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Reverse interferon signature is characteristic of antigen-presenting cells in human and rat spondyloarthritis

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Abstract

Objective—In rats transgenic for HLA-B27 (B27 rat), the development of a disorder mimicking spondyloarthritis (SpA) is tightly correlated with DCs dysfunction. To dissect the underlying mechanisms, we studied the transcriptome of *ex vivo* purified CD103⁺CD4⁺ DCs.

Methods—Transcriptome analysis was performed on splenic CD103⁺CD4⁺ DCs from B27 and control rats. Transcriptional changes for selected genes were confirmed by quantitative reverse transcriptase–polymerase chain reaction. A meta-analysis was further performed, between the rat data and published gene expression analysis performed on macrophages from ankylosing spondylitis (AS) patients.

Results—IFN signaling was the most significantly affected pathway in B27 rat DCs, with a majority of genes connected to IFN being under-expressed, as compared to controls. This pattern was already present at disease onset, persisted over time, and was conserved in two disease-prone B27 rat lines. In B27 rat DCs, we further found an upregulation of suppressor of cytokine

signaling-3 that may account for reverse IFN signaling, and a down-regulation of IL-27, a cytokine that opposes Th17 differentiation and promotes regulatory T cells. The meta-analysis between rat conventional DCs and human monocyte-derived macrophages data revealed 7 IFN-regulated genes that were negatively regulated both in human and rat SpA: IRF1, STAT1, CXCL9, CXCL10, IFIT3, DDX60 and EPSTI1.

Conclusion—Our results suggest that expression of HLA-B27 leads to a defect in IFN- γ signaling in antigen-presenting cells, shared between B27 rats and SpA patients, which may result in Th17 expansion and regulatory T cell alteration, as shown in B27 rats, and contribute to disease pathogenesis.

Keywords

spondyloarthritis; interferon; dendritic cell; HLA-B27; IL-27

Spondyloarthritis comprises a spectrum of inflammatory disorders predominantly affecting spinal and sacroiliac joints, the prototypical form of which is AS. Other characteristic manifestations that may variably combine with each other include peripheral joint arthritis and enthesitis, and several extra-articular features, including psoriasis, uveitis and inflammatory bowel disease (1). The precise molecular and cellular basis underlying the strong association of SpA with the MHC class I antigen HLA-B27 remains still uncertain, forty years after its initial description, despite the ongoing efforts of many groups to explain it (2, 3).

Important advance in understanding this relationship was gained through studies conducted in HLA-B27/human β 2-microglobulin (h β 2m) transgenic rats (B27 rats). Several lines of B27 rats have proven to be suitable model for SpA, since they develop a spontaneous multisystem inflammatory disease closely resembling the human disease, including arthritis, colitis and psoriasiform skin lesions in rats with an intact gut microbiome (4, 5).

In this model, cell transfer experiments have shown that bone marrow-derived cells, thought to be antigen-presenting cells (APCs), expressing high levels of HLA-B27 were necessary and sufficient to induce SpA in healthy rats (6), and that disease expression required the intervention of CD4⁺ T cells (7). Interestingly, professional APCs such as dendritic cells (DCs), from disease-prone B27 rats exhibited several strikingly abnormal functions, including impaired capacity to stimulate T cell responses, altered cytoskeletal dynamics, reduced expression of MHC class II molecules and enhanced apoptotic death (4, 8–10). A strict correlation was established across several transgenic lines between altered DC function, high expression of HLA-B27/h β 2m transgene and disease susceptibility (11). Finally, aberrant function was observed in DCs from premorbid B27 rats and from rats expressing disease-associated HLA-B27/h β 2m transgenic locus on a genetic background protective against disease development, thereby demonstrating that this defect was not a consequence of disease and could be a principal factor in the spontaneous development of SpA, for example by altering the interaction of DCs with CD4⁺ T cells (4).

Consistent with the foregoing hypothesis, CD4⁺ T cells expressing a pro-inflammatory Th17 profile accumulate in B27 rats in parallel with disease development (12) and DCs from B27

rats were shown to induce biased expansion of Th17 cells which could play a pathogenic role in SpA (13). Moreover, we recently observed that regulatory T cell function was altered in B27 rats, as shown by a decreased interleukin (IL)-10/IL-17 production ratio, and that such imbalance favoring pro-inflammatory over anti-inflammatory cytokine production was driven by interaction of CD4⁺ T cells with DCs (14).

Altogether, these data provide a plausible link between aberrant DC function and the development of SpA in B27 rat. However, the molecular events resulting from HLA-B27 expression in DCs that lead to their functional impairment remains still incompletely understood.

In an attempt to more thoroughly investigate such issue, we performed transcriptomic analysis, comparing *ex vivo* purified CD103⁺CD4⁺ splenic DCs between B27 rats and control HLA-B7/hβ₂m transgenic (B7) and nontransgenic (NTG) rats. Next, we compared our results with those obtained on monocyte-derived macrophages from AS patients (15). Strikingly, we observed that several genes induced by interferon (IFN) were similarly down-regulated in disease-prone B27 rats and SpA patients, thereby highlighting a coordinate dysregulation of IFN pathway in APCs that might be critical for disease development.

