The Expression of Interferon-Induced Protein with Tetracopeptide Repeats 1 in Monocytes, a Shortcut to the Interferon Signature in Lupus

Olga Sánchez-Pernaute, María Pérez-Ferro, Antonio Gabucio and Raquel Benítez

Bone and Joint Research Unit, Division of Rheumatology, Jiménez Díaz Foundation Health Research Institute and University Hospital, Madrid, Spain

Corresponding author:
Olga Sánchez-Pernaute
osanchez@fjd.es

Division of Rheumatology, Jiménez Díaz Foundation Health Research Institute & University Hospital, Avda Reyes Católicos 228040 Madrid, Spain

Tel: +34 915 504 800

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Abstract

Virtually all immune cells show alterations in lupus. Since the activation of small cell subpopulations can be missed in gene expression studies of pooled mononuclear cells, we separately assessed the expression of six relevant type I interferon-stimulated genes in monocytes, T cells and B cells from 35 lupus patients and 11 healthy donors, using quantitative PCR. We observed an enhancement of five of the selected molecules in the immune cells from lupus patients, although their up-regulation was not uniform in the different cell subsets. In monocytes, the induction of the IFN-stimulated genes was associated to plasmablast counts, and skin disease, but not to antibody titres or complement levels. The expression of some, but not all, of these molecules increased during activity. Interestingly, the appearance of the IFN-stimulated genes in lymphocytes was a distinct feature of the lupus cohort, and their induction in B cells was associated to complement decrease. We suggest that the enhancement of the IFN-stimulated genes in each cell subset appears to draw distinct information, which could be complementary to other disease biomarkers. Globally, our result point to expression levels of interferon-induced protein with tetracopeptide repeats 1 in monocytes as a biomarker of the IFN signature during active lupus.

Keywords: Systemic lupus erythematosus, Type 1 interferon, Mononuclear cell subpopulations, Biomarkers, Innate immune response

There are, however, some hurdles to the use of ISG scores as biomarkers in clinical practice. Even though monocytes display the full repertoire of innate responses, type I IFN also contributes to the abnormal activation of T and B cells in lupus [7]. In this regard, since PBMC subpopulations are not necessarily even between patients, the contribution of each subset to the IFN signature cannot be estimated in pooled PBMC, as has been already suggested [7,8]. Furthermore, the activation of small cell subsets can be hidden by gene expression profiles of larger subpopulations. This might be particularly important considering the characteristic occurrence of lymphopenia in lupus. Finally, since both T dependent and independent processes can trigger disease flares, to have cell-type specific information is probably critical in the selection of candidates for tailored therapies. In two recent studies, the transcriptional signature of isolated circulating mononuclear subpopulations from patients with lupus...
was studied using high-throughput techniques [7,8]. In both studies, the up-regulation of ISG in the patients was qualitatively and quantitatively cell-type specific, therefore suggesting that ISG should be better assessed in purified subpopulations. As principal drawbacks, these studies were based on small groups of patients and little clinical background was available for associations. In the present study we have determined mRNA expression levels of 6 ISG in each of the major PBMC from patients with lupus and from healthy subjects, using quantitative PCR. We aimed at replicating in our cohort of patients the up-regulation of some of the ISG showing a robust association to the IFN signature in previous approaches [7-10]. In addition, we used this strategy as a way to try and find a simpler approach to the IFN signature for clinical purposes.

Material and Methods

Study population

We recruited 35 patients fulfilling ACR 1997 lupus classification criteria and 11 healthy donors. The study had the approval of our Institution’s Ethics Committee, and written informed consent was obtained from all participants. All clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki. All former disease-related features and demographic data were recorded. At the time of the evaluation, patients had been on stable medication for the last 90 days. They underwent a full clinical and analytical assessment. Subjects with signs of active infection were not allowed in the study. Disease activity was measured with Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) and British Isles Lupus Assessment Group’s Disease Activity Index (BILAG-2004) [11,12]. Active lupus was defined by a ≥ 4 SLEDAI score plus a ≥ 2 point increase in the last 3 months, BILAG categories A or B, or BILAG C plus a ≥ 4 SLEDAI score. Patients were considered to have active lupus nephritis in case of ≥ 0.5 gr increase in 24 hour urinary proteins in the last 3 months, a sterile active sediment, or past history of lupus nephritis not achieving remission. Cohort characteristics are summarized in Table 1.

