(Anti-)TNF alpha matters in rheumatoid arthritis

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Chapter 9

Rheumatoid arthritis subtypes identified by genomic profiling of peripheral blood cells: Assignment of a type I interferon signature in a subpopulation of patients.

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ABSTRACT

Objective.
Rheumatoid arthritis (RA) is a heterogeneous disease with unknown cause.

Aim.
To identify peripheral blood (PB) gene expression profiles that may distinguish RA subtypes.

Methods.
Large-scale expression profiling by cDNA microarrays was performed on PB from 35 patients and 15 healthy individuals. Differential gene expression was analyzed by significance analysis of microarrays (SAM), followed by Gene Ontology analysis of the significant genes. Gene set enrichment analysis was applied to identify pathways relevant to disease.

Results.
A substantially raised expression of a spectrum of genes involved in immune defense was found in the PB of patients with RA compared to healthy individuals. SAM analysis revealed a highly significant elevated expression of interferon (IFN) type I regulated genes in patients with RA compared to healthy individuals, which was confirmed by gene ontology and pathway analysis, suggesting that this pathway was activated systemically in RA. A quantitative analysis revealed that increased expression of IFN-response genes was characteristic of approximately half of the patients (IFN\textsuperscript{high} patients). Application of pathway analysis revealed that the IFN\textsuperscript{high} group was largely different from the controls, with evidence for upregulated pathways involved in coagulation and complement cascades, and fatty acid metabolism, while the IFN\textsuperscript{low} group was similar to the controls.

Conclusion.
The IFN type I signature defines a subgroup of RA patients, with a distinct biomolecular phenotype, characterised by increased activity of the innate defense system.
Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the joints. There is growing evidence that patients with RA, as defined by the American College of Rheumatology Classification Criteria,[1] represent a highly heterogeneous group. However, the clinical approach to disease classification could erroneously suggest that criteria are applied to classify one disease entity. The heterogeneity of RA is reflected by marked variability in clinical presentation, and the presence of distinct autoantibody specificities, like rheumatoid factor and anti-cyclic citrullinated peptide antibodies (ACPA) in the serum.[2,3]

Disease heterogeneity is also apparent in histological features of the synovium, displaying different complexity levels of lymphoid organization in subsets of patients.[4,5] Moreover, gene expression profiling of synovial tissue from RA and osteoarthritis (OA) patients revealed marked variation in gene expression profiles that allowed us to identify molecularly distinct forms of RA synovium.[6,7] The wide variation in responsiveness to virtually any treatment in RA is also consistent with the heterogeneous nature of the disease.[8-10] Together these findings suggest that distinct disease mechanisms are at play in RA pathology. The relative contribution of the different mechanisms may vary among patients and, perhaps, in different stages of disease.

The heterogeneity most likely has its origin in the multifactorial nature of the disease, whereby specific combinations of environmental factors and a varying polygenic background are likely to influence not only susceptibility but also the severity and disease outcome. Findings from genetically identical twins, where the concordance rate is far less than complete, are indicative for a major role of an environmental factor in the risk of developing RA.[11,12]

Given the heterogeneous nature of RA, and its systemic features, we investigated whether this heterogeneity is reflected in peripheral blood cells, because it can be anticipated that (etio)pathogenic events in the host are reflected as phenotypic changes in the host cells. Large-scale gene expression profiling of peripheral blood cells from RA patients could thus provide a molecular portrait that reflects the contributions of diverse cellular responses that are associated with RA in general and with disease subtypes, and thus defines the samples’ unique biology.

**MATERIAL AND METHODS**

Patients and controls.

Peripheral blood (PB) was obtained in PAXgene RNA isolation tubes (PreAnalytix, GmbH, Germany) from 35 patients. From all 35 patients, 25 used methotrexate (MTX), and 10 patients were MTX and other DMARD naïve. All patients fulfilled the revised American College of Rheumatology 1987 criteria for RA,[1] except for 3 patients in the MTX naïve group, which were diagnosed with probable RA, with a disease duration of 3-12 months, a mono-arthritis and positive for ACPA. Two patients have been diagnosed with RA after 6 and 12 months. Table 1 summarizes
the characteristics of these patients. The control group consisted of 15 healthy individuals (9 females, 6 males, mean age: 43 years, ranging from 27-63). In all comparisons mentioned, the groups were age- and sex-matched. All patients and controls gave their informed consent, and the study protocol was approved by the Medical Ethics Committees from the Academic Medical Center and VU medical center.

Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>Disease characteristics</th>
<th>receiving MTX treatment n=25</th>
<th>Not receiving MTX treatment n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (range)</td>
<td>49 (23-63)</td>
<td>49 (25-67)</td>
</tr>
<tr>
<td>Sex: female, male</td>
<td>17, 8</td>
<td>8, 2</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>19 (3-76)</td>
<td>ND</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>27 (2-82)</td>
<td>41 (13-70)</td>
</tr>
<tr>
<td>Rheumatoid factor titre</td>
<td>114 (1-516)*</td>
<td>123 (12-832)</td>
</tr>
<tr>
<td>ACPA titre (kU/L)</td>
<td>935 (1-6264)*</td>
<td>1210 (1-4904)</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>113 (8-417)</td>
<td>7 (1-12)</td>
</tr>
<tr>
<td>Erosions present (n)</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>DAS28</td>
<td>5.5 (3.4-7.2)</td>
<td>5.1 (3.1-7.4)</td>
</tr>
<tr>
<td>MTX dose, mg/week (range)</td>
<td>21 (7.5-30)</td>
<td>0</td>
</tr>
<tr>
<td>Prednisone ≤10 mg/day (n)</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

ACPA= anticyclic citrullinated peptide antibodies; CRP= C-reactive protein; DAS28= Disease Activity Score 28; ESR= erythrocyte sedimentation rate; MTX= methotrexate; ND= not determined; RA= rheumatoid arthritis.

Values are given as mean (range) unless otherwise specified. * Available for 23 of 25 patients receiving MTX treatment.

Sample preparation, labeling and hybridization.

This procedure was performed as previously described.[13] In short, total RNA was isolated from PB using the PAXgene RNA isolation kit. Amplified RNA was labeled with aminoallyl-dUTP during cDNA synthesis, followed by chemical coupling of the aminoallyl group to Cy3 or Cy5 for the experimental and reference samples, respectively. The labeled cDNA transcripts were hybridized together on human cDNA microarrays with 42,000 elements, representing ~24,000 genes, generated at Stanford University, as described.[14]

Data filtering and analysis.

Data were stored and pre-analyzed in the Stanford Microarray Database (SMD) [15] at http://genome-www.stanford.edu/microarray. Data are expressed as log2 ratios of fluorescence intensities of the experimental and the common reference sample. Intensity-dependent normalization using local estimation (“Loess”) was performed separately on each sector of the array. Spots were included in the analysis when in at least 80% of the microarrays a reliable data point was obtained for that element (defined by a regression correlation coefficient R > 0.6 for Cy3 and Cy5 pixel
identifying RA subtypes by genomic profiling of peripheral blood

1. intensities, and a signal intensity of 2.5 times the local background for both Cy3 and Cy5). The use of a common reference allows comparison of the expression levels across all samples.[13] Therefore, the expression levels (as log2 ratio's) were median centered, i.e. each spotted element was expressed relative to the median expression level of that element across all samples. We corrected for array batch differences by applying Single Value Decomposition.[16] Genes represented more than once on the microarrays were averaged in SMD from sequences with the same Unigene identifier.

2. Statistical analysis.

3. Statistical analysis on microarray data was performed using Significance Analysis of Microarray data (SAM).[17] Genes that were expressed at significantly different levels between patients and controls, defined by a q-value of less than 5%, were analyzed by supervised hierarchical clustering [18] to visualize the correlation of co-expressed genes in Treeview (available at http://rana.lbl.gov/EisenSoftware.htm).

