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Microarray-based identification of novel biomarkers in IL-1 mediated diseases

Florence Allantaz[1], **Damien Chaussabel**[1,2], **Jacques Banchereau**[1], and **Virginia Pascual**[1,2]
[1] Baylor Institute for Immunology Research and Baylor Research Institute, Dallas, TX

Summary

Interleukin 1b (IL-1b) is emerging as mediator of a wide range of human diseases. Availability of IL-1 blockers that result in clinical benefits to patients with these diseases is creating a demand for biomarkers to diagnose as well as to predict and follow responses to therapy. Blood gene expression profiling can be used to identify such biomarkers. This review will summarize recent studies in the field and will discuss some of the challenges raised by the use of this technology in biomarker discovery.

Introduction

Also known as the endogenous pyrogen, Interleukin-1b has been reported to cause inflammation in a wide variety of disease states ranging from infections to Alzheimer's. While animal models supported a fundamental role for this cytokine in the pathogenesis of chronic inflammatory arthritis, clinical trials in humans with rheumatoid arthritis using a recombinant soluble receptor antagonist did not yield the expected beneficial results, casting doubts about its potential a therapeutic target in human autoimmune diseases.

The discovery less than 10 years ago that several “hereditary autoinflammatory syndromes”, a group of genetic disorders characterized by recurrent attacks of fever and multi-systemic inflammation, could be traced down to mutations in genes controlling the production of IL-1b brought this cytokine back into the spot light. The spectrum of IL-1 mediated diseases has since then grown to include a wide array of hereditary and non-hereditary conditions displaying symptoms ranging from systemic to highly tissue-restricted. Indeed, pilot trials using IL-1 blockers have resulted in clinical benefits in the many of these diseases.

Measurement of serum IL-1b is not a reliable indicator of a role of this cytokine in disease. Therefore, as the number of IL-1b mediated diseases and the availability of new therapeutic agents to block this cytokine grow, so does the demand for biomarkers to diagnose, predict and follow patient responses to therapy.

The IL-1 Family

The IL-1 family plays an important role both in inflammation and host defense. Up to 11 members of this family have been identified to date [1]. Of those, only five have been thoroughly studied: IL-1a, IL-1b, IL-18, IL-1Ra and the recently reported IL-33. The remaining

2 Corresponding authors: Virginia Pascual (Virginip@Baylorhealth.edu) and Damien Chaussabel (DamienC@Baylorhealth.edu).

six (IL-1F5; IL-1F6; IL-1F7; IL-1F8; IL-1F9; IL-1F10) have been shown to be expressed in various cell types or tissues, but their functions remain to be determined.

IL-1a and IL-1b are proinflammatory cytokines. Both are synthesized as precursor molecules (pro-IL1a and pro-IL1b) by many different cell types. Pro-IL1a is biologically active and needs to be cleaved by calpain to generate the smaller mature protein. By contrast, pro-IL1b is biologically inactive and requires enzymatic cleavage by caspase-1 in order to become active. IL-1a is primarily bound to the membrane whereas IL-1b is secreted and thus represents the predominant extracellular form of IL-1 (reviewed in [2]).

There are two transmembrane IL-1 receptors, types I and II. IL-1RI binds IL-1b and this complex recruits the IL-1-receptor accessory protein (IL-1RAcP) leading to the initiation of the signal. IL-1RII is a decoy receptor. Thus, binding of IL-1b to IL-1RII results in its neutralization. All three receptor molecules, IL-1RI, IL-1RII and IL-1RAcP can be shed from the cell membrane and therefore exist in soluble forms (sIL-1RI, sIL1-RII and sIL-1RAcP). sIL-1RII and sIL-1RAcP both function as inhibitors of IL-1-mediated signal transduction by sequestering pro-IL-1b and IL-1R1 respectively. IL-1b bound to IL-1RII may also lead to recruitment of IL-1RAcP, therefore depriving the type 1 receptor of IL-1RAcP (reviewed in [2,3]).

IL-1Ra is an endogenous receptor antagonist. It exists as 3 intracellular isoforms (icIL-1Ra1, icIL-1Ra2 and icIL-1Ra3) and a secreted isoform (sIL-1Ra). IL-1Ra is predominantly produced by activated monocytes and macrophages. sIL-1Ra binds both type I and type II IL-1 receptors, thereby preventing binding and signal transduction by IL-1a and IL-1b. The functions of icIL-1Ra remain unclear (reviewed in [2]).

IL-33 is a new member of the IL-1 family. It is produced as a propeptide that requires cleavage by caspase-1 and binds to IL-1R4 (ST2). IL-33 has been shown to stimulate T helper 2 (TH2) responses [4].

Regulation of IL-1 Production and Activity

The production and biological activity of IL-1 are regulated at multiple levels, including transcription, translation, cleavage and cellular release.

