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Hypothesis: Sarcoidosis is a STAT1-mediated disease

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Abstract

Immunologic pathways involved in sarcoidosis pathogenesis are largely unknown. We hypothesized that patients with sarcoidosis have characteristic mRNA profiles. Microarray analysis of gene expression was done on peripheral blood (12 patients,12 controls), lung (6 patients, 6 controls) and lymph node (8 patients, 5 controls). Comparing peripheral blood from patients with sarcoidosis to controls, 872 transcripts were upregulated and 1039 were downregulated at ≥ 1.5-fold change and a significant q value. Several transcripts associated with interferon and STAT1 were upregulated. Lung and lymph node analyses also showed dramatic increases in *STAT1* and *STAT1*-regulated chemokines. Granulomas in lymph nodes of patients with sarcoidosis expressed abundant STAT1 and phosphorylated STAT1. STAT1 might play an important role in sarcoidosis. This novel hypothesis unites seemingly disparate observations with regard to sarcoidosis including implication of a casual role for interferons, a suspected infectious trigger, T_H1 predominating lymphocytes in bronchoalveolar lavage, and the association with hypercalcemia.

Keywords

Gene expression profiling; microarray analysis; sarcoidosis; uveitis

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INTRODUCTION

Sarcoidosis is a granulomatous disease presumably induced by a harmful immune response. Sarcoidosis can be present in many organ systems including the lung, lymph node, eye, skin, joint, heart, liver, and brain. Although the trigger for the immunological damage is unknown, an infectious or environmental precipitant is strongly suspected [1]. Genetic factors also contribute substantially to the development of this disease [2].

Microarray-based assays have allowed the detection of thousands of mRNA transcripts from relatively small samples. The study of gene expression has been especially informative in the analysis of malignant tissue such as lymphoma [3], melanoma [4], or breast cancer [5]. In these examples, the pattern of gene expression provides diagnostic and prognostic information which cannot be obtained by histological analysis.

Microarray analysis has contributed to elucidating the pathogenesis of immune-mediated diseases. For example, many but not all patients with systemic lupus erythematosus (SLE) have an upregulation of genes induced by type I interferons [6]. Peripheral blood RNA also has distinct expression patterns in immune mediated diseases such as rheumatoid arthritis [7], multiple sclerosis [8], and dermatomyositis [9].

In order to derive clues about the pathogenesis of sarcoidosis, we performed microarray analysis of gene expression using peripheral blood samples from patients with sarcoidosis. We compared gene expression in peripheral blood with gene expression detected in either lung or lymph node from patients with this disease. Our study indicates that many genes under the regulation of the transcription factor, STAT1 (signal transducer and activator of transcription 1), have increased peripheral blood expression in sarcoidosis. The STATs are a family of transcription factors that regulate a set of genes involved in the inflammatory response [10]. STAT1, in particular, is induced by interferons that could be stimulated by viral or mycobacterial infection, potential triggers for sarcoidosis. Furthermore, we found that genes regulated by STAT1 were also upregulated in the lymph node from patients with sarcoidosis and that mRNA for at least 3 STAT1 regulated chemokines, CXCL9, CXCL10, and CXCL11, were markedly upregulated in either the lung or lymph node of patients with sarcoidosis. Finally, both STAT1 and the activated form of STAT1 (phosphorylated STAT1) were abundantly expressed in the lymph node granulomas of patients with sarcoidosis STAT1 may be a novel and promising target for pharmacotherapy of this disease since sarcoidosis is inconsistently responsive to immunosuppressive therapy [11].

METHODS

This study involved collaboration of two separate institutions which used slightly different microarray methodologies. All microarray studies on peripheral blood were performed at the Oregon Health & Science University while all solid tissue microarray studies were performed at Ohio State University Medical Center. The study received approval by the OHSU and OSUMC local institutional review boards for the blood and solid tissue studies respectively. Informed written consent was obtained from all patients and control subjects.

Human subject selection and diagnosis

Patients whose peripheral blood gene expression was analyzed had symmetric hilar adenopathy as judged either by chest x-ray or by computerized tomographic scan (CT). Since the combination of symmetric hilar adenopathy and uveitis is considered specific for a diagnosis of sarcoidosis [12], five of the seven patients with uveitis did not have biopsy confirmation of the diagnosis. Four of the five patients without uveitis had lung biopsy confirmation of the presence of non-caseating granuloma. The diagnosis of uveitis was confirmed on a dilated eye

examination at a clinic which specializes in the care of patients with uveitis. Healthy controls were attending an ophthalmology clinic for routine eye care and were known to have no current or prior evidence for uveitis. Since medications can markedly affect gene expression, all blood and solid tissue samples were obtained from patients who were not receiving oral corticosteroids or immunomodulatory therapy.

The portion of the study involving peripheral blood included 12 patients with sarcoidosis, 7 of whom also had active uveitis and 12 healthy controls. Healthy individuals were attending an ophthalmology clinic and were thus known to have no active ocular or systemic inflammatory disease. The average age for patients with sarcoidosis was 53.9 ± 12.2 years at the time of enrollment and 48.3 ± 10.9 years at diagnosis. The controls had a mean age of 48.8 ± 21.4 years, which was not statistically different from the patients. Details on gender, race, and methodology for diagnosis are shown in Table 1.