4. For an interpretation of the biological processes that are represented by the genes that show a significantly different level of expression in RA patients compared to the controls, we applied Gene Ontology analysis in the PANTHER database at http://PANTHER.appliedbiosystem.com. [19] PANTHER uses the binomial statistics tool to compare our gene list to a reference list (NCBI: Homo sapiens genes) to determine the statistically significant over-representation of functional groups of genes. A Bonferroni correction was applied to adjust for multiple comparisons. P values < 0.05 were considered significant.

5. For pathway analysis, we used Gene Set Enrichment Analysis (GSEA) [20] at http://www.broad.mit.edu/gsea/. Like SAM, it utilizes data permutation to adjust for multiple testing, indicated by a false discovery rate. A Total of 408 pathways from the Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg) and Biocarta (http://www.biocarta.com) are applied in this analysis. The same gene may be present in more than one pathway or biological process. In addition, we incorporated several IFN-response gene sets from published data.[21,22] A minimal gene set size of 20 genes per pathway was applied, and pathways with a P-value < 0.05 and a false discovery rate (FDR) of < 0.25 were considered significant, according the authors’ suggestions.[20] For the comparison of mean gene expression levels in different gene sets, a Student’s T test was used.
RESULTS

Gene expression profiling in peripheral blood cells of RA patients.

Gene expression profiling of PB cells from 32 RA, 3 probable RA patients, and 15 age and sex-matched healthy controls was performed on microarrays with a complexity of ~20K unique genes (43K elements). Data were analyzed as two class, unpaired data using SAM.[17] A total of 577 genes, of which 259 were upregulated, and 318 genes downregulated, were selected whose transcript levels were expressed at significantly different levels between the two groups. The significant gene expression differences between RA patients and healthy controls were visualized in a heatmap (Figure 1A).[18]

Genes upregulated in RA.

A global view of the significantly differential expressed genes revealed a prominent cluster of IFN-inducible genes that was upregulated in RA patients. This cluster, highlighted in Figure 1B, contains highly correlated genes such as IFRG28 (28kD interferon responsive protein), IFI35 (interferon-induced protein 35), IFI44L (interferon-induced protein 44-like), IFIT1 (interferon-induced protein with tetratricopeptide repeats 1), IFIT2, IRF2 (interferon regulatory factor 2), IRF7, GIP2 (interferon alpha-inducible protein 2), GIP3, SERPING1 (serine proteinase inhibitor clade G member 1, C1 inhibitor), OAS1 (2'-5'-oligoadenylate synthetase 1), OAS2, MX1 (Myxovirus resistance 1), G1P2/ISG15 (Interferon-induced protein 15), and RSAD2 (radical S-adenosyl methionine domain containing 2).

In addition, all patients showed increased expression of several inflammatory mediators including the chemokines CXCL12, CXCL9, CCL15, CCL19, CCL7, CXCL12, CCL19, CCL7, CXCL3, and CCL8 as well as interleukin (IL)-19 and the S100 family proteins S100 calcium-binding protein A8 (S100A8), S100A11, and S100A12. Other genes that were upregulated in RA patients were members of the antioxidant metallothionein family and the anti-inflammatory IL-1 receptor antagonist.

Genes downregulated in RA.

Genes that showed a lower expression in RA included: CD3 zeta, TCR beta chain, TARP/TCRgammaV9, granzyme M, runx3, and KLRB1, which are involved in cytotoxic functions, and many other genes with unknown function. Gene Ontology analysis of genes with significant differential expression in RA