IL-1a and IL-1b transcription is induced by a wide array of stimuli including bacterial and viral products, cytokines that include IL-1b itself, etc. The activation of caspase 1 is mediated by a multiprotein complex known as the “inflammasome”. Two main types of inflammasome have been described to date-the NALP1 inflammasome, composed of NALP1, the adaptor protein ASC, and caspases 1 and 5, and the NALP2/3 inflammasome that contains NALP2 or NALP3 (also known as CIAS1 or cryopyrin) as well as the caspase recruitment domain (CARD)-containing protein Cardinal, ASC and Caspase-1 [5]. Until recently, the mechanism(s) of activation of this complex remained unknown. $CIAS^{-/-}$ mice have revealed however that the NALP3 inflammasome can be directly activated by bacteria (*L. monocytogenes* and *S. aureus*), the purine analogs R848 and R837, bacterial mRNA, double-stranded/viral RNA and uric acid crystals [6-9]. Inflammasome activation leads to the conversion of pro-caspase-1 into caspase-1 and subsequent cleavage of pro-IL-1b into mature IL-1b (reviewed in [2]). Release of mature IL-1b depends on a second signal provided by the nucleotide P2X7 receptor, which can be activated by the human cathelicidin-derived peptide LL37 or ATP, leading to an efflux of potassium from the cell [10]. Potassium efflux is responsible for phosphatidylcholine-specific phospholipase C induction, which in turn allows the rise in intracellular free calcium concentration required for activation of phospholipase A(2). This activation is ultimately responsible for lysosome exocytosis and IL-1 beta secretion [10]. Recently, a model in which

K^+ efflux is the common and specific trigger of NALP1 and NALP3 activation induced by all reported ligands through the involvement of K^+ channel(s) has been proposed [11].

As described above, once IL-1b is released there is a tight regulation of its biological effects by a series of inhibitory molecules including IL-1Ra, sIL-1RI, sIL-1RII and sIL-1RAcP. Dysregulation of any of these steps might lead to increased IL-1b bioavailability and IL-1 mediated inflammation.

IL-1b Mediated Diseases

Il-1b is a major mediator of inflammation that plays a fundamental role in tissue injury repair as well as in the defense against microbial pathogens. In these situations, local (i.e. endothelial) and/or systemic (i.e. bone marrow) responses to this cytokine are responsible for beneficial effects, including among others cellular infiltration and neutrophil mobilization respectively. An excess of this cytokine, however, may have deleterious effects on a variety of cells and tissues. Injection of recombinant IL-1b in humans and in experimental animal models induces fever, anorexia and pain hypersensitivity through direct effects on the CNS. This cytokine also has important effects on endothelial cells that may lead to vasculitis and promote thrombosis. It plays a role in destructive joint and bone disease, and is toxic for insulin-producing β cells in the pancreas [12].

Given these protean local and systemic effects, it would not be surprising that IL-1b also mediates pathological inflammatory cascades in a variety of human diseases. These could include febrile diseases of non-infectious origin, vasculitis, arthritis and diabetes. Conventional approaches such as cytokine measurement in the serum of patients, have failed however to establish such connection.

IL-1b mediated diseases resulting from inflammasome dysregulation: the usual and the unusual suspects

As discussed above, IL-1b production is tightly controlled at distinct steps. Most IL-1b mediated human diseases identified thus far have been linked however to abnormal activation of this cytokine by the inflammasome [13]. The clinical hallmark of these diseases, which are also known as “periodic fever” or “autoinflammatory” syndromes”, is the presence of recurrent symptoms which are easily explained by an excessive production of IL-1b: fever and inflammation predominantly affecting, but not limited to, the serosal membranes, joints and skin. Their underlying genetic defects were identified through genome-wide linkage studies using multi-case families and controls. Among them, Familial Cold Autoinflammatory Syndrome (FCAS, MIM 120100), Muckle-Wells syndrome (MWS; MIM 19100), and Neonatal-Onset Multisystem Inflammatory Disease (NOMID, MIM 607115) result from mutations in the NALP3 or cryopyrin gene. Mutations in genes encoding proteins that interact with inflammasome components give rise to Familial Mediterranean Fever (FMF, MIM 249100), and the syndrome of Pyogenic Sterile Arthritis, Pyoderma gangrenosum and Acne (PAPA, MIM 604416) [14]. Yet, many patients fulfilling diagnostic criteria for some of these diseases do not display mutations in the corresponding genes. For example, no mutations have been identified in up to 50% of patients with clinically diagnosed FCAS, MWS or CINCA [15].

Inflammasome-mediated diseases, however, do not necessarily present with fever or systemic inflammation and do not always have a well-defined genetic basis. A combination of biochemical approaches and inflammasome gene knock-out in mice demonstrated that activation of the NALP3 inflammasome with contact sensitizers or uric acid crystals triggers the inflammatory cascades underlying contact hypersensitivity and gout/pseudogout

respectively [9,16-18]. These diseases affect considerably larger patient populations than the rare familial periodic fever syndromes.