MATERIALS AND METHODS

Animals

The HLA-B/hβ₂m transgenic rat lines used in this study were originally produced at University of Texas Southwestern Medical Center (Dallas, TX). The disease-prone rats of the HLA-B27/hβ₂m transgenic line 33-3, bearing 55 copies of HLA-B*2705 and 28 copies of hβ₂m, and the disease-free HLA-B7/hβ₂m transgenic line 120-4 rats, bearing 52 copies of HLA-B*0702 and 26 copies of hβ₂m were both on a F344 background (in this study, the number of hβ₂m transgene copies in 33-3 and 120-4 lines was re-evaluated using a more sensitive qPCR method than the previously reported dot-blot estimation (16)). The HLA-B27/hβ₂m transgenic line 21-3 and the hβ₂m transgenic line 283-2 were crossed to obtain the disease-prone (21-3 × 283-2) F1 rats, bearing 20 copies of HLA-B*2705 and 50 copies of hβ₂m, and disease-free 283-2 rats, bearing 35 copies of hβ₂m, both on a Lewis background. NTG F344 and Lewis littermates were used as controls. All rats were bred and housed under conventional conditions. Age- and sex-matched rats (2–12 months of age) were used in each experiment. All animal procedures were approved by the institutional Animal Experimentation Ethical Committee (CEB-26-2012).

CD103⁺CD4⁺ splenic DCs isolation

DCs were purified *ex vivo* by following methods derived from Josien et al. (17). Spleens were digested with 2 mg/ml collagenase D (Roche Diagnostics) for 20 min at 37°C in presence of EDTA at 10 mM during the last 5 min. The cell suspension was washed and resuspended in PBS/0.5 mM EDTA/1% BSA and low-density cells, containing most of the conventional CD103⁺ DC, were obtained after centrifugation on a 14.5% Nycodenz gradient (Nycomed).

For magnetic sorting, T and B cells were depleted by incubating low-density cells with anti-rat CD45RC (OX-22, Santa Cruz Biotechnology), anti-rat Ig κ chain (OX-12, Santa Cruz Biotechnology), anti-rat $\alpha\beta$ T-Cell Receptor (R73, BD Pharmingen) and anti-rat CD25 (OX-39, BD Pharmingen) mAbs at 4°C for 20 min. Negative selection of CD103⁺ cells was performed with goat anti-mouse IgG MicroBeads on CS columns following the manufacturer's instructions (Miltenyi Biotec). Cells were then incubated with anti-CD4 MicroBeads (Miltenyi Biotec) at 4°C for 20 min. Positive selection of CD103⁺CD4⁺ DC was performed on MS columns (Miltenyi Biotec). Purity was routinely around 70–80%.

For FACS-sorting, low-density cells were incubated with anti-CD103⁺ (OX-62) MicroBeads (Miltenyi Biotec) at 4°C for 20 min. Positive selection was performed using automated cell sorting (autoMACS Pro, Miltenyi Biotec). Cells were then stained with anti-rat CD103-FITC (OX-62, Santa Cruz Biotechnology), anti-rat CD4-APC (OX-35, BD Pharmingen) and anti-rat CD45RC-PE (OX-22, BD Pharmingen) mAbs, and CD103⁺CD4^{high} DC were sorted on a BD FACSaria III cell sorter (BD Biosciences) after excluding OX-22⁺ cells (including plasmacytoid dendritic cells). Purity was routinely 98%.

RNA isolation

For gene-expression studies, total RNA was isolated from DC pellets using the RNeasy kit (Qiagen), genomic DNA was eliminated by deoxyribonuclease treatment (RNase-Free DNase set; Qiagen) and samples were immediately stored at –80°C. The RNA quality was assessed using a Bioanalyzer 2100 (Agilent Technologies) and samples were quantified using a NanoDrop Spectrophotometer (Thermo Scientific NanoDrop) and their RNA Integrity Number (RIN) was 7.5–10.

Hybridization and microarray analysis

Microarray experiments were performed using an Affymetrix GeneChip®RatGenome 230.2.0 genome-wide array containing 31,100 probes. Total RNA from magnetically-sorted CD103⁺CD4⁺ DCs was reverse transcribed following the Affymetrix GeneChip® 3' IVT Express Kit. Briefly, the resulting double strand cDNA was used to synthesize biotin labeled cRNA. After purification, cRNA was fragmented and hybridized to chips. After overnight hybridization, chips were washed on the fluidic station FS450 following dedicated protocol and scanned using the GCS3000 7G (Affymetrix). The image was then analyzed with Expression Console software (Affymetrix) to obtain raw data (cel files), and metrics for Quality Controls. The microarray data accession number is E-MEXP-3808.

Gene expression levels were normalized using the GC-RMA algorithm and flags were computed using MAS5. Quality assessment of the chips has been performed with affyQCReport R package. Expression of genes was compared between B27 and control rats. For statistical analyses, *t*-test was used to calculate *P* values (significance threshold set at *P* 0.05). We used mean centered data to perform samples and genes classification by hierarchical clustering, using the Spearman correlation similarity measure and average/ward linkage algorithm. Heat maps source files were done by R software and pictures were produced by java treeview software.

For probes sets, annotation and examination of the gene networks, including canonical pathways, molecular functions, and genetic networks, the data were analyzed using IPA tools (<http://www.ingenuity.com>).

Metanalysis of human and rat micro-array data

A meta-analysis was performed, as previously described (18) between the rat data and published gene expression study performed on macrophages from 8 AS patients and 9 healthy control subjects (15). Human data extraction, normalization and analysis were performed with the same method and cut-off values as for the rat study.

We further merged human and rat expression data to perform an unsupervised clustering analysis, in order to unveil possible correspondences between samples from both species. For this, we identified differentially expressed genes common between human and rat chips (251 orthologous genes). The corresponding probes were then selected and grouped by gene using their mean to obtain one single intensity measure by gene and by sample. We merged the human and rat coded data sets and submitted it to a hierarchical clustering performed using the Spearman correlation similarity measure and average linkage in clustering algorithm.

RT-qPCR

The RT-qPCR experiments were conducted according to the recommendations of Minimum Information for Publication of Quantitative Real-Time PCR Experiments (19). Due to the large amount of data generated by our study, the detailed data obtained for each target gene are not shown but available on request.