To systematically categorize the 577 genes with significant differential expression into functional groups we used the PANTHER database consisting of a large collection of protein families that have been subdivided into functionally related subfamilies.[19] The differentially upregulated genes represented 7 significant functional biological processes. (Table 2). There were no
Figure 1. (A) Cluster diagram of the expression of 577 significantly different expressed genes in 35 patients and 15 healthy individuals. Genes are organized by hierarchical clustering based on overall similarity in expression patterns.[18] Red represents relative expression greater than the median expression level across all samples, and green represents an expression level lower than the median. Black indicates intermediate expression. Grey indicates missing data. Colored bars to the right identify the locations of a category of clustered genes, with a correlated expression profile and related function. (B) Representation of the IFN-response gene cluster with an enhanced expression in the group having patients with rheumatoid arthritis (RA). An expanded view of the genes in the IFN-response cluster of (A) is shown. Genes are either known genes with a unigene symbol characteristic for the defined gene cluster, or genes are unknown, indicated by an accession number or unigene cluster ID. (C, D) The IFN-response program is present in patients with RA irrespective of treatment. Representation of genes that are expressed at significantly different levels between patients with RA undergoing (C) or patients not undergoing (D) methotrexate (MTX) treatment and age-and sex-matched healthy controls. A selection of genes with a correlated expression profile that are indicative for an IFN-response program is shown. See color figures page 218.
significant downregulated ontology groups. The “Immunity and Defense” ontology group represents a broad composite family that consists of more specified ontology subgroups. Within these subgroups the most significant upregulated process that distinguished RA patients from controls was “Interferon-Mediated Immunity”

Pathway analysis

In addition, we performed Gene Set Enrichment Analysis (GSEA) [20] to identify pathways relevant to RA. In contrast to ontology analysis this algorithm is based on the usage of all available gene expression data and derives it power from the analysis of sets of genes that are coordinately regulated in a defined biological process or pathway, while it uses data permutation to adjust for multiple testing. In addition to the intrinsic GSEA pathway gene sets, we included previously reported IFN-response sets in our analysis.[21,22] The results revealed that besides the five GSEA intrinsic gene sets (Table 3), the previously described type I IFN-induced genes by Baechler et al.[22] (in their supplementary data), and the IFNα-induced genes were both significantly increased in RA patients.

IFN-induced genes in RA.

We confirmed expression of key genes of the IFN pathway, RSAD2 and G1P2, in all samples by real-time PCR, which showed a high correlation with the microarray data ($r = 0.78$ and 0.87 respectively, $P < 0.0001$ in both cases, data not shown). To rule out an effect of MTX treatment on the IFN-induced genes, we made a comparison of patients with (n=25) and without MTX treatment (n=10) with the appropriate age and sex-matched controls. SAM revealed that both

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**Table 2.** Ontology analysis of the genes that were expressed at significantly different levels between all 35 patients, or the subgroups consisting of 20 IFN$^\text{high}$ and 15 IFN$^\text{low}$ patients and healthy controls.

<table>
<thead>
<tr>
<th>Biological Process</th>
<th>All patients p Value</th>
<th>IFN$^\text{high}$ p Value</th>
<th>IFN$^\text{low}$ p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunity and defense</td>
<td>1.61E-11</td>
<td>3.41E-15</td>
<td>NS</td>
</tr>
<tr>
<td>Interferon-mediated immunity</td>
<td>1.09E-06</td>
<td>3.38E-08</td>
<td>NS</td>
</tr>
<tr>
<td>Macrophage-mediated immunity</td>
<td>6.83E-05</td>
<td>1.27E-04</td>
<td>NS</td>
</tr>
<tr>
<td>Lipid and fatty acid transport</td>
<td>1.54E-04</td>
<td>ns</td>
<td>NS</td>
</tr>
<tr>
<td>Transport</td>
<td>4.82E-04</td>
<td>3.33E-02</td>
<td>NS</td>
</tr>
<tr>
<td>Cytokine/chemokine-mediated immunity</td>
<td>1.63E-03</td>
<td>2.61E-05</td>
<td>NS</td>
</tr>
<tr>
<td>Ligand-mediated signalling</td>
<td>3.52E-03</td>
<td>1.92E-02</td>
<td>NS</td>
</tr>
<tr>
<td>Cell motility</td>
<td>NS</td>
<td>4.24E-03</td>
<td>NS</td>
</tr>
<tr>
<td>Blood clotting</td>
<td>NS</td>
<td>5.30E-03</td>
<td>NS</td>
</tr>
<tr>
<td>Oncogenesis</td>
<td>NS</td>
<td>1.04E-02</td>
<td>NS</td>
</tr>
<tr>
<td>Cell structure and motility</td>
<td>NS</td>
<td>1.55E-02</td>
<td>NS</td>
</tr>
<tr>
<td>Inhibition of apoptosis</td>
<td>NS</td>
<td>1.66E-02</td>
<td>NS</td>
</tr>
<tr>
<td>Signal transduction</td>
<td>NS</td>
<td>3.21E-02</td>
<td>NS</td>
</tr>
<tr>
<td>Proteolysis</td>
<td>NS</td>
<td>3.97E-02</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, non-significant. Processes with a p value<0.05 were considered significant (with Bonferroni correction).
groups of patients showed a prominent IFN-induced gene expression signature (Figure 1C and
D). Thus, the IFN expression signature was present in RA patients irrespective of MTX treatment.