In agreement with the notion that the inflammasome ultimately regulates the secretion of IL-1, blocking this cytokine with a recombinant IL-1 receptor antagonist has emerged as a successful form of therapy for many of these disorders (Supplemental Table I).

Novel IL-1b mediated diseases identified using blood gene expression profiling

Rationale for profiling the blood transcriptome—Blood is a valuable source of biomarkers. It is an accessible tissue amenable to sequential sampling, and can therefore be used not only for differential diagnosis or prognosis but also for monitoring disease progression and response to treatment. Thus far, biomarker discovery efforts have focused on the measurement of serologic markers. As the “pipeline of the immune system”, blood carries cells exposed to factors present systemically in the circulation, or locally in peripheral sites from which immune cells migrate to and from. Therefore, biomarkers can also potentially be identified through the analysis of the cellular responses to these factors.

Assessing gene expression changes in circulating blood cells represents an attractive approach for the following reasons: 1) Serum protein or lipids have been mined for decades, but the blood transcriptome constitutes a new source of potential markers; 2) Response to factors undetectable in the serum may be measured in circulating immune cells. These cells may, for example, be locally exposed to cytokines at the disease site before recirculating in the bloodstream. Alternatively, the cytokines may be released in the circulation in limiting amounts and rapidly consumed by immune cells. An example is the presence of type I interferon signatures in circulating blood cells from SLE patients even though detectable levels of members of this cytokine family are not found in the serum of many of the same patients [19,20]; 3) Considerable progresses have been made in the field of microarrays. Various commercial sources are available which show increased reproducibility when compared to “home-made” microarrays. It has also become relatively inexpensive to measure blood transcriptional profiles on an unprecedented scale using mainstream microarray technology. As a result, a number of studies have profiled the blood of patients, including in the context of IL1-mediated diseases.

Microarray analyses aiming at the discovery of diagnostic and prognostic biomarker signatures were first developed in the field of cancer [21,22]. These landmark studies, which focused on the analysis of tumor tissue, paved the way for a wider use of this technology in clinical research. Blood transcriptional signatures were reported only a few years later in patients with autoimmune diseases, including systemic lupus erythematosus [19,23,24], multiple sclerosis [25-27], rheumatoid arthritis [28-31], inflammatory bowel disease [32], psoriasis [33] and dermatomyositis [34,35]. Blood profiling studies extended rapidly to other diseases also characterized by a strong inflammatory component, especially infections caused by viruses like HIV [36], adenovirus [37], influenza [38], or dengue [39]; bacteria like *Staphylococcus aureus*, *Streptococcus pneumoniae* or *Escherichia coli* [38]; *Mycobacterium tuberculosis* [40]; and parasites *(Plasmodium)* [41]; as well as in patients with sepsis [42]. The blood of transplant recipients has also been profiled, in kidney [43], liver [44], heart [45], and hematopoeitic cell transplant recipients [46]. Furthermore, disease signatures have been detected in the blood of patients with diabetes [47,48], cardio vascular diseases [49], and non-hematological malignancies [50,51].

Exploiting the potential of blood microarray transcriptional analysis to understand Systemic onset Juvenile Idiopathic Arthritis (SoJIA)—SoJIA, one of the six major types of chronic inflammatory arthritis in children [52], is unique in terms of clinical manifestations, prognosis and lack of response to available therapies. The clinical

diagnostic hallmark of the disease is high, spiking fever, a salmon-color rash that follows the fever spikes and arthritis, although this manifestation is not always found at the time of disease initiation. Thus, SoJIA shares many clinical features with periodic fever syndromes. Although the symptoms are characteristically more persistent than those of periodic fever syndromes, many SoJIA patients experience an intermittent or polycyclic course with flares and remissions. Similarly to patients with autoinflammatory diseases, SoJIA patients usually lack autoantibodies and autoreactive T cells.

From the early 1990s several pro-inflammatory cytokines, especially IL-6 and TNFa, were postulated to play a role in SoJIA based on the detection of elevated levels in the serum or synovial fluid (SF) of these patients [53]. Interleukin-6 (IL-6) levels are elevated in SoJIA serum and to correlate with disease activity, including the severity of joint involvement, platelet counts, CRP and fever spikes. Synovial fluid levels of IL-6 are markedly elevated in SoJIA and significantly higher than in patients with other types of JIA or adult rheumatoid arthritis.

