Interferon Expression Profiling as a Detection Marker in Early Systemic Lupus Erythematosus

Paul van der Leest
June 30th, 2017
Rheumatology and Clinical Immunology
Supervised by Johanna Westra

Abstract

In recent years, there is growing interest for the interferon (IFN) signature in patients with systemic lupus erythematosus (SLE), due to diagnostic value and its link with disease activity. However, there are no generally accepted methods and study conditions to determine the IFN signature. Therefore, the initial objective in this project consisted of the selection of IFN-related transcripts, regulated by both IFN type I and type II, based on literature analysis. After transcript selection, the aim of this study is to compare the IFN signature in multiple biological substances based on a frequently used method called the IFN-score. For that reason, the RNA expression profiles of IFN-related transcripts have been measured in monocytes and PBMCs using quantitative real-time polymerase chain reaction (RT-PCR). Hereafter, the same transcripts were used to determine whether whole blood samples could be used as an easy substance to determine IFN positivity in SLE patients and patients prone to develop SLE, called incomplete SLE (iSLE). As a result, higher significance and better separation of relative expressions was found in the monocytes compared with PBMCs. Both substances display similar type I IFN-scores, which does not apply for IFN type II. The IFN-score based on the three most contributing transcripts, referred to as the 3-gene-based IFN-score, showed to be a suitable substitute for the type I IFN-score. In terms of diagnostic value, similar IFN positivity have been detected in whole blood samples compared with monocytes in iSLE and SLE patients. Altogether, for determination of positivity for the type I IFN signature is required, as is conventional in current literature, measuring the 3-gene-based IFN-score interchangeable in monocytes, PBMCs or whole blood samples would suffice. To gather more information on disease activity, pathogenesis and the predictive value of the IFN signature in SLE patients, monocytes seem to be the most reliable biological substance.

Table of contents

Introduction

Throughout history, accumulation of knowledge on healthcare and medicine resulted in the decrease of many pathogenic threats and infectious diseases. This progression is a major contributor to the increase of both lifespan and health span of people. However, because of the changes in lifestyle and increasing hygienic solutions people may also be exposed to factors that influence the immune system negatively. According to the hygiene hypothesis proposed by Strachan, there is an inverse correlation between the exposure to infectious agents and autoimmunity[1]. Epidemiologically, the distribution of autoimmune disease is a mirrored representation of the distribution of high incidence of infectious diseases, with autoimmune diseases having the highest prevalence in the industrialized western countries. One of the autoimmune diseases associated with this hygiene hypothesis is systemic lupus erythematosus (SLE). The lack of infections from pathogenic microbes is known to be associated with SLE occurrence and could have aided to the threefold increase of SLE incidence in the second half of the last century[2].

SLE, also referred to as lupus, is a chronic inflammatory connective tissue disease characterized by the production of self-reactive antibodies[3][4]. This systemic autoimmune disease affects multiple organs by causing inflammation, due to immune complexes and activation of both the innate and adaptive immune response[3][5][6]. The influence of the immune system is manifest in disturbances in various immunological processes, including apoptotic cell clearance, production of cytokines, B-cell immunity and T-cell signaling[7]. Although there is little knowledge concerning genetic predispositions in lupus, evidence of genetic susceptibility is provided. In 2012, more than 30 loci associated with SLE have been identified[8]. However, less than 10% of the genetic heritability is described by these loci. Approximately 90% of the lupus patients are female, whether that is due to hormonal or genetic factors is not clear yet[5][6][8]. Besides, SLE is up to five times more prevalent amongst black people and, in line with the hygiene hypothesis, SLE occurrence is much higher in African Americans compared to West Africans[1][5][6].

Currently, one of the challenges in SLE is early and correct diagnosis. Diagnosis of SLE is difficult due to clinical and serological heterogeneity and a wide profile of autoantibodies[9]. Nowadays, diagnosis of SLE is based on a combination of criteria, most often including the presence of antinuclear antibodies (ANA)[4]. In attempt to generalize the diagnosis procedure, two sets of classification criteria have been composed. These classification criteria, being the American College of Rheumatology (ACR) and Systemic Lupus International Collaborating Clinics (SLICC), are based on clinical and serological manifestations, and a patient is fulfilling these criteria when over four criteria are present[4][9]. However, the development of SLE usually has started long before the manifestation of clinical symptoms[4]. When an individual displays a mild form of lupus which might precede SLE, it is classified as incomplete SLE (iSLE). The cohort of iSLE patients consist of a very variable group of individuals, ranging from enhanced genetic risk to develop SLE to people with autoantibodies and some clinical features who do not meet the disease classification criteria[10]. Estimates indicate that 10-50% of iSLE patients will progress to SLE[11]. Nowadays there are no good biomarkers to predict progression and even more to select those iSLE patients who should receive early treatment, even though the clinical manifestations can be severe[11]. Since the current SLE classification criteria do not apply to people with potential or early lupus, there is growing desire for new pre-clinical insights in etiology, pathogenesis and natural disease history as potential targets for early detection and intervention[4].

Since 2003, there is increasing interest in the link between interferons (IFNs), which are hallmark inflammatory cytokines, and SLE. In that year, Baechler *et al.* showed that genes in the IFN pathway

were dysregulated in peripheral blood mononuclear cells (PBMCs) of SLE patients with active disease[3]. IFNs are subset of cytokines secreted by several immune cells, especially dendritic cells, and are involved in the inflammatory process and tissue damage in SLE patients[7][12]. In general, IFNs are involved in maintaining the viral immunity and mediate the Th1 immune response. There are different types of IFNs, predominantly type I and type II IFNs[13]. Since type I IFNs are related to the activation of the innate immune system, especially IFNα, type I IFNs have been included in the pathogenesis of SLE[14][15]. As measuring IFN-α from serum or plasma is difficult and not reliable since the concentration is very low, it is generally accepted to measure the expression of IFN related genes[16].

Growing evidence indicates that IFN-related genes are overexpressed in SLE patients. Many studies show that 75-80% of the SLE patients show upregulation of IFN-related genes[5][17][18][19]. However, the methods differ in these studies, as there are different subsets of IFN-related genes detected and in different biological substances. Instead of being obligated to measure every IFN-related transcript, which code for over 200 genes, selection of a couple transcripts is implied to be sufficient. Analysis using a subset of IFN-regulated genes showed that the expression profile of a small selection of transcripts could already be representative for the expression profile of all genes, up to a correlation of 96%[20]. The IFN-related transcripts that describe the IFN activity is called the IFN signature[16]. In recent years, a commonly used way to express the IFN signature is by calculating the IFN-score. The IFN-score can be described as the sum of the amount of standard deviations the IFN-related transcripts are differentially expressed in SLE patients compared to healthy controls[21]. Using this calculation, it would be possible to combine the expression profiles of the different transcripts while taking the relative contributions into account.

Because there are many IFN-related transcripts, determination of the IFN signature could be performed using countless different selections of transcripts. Many genes are involved in the IFN pathways and selecting them is rather difficult, since IFN-stimulated genes take on a wide range of activities. A lot of these genes control the immunological response during viral, bacterial and parasitic infection, and therefore affect a great diversity of transduction pathways[22]. Besides the effector functions, IFN-stimulated genes could be activated differently by all three IFN types. Currently many studies focus specifically on the type I IFN signature, which is mainly driven by IFNα and IFNβ activation, even though there are no conventional definition of selection criteria for IFN-related transcripts, materials or techniques yet[12][16].

Fortunately, transcript selection has been facilitated by modular analysis studies performed in recent studies[17][23]. In 2014, Chaussabel and Baldwin published their dataset analysis of every gene related to the entire immune response, dividing it into different subsets referred to as modules[23]. These modules consist of transcripts who are tightly clustered together in excessive dataset analysis and are thereby considered to belong in the same co-clustering network. By using nine different databases, all 14,000 immune response related transcripts were distributed into 260 different modules and distinguish the modules based on associative strength and functional analysis[23]. In this way, out of the 260 modules three IFN modules where determined consisting of a total of 160 unique transcripts (content is available at: http://www.biir.net/public_wikis/module_annotation/G2_Trial_8_Modules). The IFN modules detected, being 1.2, 3.4 and 5.12, are generally upregulated in patients compared with healthy controls[23]. Even more striking is that the modules 1.2, 3.4 and 5.12 are all highly upregulated in 94%, 85% and 67% of the tested SLE patients, respectively[17]. The modular analysis facilitates the selection of IFN related transcripts by combining the transcripts into three modules. The only requirement left is to find a selection of representative transcripts to determine a possible

dysregulation of gene expression within the different IFN modules. It should be kept in mind that not every IFN-related transcript is included in the IFN modules, so the modules are not a definitive representation of the IFN signature in SLE patients. In literature, most selected transcripts to determine the IFN signature belong to module 1.2 (see Table 1). It is however doubtful whether it is desirable to focus solely on IFN module 1.2. Besides the different responses to type I and type II IFNs, the three IFN modules seems to be activated chronologically[17]. If SLE patients display only one upregulated IFN module, module 1.2 is always the upregulated module. When two IFN modules are overly active, it is always a combination of module 1.2 and 3.4. The last activated module is 5.12 in every case. Besides, module 5.12 is significantly more correlated with disease activity than the other two IFN modules[17]. This could imply that module 1.2 is more a generally activated IFN module in disease, in our case SLE, and when all IFN modules are activated, there is more often increased disease activity[29].

Besides selection of which IFN-related genes should be measured, choice of biological substance is also of major importance. In previous studies whole blood samples, PBMCs, isolated monocytes and lymphocytes have been used for IFN signature determinations (see Table 1)[16]. During analysis of separated leucocyte subtypes, Flint *et al.* showed that upregulation of type I IFNs differs between lymphocytes and myeloid cell types[30]. Even more specific, CD8+ and CD4+ T-cells, and monocytes display considerably more type I IFN high results than neutrophils. Flint and colleagues therefore recommend the use of at least PBMCs and imply that the use of separated T-cells might be even better[30]. Rather contradictory, Strauß *et al.* revealed significant higher IFN signatures in neutrophils and monocytes compared with T helper cells, cytotoxic T cells and B cells[16]. Furthermore, myeloid cells have been described to exhibit the greatest number of differently expressed transcripts, and share only few similarities in expression levels with lymphocytes[31]. The use of whole blood samples is discouraged due to the abundance of neutrophils and their nonspecific and biased contribution to the IFN signature[16][30]. Nevertheless, as displayed in Table 1, most previous studies rely on whole blood patient samples when determining the IFN signature.

Lastly, there are different approaches to determine the IFN signature. In literature, quantitative real-time polymerase chain reaction (RT-PCR) and microarray assay are both frequently used for IFN-related gene detection. Even though quantitative RT-PCR and microarray assay can collect the same type of data, quantitative RT-PCR is often used to verify microarray results.

In the current project, a subset of IFN-related genes will be selected based on the current literature and their expression profiles in different biological substances will be analyzed. The main goal is to generate more knowledge on the IFN signature in SLE patients, in attempt to lay a fundament for the use of the IFN signature as an early marker to detect which people are prone to develop SLE. Since studies that focus on the IFN modules 3.4 and 5.12 are lacking, transcripts from all three IFN modules will be included in this study. A possible upregulation of the transcripts will be determined in PBMCs and isolated monocytes. Hereafter, the obtained knowledge will be applied to verify whether whole blood samples could act as a substitute biological substance to determine the IFN signature in iSLE and SLE patients, compared with the most reliable biological substance identified. Since studies using microarray analysis always verify their data using quantitative RT-PCR, in this project only the latter is used. In this way, multiple comparisons between different study conditions could be analyzed, contributing to the knowledge which is required for standardization of IFN signature determination in SLE.

