such as RA) and because IFN-α plays a pivotal role in another set of diseases (including SLE), the cross-regulation of TNF and IFN might have clinical relevance for the blockade of TNF in, for instance, patients with RA. It is speculated that these results provide a mechanistic explanation for the development of anti-double-stranded DNA antibodies and lupus-like syndrome in patients undergoing anti-TNF therapy. However, recent gene expression studies in whole blood of RA patients before and 1, 2 and 3 months after the start of TNF blockade (infliximab) revealed a variable effect on the expression of IFN response genes upon treatment. Therefore, the positive effect of TNF blockade on IFN is not consistently observed in RA [57].

Conclusion

Genomic profiling approaches have fuelled insight to the possibility of finding expression patterns that correlate with disease characteristics and therefore provide a promising tool for future clinical applications. Molecular profiling of blood cells and affected target tissues has already revealed important pathways that contribute to the spectrum of rheumatic diseases (Figure 2). Both disease-specific and subgroup-specific signatures and common signatures are emerging. The latter is reflected by the observation that clinically distinct rheumatic diseases, and even autoimmune diseases in general, all show evidence of a dysregulation of the type I IFN response pathway. Together, the developments support the notion that there is a basis for a molecular subcategorization of clinically defined rheumatic diseases. Moreover, the results indicate that innate immune pathways remain of critical importance throughout the course of rheumatic diseases. The clinical implications of these observations require further definition and independent validation.

Pharmacogenomics studies are just emerging, and the results obtained thus far indicate promise for the future. The finding of biomarkers and gene signatures before the start of targeted therapies paves the way to more individualized treatment strategies. However, caution must be exercised in the interpretation of these results because of small sample sizes and differences in measures of treatment response. To increase the sample sizes, collaborative efforts from different groups are essential. Moreover, agreement on usage of standardized objective measures of treatment responses is of

The Scientific Basis of Rheumatology: A Decade of Progress

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critical importance because this will make data from different studies comparable.

To maximize the usage of information from different laboratories, full and open access to genomics data is important. Moreover, standardization of sample processing procedures and use of the technology, and data analysis and algorithms used are of critical importance. This will ultimately allow a systems biology approach, whereby genomics, proteomics and clinical datasets from different sources are integrated to assign and validate clinically relevant markers that reflect disease pathogenesis (diagnosis), prognosis and heterogeneity, and will facilitate selection of patients with a high likelihood of responding to therapy.

Competing interests

The VU University Medical Center has filed a patent applications that is based on the present work (Patent file no. P086657EP00, 'Predicting clinical response to treatment with a soluble TNF-antagonist or TNF, or a TNF receptor agonist'; patent file no. EP08167570.4, 'Preclinical biomarkers for predicting the development of chronic autoimmune diseases'. CL Verweij, W Bos, LGM van Baarsen, D van Schaardenburg). CV and LvB are listed as inventors on the patent applications, and are stakeholders in Preselect Diagnostics BV.

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