TNFa levels are increased in all subtypes of JIA. In patients with active SoJIA, circulating levels of TNFa, sTNFR1 and sTNFR2 are significantly higher than in controls. The levels of sTNFR1 and sTNFR2, but not those of TNFa, are associated with the persistence and severity of systemic symptoms. There are controversial reports regarding serum levels of IL-1 in SoJIA patients. IL-1b, however, may be difficult to detect in the serum as significant amounts of proIL-lb remain inside the cell. Additionally, serum IL-1b binds to large proteins such as b-2-macroglobulin, complement, and the soluble type II IL-1 receptor [12], making its detection difficult.

We have used two different but complementary approaches that leverage microarray technology to help our understanding of the pathogenesis of SoJIA: 1) analyzing transcriptional changes induced upon culturing healthy blood mononuclear cells with serum from active patients; 2) analyzing transcriptional patterns of freshly isolated blood mononuclear cells from active patients themselves (Figure 1). Culture experiments revealed that SoJIA serum up-regulates IL-1 transcription in healthy cells. As expected, IL-1 secretion is also induced in a disease-activity dependent manner [20]. The serum effects are recapitulated in vivo, as IL-1b transcription is also upregulated in the blood mononuclear cells from the patients during the systemic phase of the disease. Indeed, genes known to be induced by IL-1b (i.e. pentraxin 3), or potentially involved in IL-1b secretion (i.e. KCNJ15 or ATP-sensitive inward rectifier potassium channel 15) are also upregulated in the majority of patients. Furthermore, as shown for patients with periodic fever syndromes, mononuclear cells from active SoJIA patients secrete an excess of IL-1b protein upon activation. In the same cultures, IL-6 and TNF production is not significantly different from controls, suggesting a specific dysregulation of the IL-1 pathway in patients with SoJIA.

Most clinical and laboratory manifestations of SoJIA can be explained based on increased IL-1 production, but the origin of this dysregulation remains unknown. An unusual microorganism could target an otherwise normal innate immune system resulting in IL-1 overproduction. There is no epidemiological evidence however for clustering of SoJIA patients, which would be expected if this were to be the case. Alternatively, as described above for inflammasome-mediated diseases, a common infectious or inflammatory trigger could lead to an excessive production of IL-1 in patients with underlying mutations in genes controlling IL-1 production. In favor of this hypothesis, non-specific activation of SoJIA PBMCs in vitro results in excessive IL-1 b secretion [20]. Since IL-1b can upregulate its own transcription, IL-1b itself could also be responsible for the serum effects described above.

In support of the role of IL-1b in SoJIA, blocking this cytokine results in remarkable clinical and hematological responses [20]. Rapid and sustained resolution of clinical symptoms

including fever, marked leukocytosis, thrombocytosis anemia, elevated ESR and arthritis has been reported in pilot trials (Supplemental Table I). Furthermore, IL-6 levels return to normal in patients after initiation of therapy, supporting that increased IL-6 production is a secondary event downstream of Il-1b (Allantaz, unpublished observations). Rapid responses to IL-1b blockade have also been described in patients with refractory adult-onset (Still's) disease (Supplemental Table I). All of these findings point toward IL-1b as a mediator of SoJIA and therefore link this disease to the family of autoinflammatory disorders.

Blood microarrays to identify SoJIA-specific diagnostic biomarkers—A major remaining challenge is how to establish the prompt diagnosis of the disease to initiate effective therapy. Gene expression patterns in blood leukocytes can also be used for this purpose. There is however a significant degree of overlap between the blood gene signatures of SoJIA patients and those of other febrile inflammatory disease groups which represent a true differential diagnosis in the clinical setting. This is especially true for Gram (+) bacterial infections and autoinflammatory syndromes in which IL-1b production is also increased [54] [55]. Indeed, when comparing gene expression profiles across more than two different diseases it is difficult to control potentially confounding factors.

Analysis of significance patterns is a strategy that has been successfully applied to this type of situations [55,56]. First, statistical comparisons are performed between each group of patients and their respective control groups composed of age-matched and gender-matched healthy donors. The p-values obtained from each comparison are then subjected to selection criteria to identify genes significantly changed in the disease of interest versus its control group, and not in any of the other diseases versus their own control groups. The advantage of this analysis is that it permits normalization of each disease group to its own matched control group, therefore avoiding biological (i.e. age, gender) or technical (i.e. array runs) confounding factors. Using this approach, a SoJIA-specific signature composed of 88 genes was identified (Figure 2). Furthermore, administration of IL-1 blockers resulted in the normalization of expression of the majority of these transcripts in the treated patients (Figure 3). Indeed, 12 highly significant genes from this analysis ($p<0.0001$ in SoJIA and >0.5 in all other groups) permitted accurate disease classification in 18/19 SoJIA patients [55]. If validated with a larger number of patients in multi-centric studies, this “mini-signature” should permit to establish an early diagnosis and initiate specific therapy. Thus, the subsequent development of long term disabilities might be prevented in these patients.