Patients who provided diseased lung or lymph node met the operational diagnosis of sarcoidosis based upon the accepted pathological criterion, i.e., samples displayed well-formed non-necrotizing epithelioid granuloma in the absence of identifiable infection or foreign body, in accordance with diagnostic criteria described in the American Thoracic Society's joint statement on sarcoidosis [13]. Samples exhibiting atypical pathological features, such as necrosis or fibrosis, were excluded. Disease-free lung tissues were obtained during surgical lung resections, bronchoscopic lung biopsy or in the immediate post-mortem period from patients who had submitted for organ donation for medical research. Each control sample had normal lung histology verified by a certified pathologist. The lymph node samples were from organ donors (normal) or patients undergoing surgical biopsies (sarcoidosis) provided from the Midwestern Division of the Cooperative Human Tissue Network.

Gene expression analysis

Blood was collected directly into PAXGene tubes (2.5 ml/tube; 4 tubes/subject), incubated for at least 2 hours at room temperature for cell lysis and RNA stabilization, and stored at −80 °C. RNA was purified with PAXGene columns and DNase treatment per the manufacturer's protocol and stored at −80 °C until needed for the microarray procedure. Initial tests confirmed the manufacturer's claim that there is negligible difference in the microarray hybridization results between samples processed immediately and those frozen in the PAXGene tubes [14].

For studies on peripheral blood, RNA samples were quantified by spectrophotometry using the SpectraMax M2 plate reader (Molecular Devices) and RNA quality was determined using Lab-on-a-Chip RNA NanoChips and the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). This is a capillary electrophoresis system to characterize size distribution. Total RNA quality was verified by the presence of two discrete electropherogram peaks corresponding to the 18S and 28S rRNA at a ratio approaching 2:1. Samples with electropherogram patterns consistent with acceptable microarray performance were selected for labeling and microarray analysis. Three μg of each total RNA were amplified and labeled using the GeneChip Globin Reduction Protocol rev. 1 (Affymetrix, Inc., PreAnalytiX). This protocol uses peptide nucleic acid (PNA) oligonucleotides complementary to human globin mRNA transcripts during the first strand cDNA synthesis reaction of the Affymetrix one-cycle cDNA synthesis/IVT amplification and labeling procedure to improve assay sensitivity by reducing the amount of cDNA generated from globin mRNA. The GeneChip Globin Reduction Protocol was selected after initial studies comparing it with GLOBINclear (Ambion, Austin, TX) and Ovation (NuGEN, SanCarlos, CA) options for minimizing adverse effects from the high globin mRNA content of whole blood (manuscript in preparation). Following cRNA amplification, 10 μg of each labeled target were hybridized with a Human Genome U133 Plus 2.0 array (Affymetrix, Inc.) using standard protocols as described in the GeneChip Expression Analysis manual (www.affymetrix.com/support/technical/manual/expression_manual.affx). The U133 Plus 2.0

array contains 54,000 probe sets designed to analyze the expression of 47,000 human transcripts and variants. Following hybridization, arrays were processed, stained, and then scanned using the GeneChip scanner 3000 (Affymetrix, Inc.). Image processing was performed with the Affymetrix GCOS version 1.4. Initial analysis of individual array performance was performed using the MAS 5.0 statistical analysis program.

Each array scan was processed using the GeneChip Operating Software (GCOS) to produce cell fluorescence intensity (.CEL) files. CEL files were imported into the R statistical language environment [15]. Perfect match (PM) probe data were corrected for background noise using the GeneChip robust multi-array analysis (GCRMA) developed by Wu and co-workers [16]. Corrected PM probe data were normalized with the algorithm based on rank invariant probes by Li and Wong [17]. Gene expression values were determined using a linear model estimated by the median polish algorithm, according to the description of Irizarry and colleagues [18].

After normalization, data sets were compared with the Significance Analysis of Microarrays (SAM) software[19]. This method of analysis is designed for relatively small data sets. It incorporates the concepts of the false discovery rate (FDR) [20] and the q value [21]. The FDR controls for the expected ratio of false positives among significantly expressed genes. The q value is a posterior Bayesian p value, and it indicates the minimum FDR at which the test detects a statistically significant difference. For pair-wise comparisons in this analysis, the FDR was set at 5 %, with a significant difference in gene expression defined as one having a q value less than 0.05. Differentially expressed genes are presented by using heatmaps. All computations were done with R and its add-on packages; “affy”, “gcrma” and “samr” that run above the R environment. The list of genes regulated by the STAT1 transcription factor was extracted from the Transfac Pro Database [22]. The blood data have also been used to illustrate an analytical approach described in a statistical methods paper [23].