RNA replicates of the same sample were reverse-transcribed using the SuperScript II Reverse Transcriptase (Invitrogen) with Random Primers (Invitrogen) and then pooled together to homogenize expression profiles. qPCR was performed using the LightCycler 480 SYBR Green I Master Kit (Roche) and the real time PCR system LC480 (Roche). Primers were designed using Primer3 tool and tested for specificity and efficiency (Supplementary Table 1). The thermal-cycling conditions were: 95°C for 5 min and then 45 cycles at 95°C (10 sec), 60°C (10 sec), and 72°C (15 sec). This was followed by the standard denaturation curve. Duplicates were run for each sample in a 96-well plate; Gapdh (encoding glyceraldehyde 3-phosphate dehydrogenase) and *Pgk1* (encoding phosphoglycerate kinase 1) were used together as endogenous reference genes according to published study (20). Each set of qPCR reactions included negative controls without RNA.

Data were analyzed using the Cq method. For each sample the mRNA abundance (Cq value) of the target genes was normalized to the reference genes and compared to a calibrator value, corresponding here to the average expression obtained with all samples (mean of all samples Cq values). The results were depicted as relative expression corresponding to the normalized relative quantity (NRQ).

Statistics

RT-qPCR data are expressed as mean $\pm$ s.e.m., and the significance of differences between two series of results was assessed using the unpaired, two-tailed Student's t-test. Values of $P < 0.05$ were considered significant.

RESULTS

Whole-transcriptome study of B27 rat CD103⁺CD4⁺ splenic DCs

We compared the transcriptome of CD103⁺CD4⁺ splenic DCs between B27 rats, and control B7 rats and NTG rats. We identified 422 probe sets differentially expressed between B27 rat and B7 rat DCs (164 upregulated and 258 downregulated in B27 rat) and 623 probe sets differentially expressed between B27 rat and NTG rat DCs (252 upregulated and 371 downregulated in B27 rat). Altogether, 255 probe sets corresponding to 172 annotated genes were differentially expressed in B27 rat DCs, as compared to both controls, with consistent direction of variation (69 upregulated and 103 downregulated genes in B27 rats) (Figure 1A). Using this list, clustering analysis readily separated B27 rats from both control groups (Figure 1B).

Global functional analysis conducted with Ingenuity Pathway Analysis (IPA) software indicated IFN signaling as the most significantly affected pathway (Supplementary Figure 1). Indeed, several of the most profoundly downregulated genes in B27 rat DCs were connected to IFN pathway, indicating a “reverse” (i.e., downregulated) IFN signature. For example, *Irf1*, *Irf7*, *Ifit2*, *Ifit3*, *Mx1*, *Ly6e*, *Cxcl9*, *Cxcl10* and *Cxcl11* genes known to be upregulated by IFN, were downregulated in DCs from B27 rats (Supplementary Table 2). Altogether, 36 (35%) of the genes downregulated in B27 rat were directly connected to IFN- γ pathway (including *Ifng* itself), and a large fraction of them to IFN- α as well (Supplementary Table 3). Surprisingly, the most upregulated genes in B27 rats were involved in antimicrobial peptides/innate immunity pathways such as genes of the defensin family associated to neutrophil function (*Defa*, *Np4*, *RatNP-3b*), mastocyte proteases (*Mcpt1*, *Mcpt8*, *Cpa3*), macrophage receptors (*Cd163*, *Camp*, *Pglyrp1*), or inflammatory pathway genes (*S100a8*, *S100a9*), (Supplementary Table 2), raising the possibility of a contamination of DC samples by other cells (see below).

We further measured on the same samples the expression of several differentially expressed genes by RT-qPCR: one group of genes downregulated in B27 rat which included principally genes linked to IFN pathway and IL-10, and two groups of upregulated genes corresponding to genes associated with innate/antimicrobial immunity or with endoplasmic reticulum (ER) stress (Supplementary Table 4). We confirmed significant differences between B27 DCs and control samples for most of the genes (Supplementary Figure 2).

Defective IFN signaling is consistent across ages in B27 rat DCs

To replicate and monitor variations of selected genes across ages, we measured their expression by RT-qPCR in CD103⁺CD4⁺ splenic DCs from B27 and control rats at ages of 2 months, 6 months, and 1 year. The reduced expression of several cytokines/chemokines and transcriptional factors associated with the IFN pathway (Figure 2) and up-expression of

ER-stress (Supplementary Figure 3A) and innate/antimicrobial immunity (Supplementary Figure 3B) genes was detected in DCs from B27 rats aged of 2 months (i.e. at disease onset) and persisted until the age of one year. Since no major variations in gene expression patterns were observed at different ages, we grouped together all time-points for statistical analysis. There was a statistically significant differential expression of most of the genes studied between B27 and control rats (Figure 2 and Supplementary Figure 3). Because such changes in DCs from B27 rats were already present at the time of disease onset and persisted throughout disease course, it is conceivable that at least some of them could contribute to the chronic inflammatory process in those animals. Some of the genes down-regulated in B27 DCs (i.e. *Cxcl10*, *Il10*, *Irf7* and *Stat1*; Figure 2) were significantly decreased in the B7 transgenic, as compared to the nontransgenic controls, albeit to a lesser extent than in the B27 rats.

Defective IFN signaling signature is shared with SpA patients

We have previously reported differences in the transcriptome of monocyte-derived macrophages between AS patients and healthy controls, finding a “reverse” IFN signature (15). Given the parallels between these datasets, we performed a meta-analysis of the human and rat transcriptome data.

The combined rat and human hierarchical clustering performed with 251 orthologous genes was able to separate two groups of samples: all the patients and B27 rats ($n = 16$) on one side and 19 of the 22 human and rat controls on the other (Figure 3A). The meta-analysis between rat and human data revealed 9 differentially expressed genes shared by AS patients and B27 rats, as compared to all controls (Figure 3B). The direction of variation was similar between patients and B27 rats for 7 of them, all indicative of a “reverse” IFN signature, and divergent for the 2 remaining (Table 1).