Blood microarrays support the role of IL-1 b in the pathogenesis of type 1 and type 2 diabetes—IL-1b has been implicated as an effector molecule of inflammatory beta-cell destruction leading to diabetes. In recent years however this hypothesis has remained quite controversial. Reports that high glucose–induced β-cell apoptosis occurs in both type 1 and type 2 diabetes and involves IL-1β production by β-cells, nuclear factor-kB (NF-kB) activation, and death via Fas-FasL [57] have been questioned by other groups reporting that neither high glucose in vitro nor the diabetic state in vivo induces IL-1β production or NF-kB activation in human islets [58].

Blood microarray analysis of children with newly diagnosed type 1 and type 2 diabetes show that 5 of the 10 most highly overexpressed genes in these patients are also overexpressed in patients with SoJIA and/or in healthy PBMCs incubated with SoJIA serum, one of the most relevant being IL-1b [48]. These data would support that type 1 and type 2 diabetes share a common pathway for beta cell dysfunction that includes secretion of IL-1b and downstream proteins that may exacerbate pre-existing beta cell dysfunction and contribute to further hyperglycemia.

Indeed, the blockade of interleukin-1 with recombinant soluble receptor antagonist improves glycemia and beta-cell secretory function and reduces markers of systemic inflammation in patients with type 2 disease [59]. Given the overall safety clinical record of IL-1 blockers, similar studies should be conducted in newly diagnosed type 1 diabetes patients to evaluate the value of this form of therapy in preserving beta cell function and reduce exogenous insulin requirement in this disease.

Blood Microarrays as a Pipeline for Biomarker Discovery: Challenges and Opportunities

As discussed above, transcriptional profiling studies provide excellent opportunities for the discovery of novel biomarkers, but they also raise some interesting challenges [60]. Indeed, one of the major challenges is how to identify the few markers out of all the data generated that will truly be relevant clinically. To this end, three main issues need to be addressed: 1) Noise- microarray platforms can simultaneously measure a large number of parameters for a given sample. However, this defining feature becomes a liability when differences in gene expression for each one of these parameters are tested for two or more study groups. Without a doubt, multiple comparisons carried out on such a scale inevitably generate false positive results (i.e. noise). Such noise can in turn affect the stability of biomarker signatures, and it may also lead to spurious biological interpretations [61,62]. 2) Technical variability- sample collection, processing and analysis involve many steps that can each contribute to increased data variability [63]. Technical variables can significantly affect data analysis and explain at least in part the poor reproducibility of microarray data across various microarray platforms and between laboratories [64]. 3) Biological variability-compounding noise and technical variability, patient-based studies also face considerable inter-individual variability that may be attributed to differences in genetic background, environmental influences and disease heterogeneity (different forms of the disease, different stages, co-morbidities, treatments etc…) [65,66].

These issues may limit the potential of patient blood transcriptional studies for biomarker discovery, and solutions must be sought in order to address them: 1) False positive results can be kept in check by multiple comparison correction strategies (family-wise error rate control). These tend however to act at the detriment of the biological signal (true positive); and more recently, mixture-model methods have been devised as a means to estimate false discovery rates from microarray data results [67]. Because noise can never be totally controlled, it is important to independently validate microarray results obtained during the discovery phase (training) in separate sample sets (testing). 2) Variability can be introduced by technical factors prior to the microarray analysis step. Transcriptional activity has, for instance, been found to change in blood samples kept at room temperature at different time intervals [68,69]. Also, standardization of sample collection procedures and the use of stabilizing reagents to “freeze” transcriptional profiles immediately upon blood collection are essential [70]. Variability introduced during the microarray analysis step also contributes significantly to the lack of reproducibility of microarray data observed across laboratories [71]. This is particularly problematic as microarray expression levels are not measured quantitatively but rather as an absolute intensity. Indeed, intensity levels may vary from batch to batch; and it is therefore essential to include all the controls in each of the runs. Maximizing the number of samples processed per run, adopting strict operating procedures and relying as much as possible on automation for sample processing can help control technical variability. An alternative technology platform, such as real-time PCR, can also be used to confirm the most salient results on a limited scale. 3) Even the most accurate results may still be biased as a result of an insufficient sample size. An adequate sample size, however, cannot be determined a priori. Biological variability, which is rampant in patient-based studies, can only be addressed by increasing the number of biological samples analyzed for both the discovery and independent assessment of biomarkers. Fewer samples can be used, for instance, when large differences are

measured between homogenous groups rather than when small differences are measured between heterogeneous groups of subjects. Thus, estimating sample size requires using preliminary data to estimate biological variability as well as differences in gene expression levels between study groups [72,73]. Longitudinal follow up of disease progression offers an alternative means to manage biological variability by focusing the analysis on changes occurring over time in the same individual.