Variations in Techniques for Solid Tissue Studies

Frozen tissue was maintained at −80°C until the day before total RNA isolation, at which time the sample was soaked overnight in RNAlater-ICE(Ambion, Applied Biosystems, Foster City, CA) at −20°C. The samples were then removed from RNAlater-ICE and total RNA was isolated using TRIzol reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer’s protocol. The RNA was cleaned using the QIAGEN RNeasy Mini Kit (Qiagen Inc., Valencia, CA). The integrity of total RNA samples was assessed qualitatively on an Agilent 2100 Bioanalyzer as above. Following array hybridization, housekeeping genes, β-actin and GAPDH, were used to assess the quality of the synthesized, labeled cRNA. Samples were excluded from gene array analysis if the ratio between the 3′ and 5′ signals exceeded 4, with ideal values being between 1 and 2. Statistical analysis was done by using modified t-test with random variance model using BRB Array Tools software [24]. Q values and false discovery rate adjusted p values are equivalent but differ slightly as the q value uses Bayesian analysis. Studies on blood samples relied on q values and p values were calculated for solid tissue studies because the two centers used different software for normalization and statistical analysis. Additional experimental details and data from the lymph node and lung analysis are in press [25].

Immunohistochemistry—Formalin-fixed, paraffin-embedded lymph nodes were obtained from Oregon Health & Science University pathology archives. Phosphorylated STAT1 (pSTAT1) and non-phosphorylated STAT1 expression were determined by immunohistochemistry on 5 μm sections with purified rabbit polyclonal antibodies detecting either human STAT1 or pSTAT1 at the phosphorylation site of tyrosine 701 (GenScript Corp., Piscataway, NJ, USA). Antigen retrieval was achieved by boiling sections in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 9.0) for 10 minutes. Sections were incubated for 1 hour in

blocking solution (4.5% goat serum, 0.36% Triton X-100, 0.1% bovine serum albumin) and then overnight at 4 °C with primary antibodies or control rabbit IgG diluted 1:80 in blocking solution. After several washes, sections were incubated with pre-absorbed, alkaline phosphatase-conjugated anti-rabbit IgG antibody for 1 hour (1:200, GeneTex Inc., San Antonio, TX, USA). Immunostaining was visualized by incubating with Fast Red developer (BioGenex, San Ramon, CA, USA) until coloration was apparent (approx. 2 min.). Sections were counterstained with hematoxylin.

RESULTS

Microarray analysis detected 1187 probe sets (1039 transcripts; many genes in the U133 Plus 2.0 array are represented more than once with different probe sets detecting different portions of the transcript) among the patients with sarcoidosis that were significantly upregulated with q-value less than 0.05 and by a factor of at least 1.5 fold relative to the controls. Twelve hundred eighty-one probes sets (872 transcripts) were downregulated in the sarcoidosis group relative to the controls using the same criteria. The lists of the significantly up- and downregulated genes with at least a 1.5-fold change are shown in Supplementary Tables 1 and 2. The difference between patients with sarcoidosis and controls is shown visually for the probe sets with a minimum of a 2-fold change using a display approach called a heat map which is used in most gene expression studies (Figure 1).

Our attention focused initially on STAT1 because: 1) the upregulation of the STAT1 transcript could be validated by seven different probe sets; 2) all q values for these probe sets were < 0.002; 3) the average fold change for all STAT1 probe sets was 1.99 with most probe sets showing more than a two-fold increase; and 4) STAT1 is known to be a critical transcription factor in the inflammatory response.

Consequently, we searched the TRANSFAC database to identify genes directly regulated by STAT1. As shown in Figure 2, thirteen of the 18 genes under the regulation of STAT1 were upregulated based on a q value less than 0.05 (Table 2).

We additionally analyzed other STATs, JAKs (the kinase known to activate STATs), and interferon receptors and noted that many were consistently upregulated in the patients with sarcoidosis relative to controls (Table 3). The interferon regulatory factors (IRFs) are a family of 9 transcription factors which are activated by interferons and other inflammatory mediators. Eight of the 9 known IRFs are detected by the microarray employed in our study. Transcripts for six of the 8 detectable IRFs (IRF 1, 2, 4, 5, 6, and 7) were upregulated (range 1.13 to 2.39 fold) with a q value <0.05 for each in the peripheral blood of sarcoidosis patients compared to controls.

We included two additional checks on the validity of our analysis. We studied a group of 8 patients with idiopathic uveitis. All patients in this group had active intraocular inflammation, but they also presumably represent inflammation that has resulted from a variety of different etiologies. In this group, we found only 6 statistically significant differences in gene expression between patients and the control group. The upregulation of 6 transcripts is possibly not pathogenetically significant, as one would expect to find by chance at least 6 differences between data sets based solely on the number of statistical comparisons performed. We also studied patients with ankylosing spondylitis (n = 12; 7 with uveitis and 5 without uveitis). While these patients had a gene expression pattern that differs from controls, it did not reflect a pattern of genes regulated by STAT1 (manuscript in preparation).