Table 1. Choice of biological substance and transcript selection in current literature

Author		**Brkic, 2014[24]**	**Feng, 2006[21]**	**Kalunian, 2016[19]**	**Kirou, 2005[25]**	**Landolt, 2008[26]**	**Maria, 2014[18]**	**McBride, 2012[27]**	**Reynolds, 2016[28]**	**Current study**
Disease		SLE	SLE	SLE	SLE	SLE	Sjögren	SLE	SLE/Sjögren	SLE
Biological substance	WB									
	CD14									
	PBMC									
Module	***Gene***									
1.2	CXCL10									
	EIF2AK2									
	EPSTI-1									
	HERC5									
	IFI44									
	IFI44L									
	IFIT1									
	IFIT3									
	ISG15									
	LY6E									
	MX1									
	OAS1									
	OAS2									
	OAS3									
	OASL									
	RSAD2									
	SERPING1									
	XAF1									
3.4	AIM2									
	IFIT2									
	IFITM1									
	IRF7									
	STAT1									
5.12	C1QA									
	CXCL2									
	IFI16									
	IRF9									

A selection of published studies investigating the IFN signature in either SLE or Sjögren's syndrome patients. The grey boxes above the dark grey line indicate which biological substances were used, underneath the same for the IFN-related transcripts. To which module the transcripts belong is represented on the left. The selection for the current study is depicted in the last column.

SLE = systemic lupus erythematosus; WB = whole blood sample; CD14 = monocyte; PBMC = peripheral blood mononuclear cell. For the full names of the transcripts used in the current study, see Appendix 4.

Material and methods

Transcript selection. To facilitate the selection of IFN-related transcripts representative for the IFN signature, the previously described IFN modules were taken under consideration. To receive an overview of the relative contribution of all modules and to be able to relate the discoveries to previous studies, at least 4 transcripts of the modules 1.2, 3.4 and 5.12 were implemented in this project. Use in literature and functional analysis were important criteria for transcript selection. As displayed in Table 1, the choice of transcripts of module 1.2 was mainly based on previous studies. Since there is insufficient attention for modules 3.4 and 5.12 in literature, functional analysis was the prominent factor influencing the transcript selection for these modules.

Patient characteristics. For the monocyte and PBMC study, 24 quiescent SLE patients (SLE disease activity index (SLEDAI) ≤ 4) and 2 active SLE patients (SLEDAI > 4) between the ages of 21 and 73 years were included in this study, as well as 24 age- and sex-matched healthy controls (see Table 2). 46% of the SLE patients received prednisone treatment during the sample collection; eight of these patients were treated with either hydroxychloroquine or azathioprine as well (four and four, respectively). Only one patient received all three types of medication. Of the patients treated with hydroxychloroquine (54%), two patients received methotrexate and one patient azathioprine simultaneously. 8% of all patients received solely azathioprine as medication.

For the whole blood study, 9 healthy controls, 17 iSLE patients and 18 SLE patients were included. Patient characteristics were not available for the whole blood cohort.

PBMCs of both healthy controls and SLE patients have been collected between October 2005 and March 2006 and were stored in liquid nitrogen at -180˚C since. The collection of whole blood samples of healthy controls, iSLE patients and SLE patients started at the beginning of 2017 and remained at -20˚C. Because of the wide interval between the times of collection, there are no matched patient samples in the PBMC and CD14+ monocyte group and the whole blood sample group.

Table 2. Characteristics of the healthy controls and patients

	Healthy controls	SLE patients	P-value
Age, range	23-58	21-73	
Age, mean ± SD years	40,4 ± 10,4	45,3 ± 15,2	NS
Sex, (%) female	71	89	NS
SLEDAI, median (range)	-	2 (0-8)*	
Treatment			
Prednisone, no. (%)	-	12 (46%)	
Dosage, median (range) mg/day	-	5 (2,5-10)	
Hydroxychloroquine, no. (%)	-	14 (54%)	
Dosage, median (range) mg/day	-	400 (200-800)	
Azathioprine, no. (%)	-	7 (27%)	
Dosage, median (range) mg/day	-	100 (75-150)	
Methotrexate, no. (%)	-	2 (8%)	
Dosage, median (range) mg/week	-	15 (15)	

* Two patients had a score of > 4 on the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).
Statistical analysis has been performed using the student T-test.
NS = not-significant.

Cell isolation. Previously isolated PBMC were defrosted and resolved in PBS with 10% FCS according to protocol (appendix 1.1). Cell counts and the percentage of living cells have been determined before lysation. After defrosting, PBMCs were directly lysed by addition of Trizol, monocytes were isolated using Dynabeads CD14 according to the supplied manual (summarized in appendix 1.1). After the isolation, the monocytes were too lysed using Trizol. Whole blood samples were drawn from patients and collected in PAXgene RNA tubes for RNA conservation. The PAXgene RNA isolation procedure has been performed as descripted in appendix 1.2.

RNA isolation. For the in Trizol lysed cell samples, being the PBMCs and the CD14+ monocytes, the RNA had to be isolated in contrast to the PAXgene Blood RNA procedure. The proceedings have been performed as described in appendix 2, for both cell samples in an identical manner.

cDNA synthesis. After RNA isolation, cDNA has been synthesized using all three biological substances. The initial volume preceding the RT-PCR procedure was corrected for depending on the RNA concentration determined during the RNA isolation procedure. The performed actions are defined in appendix 3.1.

Quantitative RT-PCR. The cDNA was quantitatively analyzed using an Applied Biosystems Taqman 7900HT Fast Real-Time PCR System. The outcome measure consisted of the cycle threshold (Ct) value which, when related to a housekeeping gene, could be used to quantify the presence of the transcripts and enables multiple calculations related to the IFN signature.

Data analysis. The expression of all selected transcripts was calculated using the housekeeping gene GAPDH. GAPDH Ct values ranging from 18 to 29 for monocytes and 17 to 25 for PBCMs and whole blood samples were considered to contain sufficient cDNA for accurate relative expression determination. The relative expression (referred to R.E. in the formulas) is calculated via the formula:

$$R.E. = 2^{-(Ct_{gene} - Ct_{GAPDH})}$$

The formula is often described as $2^{\Delta\Delta CT}$. Statistical analysis between healthy controls and SLE patients was perform using a student t-test. All correlations have been calculated and imaged using GraphPad Prism 5.

To be able to combine the relative expression of the transcripts per module to determine the IFN signature, the IFN-score was calculated as follows:

$$IFN\ score = \sum \frac{Log(R.E._{SLE}) - MEAN\ R.E._{HC}}{SD\ R.E._{HC}}$$

The logarithmic transformation has been applied since it has been recommended in current literature[32][33]. Statistical analysis between healthy controls and SLE patients was perform using a student t-test. Determination of IFN positivity was concluded when the IFN-score was higher than the median of the healthy controls with two standard deviations added (generally accepted in literature[34]). Significance between the number of positive IFN-score in the monocyte group and the PBMC group has been calculated using a chi-squared test.

The relative contribution of a transcript to the modular or tot IFN-score was determined by calculating the average IFN-score per transcript and calculate the contribution to the average of the IFN-score of interest. Statistical analysis between monocytes and PBMCs was perform using a chi-square test.

Results

Literature analysis to select 14 transcripts for IFN signature determinations

First, it was required to determine a subset of IFN-related genes to be able to detect the so called IFN signature. Based on the current knowledge in literature concerning the IFN signature and the association of IFN-related genes and disease activity, we selected the transcripts. Hereby the modular analysis performed by Chaussabel and Baldwin fulfills a crucial role. As previously mentioned, most recent studies mainly focus on transcripts from module 1.2, which is considered as a stably activated IFN module. However, module 5.12 is most strongly correlated with disease activity. Therefore 14 transcripts of IFN-related genes have been selected for IFN signature determinations from the different modules, six from module 1.2 and four each belonging to modules 3.4 and 5.12 (see Table 1). In this way, there should be sufficient transcripts per module to make statements concerning the influence of IFN on the different modules in our SLE patient cohort. Furthermore, functional considerations of the IFN-related genes were a major factor in the selection process. For instance, the transcripts C-X-C motif chemokine ligand 10 (CXCL10) and myxovirus resistance 1 (MX1) have been included since their translational products IP-10 and MxA, respectively, could be measured in serum using ELISA or another type of immunoassay for comparing purposes[35]. Furthermore, these proteins have been described to be upregulated in patients with SLE and iSLE[36][37][38]. Complement C1q A chain (C1QA) and serpin family G member 1 (SERPING1) are both included because of their antagonistic functionality, where C1QA codes for C1q and SERPING1 is a famous C1-inhibitor[39]. See Appendix 4 for all functional properties of the selected genes.

Upregulated relative expression of most IFN-related genes in monocytes and PBMCs

Following transcript selection, verification whether the transcripts showed different expression profiles in SLE compared to healthy controls is required. The relative expression of every transcript in both monocytes and PBMCs are depicted in Table 3. In monocytes, a significant upregulation of transcript expression in SLE compared to healthy controls is determined for most transcripts, except for absent in melanoma 2 (AIM2) and C-X-C motif chemokine ligand 2 (CXCL2). Quiet similarly, eleven out of fourteen transcripts show upregulated expression in the PBMCs of SLE patients, except for AIM2, CXCL2 and lymphocyte antigen 6 complex, locus E (LY6E). Figure 1 represents the difference in relative expression of LY6E in monocytes and PBMCs. In general, although both significant, monocytes show better separation and higher significance in relative expression than PBMCs. In Figure 2 a specific example that images this observation is depicted, being IFN regulatory factor 7 (IRF7). To determine whether the transcripts display similar activation profiles within their modules, the correlations have been reviewed (see Table 4). It becomes evident that the transcripts correlate more often in PBMCs than monocytes. When transcripts correlate in monocytes, the same correlation is always found in PBMCs as well.