Conclusion

Blood gene expression profiling studies have permitted to identify the presence of transcriptional signatures in a wide range of human diseases. These studies have shown that diseases with diverse pathogenesis and clinical manifestations may share common immune mediators, which represent therapeutic targets for intervention. An IL-1 b signature, for example, has been found in systemic onset juvenile arthritis and type 2 diabetes. Pilot clinical trials using IL-1 blockers have resulted in clinical benefits in both diseases, supporting the validity of blood microarrays to identify therapeutic targets. Blood microarrays can also be used to discover transcriptional biomarkers to diagnose and follow disease activity and response to therapy in the clinical setting. Thus far, progress in the identification of robust biomarkers has been hampered by problems inherent to system-wide studies. Solutions now exist, however, to account for noise and to better control technical variability. Blood transcriptional studies are therefore ready to move to the next level and tackle inter-individual variability in large scale multi-centric studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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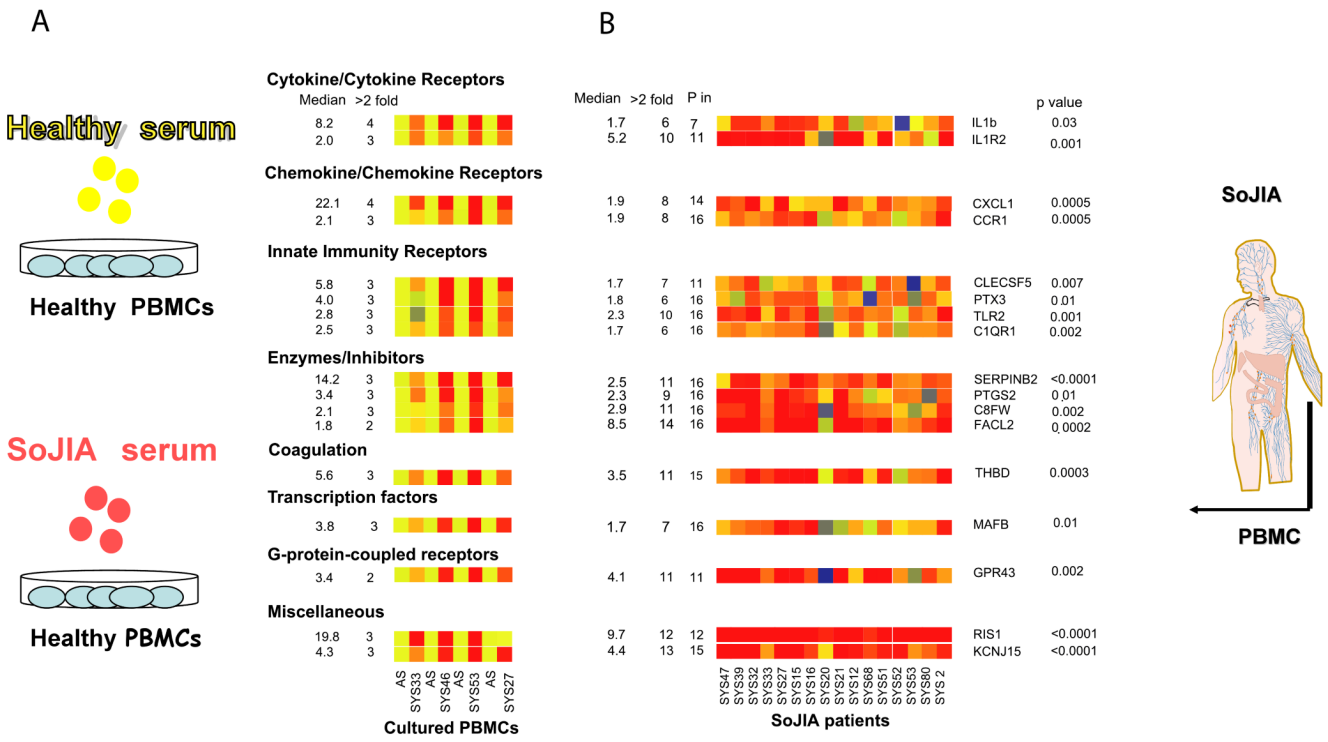

Figure 1.