Studies based on relatively small numbers of subjects and involving multiple statistical comparisons are fraught with the detection of differences that are not reproducible (i.e., type 1 statistical errors). The finding that many transcripts regulated by STAT1 are also increased

strongly suggests that increased STAT1 mRNA is not an artifact. However, as an additional validation, we compared the STAT1 signature in peripheral blood with gene expression in two tissues classically affected in sarcoidosis, lung and lymph node. Demographics for these patients are shown in Table 4. In addition to upregulation of STAT1 itself, six of the 18 STAT1 regulated genes as identified by the TRANSFAC database showed increased expression in lymph node based on FDR adjusted $p<0.05$ for each comparison. The upregulated transcripts were CXCL9, IRF-1, A2M, WARS, c-fos, and SOAT1. The transcript for the chemokine, CXCL9 was upregulated 9 fold. This same transcript was upregulated 9.5 fold in the lung. The TRANSFAC database used to analyze the data from peripheral blood does not include CXCL10 and CXCL11 in its listing of STAT1 regulated genes, although CXCL10 is γ-interferon inducible protein 10 (IP10) and CXCL11 is γ-interferon inducible T cell alpha chemoattractant (I-Tac). Table 5 summarizes the upregulation of STAT1 and the transcripts for CXCL9, CXCL10, and CXCL11 as found in blood, lymph node, and lung relative to the appropriate control tissue.

Although STAT2 and STAT3 were slightly upregulated in peripheral blood, we were unable to show that these two specific transcription factors or other STATs were up regulated in the lymph nodes from patients with sarcoidosis.

All of the above studies are based on the measurement of mRNA. In order to determine if the alteration in mRNA expression correlated with a change in protein expression, we examined the presence of both STAT1 and activated STAT1 (phosphorylated STAT1) in lymph nodes from 6 patients with sarcoidosis and 6 control subjects with follicular hyperplasia. Both STAT1 and pSTAT1 were much more abundant in the lymph nodes of patients with sarcoidosis than in control lymph nodes (Figure 3). In addition, the expression of STAT1 was predominantly in the granulomas themselves suggesting that it is directly involved in the pathogenesis of the granulomas.

DISCUSSION

Our results indicate that RNA for the major transcription factor, STAT1, is upregulated in the peripheral blood of patients with sarcoidosis compared to healthy controls. In addition, mRNAs for 13 of the 18 genes directly regulated by STAT1 have a statistically significant increase in the blood of patients with sarcoidosis. There are 7 known STATs which join in various combinations of homo- or heterodimers [10]. The STATs are activated by Janus protein tyrosine kinases (JAKs). Although signaling through many receptors is dependent on JAK-STAT activation, interferons are especially dependent on this pathway. γ-interferon binds to a specific receptor and induces gene expression via activation of STAT1 homodimers.

A limitation of our study is that the sample size is relatively small and our analysis involves multiple statistical comparisons. However, the upregulation of this transcript is confirmed by multiple independent probe sets which are included in the microarray. All but one of these probes indicated that the transcript for STAT1 was significantly upregulated. More importantly the proposed role of STAT1 is strongly supported by RNA data from lung and lymph node and the detection of activated STAT1 protein in granulomas from lymph nodes of patients with sarcoidosis. Although STAT1 has not been implicated previously in the pathogenesis of sarcoidosis, it may be a major contributor to many clinical characteristics. First, the increase in STAT1 fits well with a possible infectious trigger such as mycobacteria for this disease.

Catalase-peroxidase derived from *M. tuberculosis* was found in affected tissue of 55% of patients with sarcoidosis and in no control tissue [26]. Mycobacterial antigens strongly induce the production of γ-interferon [27]. Polymorphisms in the STAT1 gene influence susceptibility to mycobacterial infection [28]. Mice which lack STAT1 are especially susceptible to

mycobacterial infection in the lung [29]. Mice infected with the parasite, *Angiostrongylus cantonensis*, have increased STAT1 in their granulomatous brains [30]. Second, activated expression of STAT1 explains why sarcoidosis is generally considered to be a T_H1-mediated disease. T cell subsets are grouped on the basis of the most abundant cytokines which are produced. T cells which best express γ-interferon are designated T_H1. This subset of T cells predominates in the broncho-alveolar lavage of patients with sarcoidosis [31;32;33]. Since γ-interferon induces STAT1 expression, a T_H1 mediated disease should be associated with increased expression of STAT1. On the other hand, T_H1 expressing lymphocytes do not predominate in the peripheral blood of patients with sarcoidosis [32]. Accordingly our peripheral blood measurements likely reflect sources in addition to T cells. Third, hypercalcemia, a well described complication of sarcoidosis, can be explained by an increased expression of STAT1 [34], which enhances the conversion of 25-hydroxy vitamin D to its more active form, 1, 25 di-hydroxyvitamin D. Fourth, independent studies have already noted an increase in protein in the serum of patients with sarcoidosis for several chemokines regulated by STAT1 including CXCL9 (MIG or monokine induced by γ-interferon) and CXCL10 (IP10, interferon inducible protein 10) [35]. Fifth a published study that used microarray to study bronchoalveolar lavage cells from 3 patients with sarcoidosis found elevation of TYK2 and p21Waf1/Cip1 [36]. Both of which are regulated by γ-interferon and thus are consistent with our observations. Finally, more than 60 patients have developed granulomatous disease subsequent to treatment with various interferons [37;38;39]. This clinical condition mimics sarcoidosis.