Table 3. Relative expressions of the transcripts in monocytes and PBMCs for healthy controls and SLE patients

Module	Transcript	Biological substance		Relative expression	P-value
1.2	CXCL10	CD14	HC	4.73 (0.486 – 19.6)	**
			SLE	14.5 (1.44 – 36.0)	
		PBMC	HC	1.91 (0.145 – 50.8)	**
			SLE	15.9 (0.282 – 187)	
	IFI44L	CD14	HC	5.17 (0.673 – 250)	***
			SLE	78.8 (2.46 – 267)	
		PBMC	HC	3.70 (0.430 – 54.6)	***
			SLE	58.2 (0.395 – 415)	
	IFIT3	CD14	HC	6.27 (0.338 – 21.4)	***
			SLE	16.3 (4.90 – 227)	
		PBMC	HC	1.99 (0.0698 – 23.7)	*
			SLE	11.8 (0.0884 – 34.0)	
	LY6E	CD14	HC	24.3 (5.07 – 239)	***
			SLE	206 (10.1 – 570)	
		PBMC	HC	11.5 (1.96 – 323)	NS
			SLE	365 (3.89 – 407)	
	MX1	CD14	HC	6.19 (1.47 – 505)	**
			SLE	28.0 (12.0 – 839)	
		PBMC	HC	15.4 (0.595 – 141)	***
			SLE	144 (0.508 – 384)	
	SERPING1	CD14	HC	3.14 (0.479 – 108)	***
			SLE	34.3 (1.94 – 164)	
		PBMC	HC	3.65 (0.784 – 56.1)	***
			SLE	57.2 (0.866 – 358)	
3.4	AIM2	CD14	HC	2.65 (0.0881 – 14.6)	NS
			SLE	4.57 (0.265 – 26.6)	
		PBMC	HC	3.01 (1.03 – 130)	NS
			SLE	8.85 (0.634 – 16.8)	
	IFITM1	CD14	HC	34.7 (2.49 – 392)	**
			SLE	143 (7.02 – 4730)	
		PBMC	HC	278 (48.7 – 38300)	**
			SLE	4900 (20.8 – 59300)	
	IRF7	CD14	HC	15.0 (4.49 – 96.7)	***
			SLE	65.1 (12.2 – 178)	
		PBMC	HC	7.57 (1.07 – 67.6)	*
			SLE	20.9 (4.43 – 64.1)	
	STAT1	CD14	HC	19.9 (3.03 – 94.9)	***
			SLE	59.1 (1.75 – 150)	
		PBMC	HC	14.4 (0.0322 – 495)	**
			SLE	50.8 (13.9 – 4800)	
5.12	C1QA	CD14	HC	4.36 (0.902 – 145)	**
			SLE	11.2 (1.77 – 515)	
		PBMC	HC	1.84 (0.00155 – 47.7)	**
			SLE	12.0 (0.103 – 225)	
	CXCL2	CD14	HC	102 (18.4 – 981)	NS
			SLE	188 (30.5 – 1260)	
		PBMC	HC	88.1 (7.68 – 351)	NS
			SLE	157 (10.2 – 820)	
	IFI16	CD14	HC	21.4 (1.06 – 84.0)	*
			SLE	43.7 (2.00 – 981)	
		PBMC	HC	17.7 (2.39 – 94.2)	*
			SLE	56.9 (0.763 – 168)	
	IRF9	CD14	HC	20.3 (1.38 – 386)	**
			SLE	44.9 (8.57 – 903)	
		PBMC	HC	12.1 (1.45 – 142)	**
			SLE	125 (1.10 – 254)	

The differences in relative expressions between healthy controls and SLE patients for each transcript in both biological substances are displayed. Relative expressions are presented as medians and range as values x 10^{-3}. To compare medians, a student t-test has been performed.
* = P < 0.05; ** = P < 0.01; *** = P < 0.001; NS = not-significant

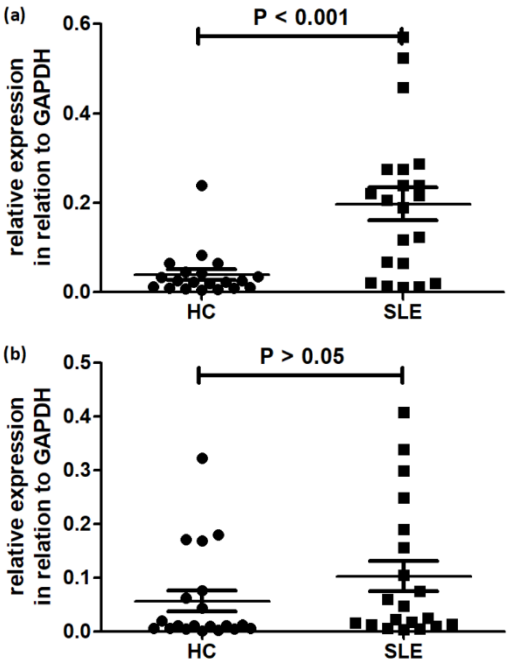

Figure 1. The relative expression of LY6E in (a) monocytes and (b) PBMCs in relation to the housekeeping gene GAPDH. Each symbol represents an individual patient sample; the horizontal line represents the median. Statistical analysis has been performed using a student t-test. P-values are shown and considered significant when P < 0.05.
HC = healthy control; SLE = systemic lupus erythematosus.

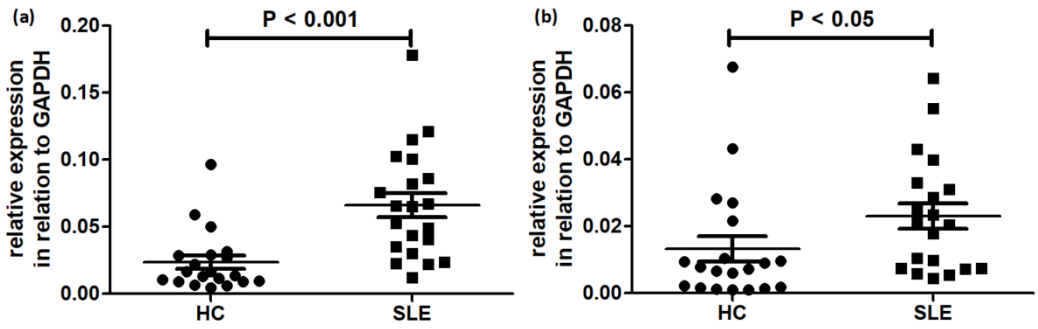

Figure 2. The relative expression of IRF7 in (a) monocytes and (b) PBMCs in relation to the housekeeping gene GAPDH. Each symbol represents an individual patient sample; the horizontal line represents the median. Statistical analysis has been performed using a student t-test. P-values are shown and considered significant when P < 0.05. HC = healthy control; SLE = systemic lupus erythematosus.

Table 4. Correlations of transcripts per modules in monocytes and PBMCs for SLE patients

(a)	CXCL10		IFI44L		IFIT3		LY6E		MX1		SERPING1	
Biological substance	CD14	PBMC	CD14	PBMC	CD14	PBMC	CD14	PBMC	CD14	PBMC	CD14	PBMC
CXCL10			***	***	NS	**	NS	**	NS	NS	NS	NS
IFI44L	0.662	0.809			NS	***	NS	***	NS	NS	NS	NS
IFIT3	0.229	0.603	0.252	0.755			***	**	NS	*	NS	NS
LY6E	0.127	0.592	0.266	0.738	0.755	0.608			NS	NS	NS	NS
MX1	0.169	0.349	-0.186	0.265	0.106	0.450	-0.235	-0.158			***	**
SERPING1	0.291	-0.045	-0.051	0.140	0.190	0.328	-0.249	-0.035	0.656	0.580		

(b)	AIM2		IFITM1		IRF7		STAT1	
Biological substance	CD14	PBMC	CD14	PBMC	CD14	PBMC	CD14	PBMC
AIM2			NS	NS	NS	NS	NS	NS
IFITM1	0.316	0.215			NS	**	NS	***
IRF7	0.427	0.335	0.358	0.677			NS	NS
STAT1	0.210	0.253	0.312	0.737	0.309	0.349		

(c)	C1QA		CXCL2		IFI16		IRF9	
Biological substance	CD14	PBMC	CD14	PBMC	CD14	PBMC	CD14	PBMC
C1QA			NS	NS	NS	***	NS	***
CXCL2	0.178	0.183			*	*	NS	NS
IFI16	0.194	0.842	0.575	0.471			*	***
IRF9	0.275	0.821	0.038	0.217	0.523	0.798		

The correlations of transcripts in monocytes and PBMCs of SLE patients are displayed for (a) module 1.2, (b) 3.4 and (c) 5.12. Underneath the grey line the correlation coefficients are displayed; above the statistical value. Correlation coefficients have been determined using a nonparametric Spearman's rank correlation coefficients test.
* = P < 0.05; ** = P < 0.01; *** = P < 0.001; NS = not-significant.

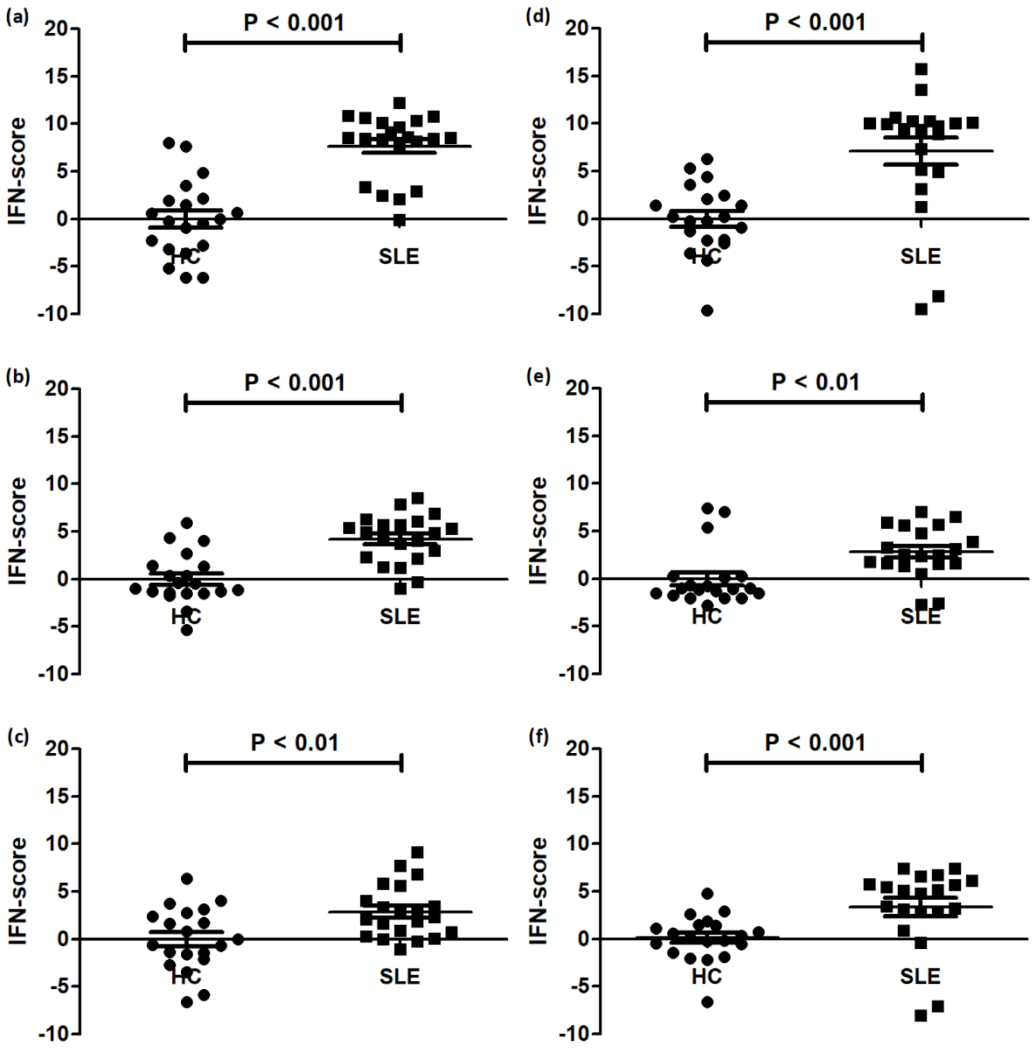

Figure 3. The modular IFN-score for module 1.2, 3.4 and 5.12 (a, b and c, respectively) in monocytes and in PBMCs (d-f). Each symbol represents an individual patient sample; the horizontal line represents the median. Statistical analysis has been performed using a student t-test. P-values are shown and considered significant when $P < 0.05$.
HC = healthy control; SLE = systemic lupus erythematosus.