Defective IFN signaling is confirmed in FACS-sorted B27 rat DCs

Since a possible contamination of magnetically-sorted rat DC samples by other cell populations was a concern, as mentioned above, we repeated RT-qPCR experiments on FACS-sorted CD103⁺CD4⁺ splenic DCs with higher purity (> 98%). These data confirmed the significant down-expression of most of the genes connected to the IFN pathway in DCs from B27 rats, as compared to NTG rats (Figure 4A and B), including those shared between human and rat data, including *Cxcl9*, *Cxcl10*, *Ifit3*, *Irf1*, *Stat1*, and also of *Il10*. Upregulation of genes linked to ER-stress in B27 rat DCs was also confirmed (Figure 4C).

Considering the genes related to innate/antimicrobial immunity only low-level expression was observed both in B27 and NTG rats, confirming that their overexpression, as detected in magnetically-sorted B27 rat DCs, most likely reflected contaminant cell population(s) (Supplementary Figure 4).

It has recently been shown that engagement of IFN- γ pathway in DCs induces IL-27 while inhibiting osteopontin expression, leading to suppression of IL-17 production and induction of IL-10 from T cells (21). Interestingly, we found down-regulated *Il27* gene expression in highly purified CD103⁺CD4⁺ B27 rat DCs (Figure 4A). Additionally, we investigated the

expression of suppressor of cytokine signaling-1 and 3 (*Socs1* and *Socs3*) genes, known for their crucial role in limiting cytokine-mediated inflammatory responses (including IFN- γ related responses), in those cells. No difference was observed in *Socs1* expression between B27 and NTG rats. In contrast, *Socs3* was overexpressed in DCs from B27 rats, providing a possible underlying explanation for the down-regulated IFN pathway (Figure 4D).

Defective IFN signaling is common to SpA-prone B27 rats DC

To investigate the putative role of unfolded protein response and ER stress in SpA, rats of the 21-3 transgenic line that express moderate levels of HLA-B27/h β 2m and remain free of arthritis have been crossed with rats of the 283-2 line transgenic for h β 2m, both on Lewis background. This resulted in F1 offspring in which the males developed a high incidence of severe arthritis and spondylitis, despite evidence for higher HLA-B27 folding efficiency and reduced ER stress (22). This phenotype differs from that seen in the 33-3 transgenic line used above, in the following ways: the (21-3 $\times$ 283-2) F1 disease is male-specific, has more severe and more prevalent peripheral arthritis and much more prevalent spondylitis, and lacks any gastrointestinal inflammation.

We have previously shown that splenic DCs from (21-3 $\times$ 283-2) F1 males were as defective in their capacity to stimulate T cell proliferation as those from 33-3 rats (11). Here, we examined whether HLA-B27 expression would also be associated with defective IFN signaling in (21-3 $\times$ 283-2) F1 males. This was indeed the case: several genes of the IFN pathway (*Cxcl10*, *Ifit2*, *Ifit3*, *Irf7*) were significantly down-regulated in (21-3 $\times$ 283-2) F1 males, as compared to NTG and/or h β 2m transgenic rats from the 283-2 line (Figure 5A). Interestingly, among ER stress response genes, only *Pdia4* was moderately overexpressed in (21-3 $\times$ 283-2) F1, a result consistent with the previously established reduction of HLA-B27 misfolding in this line (Figure 5B). Finally, significant up-regulation of *Socs3* and down-regulation of *Il27* genes were evidenced in (21-3 $\times$ 283-2) F1, as in the 33-3 line (Figure 5A and C).

DISCUSSION

Several hypotheses have been proposed over the years to explain the striking association between SpA and HLA-B27. Investigations performed in B27 rat lines allowed us to address some of them. For example, the classical arthritogenic peptide hypothesis (23) was rendered unlikely, given that CD8⁺ T cells are not required for disease expression in the rats (7, 24, 25). Results of cell-transfer experiments led us to speculate that APCs expressing high levels of HLA-B27 were required for disease induction, by interacting with CD4⁺ T cells (6, 7). It has also been proposed that pro-inflammatory Th17 cells are key effectors, since these cells accumulate in lymphoid and target organs in parallel with disease development (12, 13, 26). Such interpretation is consistent with recent experiments demonstrating that DCs from B27 rats favor a biased expansion of Th17 cells (10, 13). Moreover, B27 rat DCs seem to affect regulatory T cell differentiation and activation in a way that they produce less IL-10 and more IL-17 (14). These results provide a plausible explanation as for why HLA-B27 may favor a Th17-driven disease, an interpretation consistent with other recent data in SpA (27).

However, the molecular mechanism by which B27 rat DCs may influence the differentiation of CD4⁺ T cells, leading to such pro-inflammatory bias remains to be understood. We previously reported that DCs from disease-prone rat lines exhibited altered functions (4). A consistent abnormality was their decreased capacity to support T cell stimulation, even in antigen-independent assays (8). This phenomenon was linked to impaired formation of an immunological synapse between B27 rat DCs and CD4⁺ T cells, which could itself be attributed to several molecular abnormalities, including a defective engagement of costimulatory molecules expressed on DCs with their T cell partners, altered cytoskeletal dynamics and reduced expression of MHC class II molecules (8). We have also found evidence that the expression of the HLA-B27 molecule on the DC surface was likely responsible for these molecular abnormalities (8). Similar defective T cell stimulation capacity has now been shown using monocyte-derived DCs from AS patients (28).