The potential importance of STAT1 in the pathogenesis of sarcoidosis is supported by observations in both lymph node and lung. However, the findings in solid tissue are not identical to those from blood, i.e., not all STAT1 regulated transcripts showing upregulation in blood showed statistically significant upregulation in solid tissue. Since cellular composition and local factors within specific organs or tissues will modify gene expression, these differences are not surprising. Gene expression in blood resembles gene expression in lymph node more than lung, consistent with greater cell trafficking between blood and lymph node. Tissue response to a cytokine such as interferon obviously depends on cells which are present in that tissue. For example, even adjacent cells such as astrocytes and microglia differ in their synthesis of CXCL9 and CXCL10 [40]. Nonetheless, STAT1 regulated genes are strongly represented in all the sarcoidosis tissues that we examined.

CXCL9 was consistently upregulated in blood, lymph node, and lung. Like CXCL10, CXCL9 binds to a receptor known as CXCR3. CXCL9 has been strongly implicated in granuloma formation in primates [41]. CXCL9 plays a major role in several inflammatory diseases including autoimmune diseases of the skin [42;43], inflammatory bowel disease [44], forms of arthritis [45], demyelinating disease [46], and several infections [47;48].

As indicated in Supplementary Table 1, STAT1 is far from the only gene upregulated in the blood of patients with sarcoidosis. For example, we found upregulation of the receptor for epidermal growth factor, which has been associated with mycobacterial-induced granulomas [49]. Our analysis does not negate the importance of other genes or gene networks in the pathogenesis of this disease. Rather, our findings clearly support multiple factors in the pathogenesis.

Sarcoidosis is not the first immune mediated disease for which interferon regulated genes have been implicated. Gene expression studies have also suggested a role for genes regulated by either interferon type 1or γ-interferon in immunological diseases which include rheumatoid arthritis, inflammatory myopathies, and SLE [6;9]. STAT1 is upregulated in the affected skin of patients with psoriasis [50] and in the synovium of patients with rheumatoid arthritis [51]. Polymorphisms in STATs have also been linked to susceptibility of several immune-mediated

diseases including Graves' disease, SLE, and rheumatoid arthritis [52;53]. The role of STAT1 polymorphisms in the susceptibility to sarcoidosis has not been thoroughly studied.

The pattern of gene expression could conceivably have diagnostic, prognostic, and therapeutic implications. Further study will determine how therapy impacts gene expression and if gene expression predicts response to a specific intervention. Additional study is indicated to determine which cells are most responsible for the detection of mRNA for STAT1 in our studies. Certainly if a role for STAT1 is confirmed, medications that target STAT1 could be considered to treat sarcoidosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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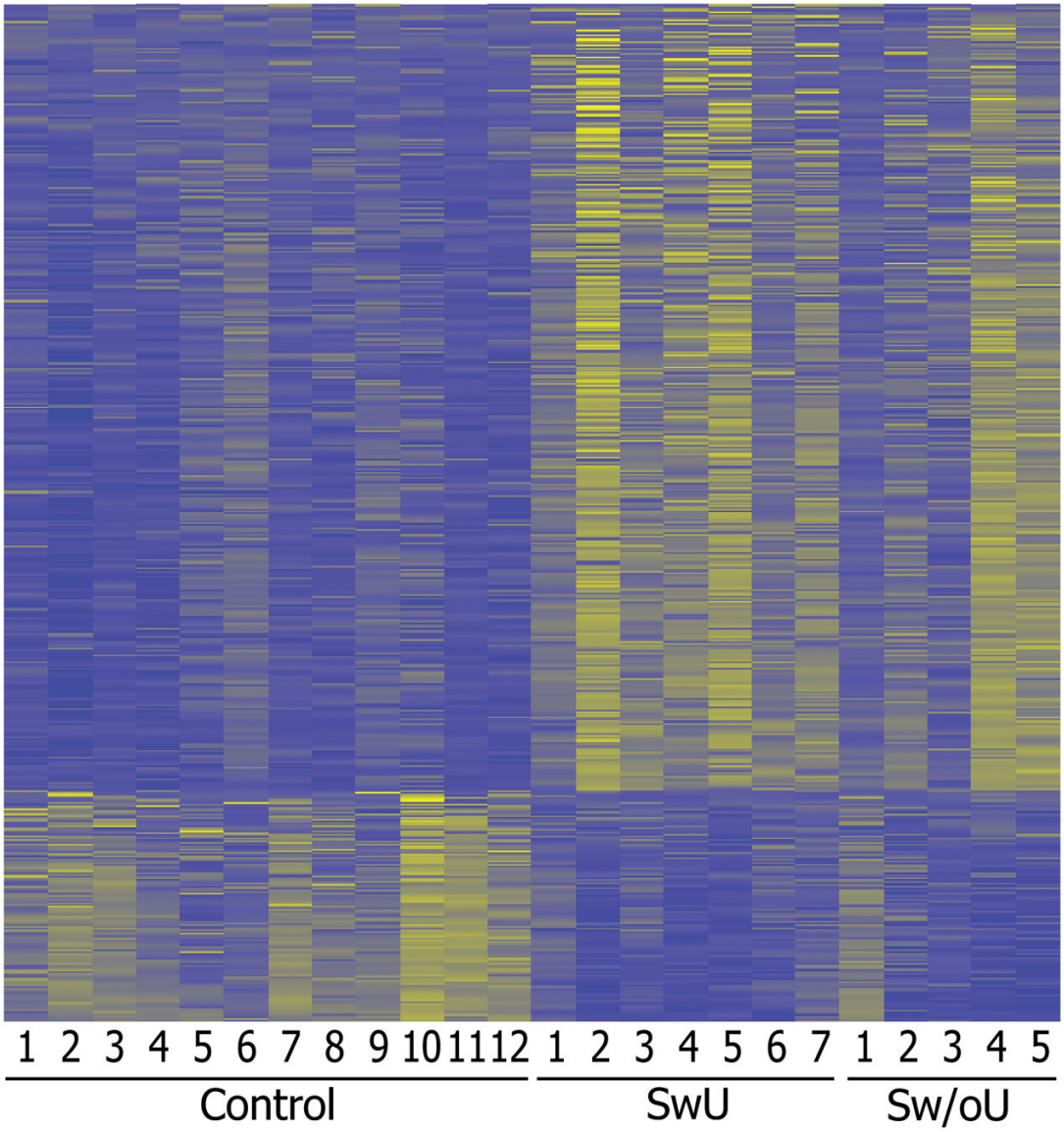