Table 3. Pathways which are overexpressed in all patients, and in the subgroups of IFN\(^{\text{high}}\) and IFN\(^{\text{low}}\) patients, all compared with healthy controls, analysed by gene set enrichment analysis.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>No. of genes</th>
<th>All patients P Value</th>
<th>IFN(^{\text{high}}) P Value</th>
<th>IFN(^{\text{low}}) P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I IFN-induced genes [^{[22]}]</td>
<td>83</td>
<td>0.004</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>IFN(^{\alpha})-induced [^{[21]}]</td>
<td>36</td>
<td>0.022</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>IFN(^{\beta})-induced [^{[21]}]</td>
<td>51</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Cytokine-cytokine receptor interaction</td>
<td>129</td>
<td>0.006</td>
<td>0.005</td>
<td>NS</td>
</tr>
<tr>
<td>Neuroactive ligand-receptor interaction</td>
<td>90</td>
<td>0.009</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Jak-STAT signalling pathway</td>
<td>86</td>
<td>0.016</td>
<td>0.008</td>
<td>NS</td>
</tr>
<tr>
<td>Complement and coagulation cascades</td>
<td>44</td>
<td>0.025</td>
<td>0.009</td>
<td>NS</td>
</tr>
<tr>
<td>Fatty acid metabolism</td>
<td>41</td>
<td>0.027</td>
<td>0.015</td>
<td>NS</td>
</tr>
<tr>
<td>IFN(^{\gamma})-induced [^{[21]}]</td>
<td>34</td>
<td>NS</td>
<td>0.019</td>
<td>NS</td>
</tr>
</tbody>
</table>

IFN= interferon; NS= non-significant. Pathways are shown with a p value <0.05, and a false discovery rate value <0.25.

The expression profiles of the three probable RA patients, within the MTX naive group, did not differ from the other MTX-naive patients. Meanwhile, two of the probable RA patients have been diagnosed with “definite” RA at 6 and 12 months after Paxgene blood sampling, respectively, suggesting that the RA signature is present in the blood prior to diagnosis.

Selective upregulation of type I IFN-response genes in RA.

Type I IFNs are mainly produced directly after viral infection whereas type II IFNs are secondary produced by activated T- and natural killer (NK) cells. Type I and type II IFN response programs share many of their genes. To disclose information on the inducing type of IFN, we obtained a specific type I IFN- and type II IFN-response gene set \[^{[21]}\] (Supplementary Table 1). The type I IFN-response set consists of 5 genes that respond to both IFN\(^{\alpha}\) and IFN\(^{\beta}\), but not to IFN\(^{\gamma}\). The type II IFN-response set consists of 13 genes responding specifically to IFN\(^{\gamma}\).\[^{[21]}\] To investigate the relative contribution of either gene set to the RA gene expression profile we calculated for each gene set the mean gene expression level (log2 ratio) per patient and healthy control and compared the two groups with each other (Figure 2). This analysis showed that the mean gene expression level of the type I IFN gene set was significantly higher in the RA patient group (P = 0.0004), whereas the mean gene expression level of the type II IFN genes was similar between patients and controls. Hence, these findings provide evidence that type I IFNs rather than type II IFNs are responsible for the increased expression of IFN-induced genes.

The IFN signature defines a subgroup of RA patients.