In the present study, we further investigated the DC dysregulation linked to HLA-B27 expression. We focused our study on *ex vivo* sorted CD103⁺CD4⁺ splenic DCs, given that this population has been implicated in T cell activation (29). Our transcriptomic findings revealed coordinate aberrant gene expression pattern in DCs from B27 rats of the 33-3 line, with striking evidence for a “reverse” IFN signature. This result was confirmed in several instances: using rats of various ages, starting at 2 months, i.e. at disease onset, then in highly purified DCs and finally in another HLA-B27 transgenic line, i.e. the (21-3 × 283-2) F1 rats, in which the phenotype closely mimics AS without gut inflammation. Moreover, we found that this B27 rat DC signature was shared with monocyte-derived macrophages from AS patients. This strengthened our observation and provided us with more evidence that the B27 rat is a faithful model of SpA.

We further observed an overexpression of genes involved in ER stress process in B27 rats of the 33-3 line, i.e. *Calr*, *Pdia3*, *Pdia4*, *Pdia6*. This corroborated our previous proteomic study on CD103⁺ splenic DCs from the same line of rats that showed enhanced expression of calreticulin and PDIA3 (9). It is consistent with cells having survived an unfolded protein response, such as initially described in bone marrow-derived macrophages from 33-3 rats (22). HLA-B27 misfolding and the ensuing unfolded protein response have been proposed as triggering events explaining HLA-B27 pathogenicity in B27 rat, by facilitating the transcription of IFN- β and/or IL-23p19 (22). However, such a mechanism would not directly account for the aberrant DC function studied herein, since we found no evidence for upregulation of those cytokines. Moreover, the evidence for ER stress was much weaker in DCs from the arthritic (21-3 × 283-2) F1 rats, consistent with previous experiments showing an attenuated unfolded protein response in this line, presumably as a consequence of enhanced h β 2m expression(22).

A group of innate immunity genes was also upregulated. However, when FACS was used instead of magnetic selection to improve the DCs purification, this signature disappeared, indicating that it was secondary to contamination of DCs samples by other cells. Similar signatures, attributed to tissue infiltration by neutrophils, macrophages and mast cells have been shown in human SpA studies, suggesting that it reflected the ongoing disease process, and could likely be attributed to Th17-mediated inflammation (30).

Thus, the down-regulated IFN pathway including the IFN- γ gene itself was the most relevant DC signature for SpA pathogenesis. This, combined with a decreased IL-10 production by B27 rat DCs, might contribute to drive the differentiation of CD4⁺ T cells into pathogenic Th17 cells, and might also affect regulatory T cells by decreasing their IL-10/IL-17 production ratio, thereby contributing to disease induction (31, 32). Hence, enhanced IL-17-mediated inflammation has been shown to result from IFN- γ signaling deficiency in DCs through reciprocal modulation of osteopontin and IL-27 (21). Thus, IFN- γ induces IL-27 and conversely inhibits osteopontin production, both effects contributing to suppress IL-17 production while inducing IL-10 from T cells. Consistent with such interpretation, we observed a decreased expression of *Il27* mRNA in B27 rat DCs. Moreover, increased osteopontin was reported in AS (33) and the *IL27A* gene has recently been shown as associated with AS susceptibility (34).

As another putative consequence of reverse IFN signature in DCs, B27 rat might have altered capacity to raise appropriate immune response to infectious agents (35). Indeed, their heightened vulnerability was previously shown to pathogenic *Listeria monocytogenes* (36). Such mechanism could also account for the dysregulated microbiota that has been documented in the 33-3 line. Although in this line it remains to be determined whether it is a cause or consequence of gut inflammation (37), recent data indicate that the (21-3 $\times$ 283-2) F1 rats, which show no gut inflammation, also harbor a microbiome that is distinctly different from nontransgenic rats (38) (J.D. Taurog et al., unpublished data).

We examined *Socs1* and *Socs3* expression levels, since both are essential inhibitors of Toll-like receptors (TLR) and cytokine receptors cascades, including IFN receptor-mediated signal transduction. Hence, STAT1 is hyperactivated and *Ifng* and IFN-regulated genes are upregulated in SOCS1-deficient DCs. Conversely, SOCS3-transduced DCs express low levels of IFN- γ (39). We found no differences in *Socs1* expression between DCs from B27 and control rats, but we did observe an overexpression of *Socs3* in B27 rats DCs, thereby providing a possible explanation for the reverse IFN signature. Consistently, our transcriptomic analysis exhibited a moderate but significant down-expression of several other genes associated with TLR and cytokine signaling (*Irak2*, *Mp4k4*, *Traf3*) in DCs from B27 rats (data not shown).

Whether there is causal link between HLA-B27 expression in DCs and *Socs3* induction remains to be established. SOCS are induced in response to TLR and cytokine signaling by a negative feedback loop that engages signaling by TAM receptor protein kinases (40). In addition to their canonical function of antigen presentation, MHC class I molecules can be signaling receptors themselves to mediate reverse signaling via association with other receptors or directly through aggregation, and exert nonclassical functions (41,42). Interestingly, MHC class I molecules expressed in APCs are involved *via* reverse signaling, in negative regulation of TLR-triggered inflammatory responses mediated by the signaling molecule TRAF6 (43). Thus, the singular biochemical properties of HLA-B27, including a tendency to form oligomers of heavy chains at the cell surface, as previously shown in B27 rat DCs (43), could theoretically affect MHC-dependent feedback and influence downstream events, resulting in heightened SOCS3 induction.