Microarray technology to help understanding the pathogenesis of SoJIA: A) Analysis of transcriptional changes induced upon incubation of healthy PBMCs with autologous sera (AS) or with sera from four patients with active SoJIA (SYS33, SYS46, SYS53, and SYS27). Sera from patients induced the up-regulation of 46 genes. Median fold up-regulation by the four SoJIA sera incubation is depicted on the left column. The number of SoJIA sera that induced greater than twofold up-regulation is shown in the next column. B) Expression of a set of gene probes from Fig.1a in the PBMCs of 16 active SoJIA patients. The patient PBMCs expression data were normalized to the median expression of the same gene probes in the PBMCs of 12 healthy children. Median gene expression and number of samples with greater than twofold up-regulation are depicted in the first two columns. The third column represents the number of samples with a P (present) flag according to Affymetrix MAS 5.0 scaled gene expression data. p-values (Mann-Whitney test) are given next to these genes. Adapted from reference #20

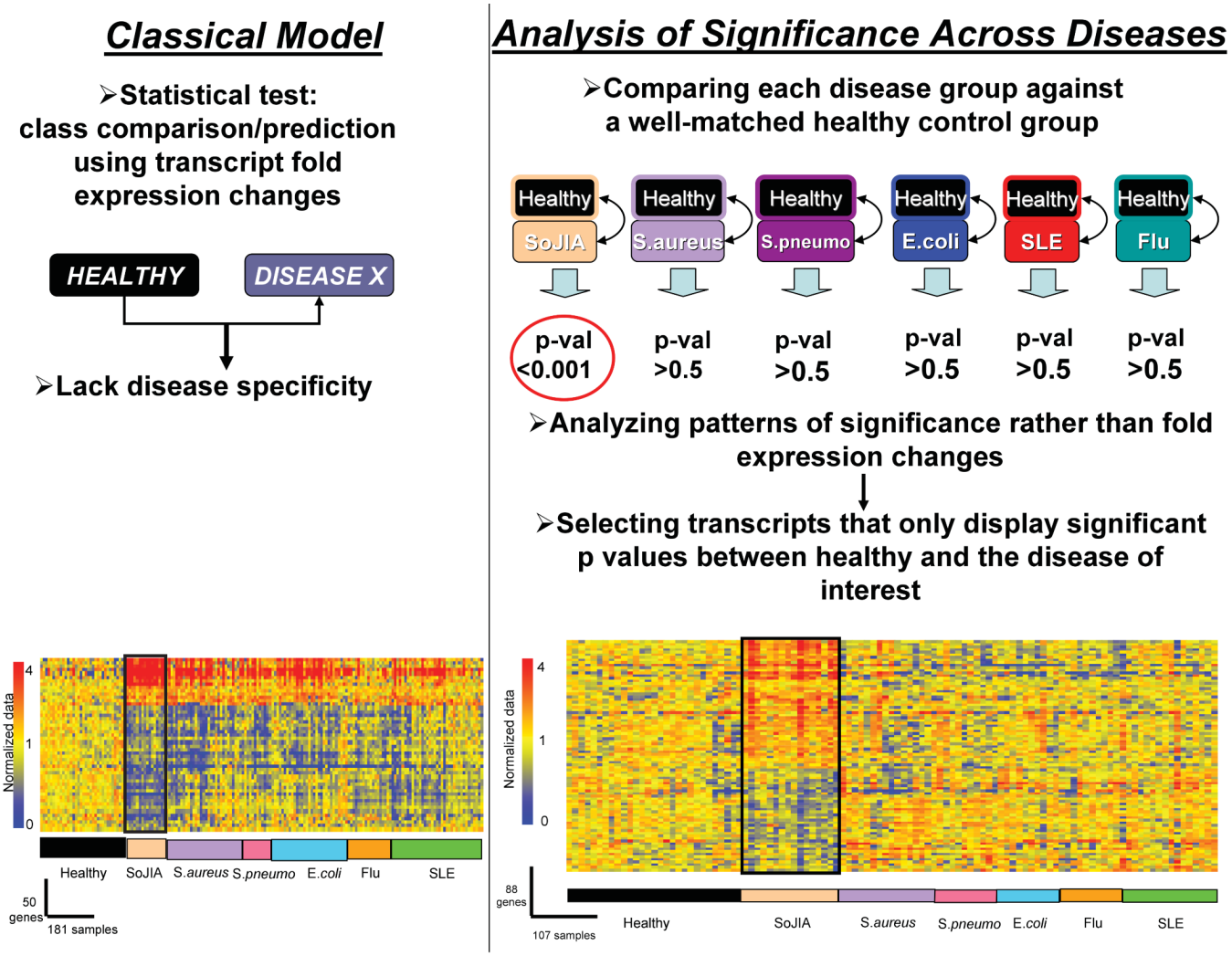

Figure 2. Analysis of significance across diseases identifies 88 SoJIA-specific transcripts

(A) Eight healthy and eight SoJIA samples were used as training set to generate a list of 50 classifier genes displaying the best ability to discriminate SoJIA patients from healthy controls. Those classifier genes were hierarchically clustered in a test set composed of 35 healthy controls, 16 SoJIA, 31 *S. aureus*, 12 *S. pneumoniae*, 31 *E. coli*, 18 Influenza A and 38 SLE patients. (B) Genes expressed at statistically different levels in SoJIA patients compared to healthy volunteers ($p<0.01$, Wilcoxon-Mann-Whitney test) were selected (4311 probe sets). Out of those, 88 were found expressed at statistically different levels in SoJIA patients compared to healthy volunteers ($p<0.01$, Wilcoxon-Mann-Whitney test) but not in all the other groups ($p>0.5$, Wilcoxon-Mann-Whitney test). The 88 genes are hierarchically clustered in the 107 samples from different disease groups used in (A). Expression values or the genes are normalized per-gene to the healthy group (Adapted from Reference # 55).