Figure 1.

Heat map differentially expressed transcripts between control and sarcoidosis patients (fold difference ≥ 2 and q ≤ 0.05).436 up- and 128 down-regulated in sarcoidosis groups. Refer to Supplementary Tables 1 and 2 for the complete gene lists.

SwU indicates sarcoidosis with uveitis and Sw/oU indicates sarcoidosis without uveitis.

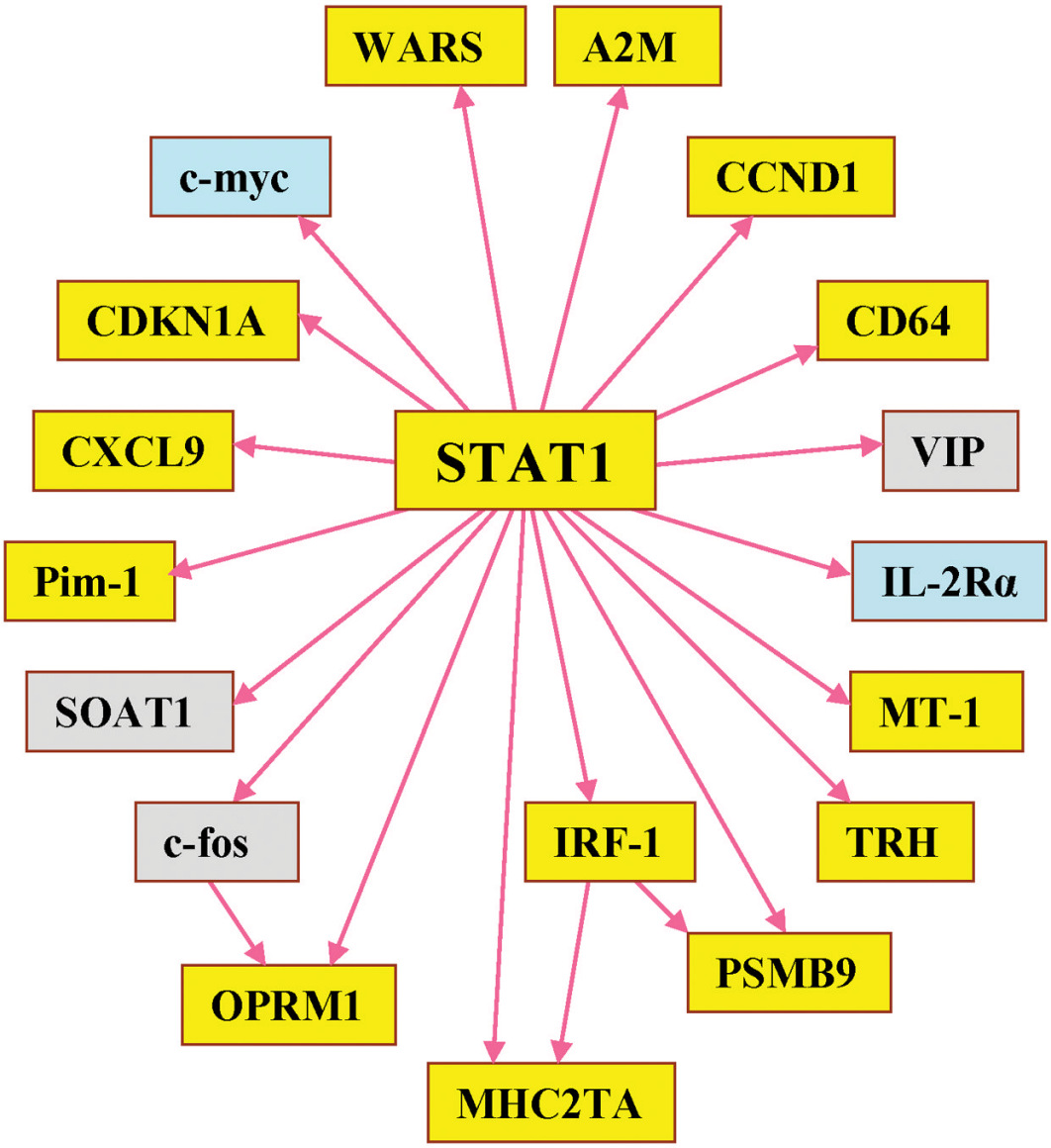

Figure 2.
STAT1 downstream genes: yellow genes were significantly upregulated (q-value < 0.05), light blue genes were downregulated, and gray genes were not differentially expressed in the sarcoidosis group compared to the control group.