In summary, our findings indicate that expression of HLA-B27 in APCs may lead to a defect in IFN signaling, secondary to *Socs3* induction. Given the critical role of IFN pathway in control of both inflammatory and regulatory responses by APCs, we postulate that this deficiency can bear fundamental consequences and implications for SpA pathogenesis and treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. van Tubergen A, Weber U. Diagnosis and classification in spondyloarthritis: identifying a chameleon. *Nat Rev Rheumatol.* 2012; 8(5):253–61. [PubMed: 22450552]
2. Taurog JD. The role of HLA-B27 in spondyloarthritis. *J Rheumatol.* 2010; 37(12):2606–16. [PubMed: 21123333]
3. McHugh K, Bowness P. The link between HLA-B27 and SpA—new ideas on an old problem. *Rheumatology (Oxford).* 2012; 51(9):1529–39. [PubMed: 22513152]
4. Hacquard-Bouder C, Falgarone G, Bosquet A, Smaoui F, Monnet D, Ittah M, et al. Defective costimulatory function is a striking feature of antigen-presenting cells in an HLA-B27-transgenic rat model of spondylarthropathy. *Arthritis Rheum.* 2004; 50(5):1624–35. [PubMed: 15146433]
5. Breban M, Hacquard-Bouder C, Falgarone G. Animal models of HLA-B27-associated diseases. *Curr Mol Med.* 2004; 4(1):31–40. [PubMed: 15011957]
6. Breban M, Hammer RE, Richardson JA, Taurog JD. Transfer of the inflammatory disease of HLA-B27 transgenic rats by bone marrow engraftment. *J Exp Med.* 1993; 178(5):1607–16. [PubMed: 8228809]
7. Breban M, Fernandez-Sueiro JL, Richardson JA, Hadavand RR, Maika SD, Hammer RE, et al. T cells, but not thymic exposure to HLA-B27, are required for the inflammatory disease of HLA-B27 transgenic rats. *J Immunol.* 1996; 156(2):794–803. [PubMed: 8543835]
8. Hacquard-Bouder C, Chimenti MS, Giquel B, Donnadieu E, Fert I, Schmitt A, et al. Alteration of antigen-independent immunologic synapse formation between dendritic cells from HLA-B27-transgenic rats and CD4+ T cells: selective impairment of costimulatory molecule engagement by mature HLA-B27. *Arthritis Rheum.* 2007; 56(5):1478–89. [PubMed: 17469106]
9. Dhaenens M, Fert I, Glatigny S, Haerinck S, Poulain C, Donnadieu E, et al. Dendritic cells from spondylarthritis-prone HLA-B27-transgenic rats display altered cytoskeletal dynamics, class II major histocompatibility complex expression, and viability. *Arthritis Rheum.* 2009; 60(9):2622–32. [PubMed: 19714626]
10. Utriainen L, Firmin D, Wright P, Cerovic V, Breban M, McInnes I, et al. Expression of HLA-B27 causes loss of migratory dendritic cells in a rat model of spondylarthritis. *Arthritis Rheum.* 2012; 64(10):3199–209. [PubMed: 22674414]
11. Fert I, Glatigny S, Poulain C, Satumtira N, Dorris ML, Taurog JD, et al. Correlation between dendritic cell functional defect and spondylarthritis phenotypes in HLA-B27/HUMAN beta2-microglobulin-transgenic rat lines. *Arthritis Rheum.* 2008; 58(11):3425–9. [PubMed: 18975325]

12. DeLay ML, Turner MJ, Klenk EI, Smith JA, Sowders DP, Colbert RA. HLA-B27 misfolding and the unfolded protein response augment interleukin-23 production and are associated with Th17 activation in transgenic rats. *Arthritis Rheum.* 2009; 60(9):2633–43. [PubMed: 19714651]
13. Glatigny S, Fert I, Blaton MA, Lories RJ, Araujo LM, Chiocchia G, et al. Proinflammatory Th17 cells are expanded and induced by dendritic cells in spondylarthritis-prone HLA-B27-transgenic rats. *Arthritis Rheum.* 2012; 64(1):110–20. [PubMed: 21905004]
14. Krause L, Fert I, Labroquere K, Andrieu M, Chiocchia G, Breban MA. Regulatory T Cells in Spondyloarthritis (SpA) Animal Model and Modulatory Role of Inducible Costimulator (ICOS). *Arthritis and Rheumatism.* 2012; 64(10):S260–S1.
15. Smith JA, Barnes MD, Hong D, DeLay ML, Inman RD, Colbert RA. Gene expression analysis of macrophages derived from ankylosing spondylitis patients reveals interferon-gamma dysregulation. *Arthritis Rheum.* 2008; 58(6):1640–9. [PubMed: 18512784]
16. Taurog JD, Maika SD, Satumtira N, Dorris ML, McLean IL, Yanagisawa H, et al. Inflammatory disease in HLA-B27 transgenic rats. *Immunol Rev.* 1999; 169:209–23. [PubMed: 10450519]
17. Josien R, Heslan M, Soulillou JP, Cuturi MC. Rat spleen dendritic cells express natural killer cell receptor protein 1 (NKR-P1) and have cytotoxic activity to select targets via a Ca²⁺-dependent mechanism. *J Exp Med.* 1997; 186(3):467–72. [PubMed: 9236200]
18. Cros J, Cagnard N, Woollard K, Patey N, Zhang SY, Senechal B, et al. Human CD14^{dim} monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity.* 2010; 33(3):375–86. [PubMed: 20832340]
19. Bustin SA, Beaulieu JF, Huggett J, Jaggi R, Kibenge FS, Olsvik PA, et al. MIQE precis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. *BMC Mol Biol.* 2010; 11:74. [PubMed: 20858237]
20. Pohjanvirta R, Niittynen M, Linden J, Boutros PC, Moffat ID, Okey AB. Evaluation of various housekeeping genes for their applicability for normalization of mRNA expression in dioxin-treated rats. *Chem Biol Interact.* 2006; 160(2):134–49. [PubMed: 16466705]
21. Murugaiyan G, Mittal A, Weiner HL. Identification of an IL-27/osteopontin axis in dendritic cells and its modulation by IFN-gamma limits IL-17-mediated autoimmune inflammation. *Proc Natl Acad Sci U S A.* 2010; 107(25):11495–500. [PubMed: 20534530]
22. Colbert RA, DeLay ML, Klenk EI, Layh-Schmitt G. From HLA-B27 to spondyloarthritis: a journey through the ER. *Immunol Rev.* 2010; 233(1):181–202. [PubMed: 20193000]
23. Benjamin R, Parham P. Guilt by association: HLA-B27 and ankylosing spondylitis. *Immunol Today.* 1990; 11(4):137–42. [PubMed: 2187471]
24. May E, Dorris ML, Satumtira N, Iqbal I, Rehman MI, Lightfoot E, et al. CD8 alpha beta T cells are not essential to the pathogenesis of arthritis or colitis in HLA-B27 transgenic rats. *J Immunol.* 2003; 170(2):1099–105. [PubMed: 12517979]
25. Taurog JD, Dorris ML, Satumtira N, Tran TM, Sharma R, Dressel R, et al. Spondylarthritis in HLA-B27/human beta2-microglobulin-transgenic rats is not prevented by lack of CD8. *Arthritis Rheum.* 2009; 60(7):1977–84. [PubMed: 19565478]
26. van Duivenvoorde LM, Dorris ML, Satumtira N, van Tok MN, Redlich K, Tak PP, et al. Relationship between inflammation, bone destruction, and osteoproliferation in the HLA-B27/human beta2-microglobulin-transgenic rat model of spondylarthritis. *Arthritis Rheum.* 2012; 64(10):3210–9. [PubMed: 22736144]
27. Sherlock JP, Joyce-Shaikh B, Turner SP, Chao CC, Sathe M, Grein J, et al. IL-23 induces spondyloarthropathy by acting on ROR-gamma⁺ CD3⁺CD4⁺CD8[−] enthesal resident T cells. *Nat Med.* 2012; 18(7):1069–76. [PubMed: 22772566]
28. Bonilla N, Breban M, Chiocchia G. Heightened HLA molecules upregulation and decreased CD4⁺ T cells stimulation in monocytes-derived dendritic cells (DCs) from ankylosing spondylitis (AS) patients. *Arthritis Rheum.* 2009; 60(S1432)
29. Voisine C, Hubert FX, Trinite B, Heslan M, Josien R. Two phenotypically distinct subsets of spleen dendritic cells in rats exhibit different cytokine production and T cell stimulatory activity. *J Immunol.* 2002; 169(5):2284–91. [PubMed: 12193693]