Consistent with the heterogeneous nature of RA we observed that the IFN-response showed a large variation between RA patients (Figure 2 and 3). To obtain more insight into the differential
expression of IFN-induced genes in individual patients, we calculated for each individual, the average expression of the IFN-cluster genes, that were upregulated in RA patients, as described in Figure 1B. Next we defined which patients show an altered IFN-response, by calculating the 95 % limits of the controls (normal values, defined as the mean expression of the 43 IFN genes, plus or minus 1.96 times the standard deviation). We identified 20 patients with an average expression level above normal values, further defined as the IFNhigh group, while the remainder of the patients, with an expression level equal to controls, were defined as IFNlow (Figure 3).

Distinct characteristics of the IFNhigh group.

To further characterize the IFNhigh group, we performed SAM analysis, which revealed that 484 genes were upregulated in IFNhigh patients, compared to the healthy controls, while 229 genes were downregulated. The same analysis for the IFNlow patients revealed only 57 upregulated genes and 93 downregulated genes. These data indicate that within the RA patients, the patients with an IFN signature represent the most distinct group compared to normal controls.

When we applied Gene Ontology analysis, we found that nearly all of the processes that were identified as upregulated in the whole RA group, were also upregulated in the IFNhigh group. Moreover, an additional group of 10 biological processes were selectively upregulated in the IFNhigh group. (Table 2). No downregulated processes were identified. Gene Ontology analysis of the IFNlow group revealed no significant down or upregulated processes.

Figure 2. Type I interferon (IFN)-induced genes are overexpressed in rheumatoid arthritis (RA). Each square indicates the mean expression levels of genes known to be specifically induced either by type I (13 genes) or type II IFN (5 genes) per individual patient or per healthy control (HC). These genes are extracted from the gene sets used for pathway analysis in table 3.[21]
Figure 3. A subgroup of patients with rheumatoid arthritis (RA) show increased expression of IFN-response genes (IFN$^{\text{high}}$). Each square represents a single individual with the average expression ratio of all 43 IFN-response genes, which are shown as a distinct cluster in fig 1A and B. The shaded box indicates the normal range within the 95% confidence limits. Patients with RA outside the shaded box are defined as the IFN$^{\text{high}}$ group. HC, healthy control. See figure A in color section page 219.
In accordance with Gene Ontology analysis, pathway analysis by GSEA revealed that the IFN<sup>high</sup> patients were responsible for the upregulated pathways in the overall RA group (Table 3). This was particularly clear for the IFN-type I induced gene sets, complement, and coagulation cascades. On the other hand, the IFN<sup>low</sup> group was associated with increased expression of the “Neuroactive ligand-receptor interaction” pathway. We did not identify any downregulated pathways in either group of RA patients. Overall, these analyses indicate that within the whole group of patients, the IFN<sup>high</sup> group is more distinct from controls than the IFN<sup>low</sup> group. The molecular stratification of RA was not associated with clinical parameters that are described in Table 1.

**DISCUSSION**

Since RA is a systemic disease, several investigators addressed the question whether disease characteristics are reflected by changes in gene expression levels in PB cells. Whereas these studies provided insight into the genes that were differentially expressed between RA patients and healthy controls, the issue of transcript-based disease heterogeneity has not been addressed so far, except for a comparison between recent onset arthritis and longstanding disease.[23]

Using large-scale gene expression profiling, we identified a large number of genes, including genes involved in the immune/inflammatory response, such as the previously described calcium-binding proteins S100A8, S100A12, and IL1RA. [24,25] Pathway level analysis was used to classify gene expression data in biological processes and pathways. The clear induction of IFN-response genes in RA patients prompted us to incorporate several IFN-response gene sets from published data [21,22] in the analysis. This analysis revealed that the type I IFN-mediated immunity was the most significantly upregulated pathway in RA patients, independent of MTX treatment. Albeit, that inclusion of the type I IFN gene set is a biased decision, this approach provides a method to demonstrate the significance of the type I IFN response program in RA.