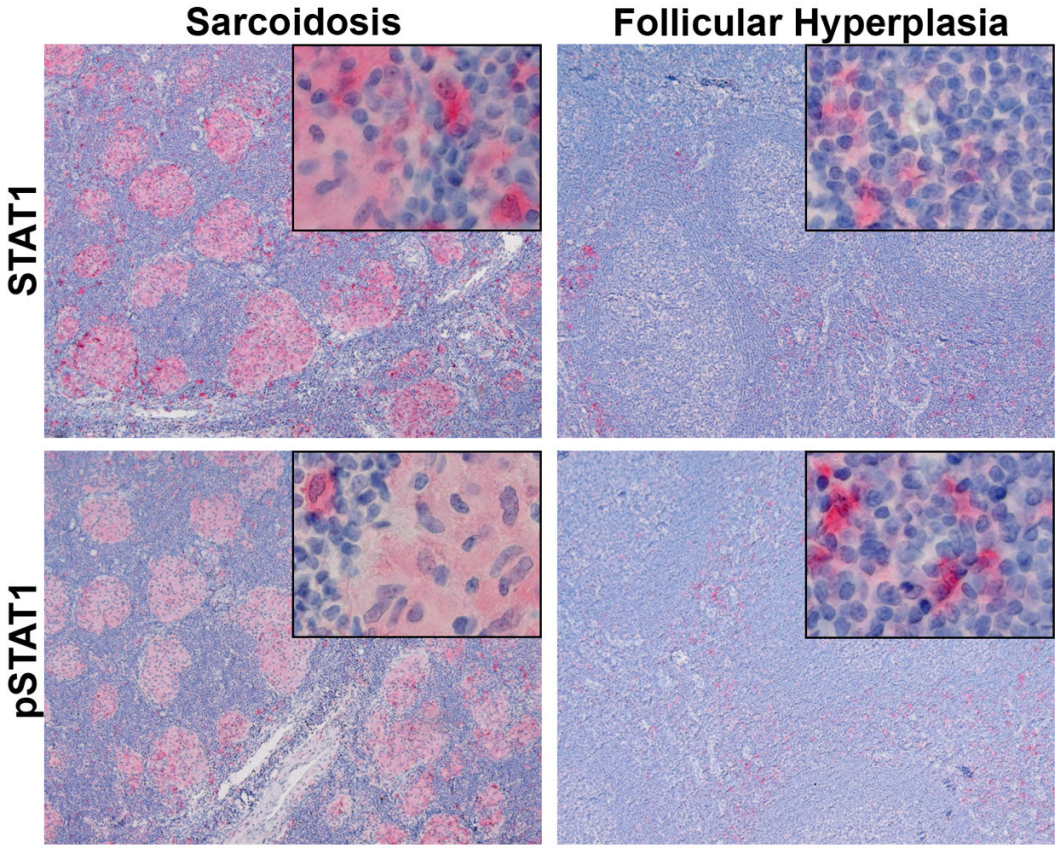

Figure 3.

Sarcoidosis patients have abundant STAT1 and pSTAT1 in lymph node granulomas. Sections of lymph nodes from patients with sarcoidosis or follicular hyperplasia (controls) were immunostained for STAT1 or pSTAT1. Both sarcoid and control lymph nodes had scattered cortical STAT1 and pSTAT1 positive cells. In addition, granulomas in the sarcoid samples were strongly positive for both forms of STAT1. No staining was evident when control IgG was substituted for the primary antibodies (not shown). Original magnification main panels 100X; inserts 400X.

Table 1

General characteristics of study subjects

Subject	Age at diagnosis of sarcoidosis	Age at study	Gender	Race	History of uveitis	Sarcoidosis diagnosis			Systemic symptoms
						Chest x-ray*	Chest CT*	Biopsy*	
Sarcoidosis group									
S1	46.9	47.5	F	Caucasian	Yes	N/A	+	N/A	Pulmonary
S2	49.3	49.3	M	Asian	Yes		+	N/A	None
S3	51.7	69.6	F	Caucasian	Yes	N/A	+	N/A	Joint
S4	39.3	41.1	F	Caucasian	Yes		+	N/A	None
S5	64.2	68.2	F	Caucasian	Yes	+	+	+	Pulmonary
S6	37.6	59.1	F	Caucasian	Yes	+	N/A	N/A	Pulmonary
S7	25.2	27.9	M	African American	Yes			+	None
S8	47.0	58.9	F	Caucasian	No	N/A	N/A	+	Pulmonary/CNS
S9	58.6	63.2	F	Caucasian	No	+	+	+	Pulmonary
S10	44.1	44.6	M	Caucasian	No		+	+	Pulmonary
S11	54.9	56.9	F	Caucasian	No	N/A	+	+	Pulmonary
S12	60.3	60.4	F	Caucasian	No	+	+	N/A	None
Control group									
C1	-	41.5	M	Caucasian	No				
C2	-	35.7	F	Caucasian	No				
C3	-	68.2	F	Caucasian	No				
C4	-	31.9	M	Caucasian	No				
C5	-	25.3	F	Caucasian	No				
C6	-	83.1	F	Caucasian	No				
C7	-	59.5	F	Caucasian	No				
C8	-	21.8	F	Caucasian	No				
C9	-	22.9	F	Asian	No				
C10	-	69.6	M	Caucasian	No				
C11	-	68.6	F	Caucasian	No				
C12	-	57.0	M	Caucasian	No				