Table 5. Numbers of positive IFN-scores per module of iSLE and SLE patients for monocytes and PBMCs

	IFN positive in monocytes	IFN positive in PBMCs	P-value
IFN-score module 1.2	15/21 (71%)	13/20 (65%)	0.658
IFN-score module 3.4	11/21 (52%)	5/20 (25%)	0.072
IFN-score module 5.12	3/21 (14%)	10/20 (50%)	0.014
3-gene-based IFN-score	14/21 (67%)	15/20 (75%)	0.558

Number of IFN positive patients is displays compared to the total number of patients per biological substance, with the corresponding percentages. The statistical analysis has been performed using a chi-squared test. P-values are considered significant when $P < 0.05$.

Positive IFN-scores for each module in monocytes and PBMCs

Using the previously mentioned calculation (see Materials and Methods), an IFN-score is determined for each module, utilizing all transcripts belonging to that module. On average, all modules have a significantly higher IFN-score in SLE patients compared to healthy controls in both monocytes and PBMCs (see Figure 3). However, the IFN-score is a statistical calculation which can determine a positive or negative score on an individual level. Therefore, it is more interesting to consider the number of positive IFN-scores that could be identified using the different modules in monocytes and PBMCs, as is imaged in Table 5. This IFN-score will be referred to as the modular IFN-score. 71% and 65% of the SLE patients has a positive IFN-score for module 1.2 in monocytes and PBMCs, respectively. For module 3.4, eleven out of twenty-one SLE patients have a positive IFN-score compared to only five out of twenty in PBMCs. Controversially, in module 5.12 three SLE patients showed a positivity in monocytes, while half of the patients were determined positive based on PBMC samples. For module 5.12 the results gathered from monocytes and PBMCs differed significantly.

When analyzing the IFN-score using the transcripts per module, the relative contributions of the transcripts to the modular IFN-score have been determined (see Materials and Methods for detailed information). The results are depicted in Figures 4 a, b and c for modules 1.2, 3.4 and 5.12, respectively, and are ordered from highest contribution at the bottom to the lowest at the top. It is clearly visible that the order differs between monocytes and PBMCs, and that the relative contribution if each transcript is not identical for the two biological substances.

Using the same calculations used to select the transcripts for the modular IFN-scores for all fourteen transcripts (data not shown), the relative contribution of each transcript to the total IFN-score could be calculated. In this way, the three most contributing transcripts in both monocytes and PBMCs have been determined, being Interferon Induced Protein 44 Like (IFI44L),

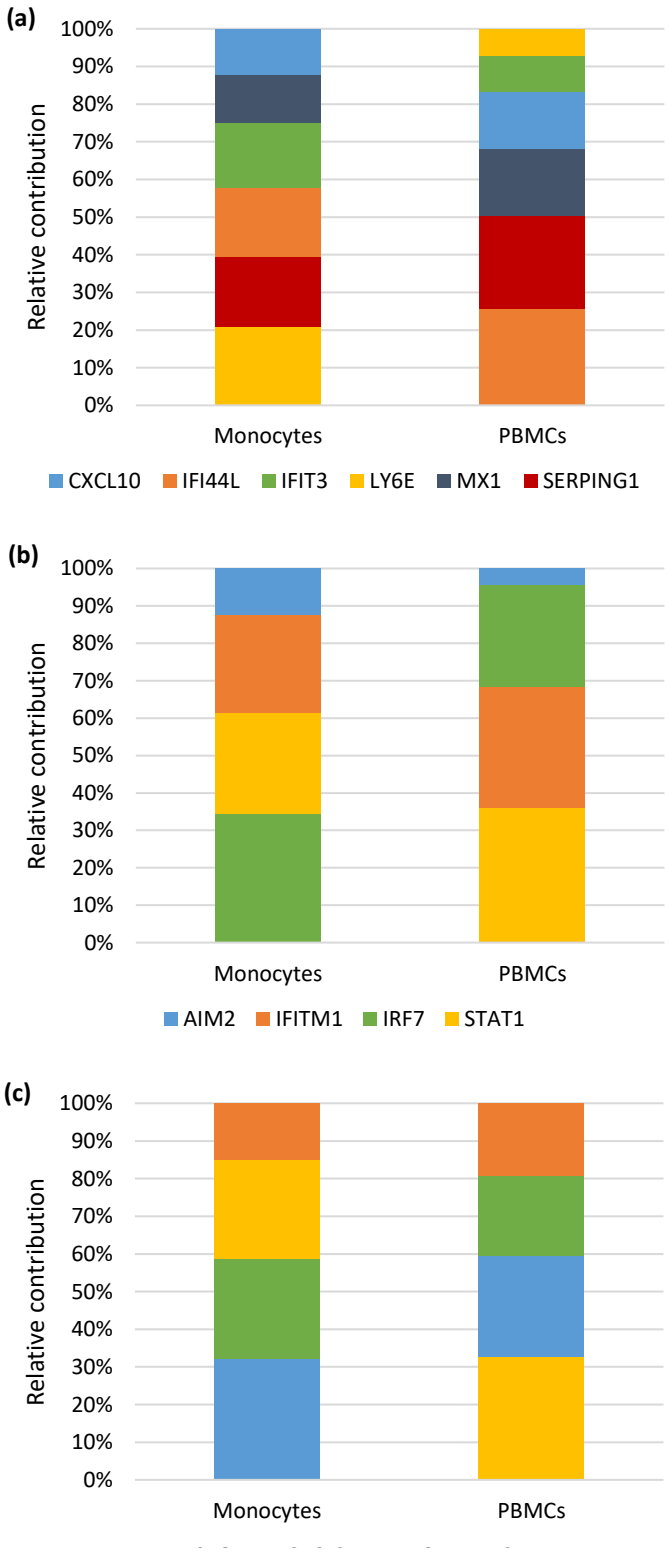

Figure 4. The relative contributions to the modular IFN-score for (a) module 1.2, (b) 3.4 and (c) 5.12. Relative contributions have been calculated as percentages of influence on the modular IFN-score, as described in the Materials and Methods section. The bars are ordered so the highest contribution is at the bottom and the lowest at the top.

SERPING1 and IRF7 (see Table 6). The correlations for this selection has been determined as well, and is displayed in Table 7. In monocytes, none of the transcripts correlate at all. In PBMCs, only IFI44L correlates strongly with IRF7. The IFN-score based on IFI44L, SERPING1 and IRF7 is from now on referred to as the 3-gene-based IFN-score and is shown in Figure 5. These three transcripts define 67% and 75% of the SLE patients as positive using monocytes and PBMCs, respectively (see Table 4). These results do not differ significantly from the percentages obtained using all transcripts from module 1.2 and correlate highly significant on individual level in monocytes and PBMCs ($P < 0.001$ in both biological substances, see Figure 6).

Table 6. Contribution of the transcripts to the total IFN-Score based on all 14 transcripts

Transcript	Monocytes	PBMCs
CXCL10	5.46	7.43
IFI44L	8.15	12.49
IFIT3	7.79	4.66
LY6E	9.30	3.40
MX1	5.67	8.57
SERPING1	8.40	11.93
AIM2	3.03	0.84
IFITM1	6.43	6.24
IRF7	22.10	14.65
STAT1	6.60	6.94
C1QA	5.49	6.12
CXCL2	2.56	4.39
IFI16	4.52	4.85
IRF9	5.50	7.48

Numbers are percentages of contribution to the IFN-score based on all 14 transcripts using the same calculation as for the modular relative contributions (see Figure 4). The three selected transcripts are among the four most contributing transcripts in both biological substances.

Table 7. Correlations of the three most contributing transcripts to the total IFN-Score based on all 14 transcripts

	IFI44L		SERPING1		IRF7	
Biological substance	CD14	PBMC	CD14	PBMC	CD14	PBMC
IFI44L			NS	NS	NS	***
SERPING1	-0.051	0.140			NS	NS
IRF7	0.374	0.589	-0.091	0.030		

Underneath the grey line the correlation coefficients are displayed; above the statistical value. Correlation coefficients have been determined using a nonparametric Spearman's rank correlation coefficients test. *** = $P < 0.001$; NS = not-significant.

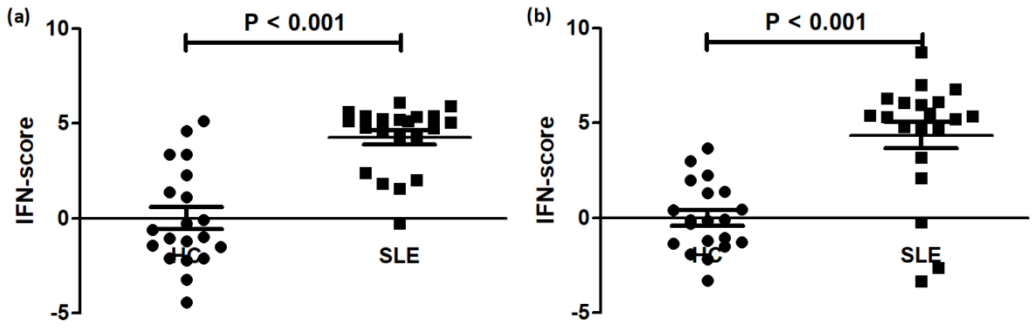

Figure 5. The 3-gene-based IFN-score in (a) monocytes and (b) PBMCs. Each symbol represents an individual patient sample; the horizontal line represents the median. Statistical analysis has been performed with a student t-test. P-values are shown and considered significant when $P < 0.05$.
HC = healthy control; SLE = systemic lupus erythematosus.

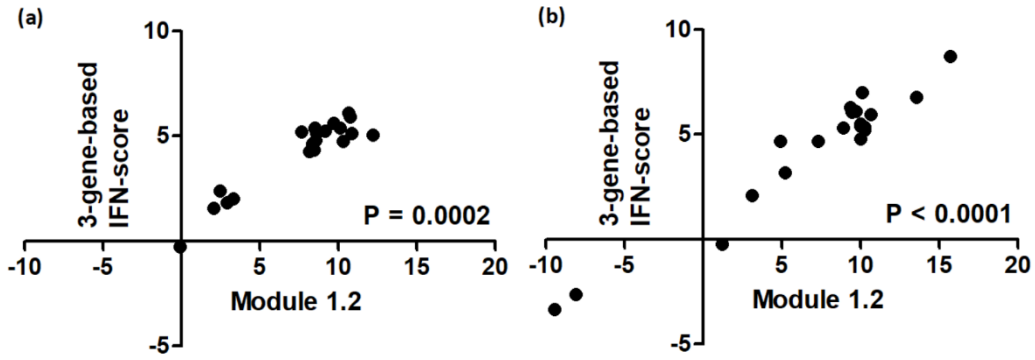

Figure 6. The correlations between the IFN-score of SLE patients for module 1.2 and the 3-gene-based IFN-score in (a) monocytes and (b) PBMCs. R-values of 0.727 and 0.795 have been found in monocytes and PBMCs, respectively. Each symbol represents an individual patient sample. P-values are shown and considered significant when $P < 0.05$.