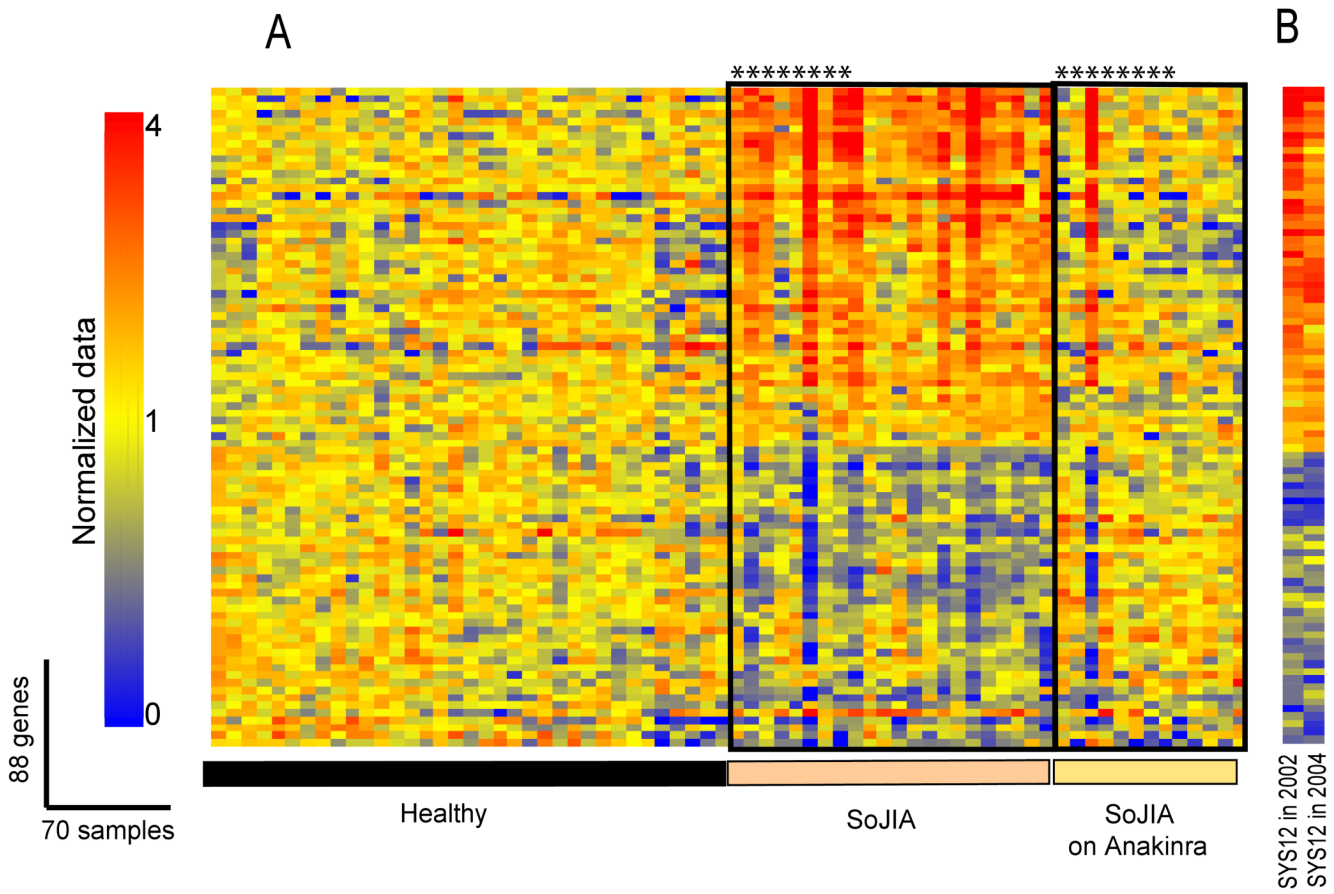

Figure 3. Treatment with IL-1Ra (Anakinra) extinguishes the SoJIA-specific signature

(A). 88 SoJIA-specific genes were analyzed in 35 healthy, 22 SoJIA patients not receiving IL-1 blockers and 14 SoJIA patients after initiation of treatment with IL-blockers. *represents the same patients before and after initiation of the therapy. **(B).** The SoJIA signature is present in a patient on two occasions taken 2 years apart. On both occasions the patient was active and not receiving IL-1 blockers.