Most interestingly, our analysis revealed a striking heterogeneity between RA patients based on the differential expression of genes involved in the innate defense system, in particular the type I IFN system. These findings suggest that different pathogenic mechanisms may contribute to the disease. The IFN<sup>high</sup> group was further characterized by gene sets reflecting increased activity of complement and coagulation cascades.[26] Next to complement activation, the other pathways associated with the IFN type I signature, such as “Fatty acid metabolism” and “Coagulation” may all contribute to the increased risk for cardiovascular disease in a subgroup of RA patients.[27]
The most significant genes from the complement and coagulation pathway are indicated in Figure 1, including Complement subcomponent C1q chain B (C1QB), Coagulation factor XII (F12), tissue plasminogen activator (PLAT) and Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1 (SERPING1). These genes are involved in activation as well as inhibitory components of the pathways. Upregulation of IFN-induced genes has also been observed in PB cells of (a subset of) patients with other autoimmune diseases like systemic lupus erythematosus (SLE), [22,28] systemic sclerosis (SSc),[29] multiple sclerosis (MS),[13] and in tissues from patients with Sjögren’s syndrome (SS),[30] type I diabetes mellitus,[31] and dermatomyositis.[32] These findings suggest that an activated IFN gene expression program is a common hallmark in chronic autoimmune diseases.

Type I IFNs, which are the early mediators of the innate immune response that influence the adaptive immune response through direct and indirect actions on dendritic cells (DC), T- and B cells, and NK cells, could impact 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.[33] This appears to be the case for SLE, but for RA both clinical and pathophysiological data have suggested that tumor necrosis factor alpha (TNFα) rather than type I IFN is essential for persistence of the disease. Hence, it is believed that mutually exclusive cytokine expression patterns are characteristic for distinct autoimmune diseases. However, since we observed an IFN type I signature in the PB of a subgroup of RA patients this could mean that cytokine profiles are a patient-specific rather than a disease-specific phenomenon.

In SLE patients, the IFN signature is related to disease severity.[22] It is at present unclear what the role of type I IFNs in RA pathogenesis could be. In analogy to SSc[29] and MS,[13] no clinical associations were found for RA so far. We have previously suggested that IFN/STAT-1 activation in RA synovium could be a reactive attempt to limit inflammation.[34] This suggestion was recently supported by a study showing that IFNβ deficiency could prolong experimental arthritis and resulted in increased activation of FLS in vitro.[35] In addition, IFNβ-competent fibroblasts were able to ameliorate arthritis in IFNβ-deficient recipients. It should be noted, however, that systemic administration of IFNβ was unsuccessful in the treatment of RA, which may be due to pharmacokinetic issues.[36]

Concerning the origin of type I IFNs, infectious and endogenous agents, such as viruses, bacteria, unmethylated CpG DNA, single- or double stranded RNA, heat shock protein 60, or fibrinogen fragments could all be proximal mediators of type I IFN production and thus lead to the more downstream activation program. In sera from SLE patients, IFNα levels correlate with IFN-response gene expression levels of PB cells.[37] It has been demonstrated for SLE and SS that immune complexes of autoantibodies and DNA- or RNA-containing autoantigens, can induce type I IFN production by PB plasmacytoid DCs (pDCs).[28,30,38,39] This response is
dependent on interaction with FcγRIIa and Toll-like receptors.[30,39] Further studies need to
determine whether the increased type I IFN-response genes in RA are the result of endogenous
or infectious factors.

Besides a role for peripheral blood cells as producers of type I IFNs, cells at the site of inflam-
mation may also be responsible for production. Cells with morphological and phenotypic
characteristics of pDC were shown to infiltrate skin lesions in SLE and actively produce type
1 IFN locally.[40] In patients with SS, numerous IFNα -producing cells were detected in the
affected salivary gland biopsies.[30] In RA, IFNβ protein has been detected in the synovium.
[41] Moreover, fibroblast-like synoviocytes (FLS) were responsible for increased levels of IFNβ in
the RA synovium.[42] The endogenous TLR3 ligand, dsRNA derived from necrotic synovial fluid
cells, has been shown to stimulate the production of IFNβ in RA FLS.[43]

In conclusion, we demonstrated that genomic profiling powers disease subclassification and
has led to the identification of subgroups of patients, based on differential expression of genes
involved in non-specific immunity.

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REFERENCES