Subject	Age at diagnosis of sarcoidosis	Age at study	Gender	Race	History of uveitis	Sarcoidosis diagnosis			Systemic symptoms
						Chest x-ray*	Chest CT*	Biopsy*	

Table 2

STAT1-regulated genes with increased expression in blood from patients with sarcoidosis.

Gene. Symbol	Gene Title	Mean FC*	q-value**	Representative functions
MHC2TA	Major histocompatibility complex, class II, transactivator	1.23	0.029	Master of regulator of MHC class II genes [51]
IRF1	Interferon regulatory factor 1	1.45	0.002	Nuclear factor and a transcriptional factor for type I IFN [52], can induce CDKN1A activity
WARS	Tryptophanyl-tRNA synthetase	1.72	0.001	Antagonist of VEGF [53]
PSMB9	Proteasome subunit, beta-type, 9	1.42	0.002	Transport protein into the endoplasmic reticulum, important in MHC class I regulation [54]
CD64	Fc fragment of IgG, high affinity	3.14	0.029	Fc fragment of IgG receptor, expressed on human monocytes and macrophages [55]
CXCL9	Chemokine CXC motif, ligand 9	1.16	0.029	T-cell chemoattractant
PIM1	Oncogene PIM1	1.39	0.009	Protooncogene in prostate cancer and hematopoietic malignancies [56]
CCND1	Cyclin D1	1.16	0.029	Function as an oncogene, related to lymphoma, leukemia [57], parathyroid tumor [58], and colorectal cancer [59]
CDKN1A	Cyclin-dependent kinase inhibitor 1A	1.36	0.029	Inhibitor of protein kinase, CDK1
A2M	Alpha-2-macroglobulin	1.15	0.029	Major plasma proteinase inhibitor
OPRM-1	Opioid receptor, mu-1	1.15	0.029	Pain-related gene
MT1E	Metallothionein 1E	1.31	0.012	Heavy metal-binding protein, Zn and Cu homeostasis
TRH	Thyrotropin-releasing hormone deficiency	1.14	0.029	Major hypothalamic mediator of thyroid stimulating hormone release

*Fold change,

**the maximum q-value in case of multiple probe sets

Table 3

Genes in JAK-STAT pathway upregulated in the peripheral blood of patients with sarcoidosis.

Gene symbols	Gene titles	Number of probe sets	Mean fold increase	q-value*
IFNAR1	interferon (alpha, beta and omega) receptor 1	3	1.25	0.029
IFNAR2	interferon (alpha, beta and omega) receptor 2	2	1.28	0.028
IFNGR2	interferon γreceptor 2 (interferon γ transducer 1)	1	1.11	0.029
JAK1	Janus kinase 1 (a protein tyrosine kinase)	3	0.88	0.039
JAK2	Janus kinase 2 (a protein tyrosine kinase)	3	1.38	0.029
STAT1	signal transducer and activator of transcription 1, 91kDa	6	1.99	0.001
STAT2	signal transducer and activator of transcription 2, 113kDa	3	1.44	0.029
STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)	4	1.38	0.029

Number of Probe Sets reflects the characteristics of the Affymetrix U133 Plus 2.0 array which detects some transcripts with discrete probes that identify different portions of the transcript.

* The maximum q-value in case of multiple probe sets

Table 4

Patient Demographics for Tissue Gene Expression Analyses

	Lung		Lymph node	
	Control (n = 6)	**Sarcoidosis (n = 6)**	**Control (n=5)**	**Sarcoidosis (n=8)**
Age ± SEM (yrs)	50.8 ± 5.2	40.7 ± 5.6	43.0 ± 6.1	40.0 ± 4.8
Gender (male/female)	3/3	2/4	3/2	3/5
Race (White/Black/Other)	4/2/0	4/2/0	3/2/0	5/3/0

Table 5

Upregulation of Transcripts for STAT1 regulated chemokines in sarcoidosis. Values indicate fold upregulation compared to gene expression in control tissue.

	STAT1	CXCL9	CXCL10	CXCL11
Blood	2.0***	1.2*	2.8**	1.1*
Lung	4.3***	9.5***	5.1**	9.8***
Lymph node	7.0***	19.5**	NS	19.2**

* p or q <0.03,

** p or q <0.01,

*** p or q <0.001 (q values for blood samples and the FDR adjusted p values for lung and lymph node samples.) NS=not significant