The IFN-score calculations applied in whole blood and monocytes of iSLE and SLE patients

As previously described, usage of whole blood samples is discouraged for IFN signature determinations mostly due to negative influences of neutrophils. However, since the RNA isolation process is notably faster and executable, it remains interesting to know whether whole blood could be applied to solely determine a positivity for the IFN-score. In iSLE patients, this could be a convenient detection method to distinguish who is prone to develop SLE and which patients are unlikely to progress. Therefore, the IFN-score in whole blood samples and monocytes, since we believe monocytes are the most suitable biological substance (see Discussion), of healthy controls, iSLE and SLE patients have been analyzed. Concerning the relative expressions of the studied transcripts, no statistically relevant differences in expression have been determined between iSLE and SLE in whole blood and monocytes (data not shown). Table 8 displays the numbers of positive IFN-scores per module in iSLE and SLE patients for whole blood and monocytes, considering positive IFN-scores in monocytes as truly positive. Even though some inequalities are found for modules 1.2 and 3.4, and the 3-gene-based IFN-score, no significant differences have been determined in either iSLE or SLE patients. However, the number of positive IFN-scores for module 5.12 does not correspond between the two biological substances in both iSLE and SLE patients, due to no positive IFN-scores in the whole blood samples. When considering the correlations between the biological substances, similar results are obtained for modules 1.2 and 3.4, and the 3-gene-based IFN-score, showing significant correlation

Table 8. Numbers of positive IFN-scores per module of iSLE and SLE patients for whole blood samples and monocyte

	IFN positive in iSLE		P-value	IFN positive in SLE		P-value
	Whole blood	Monocytes		Whole blood	Monocytes	
IFN-score module 1.2	10/17 (59%)	7/18 (39%)	0.238	8/18 (44%)	7/18 (39%)	0.735
IFN-score module 3.4	5/17 (29%)	5/18 (28%)	0.915	5/18 (28%)	7/18 (39%)	0.480
IFN-score module 5.12	0/17 (0%)	5/18 (28%)	0.019	0/18 (0%)	4/18 (22%)	0.034
3-gene-based IFN-score	10/17 (59%)	7/18 (39%)	0.238	6/18 (33%)	7/18 (39%)	0.729

Number of IFN positive patients is displays compared to the total number of both iSLE and SLE patients per biological substance, with the corresponding percentages. The statistical analysis has been performed using a chi-squared test; P-values are considered significant when $P < 0.05$.
iSLE = incomplete systemic lupus erythematosus; SLE = systemic lupus erythematosus

coefficients (see Table 9). Interestingly, module 5.12 correlates significantly between whole blood and monocytes even though they had rather different positivity numbers (0 and 5, respectively; see Table 8). To verify whether the 3-gene-based IFN-score could act as a substitute for module 1.2 based IFN-score, the individual IFN-scores of both groups have been correlated (see Figure 7). In both whole blood samples and monocytes, there are strongly significant correlations ($P < 0.001$) in iSLE and SLE patients, with all Spearman's rho values over 0.9.

Table 9. Correlations between IFN-scores in whole blood samples and monocytes of iSLE and SLE patients

	Module	Correlation coefficients	P-value
iSLE	1.2	0.914	< 0.0001
	3.4	0.850	< 0.0001
	5.12	0.586	0.0218
	3-gene-based	0.954	< 0.0001
SLE	1.2	0.859	< 0.0001
	3.4	0.541	0.0304
	5.12	0.097	0.721
	3-gene-based	0.782	0.0003

Correlation coefficients have been determined using a nonparametric Spearman's rank correlation coefficients test. P-values are considered significant when $P < 0.05$.
iSLE = incomplete systemic lupus erythematosus; SLE = systemic lupus erythematosus

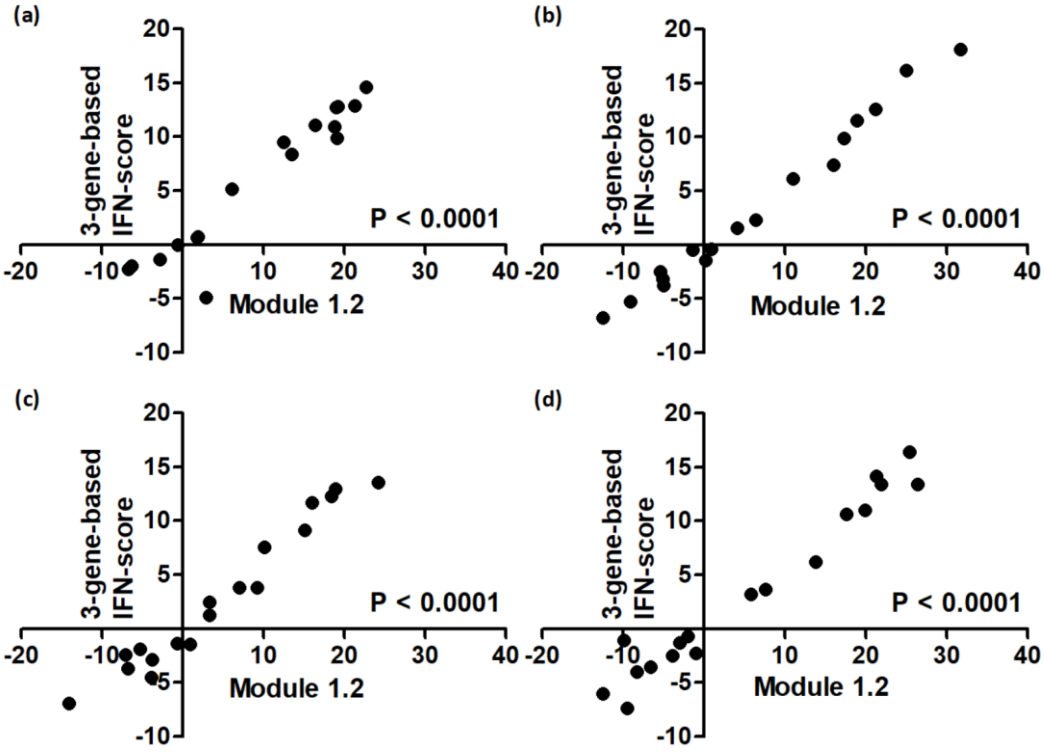

Figure 7. The correlations between the IFN-score of module 1.2 and the 3-gene-based IFN-score in (a) whole blood and (b) monocytes of iSLE patients, and (c) whole blood and (d) monocytes of SLE patients. R-values of (a) 0.929, (b) 0.988, (c) 0.967 and (d) 0.930 have been found. Each symbol represents an individual patient sample. P-values are shown and considered significant when $P < 0.05$.

Discussion

The current research provides extended information on the involvement of IFN-related genes in patients with SLE, examined in different biological substances. For type I IFN positivity determinations, monocytes, PBMCs and whole blood samples appear to be applicable interchangeably. The 3-gene-based IFN-score serves as a facile detection method for IFN positivity. In terms of disease activity, pathogenesis and predictive value, monocytes seem to be the most reliable biological substance to determine the IFN-score in SLE patients.

The IFN signature receives growing interest in autoimmune diseases, SLE in particular. However, the lack of generally accepted methods to calculate the IFN-score causes many varieties in transcript combinations and laboratory conditions. A recent overview of investigated IFN transcripts displayed that virtually all studied transcripts belong to module 1.2, only two transcripts belong to module 3.4 and none of them belong to module 5.12[16]. However, even within the module 1.2 there is no consensus on which transcripts should be used. Module 1.2, in contrast to module 3.4 and 5.12, is mostly regulated by type I IFNs instead of type II[17]. There is no difference in the effects of type I and type II IFNs on modules 3.4 and 5.12. Therefore, the IFN-score based on transcripts from module 1.2 represents the type I IFN signature. Even though the type I IFN signature is only of interest in an abundance of contemporary studies, it is doubtful whether this represents the entire IFN profile and provides enough information on the disease activity of SLE patients. There are numerous considerations that support the selection of certain transcripts, also transcripts not originating from module 1.2. As mentioned in the in the literature analysis (see Results), IP-10 and MxA concentrations could be easily detected in serum by ELISA and therefore have been a subject of interest in SLE research. IP-10 secretion is stimulated by IFN-γ, which recruits Th1-type adaptive cells to sites of inflammation[40]. IP-10 production resulting of IFN-α mediated stimulation of monocytes has been correlated longitudinally with SLE disease activity[16]. IP-10 is described as an independent predictor of disease activity of SLE patients[41]. MxA is also used as an IFN biomarker in experimental and clinical settings, since it is considered as a mediator of the early innate immune response[18]. High expression of LY6E, which regulates T lymphocyte proliferation, differentiation and development, has been associated with stronger disease activity of SLE patients, displaying higher SLEDAI scores, increased proteinuria and decreased blood C3 complement[42][43]. Besides transcripts of effector proteins, regulatory proteins could aid to knowledge on the IFN signature. In the activation of the IFN-signaling cascade of all IFN types (I, II and III), signal transducer and activator of transcription 1 (STAT1) phosphorylation precedes IFN-stimulation gene transcriptional activation[22]. Via the type I and type III pathways, phosphorylated STAT1 interacts with IFN regulatory factor 9 (IRF9), resulting in transcription of lots of IFN-stimulated genes. STAT1 and IRF9 are present at baseline, but are reinforced by the IFN response since they are also IFN-stimulated genes[22]. Furthermore, STAT1 is significantly upregulated in immunostaining synovial gland of SLE patients[44].

A vast majority of the IFN-related genes selected for this study show a higher relative expression in SLE patients compared to healthy controls in both monocytes and PBMCs (12 and 11 out of 14, respectively). This verifies the believe that IFN is more active in SLE patients and is responsible for transcription of a certain family of genes. When critically analyzing the obtained data, it is concluded that the relative expression shows better separation and higher significance in monocytes than PBMCs. For diagnostic value and additional knowledge of the effect on disease activity, calculation of the

modular IFN-scores is more relevant than just the relative expression profiles. Overall, the IFN-score of all modules in both biological substances is significantly higher in SLE patients. 71% and 65% of the SLE patients has a positive IFN-score for module 1.2, which is considered as the most accurate IFN-score in current literature, in monocytes and PBMCs, respectively. These percentages approach the 75% of IFN positive SLE patients known through recent studies, as mentioned earlier (see Introduction). Interestingly, contradictory results are found in for modules 3.4 and 5.12 in monocytes compared to PBMCs. Monocytes show higher numbers of IFN positive patients for module 3.4 than for 5.12, while PBMCs have higher module 5.12 positivity. However, it should be kept in mind that the studied cohort consists of quiescent patients, and that the modules show chronological activation and are differently correlated with disease activity, especially module 5.12[17]. It is nevertheless striking that in such a small cohort such great differences are found in positivity for module 3.4 and 5.12, with chi-square P-values of 0.072 (not significant) and 0.014 (significant), respectively. Furthermore, individual analysis showed that monocytes display a chronological activation profile, while the modular activation in PBMCs seems to be more random (data not shown). Therefore, it would be very interesting to study the modular IFN-scores of active SLE patients. Herewith the hypothesis that module 1.2 is a generally activated module in IFN positive SLE patients and modules 3.4 and 5.12 are progressively activated accompanied with disease activity could be investigated.