Cell isolation and gene expression studies

Twenty milliliters of whole blood were used to obtain PBMC by density-gradient centrifugation. B and T lymphocytes, and monocytes were sequentially isolated from PBMC with magnetic sorting (Miltenyi Biotec, Bergisch Gladbach, Germany). The respective lineage selection was done with CD19, CD3, and CD14 specific antibodies (Miltenyi). The purity of the sorted subpopulations was assessed in FACS aliquots and was found to be >92%. Total RNA was purified from sorted cells with miRVana PARIS reagents (Life Technologies, Madrid, Spain). Three hundred ng of RNA were employed for retro-transcription using the high capacity PCR reagents (Life Technologies). The gene expression analysis was carried out with quantitative PCR in a StepOne Plus Real-Time PCR System (Life Technologies) using cDNA as template and Sybr green techniques (Life Technologies). The ISG selected for this study were IFN-induced GTP-binding myxovirus resistance protein (MX) 1, E3 protein ligase with ISG 15 activity (HERC) 5, IFN-induced protein with tetracopeptide repeats (IFIT) 1, 2'-5'-oligoadenylate synthase (OAS) 3, S-adenosyl methionine domain-containing protein (RDAS) 2 and IFN-induced 10 kDa peptide (IP10). The 6 selected molecules were picked up because of their identification in previous research, mostly addressing transcriptomic analysis of PBMC from patients with lupus [1, 6-9, 13]. We were interested to test them separately, because results drawn in the mentioned studies had been diverse.

The characteristics of each molecule and primer pairs used for their transcript amplification are shown in Table 2. Results were calculated with the delta Ct method, with 18s as house-keeping gene (gene expression assay; Life Technologies, Madrid, Spain).

Statistical analysis

Frequencies are shown as percentage (and total counts), and continuous variables are expressed as median (and interquartile range). Fisher’s exact test was used to compare frequencies, and the gene expression levels were compared with Mann Whitney’s test. Correlation studies were done with the Spearman’s method. The equal distribution of each gene in the different cell subsets was analyzed with the related-samples Friedman’s two-way ANOVA. A two-side p value <0.05 was considered significant. The SPSS 20.0 software was used in the analysis.

Results and Discussion

The contribution of each cell subset to the IFN signature

As expected, monocytes were the principal source of the ISG between these mononuclear cell subpopulations. In these cells, all the tested genes showed mRNA expression both in healthy individuals and in patients. With the exception of IP10, their levels were heavily enhanced in patients compared to controls (Figure 1). As can be observed in the figure, lupus patients showed also an up-regulation of ISG in lymphocytes, where the presence of some of these molecules was mostly restricted to the lupus cohort. In particular, RSAD2 was found in T cells from 60% and in B cells from 40% of lupus patients, but was absent in these subpopulations in the healthy subjects (p 0.002, p 0.034). In B cells, expression of IFIT1 could be found in 60% of patients and no controls (p 0.008), and expression of OAS3 in 60% of patients and 10% of healthy individuals (p 0.011). Although a larger sample would be needed in order to draw solid conclusions from these findings, it appears that the study of ISG in the lymphocyte subpopulations might provide ground for tailored therapeutic strategies in patients with lupus.

The message of the ISG up-regulation in our patients

As it has been described in earlier works, induction of ISG in monocytes was found to occur in a network-like fashion [13]. Figure 2 shows the tight associations that we observed between expression levels of these genes. In this network, only IP10 showed little correlation with the others. We then tried to find out whether the IFN signature could be approached with levels of the other 5 ISG in purified monocytes. In previous research, a higher IFN signature has been found in relationship with skin rashes, as well as with major organ involvement, including kidney disease [3,10,14,15]. We could find a consistent association
Table 1 Cohort characteristics.