30. Baeten D, Breban M, Lories R, Schett G, Sieper J. Are spondylarthritides related but distinct conditions or a single disease with a heterogeneous phenotype? *Arthritis Rheum.* 2013; 65(1):12–20. [PubMed: 23288559]
31. Huber S, Gagliani N, Esplugues E, O'Connor W Jr, Huber FJ, Chaudhry A, et al. Th17 cells express interleukin-10 receptor and are controlled by Foxp3(–) and Foxp3+ regulatory CD4+ T cells in an interleukin-10-dependent manner. *Immunity.* 2011; 34(4):554–65. [PubMed: 21511184]
32. Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, Heinrich JM, et al. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity.* 2011; 34(4):566–78. [PubMed: 21511185]
33. Choi ST, Kim JH, Kang EJ, Lee SW, Park MC, Park YB, et al. Osteopontin might be involved in bone remodelling rather than in inflammation in ankylosing spondylitis. *Rheumatology (Oxford).* 2008; 47(12):1775–9. [PubMed: 18854347]
34. Cortes A, Hadler J, Pointon JP, Robinson PC, Karaderi T, Leo P, et al. Multiple novel loci harboring common and rare variants implicated in ankylosing spondylitis. *Nature Genet.* 2013; 45(7):730–8. [PubMed: 23749187]
35. MacMicking JD. Interferon-inducible effector mechanisms in cell-autonomous immunity. *Nat Rev Immunol.* 2012; 12(5):367–82. [PubMed: 22531325]
36. Warner TF, Madsen J, Starling J, Wagner RD, Taurog JD, Balish E. Human HLA-B27 gene enhances susceptibility of rats to oral infection by *Listeria monocytogenes*. *Am J Pathol.* 1996; 149(5):1737–43. [PubMed: 8909262]
37. Onderdonk AB, Richardson JA, Hammer RE, Taurog JD. Correlation of cecal microflora of HLA-B27 transgenic rats with inflammatory bowel disease. *Infect Immun.* 1998; 66(12):6022–3. [PubMed: 9826389]
38. Bach MHVGR, Taurog JD, Rosenbaum JT. The presence of HLA-B27 shapes gut microbiome composition in rats. *Arthritis Rheum.* 2012; 64(S1052–3)
39. Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. *Nat Rev Immunol.* 2007; 7(6):454–65. [PubMed: 17525754]
40. Lemke G, Rothlin CV. Immunobiology of the TAM receptors. *Nat Rev Immunol.* 2008; 8(5):327–36. [PubMed: 18421305]
41. Gur H, el-Zaatari F, Geppert TD, Wacholtz MC, Taurog JD, Lipsky PE. Analysis of T cell signaling by class I MHC molecules: the cytoplasmic domain is not required for signal transduction. *J Exp Med.* 1990; 172(4):1267–70. [PubMed: 2212953]
42. Jin YP, Korin Y, Zhang X, Jindra PT, Rozengurt E, Reed EF. RNA interference elucidates the role of focal adhesion kinase in HLA class I-mediated focal adhesion complex formation and proliferation in human endothelial cells. *J Immunol.* 2007; 178(12):7911–22. [PubMed: 17548629]
43. Xu S, Liu X, Bao Y, Zhu X, Han C, Zhang P, et al. Constitutive MHC class I molecules negatively regulate TLR-triggered inflammatory responses via the Fps-SHP-2 pathway. *Nat Immunol.* 2012; 13(6):551–9. [PubMed: 22522491]
44. Kollnberger S, Bird LA, Roddis M, Hacquard-Bouder C, Kubagawa H, Bodmer HC, et al. HLA-B27 heavy chain homodimers are expressed in HLA-B27 transgenic rodent models of spondyloarthritis and are ligands for paired Ig-like receptors. *J Immunol.* 2004; 173(3):1699–710. [PubMed: 15265899]