If transcript analysis to determine the IFN-score will be applied, a small collection of transcripts that describe the underlying process to the utmost extent is desired. When considering the correlations of relative expressions of transcripts within the modules (see Table 4), it becomes clear that some transcripts explain similar upregulation profiles with strong statistical support. In these cases, one transcript could interchangeably be used to represent their contribution to the IFN-score, instead of using both transcripts. The usage of transcripts with a low correlation is thought to describe a broader perspective of the IFN-score than strongly correlated transcripts. Combined with the relative contributions to the modular IFN-scores, it is elucidated that for modules 1.2 and 5.12 a reduction of transcripts to 2-4 transcripts would be possible to determine IFN positivity. In monocytes, a combination of IFI44L, LY6E and SERPING1, and C1QA with IFI16 would suffice to represent the entire IFN-scores for modules 1.2 and 5.12, respectively. Which transcripts contribute most to the IFN-score, however, has not been uniform in current literature. In 2013, analysis of eleven IFN inducible genes showed that 95% of the total variance was described by five genes (IFI44, IFI44L, IFIT3, LY6E and MX1) in monocytes of Sjögren's syndrome patients[45]. When the same subset of genes was tested in SLE patients, a subset of four genes, being IFI44L, IFITM1, LY6E and SERPING1, explained 95% of the total variance[24]. As previously mentioned, microarray analysis of 128 IFN-related genes revealed that just three transcripts can correlate almost perfectly with the total IFN-signature[20]. Based on these observations, it appears the most representing transcripts differ according to the varying conditions and transcript selections. To determine the most representable transcripts in this study, the relative contributions to the total IFN-score have been calculated and are depicted in Table 6. From the top-5 contributors in monocytes and PBMCs separately, the three most contributing transcripts which overlap have been selected for the 3-gene-based IFN-score, being IFI44L, SERPING1 and IRF7. These transcripts determine approximately 40% of the total IFN-score, 38.6% in monocytes and 39.1% in PBMCs. Considering the correlations between these transcripts, the selection would be optimal for a broad IFN-score in monocytes (see Table 7). Unfortunately, this differs for PBCMs since IFI44L correlates strongly with IRF7 in this biological substance. Even though, the 3-gene-based IFN-score seems to be a suitable replacement for the IFN-score of module 1.2, since the IFN positivity numbers approach each other (P-values of 0.74 and 0.49 for monocytes and PBMCs, respectively; data not

shown). Furthermore, the IFN-scores based on module 1.2 and the 3-gene-based IFN-scores strongly correlate in both monocytes and PBMCs, which indicates that the reduced selection could be applied as a substitute.

Even though the IFN-score could already be determined in inactive SLE patients, it could be even more explanatory in active SLE patients. Especially concerning the alternate activation profiles of the IFN modules, involving active SLE patients would aid to the descriptive value of the modular IFN-scores. Unfortunately, due to limitations in the studied cohort, there were only two active SLE patients included in this project and therefore none of the findings could be supported by statistical evidence. Besides, although these patients display strong modular IFN-scores, no exceptional IFN-scores or modular activation patterns have been detected in the two active SLE patients compared with the inactive cohort. Current literature suggests that there are in fact discrepancies between inactive and active SLE patients concerning the IFN signature. Chemokines produced by IFN-related transcripts have been described to serve as biomarkers of disease activity in SLE[35]. For example, serum IP-10 levels are upregulated in active SLE patients compared to inactive SLE patients. Furthermore, the IP-10 levels are strongly associated with current and future disease activity and is considered as a stand-alone biomarker of SLE activity[35]. Whole blood gene expression profiling studies suggest that SLE patients with a positive IFN-score correlated with disease activity in contrast to negative IFN-scores, thereby serving as a biomarker for active SLE[46]. In modular analysis studies, it was reported that module 5.12 displayed increased expression in only 25% of inactive SLE patients compared to 100% in patients suffering from a lupus flare[29]. Since module 1.2 showed positivity in all SLE groups, module 1.2 serves as a SLE marker whereas module 5.12 correlates with disease activity. Taken together, the IFN signature seems to have predictive value for disease activity and it would be very interesting to extensively analyze the modular IFN-score in a cohort containing active SLE patients.

Clinically it is challenging to distinguish a viral infection from a lupus flare, since IFNs are involved in the viral immune response. Defining the IFN signature with a specific selection of transcripts might be the solution to effectively discriminate a pathogenically amplified IFN signature and a typical viral response signature[37]. However, which transcripts could fulfill that purpose remains uncertain, on account of little distinctive IFN-related transcripts identified which seem to vary by cell type as well[29][37]. Besides distinguishing inactive and active disease, the IFN signature might be a predictive biomarker for progression to SLE in patients with incomplete lupus. Recently, the expression levels of type I IFN-related genes, e.g. MxA, have been determined in iSLE and SLE[36]. MX1 showed higher expression levels in the iSLE group compared with healthy controls, but no significant expression levels were found between iSLE and SLE patients. Besides expression levels, protein concentrations of IFN-related genes where measured, e.g. C1q and IP-10, who in both cases where overabundant in SLE compared with iSLE[36]. In the current study, we found no significant differences between iSLE and SLE patients for all transcripts, and varying statistically relevant upregulation of these groups compared with healthy controls. However, when applying this data to calculate the IFN-scores, the yield of IFN positive SLE patients is lower in the studied cohort compared with the previously analyzed monocytes (39% versus 71%) and as described in current literature (75%). Therefore, it is rather hard to correlate the data obtained in the whole blood study with the monocytes and PBMC experiments. The observed lower IFN positivity in the whole blood study could be due to multiple factors. First, the number of participants in this study was quite low. Since not all SLE patients display an IFN signature, it might be that the studied cohort consists of mostly IFN negative patients. Second, even though both cohorts consist of quiescent SLE patients, use of hydroxychloroquine is more prominent in the newly obtained

whole blood samples and monocytes of SLE patients. Currently, almost every SLE patient receives hydroxychloroquine treatment in North-American and Western-European hospitals (compared to half in the monocytes and PBMCs study), which strongly reduces IFNα levels in these patients[41][47]. However, when relying on the monocytes as the biological substance to determine the IFN-score correctly, it appears that whole blood samples could at least be used to determine the IFN-score based on module 1.2 and the 3-gene-based IFN-score in both iSLE and SLE. No significant differences in the number of IFN positive patient were found, accompanied with very strong correlation coefficients. The same goes for module 3.4, even though the correlation coefficients have lowered. For module 5.12, however, less IFN positivity was determined in whole blood samples of iSLE and SLE patients compared with monocytes, as well as no significant correlation between the biological substances in SLE patients. Unexpectedly, a correlation was found in iSLE patients despite the lower IFN positivity. Nevertheless, the number of IFN positive patients is the highest for module 1.2 and the lowest in module 5.12, which resembles to the chronological activation profile of the modules and the variability of the modules previously described in literature[17].

In this study, we found that the IFN signature of SLE patients could be detected using IFN-related transcripts in three different biological substances, being monocytes PBMCs and whole blood samples. When critically analyzing the results, monocytes seem to be the most reliable source to determine the IFN-scores, based on stronger significance and better separation. However, if simply a positivity determination for the type I IFN signature is desired, all three biological substances should suffice to serve that purpose for iSLE and SLE patients. In line with the current literature, module 1.2 seems to be a stably active IFN module in SLE patients. In all biological substances, module 5.12 has shown to be the hardest to determine the IFN-score accurately, displaying the most variations and the least correlation. Since module 5.12 is most of all correlated with disease activity and barely any active patients were included in this study, it would be interesting to involve active SLE patients in future studies. Furthermore, it would be enlightening for interpretation of the clinical value of the IFN-score to correlate the modular IFN-scores of iSLE and SLE patients with the clinical characteristics and perhaps serological measurements, like MxA and IP-10 ELISAs. Lastly, longitudinal studies involving iSLE and SLE patients could add to the knowledge on the relation between the IFN signature and the development to SLE, and the IFN-scores during different disease expressions, e.g. flares. Altogether, the IFN signature shows promising results to serve as a detection marker of SLE and early SLE, and might operate as a predictor of SLE development and disease activity.

Bibliography

1. Okada, H., Kuhn, C., Feillet, H., and Bach, J.-F. (2010). The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. *Clinical and Experimental Immunology*; **160**: 1-9.
2. Mu, Q., Zhang, H., and Luo, M. X. (2015). SLE: Another Autoimmune Disorder Influenced by Microbes and Diet? *Frontiers in Immunology*; **6**: 608.
3. Baechler, E. C., Batliwalla, F. M., Karypis, G., *et al*. (2003). Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *PNAS*; **100** (5): 2610-2615.
4. Costenbader, K. H., and Schur, P. H. (2015). We Need Better Classification and Terminology for "People at High Risk of or in the Process of Developing Lupus". *Arthritis Care & Research*; **67** (5): 593-596.
5. Li, Q.-Z., Zhou, J., Wandstrat, A. E., *et al*. (2007). Protein array autoantibody profiles for insights into systemic lupus erythematosus and incomplete lupus syndromes. *Clinical and Experimental Immunology*; **147** (1): 60-70.
6. Rahman, A., and Isenberg, D. A. (2008). Systemic Lupus Erythematosus. *The New England Journal of Medicine*; **358**: 929-939.
7. Lisnevkaia, L., Murphy, G., and Isenberg, D. (2014). Systemic Lupus Erythematosus. *The Lancet*; **384**: 1878-1888.
8. Guerra, S. G., Vyse, T. J., and Cunninghame Graham, D. S. (2012). The genetics of lupus: a functional perspective. *Arthritis Research & Therapy*; **14**: 211.
9. Yu, C., Gershwin, M. E., and Chang, C. (2014). Diagnostic criteria for systemic lupus erythematosus: A critical review. *Journal of Autoimmunity*; **48-49**: 10-13.
10. Robertson, J. M., and James, A. J. (2014). Preclinical systemic lupus erythematosus. *Rheumatic Diseases Clinics of North America*; **40** (4): 621-635.
11. Bourn, R., and James, A. J. (2015). Preclinical lupus. *Current Opinion in Rheumatology*; **27** (5): 433-439.
12. Ivashkiv, L. B., and Donlin, L. T. (2014). Regulation of type I interferon responses. *Nature Reviews Immunology*; **14** (1): 36-49.
13. Pollard, K. M., Cauvi, D. M., Toomey, C. B., *et al*. (2013). Interferon-γ and Systemic Autoimmunity. *Discovery Medicine*; **16** (87): 123-131.
14. Yao, Y., Higgs, B. W., Richman, L., *et al*. (2010). Use of type I interferon-inducible mRNAs as pharmacodynamic markers and potential diagnostic markers in trials with sifalimumab, an anti-IFNα antibody, in systemic lupus erythematosus. *Arthritis Research & Therapy*; **12** (supplementary 1): S6.
15. Barturen, G., and Alarcón-Riquelme, M. E. (2017). Gene expression profiling comes closer to the clinic. *Nature Reviews Rheumatology*; **13** (2): 69-70.
16. Strauß, R., Rose, T., Flint, S. M., *et al*. (2017). Type I interferon as a biomarker in autoimmunity and viral infection: a leukocyte subset-specific analysis unveils hidden diagnostic options. *Journal of Molecular Medicine*; doi: 10.1007/s00109-017-1515-7. [Epub ahead of print].
17. Chiche, L., Jourde-Chiche, N., Whalen, E., *et al*. (2014). Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. *Arthritis & Rheumatology*; **66** (6): 1583-1595.
18. Maria, N. I., Brkic, Z., Waris, M., *et al*. (2014). MxA as a clinically applicable biomarker for identifying systemic interferon type I in primary Sjögren's syndrome. *Annals of the Rheumatic Disease*; **73**: 1052-1059.
19. Kalunian, K. C., Merrill, J. T., Maciuca, R., *et al*. (2016). A Phase II study of the efficacy and safety of rontalizumab (rhuMAb interferon-α) in patients with systemic lupus erythematosus (ROSE). *Annals of the Rheumatic Disease*; **75**: 196-202.
20. Kennedy, W. P., Maciuca, R., Wolslegel, K., *et al*. (2015). Association of the interferon signature metric with serological disease manifestations but not global activity scores in multiple cohorts of patients with SLE. *Lupus Science & Medicine*; **2**: e000080.
21. Feng, X., Wu, H., Grossman, J. M., *et al*. (2006). Association of Increased Interferon-Inducible Gene Expression With Disease Activity and Lupus Nephritis in Patients With Systemic Lupus Erythematosus. *Arthritis & Rheumatism*; **54** (9): 2951-2961.
22. Schneider, W. M., Dittmann Chevillotte, M., and Rice, C. M. (2014). Interferon-Stimulated Genes: A Complex Web of Host Defenses. *Annual Review of Immunology*; **32**: (513-545).
23. Chaussabel, D., and Baldwin, N. (2014). Democratizing systems immunology with modular transcriptional repertoire analyses. *Nature Reviews Immunology*; **14**: 271-280.
24. Brkic, Z., Corneth, O. B. J., Helden-Meeuwsen, C. G. van, *et al*. (2014). T-Helper 17 cytokines and interferon type I: partners in crime in systemic lupus erythematosus? *Arthritis Research & Therapy*; **16**: R62.
25. Kirou, K., Lee, C., George, S., *et al*. (2005). Activation of the Interferon-α Pathway Identifies a Subgroup of Systemic Lupus Erythematosus Patients With Distinct Serologic Features and Active Disease. *Arthritis & Rheumatism*; **52** (5): 1491-1503.
26. Landolt-Marticorena, C., Bonventi, G., Lubovich, A., *et al*. (2009). Lack of association between the interferon-a signature and longitudinal changes in disease activity in systemic lupus erythematosus. *Annals of the Rheumatic Disease*; **68**: 1440-1446.
27. McBride, J. M., Jiang, J., Abbas, A. R., *et al*. (2012). Safety and Pharmacodynamics of Rontalizumab in Patients With Systemic Lupus Erythematosus. *Arthritis & Rheumatism*; **64** (11): 3666-3676.