|                                | Median (IQR) | n (%) |
|--------------------------------|--------------|-------|
| Number                         | 35, 33 women (94) |
| Age, years                     | 42 (18)      |
| Disease duration, years        | 9 (14)       |
| + anti DNA antibodies          | 30 (86)      |
| Renal disease                  | 23 (66)      |
| Major organ involvement        | 23 (66)      |
| Use of immunosuppressive drugs | 26 (74)      |
| SLICC damage index             | 1 (2)        |
| Active disease at the time of  | 21 (60)      |
| the study                      |              |
| SLEDAI 2K                      | 4 (7)        |
| BILAG 2004 (sum of items)      | 6 (13)       |
| Biologic activity              | 20 (57)      |
| Active lupus nephritis         | 9 (26)       |
| Low complement levels          | 23 (66)      |
| Lymphopenia                    | 14 (40)      |
| Plasmablasts (% total B cells) | 2.5 (7.04)   |
| Exanthema                      | 12 (34)      |

between these clinical traits and monocyte levels of the ISG (Table 3). In brief, HERC5, IFIT1, OAS3 and RSAD2 were higher in patients with concurrent skin disease (n=12), and the 6 patients with a BILAG A category, reflecting an active severe flare, depicted higher monocyte levels of MX1, HERC5, IFIT1, and OAS3 (Table 3). In addition, we could observe a strong association between IFIT1 expression levels in monocytes and active nephritis.

Interestingly, in our cohort the amount of plasmablasts could be taken as surrogate marker of the up-regulation of IFIT1, OAS3 and MX1 in monocytes, which is in agreement with the putative role of IFN-inducible molecules in promoting survival and differentiation of B lymphocytes [16]. On the other hand, no association was found between gene expression of any of the ISG in monocytes and titres of anti-DNA antibody or with complement levels, which are currently considered the most reliable markers of lupus activity. It appears, therefore, that the IFN signature, as approached here, could reflect a distinct pathway whose assessment in clinical practice would add information to classical disease biomarkers.

In contrast, it was interesting to find that the induction of the selected ISG in B lymphocytes in our patients with lupus was associated to complement decrease.

Better be alone than in bad company?

To be used in clinical practice, a biomarker should be easy to measure and carry a clear message. According to our results, an approximation to the IFN signature could be done with the gene expression levels of IFIT1 in isolated monocytes. The molecule showed a strong association with other ISG and with activity indexes, as well as with kidney disease.

Assuming the limitations of our small cohort-based study, our results indicate that the robust information provided by IFIT1 could be lost if analyzed together with other ISG. In this regard, MX1 is one of the ISG usually showing higher stimulation in patients with lupus, as we could also observe in our patients. However, at least in our study, MX1 levels did not differ between active and inactive lupus. Thus, the inclusion of MX1 in ISG scores might derive in a lack of association between the signature and disease activity, which is one of the drawbacks of this potentially useful biomarker [4].

In a similar way, our findings would not argue for the inclusion of IP10 in ISG scores. In fact, the expression of this molecule was not found globally enhanced in the mononuclear subsets of our patients. Nonetheless, IP10 could be increased in a distinct subgroup of patients, as is well supported by previous research.

Altogether, these findings suggest that up-regulation of the ISG do not render a single message. Positively, along with IFN-I there are many possible sources of the induction of ISG (summarized in Table 2). In particular, IP10 and RSAD2 can be triggered by IFNγ, a fact that could place these mediators in a different activation pathway.

Conclusion

With the best approach to measure the IFN signature in lupus still to be identified, we propose monocyte expression levels of IFIT1 as potential biomarker to be tested in clinical practice. Our results suggest that the addition to composite scores of other up-regulated ISG can mask some of the IFN-related traits.

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Table 2 Panel of the interferon inducible genes selected for the study.