Figure 1. B27 rat CD103+CD4+ splenic DCs whole-transcriptome study

Gene array analysis was performed on pools (2 rats/pool) of 6–10 months old male B27 rats (8 pools) that were compared to two age- and sex-matched control groups: B7 rats (6 pools) and NTG rats (7 pools). **A**, The Venn diagram represents the overlap between two comparisons of lists: B27 vs. NTG rats and B27 vs. B7 rats. Differentially expressed probe sets are distinguished according to their sense of variation in B27 rats, as compared to controls. Only two of 257 overlapping probe sets presented opposite directions of variation in both comparisons. **B**, Hierarchical clustering was performed with 255 overlapping probe sets corresponding to 172 annotated genes, exhibiting consistent variations between B27 rats

and both control groups. The blue-coded bar on the right side corresponds to transcripts downregulated and the magenta bar to transcripts upregulated in B27 rats.

Figure 2. Monitoring of IFN-signaling and *Il10* genes expression in B27 rat DCs across ages, using RT-qPCR

Gene expression of **A**, cytokines/chemokines and **B**, transcriptional factors, related to the IFN pathway was quantified on *ex-vivo* magnetically-sorted CD103⁺CD4⁺ splenic DCs from B27 rats and control NTG and B7 rats at indicated ages. PCR data were normalized to those of *Gapdh* and *Pgk1*. Data are mean $\pm$ s.e.m. Values of relative expression were calculated from 5–6 pools (2 rats/pool) per condition. Statistical analysis was performed using unpaired, two-tailed Student's t-test.

Figure 3. Combined gene expression profiling and meta-analysis of human and rat transcriptome data

A, A combined rat and human hierarchical clustering was performed using 251 orthologous genes, allowing to strikingly separate all the 16 patients and B27 rats on one side (red), from 19 of 22 human and rat controls on the other side (black). **B**, The Venn diagram represents the genes differentially expressed in DCs from B27 rats, as compared to NTG rats (blue-left), in DCs from B27 rats, as compared to B7 rats (violet-right), and in macrophages from AS patients, as compared to human healthy controls (red-middle).

Figure 4. Expression of selected-genes in FACS-sorted rat DCs using RT-qPCR

Gene expression of **A**, cytokines/chemokines and **B**, transcriptional factors connected to the IFN pathway and IL-10, **C**, ER stress response and **D**, suppressors of cytokine signaling, were quantified on FACS-sorted CD103+CD4+ splenic DCs from B27 rats and NTG control rats. qPCR data were normalized to those of *Gapdh* and *Pgk1*. Horizontal bars indicate the mean calculated from 5–6 pools (2 rats/pool). Statistical analysis was performed using unpaired, two-tailed Student's t-test.

Figure 5. Expression of selected genes in FACS-sorted DCs from (21-3 × 283-2) F1 B27 rat line RT-qPCR was used to quantify the expression of genes connected to **A**, IFN pathway and IL-10, **B**, ER stress response and **C**, cytokine suppression, on FACS-sorted CD103+CD4+ splenic DCs from 6–10 months old arthritic HLA-B27/hβ2m transgenic male rats of the (21-3 × 283-2) F1 line. Controls were hβ2m single-transgenic rats of the 283-2 line and NTG Lewis rats. qPCR data were normalized to those of *Gapdh* and *Pgk1*. Data are mean ± s.e.m. Values of relative expression are shown and were calculated from 3–4 rats per group. Statistical analysis was performed using unpaired, two-tailed Student's t-test

Table 1

Dysregulated genes common to DCs from B27 rats and macrophages from AS patients

Gene	Human		Rat				Gene product
	Patients vs. Controls		HLA-B27 vs. NTG		HLA-B27 vs. HLA-B7		
	P-value	Ratio	P-value	Ratio	P-value	Ratio	
<i>Cxcl9</i>	8,39E-03	0,28	9,58E-07	0,22	3,08E-06	0,24	chemokine (C-X-C motif) ligand 9
<i>Cxcl10</i>	7,61E-03	0,20	1,42E-06	0,16	5,65E-07	0,22	chemokine (C-X-C motif) ligand 10
<i>Ifit3</i>	6,34E-03	0,64	4,20E-05	0,10	8,59E-03	0,21	IFN-induced protein with tetratricopeptide repeats 3
<i>Irf1</i>	6,76E-03	0,32	2,62E-06	0,59	2,23E-05	0,62	IFN regulatory factor 1
<i>Stat1</i>	5,41E-03	0,46	1,57E-04	0,53	5,39E-05	0,67	signal transducer and activator of transcription 1
<i>Ddx60</i>	4,20E-02	0,56	2,37E-05	0,43	3,93E-05	0,35	DEAD box polypeptide 60
<i>Epsil</i>	1,95E-03	0,30	4,43E-03	0,61	7,37E-03	0,62	epithelial stromal interaction 1
<i>Tpds211</i>	2,94E-02	1,89	1,17E-05	0,10	1,14E-02	0,27	tumor protein D52-like 1
<i>Sell</i>	3,88E-02	0,50	2,15E-02	1,80	1,22E-02	1,97	L-selectin