28. Reynolds, J. A., Khan, M., Briggs, T. A., *et al*. (2016). A Type-I Interferon Signature Is Associated with Autoantibody Profiles in Connective Tissue Diseases: Results from the Lupus Extended Autoimmune Phenotype (LEAP) Study [abstract]. *Arthritis & Rheumatology*; **68** (supplementary 10). http://acrabstracts.org/abstract/a-type-i-interferon-signature-is-associated-with-autoantibody-profiles-in-connective-tissue-diseases-results-from-the-lupus-extended-autoimmune-phenotype-leap-study/. Accessed November 23, 2016.
29. Mackay, M., Oswald, M., Sanchez-Guerrero, J., *et al*. (2016). Molecular signatures in systemic lupus erythematosus: distinction between disease flare and infection. *Lupus Science & Medicine*; **3**: e000159.
30. Flint, S. M., Jovanovic, V., Teo, B. W., *et al*. (2016). Leucocyte subset-specific type 1 interferon signatures in SLE and other immune-mediated diseases. *RMD Open*; **2** (1): e000183.
31. Becker, A. M., Dao, K. H., Han, B. K. *et al*. (2013). SLE peripheral blood B cell, T cell and myeloid cell transcriptomes display unique profiles and each subset contributes to the interferon signature. *PLoS One*; **8** (6): e67003.
32. Kirou, K. A., Lee, C., George, S., *et al*. (2004) Coordinate Overexpression of Interferon-α–Induced Genes in Systemic Lupus Erythematosus. *Arthritis & Rheumatism*; **50** (12): 3958-3967.
33. Liu, X., Mayes, D., Tan, F. K., *et al*. (2013). Correlation of Interferon-Inducible Chemokine Plasma Levels With Disease Severity in Systemic Sclerosis. *Arthritis & Rheumatism*; **65** (1): 226-235.
34. Rodero, M. P., Decalf, J., Bondet, V., *et al*. (2017). Detection of interferon alpha protein reveals differential levels and cellular sources in disease. *The Journal of Experimental Medicine*; **214** (5): 1547-1555.
35. Bauer, J. W., Petri, M., Batliwalla, F. M., *et al*. (2009) Interferon-Regulated Chemokines as Biomarkers of Systemic Lupus Erythematosus Disease Activity. *Arthritis & Rheumatism*; **60** (10): 3098-3107.
36. Olsen, N. J., McAloose, C., Carter, J., *et al*. (2016). Clinical and Immunologic Profiles in Incomplete Lupus Erythematosus and Improvement with Hydroxychloroquine Treatment. *Autoimmune Diseases*; **2016**: 8791629.
37. Kyogoku, C., Smiljanovic, B., Grün, J. R., *et al*. (2013). Cell-Specific Type I IFN Signatures in Autoimmunity and Viral Infection: What Makes the Difference? *PLoS One*; **8** (12): e83776.
38. Abdulahad, D. A., Westra, J., Reefman, E., *et al*. (2013). High mobility group box1 (HMGB1) in relation to cutaneous inflammation in systemic lupus erythematosus (SLE). *Lupus*; **22**: 597-606.
39. Cugno, M., Zanichelli, A., Foieni, F., *et al*. (2009). C1-inhibitor deficiency and angioedema: molecular mechanisms and clinical progress. *Trends in Molecular Medicine*; **15** (2): 69-78.
40. Munroe, M. E., Lu, R., Zhao, Y. D., *et al*. (2016). Altered type II interferon precedes autoantibody accrual and elevated type I interferon activity prior to systemic lupus erythematosus classification. *Annals of the Rheumatic Disease*; **75** (11): 2014-2021.
41. Willis, R., Smikle, M., DeCeulaer, K., *et al*. (2017). Clinical associations of proinflammatory cytokines, oxidative biomarkers and vitamin D levels in systemic lupus erythematosus. *Lupus*; **0**: 1-11.
42. Yu, J., Liang, C., and Liu, S.-L. (2017). Interferon-inducible LY6E Protein Promotes HIV-1 Infection. *The Journal of Biological Chemistry*; **292** (11): 4674-4685.
43. Tang, J., Gu, Y., Zhang, M., *et al*. (2008). Increased expression of the type I interferon-inducible gene, lymphocyte antigen 6 complex locus E, in peripheral blood cells is predictive of lupus activity in a large cohort of Chinese lupus patients. *Lupus*; **17**: 805-813.
44. Nzeusseu-Toukap, A., Galant, C., Theate, I., *et al*. (2007). Identification of Distinct Gene Expression Profiles in the Synovium of Patients With Systemic Lupus Erythematosus. *Arthritis & Rheumatism*; **56** (5): 1579-1588.
45. Brkic, Z., Maria, N. I., Helden-Meeuwsen, C. G van, *et al*. (2013). Prevalence of interferon type I signature in CD14 monocytes of patients with Sjögren's syndrome and association with disease activity and BAFF gene expression. *Annals of the Rheumatic Disease*; **72**: 728-735.
46. Nikpour, M., Dempsey, A. A., Urowitz, M. B., *et al*. (2008). Association of a gene expression profile from whole blood with disease activity in systemic lupus erythaematosus. *Annals of the Rheumatic Disease*; **67**: 1069-1075.
47. Willis, R., Seif, A. M., McGwin Jr, G., *et al.* (2012). Effect of hydroxychloroquine treatment on pro-inflammatory cytokines and disease activity in SLE patients: data from LUMINA (LXXV), a multiethnic US cohort. *Lupus*; **21** (8): 830-835.

Appendices

Appendix 1.1

Protocol for defrosting and lysing PBMCs and CD14+ monocytes

Preparations:

- 10% FCS in PBS buffer (at least 12 mL/sample)

Proceedings:

- Collect the frozen PBMC containing ampoules from the liquid nitrogen vessel
- Contain them on ice
- Immerse the ampoule in warm water until the content is a little fluid
- Transfer the content in a 15 mL tube
- Add 10 mL of 10% FCS buffer to the 15 mL tube, the first 5 mL dropwise
- Spin down 6 minutes at 1400 rpm, brake 0
- Remove the supernatant by suction without disturbing the cell pellet
- Resuspend the cell pellet with 1 mL 10% FCS buffer
- Make a cell count: add 40 µL cell suspension to 20 mL counting fluid in a counting jar
- Determine the amount of living cells: add 10 µL cell suspension to 10 µL trypan blue in an Eppendorf cup, count a hundred cells in a Burker count chamber

<u>For PBMC samples:</u>

- Spin the remaining cell suspension down at 3000 g for 6 minutes
- Remove the supernatant by pipetting without interfering with the cell pallet
- Add 1 mL Trizol to the cell pallet, resuspend, and vortex until the entire pallet is dissolved
- Store when necessary at -80˚C

<u>For CD14 samples:</u>

Preparations:

- Isolation buffer: PBS containing 0.1% BSA; 2 mM EDTA (at least 5 mL/sample)
- Magnet for 5 mL tubes

Proceedings:

Prepare isolation beads

- Add 25 µL of CD14+ beads in a 5 mL tube
- Add 1 mL isolation buffer
- Place the tube into the magnet and pipet remove the supernatant by pipetting
- Remove the tube from the magnet and add 25 µL of isolation buffer to the beads

Cell isolation

- Make sure there are up to 10^7 cells/mL in the sample
- Add 1 mL of sample to the magnetic beads
- Incubate the tube for 20 minutes in cold storage (2-8˚C) while shaking lightly
- Place the tube in the magnet for 2 minutes
- Remove the supernatant by pipetting while the tube is in the magnet

- Wash the beads three times by adding 1 mL isolation buffer, followed by supernatant removal while the tube is inside the magnet
- Lyse the cells by adding 1 mL of Trizol to the beads
- Remove the supernatant (containing the RNA) while inside the magnet and store in a RNase-free Eppendorf cup
- Store if necessary at -80˚C

Appendix 1.2

PAXgene Blood RNA Kit procedure

Preparations:

- PAXgene Blood RNA Kit (50) Cat No./ID: 762164
- 96% ethanol (at least 350 µL/sample)

Proceedings:

- Defrost the blood in the PAXgene tube, make sure that it has been at room temperature for at least 2 hours (15-25°C)
- Centrifuge the PAXgene Blood RNA Tube for 15 minutes at 4.000 rpm (2800 g)
- Remove the supernatant by decanting
- Add 4 ml RNase-free water (RNFW) to the pellet, and close the tube using a fresh secondary BD Hemogard closure (supplied with the kit). When the supernatant is decanted, take care not to disturb the pellet, and dry the rim of the tube with a clean paper towel
- Vortex until the pellet is visibly dissolved, and centrifuge for 15 minutes at 4.000 rpm (2800 g)
- Remove and discard the entire supernatant (incomplete removal will inhibit lysis)
- Add 350 µl resuspension buffer (BR1), and vortex until the pellet is visibly dissolved
- Pipet the sample into a 1.5 ml microcentrifuge tube (MCT)
- Add 300 µl binding buffer (BR2) and 40 µl proteinase K (PK). Mix by vortexing for 5 seconds, and incubate for 10 minutes at 55°C using a shaking water bath at 250 rpm (max)
- Pipet the lysate directly into a PAXgene Shredder spin column (PSC; lilac) placed in a 2 ml processing tube (PT), and centrifuge for 3 minutes at 19.000 g
- Carefully transfer the entire supernatant of the flow-through fraction to a fresh 1.5 ml microcentrifuge tube (MCT) without disturbing the pellet in the processing tube (PT).
- Add 350 µl 100% ethanol; mix by vortexing, and centrifuge briefly to remove drops from the inside of the tube lid
- Pipet 700 µl sample into the PAXgene RNA spin column (PRC; red) placed in a 2 ml processing tube (PT), and centrifuge for 1 minute at 19.000 x g
- Place the spin column (PRC) in a new 2 ml processing tube (PT), and discard the old processing tube (PT) containing flow-through
- Pipet the remaining sample into the PAXgene RNA spin column (PRC), and centrifuge for 1 minute at 19.000 g
- Place the spin column (PRC) in a new 2 ml processing tube (PT), and discard the old processing tube (PT) containing flow-through
- Pipet 350 µl wash buffer (BR3) into the PAXgene RNA spin column (PRC), and centrifuge for 1 minute at 19.000 g
- Place the spin column (PRC) in a new 2 ml processing tube, and discard the old processing tube (PT) containing flow-through
- Add 10 µl DNase I (RNFD) stock solution to 70 µl DNA digestion buffer (RDD) in a 1.5 ml microcentrifuge tube (MCT)
- Mix by gently flicking the tube, and centrifuge briefly to collect residual liquid from the sides of the tube; do not vortex
- Pipet the DNase I (RNFD) incubation mix (78 µl) directly onto the PAXgene RNA spin column (PRC) membrane, and place on the benchtop (20–30°C) for 15 minutes