| Name                                              | Abbreviation | Inductors                      | Functions                                                                                                                                  | antiviral activity                                                                 | Primer pairs                                                                 |
|---------------------------------------------------|--------------|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Interferon-induced GTP-binding myxovirus resistance protein 1 | Mx1, MxA, IFI-78K | IFN-I and IFN-III, HSV1        | predominantly binds negatively charged phospholipids; triggers ER stress-mediated death in influenza-infected cells                       | many RNA viruses some DNA viruses (HSV1)                                           | as: ggg tag cca ctg gag tga s: agg tgg agc gat tct gag |
| E3 protein ligase with interferon stimulated gene (ISG) 15 activity | HERC5        | IFN-beta, LPS, TNF-alpha, ILL-beta, viral infections | ISGylation of IRF3, increasing its activity. ISGylation of viral nascent peptides, such as influenza NS1, attenuating their virulence.           | influenza, papillomavirus                                                               | as: gtt tgg tgg ctg agc ttg tt s: tgc cac ctt cca cat gct at |
| Interferon-induced protein with tetracopeptide repeats (IFIT) 1 | IFIT1, IFI-56k | IFN-I dsRNA, many viruses      | binds ssRNA bearing a 5’-3P group                                                                                                        | inhibits ssRNA viruses                                                             | as: tgc ttg acc ctg aa s: ata ggc aga gat cgc ata ccc |
| 2’-5’-oligoadenylate synthase 3                    | OAS3         | dsRNA                          | ATPase activity rendering oligoadenylates, which bind to and are degraded by RNase L. Cell growth, apoptosis, differentiation             | >15 nucleotides long dsRNA viruses                                                                                                         | as: cgc ctg aca tcc gta gat ct s: cat tga agg cag gca cca g |
| S-adenosyl methionine domain-containing protein 2  | RSAD2        | IFN-I, IFN-II bacterial lipopolysaccharide | Alters lipid rafts, activates CD4 alpha/beta T cells, promotes Th2 cytokine production, positive regulator of TLR7 and TLR9                | many RNA and DNA viruses                                                             | as: cct gat ttt ctg ctg ccc gat s: cgt gtt ttc agt gag cac cac |
| Interferon-induced 10 kDa peptide                 | IP10, CXCL10 | IFN-I, IFN-II                  | can be secreted and binds to CXCR3, chemotactic for monocytes and T cells                                                                  |                                                                                  | as: aga atc gaa ggc cat caa ga s: cct ttc ctt gct aac tgc ttt c |
### Table 3: Association of the type I interferon-stimulated genes with disease manifestations.

| CD14 MX1 | Activity measures | Other clinical traits |
|----------|-------------------|-----------------------|
|          | plasmablast count (ρ=0.407, p=0.026, n=30) | active BILAG A (p=0.041) |

| CD14 HERC5 | Activity measures | Other clinical traits |
|------------|-------------------|-----------------------|
| SLEDAI (ρ=0.388, p=0.038, n=29) | active BILAG A (p=0.016) mucocutaneous flare (p=0.021) |

| CD19 HERC5 | Activity measures | Other clinical traits |
|------------|-------------------|-----------------------|
| mucocutaneous flare (p=0.041) |

| CD14 IFIT1 | Activity measures | Other clinical traits |
|------------|-------------------|-----------------------|
| SLEDAI (ρ=0.488, p=0.007, n=29) | urinary albumin/creatinine (ρ=0.479, p=0.009, n=29) active lupus nephritis (p=0.021) active BILAG A (p=0.007) mucocutaneous flare (p=0.024) |

| CD14 OAS3 | Activity measures | Other clinical traits |
|-----------|-------------------|-----------------------|
| SLEDAI (ρ=0.393, p=0.029, n=31) | active lupus nephritis (p=0.034) active BILAG A (p=0.007) mucocutaneous flare (p=0.043) |

| CD14 RSAD2 | Activity measures | Other clinical traits |
|------------|-------------------|-----------------------|
| mucocutaneous flare (p=0.041) |

| CD19 RSAD2 | Activity measures | Other clinical traits |
|------------|-------------------|-----------------------|
| lower C’4 levels (p=0.018) |

| CD14 IP10 | Activity measures | Other clinical traits |
|-----------|-------------------|-----------------------|
| lymphopenia (p=0.018) lower CD3 counts (ρ=-0.353, p=0.044, n=33) |

| CD3 IP10 | Activity measures | Other clinical traits |
|----------|-------------------|-----------------------|
| hypocomplementemia (p=0.052) | CRP levels (p=0.016) |

| CD19 IP10 | Activity measures | Other clinical traits |
|-----------|-------------------|-----------------------|
| lower C’4 levels (p=0.014) |
The equal distribution of each molecule in the different PBMC subpopulations was analyzed with the related-sample Friedman’s ANOVA test, and p values are shown under the gene names. The associations between pairs of molecules are shown as 4-grade heatmaps (upper-right half of the figure) as follows: blue - inverse association, grey - no association, orange - significant association with p value >0.01, red - association with p value <0.01. Values for r and p are given in the lower-left section.
Figure 2  Gene expression levels of the type I interferon inducible molecules in the 3 major circulating mononuclear cell subpopulations as studied with quantitative PCR. The graphs show plotted individual values and medians of each cohort. Results are shown as fold-increase from reference levels.
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