- Pipet 350 µl wash buffer (BR3) into the PAXgene RNA spin column (PRC), and centrifuge for 1 minute at 19.000 g
- Place the spin column in a new 2 ml processing tube (PT), and discard the old processing tube (PT) containing flow- through
- Pipet 500 µl wash buffer (BR4) into the PAXgene RNA spin column (PRC), and centrifuge for 1 minute at 19.000 g
- Place the spin column (PRC) in a new 2 ml processing tube (PT), and discard the old processing tube (PT) containing flow-through
- Add another 500 µl wash buffer (BR4) to the PAXgene RNA spin column (PRC), and centrifuge for 3 minutes at 19.000 g
- Place the spin column (PRC) in a new 2 ml processing tube (PT), and discard the old processing tube (PT) containing flow-through
- Centrifuge for 1 minute at 19.000 g
- Discard the processing tube (PT) containing the flow-through
- Place the PAXgene RNA spin column (PRC) in a 1.5 ml microcentrifuge tube (MCT)
- Pipet 40 µl elution buffer (BR5) directly onto the PAXgene RNA spin column (PRC) membrane, and centrifuge for 1 minute at 19.000 g to elute the RNA
- Repeat the previous step (elution step) as described, using the same microcentrifuge tube (MCT)
- Incubate the eluate for 5 minutes at 65°C in a water bath without shaking
- After incubation, chill immediately on ice
- Measure the RNA concentration of the sample using nanodrop detection
- Store if necessary at -80˚C

Appendix 2

Protocol for RNA isolation

Preparations:

- Chloroform (0,2 mL/sample)
- Isopropanol (0,5 mL/sample)
- Ice-cold 75% ethanol (1 mL/sample)
- Ambion Kit no. 1906
- Wear gloves at any moment

Proceedings:

- Defrost the in Trizol lysed cells if required
- Add 0,2 mL chloroform, vortex for 15 seconds
- Incubate 2 minutes at room temperature
- Centrifuge the sample for 15 minutes at 10.000 rpm; 4˚C
- Transfer the water phase at the top (approximately 470 µL) into a fresh Eppendorf
- Add 0,5 mL isopropanol, vortex swiftly
- Incubate for 10 minutes at room temperature
- Centrifuge for 5 minutes at 10.000 rpm; 4˚C
- Decant the supernatant
- Wash the pellet with 1 mL ice-cold 75% ethanol, vortex swiftly
- Centrifuge for 5 minutes at 8.000 rpm; 4˚C
- Decant the supernatant
- Dry the pellet by leaving the Eppendorf open and upside-down for an hour

Continue with the DNase treatment

- Mix 22,5 µL RNase-free water + 2,5 µL DNase buffer + 1 µL DNase per sample
- Add 26 µL of the mix to the dried sample pellets
- Resuspend the pellet by flicking the tube
- Incubate for 30 minutes at 37˚C
- Vortex the inactivation reagent
- Add 5 µL inactivation reagent to the sample
- Resuspend the pellet by flicking the tube
- Incubate for 2 minutes at room temperature
- Centrifuge the sample swiftly using impulse
- Transfer the supernatant to a 0,5 mL RNase-free Eppendorf tube
- Measure the RNA concentration of the sample using nanodrop detection
- Store if necessary at -80˚C

Appendix 3.1

Protocol for Reverse Transcriptase – Polymerase Chain Reaction cDNA synthesis

Proceedings:

- Use 1 µg of RNA per RT-PCR reaction, with a total volume of 20-25 µL
- Store the RNA for 10 minutes at 65˚C, afterwards immediately store on ice
- Meanwhile, prepare the RT mix containing:
    1. ultra-pure water 6,6 µL
    2. 5x First Strand buffer 5 µL
    3. 25 mM dNTP mix 1 µL
    4. 0,1 M DTT 0,9 µL
    5. 7,5 µM oligo (dT) 0,5 µL
    6. M-MLV Reverse Transcriptase (RT) enzyme 1 µL
- Add 15 µL of the mix to the sample
- Place the sample in the Bio-Rad PCR machine following this program:
    - 1 hour at 41˚C
    - 5 minutes at 95˚C
    - Leave at 4˚C
- Store if necessary at -20˚C

Appendix 3.2

Protocol for quantitative Real-Time – Polymerase Chain Reaction

Proceedings:

- Pipet 1 µL of cDNA in a 384 wells plate, at least in duplet
- Spin down the plate for 10 minutes in a vacuum DNA speedvac
- Prepare the desired primer mix containing:
    - 0,5 µL primer of interest
    - 4,5 µL ultra-pure water
    - 5,0 µL Taqman premix
- Add 10 µL mix to the cDNA
- Cover the plate with an Optical Seal provide by Applied Biosystems
- Spin down the plate swiftly
- Set-up the Taqman instrument as follows:
    - 15 minutes at 95˚C
    - 40 repeats of 1 minute at 41˚C; 15 seconds at 95˚C
    - Volume = 10 µL

Appendix 4

A brief overview of gene functions

Module 1.2

CXCL10 – C-X-C motif chemokine ligand 10

- Antimicrobial gene that codes for the chemokine IP-10, a ligand for the CXCR3 receptor[A1].
- Binding op IP-10 to CXCR3 results in stimulation of monocytes, NK-cell and T-cell migration, and modulation of adhesion molecule expression[A1].
- May be a interferon gamma induced protein that mediates the interferon gamma response in a several cell types[A1].

IFI44L – Interferon induced protein 44 like

- Increased expression of IFI44L is a component of the type-I IFN response and part of the cellular response to viral infections[A2].
- Low antiviral activity against hepatitis C[A1].

IFIT3 – Interferon induced protein with tetratricopeptide repeats 3

- IFN-induced antiviral protein which acts as an inhibitor of cellular as well as viral processes, cell migration, proliferation, signaling, and viral replication[A1].
- Promotes antiviral gene transcription and exhibits antiproliferative activity[A1].
- Can negatively regulate the apoptotic effects of IFIT2[A1].

LY6E – Lymphocyte antigen 6 complex, locus E

- Regulates proliferation, differentiation and development of T-lymphocytes[A3].
- Overexpression is associated with poor survival and malignancy in several types of cancer[A4].
- Activated by IFN-alpha in monocytic cell line U-937 and in peripheral blood monocyte cells[A1].

MX1 – Myxovirus resistance 1

- Participates in the cellular antiviral response[A1].
- Induced by type I and type II interferons[A1].
- Antagonizes replication of several RNA and DNA viruses[A1].

SERPING1 – Serpin family G member 1

- Regulates complement activation by inhibition of C1 components[A1][A5].
- Inactivates the C1q subunit of the complement system and thereby prevents further activation of C4 and C2, possibly predisposing for systemic lupus erythematosus[A6].
- Regulates complement activation, blood coagulation, fibrinolysis and generation of kinins[A1].

Module 3.4

AIM2 – Absent in melanoma 2

- Interferon gamma inducible member of the IFI20X/IFI16 family that plays a role in tumorigenic revision and may control cell proliferation[A1].
- Involved in recognition of cytosolic dsDNA of viral and bacterial origin in macrophages[A1].

IFITM1 – Interferon induced transmembrane protein 1

- IFN-induced antiviral protein which prevents viral fusion and release of viral contents in the cytosol[A1].
- Plays a key role in the anti-proliferative action of IFN-gamma either by inhibition of transcription or arresting cell growth[A1].

IRF7 – Interferon regulatory factor 7

- Fulfills a crucial role in transcriptional regulation of the type I interferon-dependent immune response against DNA and RNA viruses[A1].
- Expression is largely restricted to lymphoid tissue[A1].
- More crucial in the late phase of the interferon gene induction than the early phase[A1].
- Regulates interferon-related gene expression by binding to an interferon-stimulated response element in their promotors[A1].

STAT1 – Signal transducer and activator of transcription 1

- An early transcription factor that is activation during monocyte to macrophage differentiation[A1].
- Forms complexes that can bind to interferon (gamma) promoter elements in response to either interferon alpha or interferon beta stimulation, which results in an increased expression of interferon-related genes and drives a cellular antiviral state[A1]
- Associates with IRF9 to form a complex transcription factor which can enter the nucleus termed ISGF3[A1].

Module 5.12

C1QA – Complement C1q A chain

- Code for complement subcomponent C1q, which associates with proenzymes to form the primary component of the complement system C1[A1].
- Deficiency of C1q has been associated with lupus erythematosus and glomerunephritis[A1].

CXCL2 – C-X-C motif chemokine ligand 2

- Produced by activated monocytes and neutrophils at sites of inflammation[A1].
- Antimicrobial gene that secretes proteins involved in the inflammatory response[A1].
- Described to suppress proliferation of hematopoietic progenitor cells[A1].

IFI16 – Interferon gamma inducible protein 16

- Interferon-inducible myeloid differentiation transcriptional activator[A1].
- Induced in monocytes by IFN-alpha and viral dsDNA[A1].
- Senses viral dsDNA in the cytosol and probably the nucleus[A1].
- Has anti-inflammatory activity, probably via association with AIM2[A1].

IRF9 – Interferon regulatory factor 9

- Transcription factor that mediates signaling by type I interferons[A1].
- Associated with STAT1 in the Jak/STAT pathway, driving the antiviral state[A1].

References

A1. Data is retrieved from the GeneCards human gene database by the Weizmann Institute of Science. The data is online available at: https://www.genecards.org.

A2. Zhang, M., Zhou, Y., Zhu, B., et al. (2016). IFI44L promoter methylation as a blood biomarker for systemic lupus erythematosus. *Annals of the Rheumatic Disease*; **75**: 1998-2006.

A3. Yu, J., Liang, C., and Liu, S.-L. (2017). Interferon-inducible LY6E Protein Promotes HIV-1 Infection. *The Journal of Biological Chemistry*; **292** (11): 4674-4685.

A4. Luo, L., McGarvey, P., Madhavan, S., et al. (2016). Distinct lymphocyte antigens 6 (Ly6) family members Ly6D, Ly6E, Ly6K and Ly6H drive tumorigenesis and clinical outcome. *Oncotarget*; **7** (10): 11165-11193.

A5. Cugno, M., Zanichelli, A., Foieni, F., et al. (2009). C1-inhibitor deficiency and angioedema: molecular mechanisms and clinical progress. *Trends in Molecular Medicine*; **15** (2): 69-78.

A6. Lintner, K. E., Wu, Y. L., Yang, Y., et al. (2016). Early Components of the Complement Classical Activation Pathway in Human Systemic Autoimmune Diseases. *Frontier in Immunology*; **7**: 36.