Dendritic cells activated by double-stranded RNA induce arthritis via autocrine type I IFN signaling

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RECEIVED JUNE 10, 2013; REVISED NOVEMBER 4, 2013; ACCEPTED NOVEMBER 19, 2013. DOI: 10.1189/jlb.0613320

ABSTRACT
Viral dsRNA can be found at the site of inflammation in RA patients, and intra-articular injection of dsRNA induces arthritis by activating type I IFN signaling in mice. Further, DCs, a major source of IFN-α, can be found in the synovium of RA patients. We therefore determined the occurrence of DCs in dsRNA-induced arthritis and their ability to induce arthritis. Here, we show, by immunohistochemistry, that cells expressing the pan-DC marker CD11c and the pDC marker PDCA-1 are present in the inflamed synovium in dsRNA-induced arthritis. Flt3L-generated and splenic DCs preactivated with dsRNA before intra-articular injection, but not mock-stimulated cells, clearly induced arthritis. Induction of arthritis was dependent on type I IFN signaling in the donor DCs, whereas IFNAR expression in the recipient was not required. Sorting of the Flt3L-DC population into cDCs (CD11c+PDCA-1−) and pDCs (CD11c−PDCA-1+) revealed that both subtypes were arthritogenic and produced type I IFN if treated with dsRNA. Taken together, these results demonstrate that viral nucleic acids can elicit arthritis by activating type I IFN signaling in DCs. Once triggered, autocrine type I IFN signaling in dsRNA-activated DCs is sufficient to propagate arthritis.

J. Leukoc. Biol. 95: 000–000; 2014.

Introduction
RA is characterized by chronic joint inflammation. Both transient and chronic arthralgias and precipitations of flares in RA may ensue viral infections [1]. Also, microbial products, such as bacterial DNA and peptidoglycans, are commonly found in the inflamed joints of RA patients [2]. Thus, a role of antimicrobial immunity in arthritis may be anticipated. dsRNA is expressed during the lifecycle of most viruses [3] and is a strong activator of innate immunity, including type I IFN [4]. RA patients, compared with osteoarthritis patients, have much higher levels of dsRNA in their sera and synovial fluid, which is indicative of ongoing subclinical viral infection [5], and the presence of synovial dsRNA in RA patients clearly correlates to an erosive disease course [5]. Experimentally, intra-articular injection of such dsRNA is highly arthritogenic by activating type I IFN signaling [6]. Thus, one way by which viral constituents in the joint may contribute to inflammatory manifestations is by induction of IFN-α production.

Most cells can produce small amounts of IFN-α upon viral infection. However, DCs, pDCs, and cDCs can produce vast amounts of IFN-α in response to various stimuli, including microbial nucleic acids [4]. DCs express an array of receptors, e.g., the TLRs, nucleotide-binding domain, leucine-rich repeat-containing protein receptors [7], and retinoic acid-inducible gene I protein-like receptors [8]. These receptors recognize foreign and endogenous molecules, indicative of infection and/or tissue damage. DCs activated by such ligands can create local inflammation, preventing spread of infection, but also cause tissue damage that can become pathological if the activation does not cease [9].

In RA patients, compared with healthy controls, DCs are depleted from the bloodstream and enriched in the inflamed joint [10], but their actual role in joint inflammation remains elusive.

Here, we confirmed that in experimental dsRNA-induced arthritis, the inflamed joint is also populated by DCs, and we show for the first time that dsRNA-stimulated DCs induce arthritis dependent on autocrine-type I IFN signaling. We further show that pDCs and cDCs harbor arthritic potential if activated by dsRNA. Thus, DCs activated by viral
dsRNA may be one way that viral infections may contribute to arthritis.

**MATERIALS AND METHODS**

**Animals**

IFNAR KO (A129) and WT (129) mice were a gift from Maries van den Broek (Zürich University, Switzerland) [11]. Animals were kept at the animal facility of Göteborg University (Sweden). Experiments were performed according to the Swedish Animal Welfare Act and approved by the University Ethical Committee.

**Immunohistochemical examination of arthritic joints**

Joint inflammation was induced by intra-articular injection in female, 5- to 7-week-old mice of 5 µg viral dsRNA (or PBS as a control), mixed with the in vivo transfection agent jetPEI, as described in ref. [6]. Three days later, joints were prepared, as described in ref. [12], and synovial expression of CD11c, MHC class II, and 120G8 (a kind gift from Schering-Plough, Kenilworth, NJ, USA) was determined by immunohistochemistry, essentially as described in ref. [13] and detailed in the figure legends.

**Generation of DCs**

Erythrocyte-free bone marrow was isolated, and Flt3L-DCs were prepared, as described earlier [14]. In brief, cells were cultured with 25 ng/ml murine Flt3L (R&D Systems, Minneapolis, MN, USA) at 1 × 10^6/ml in a total volume of 4 ml and incubated at 37°C in 5% CO₂, 95% humidity. One-half of the medium volume (2 ml) was changed on Days 3 and 6, and cells were harvested on Day 9.

**FACS analysis and sorting**

Flt3L-DCs and single-cell suspension of spleen cells were harvested, washed, and analyzed by FACS or sorted into CD11c⁺, PDCA-1⁻ (cDC) and CD11c⁺, PDCA-1⁺ (pDC) using anti-CD11c[FITC] (HL3; Becton Dickinson, San Diego, CA, USA) and biotinylated PDCA-1 (Miltenyi Biotech, Germany) plus streptavidin-allophycocyanin. The 120G8 antibody used in immunohistochemistry to detect pDCs was not used in FACS on cultured Flt3L-DCs, as expression of the 120G8 antigen decreases with time [15]. Cells were sorted on FACSaria (Becton Dickinson). Collected data were analyzed using FlowJo software (Tree Star, Ashland, OR, USA).

**DC activation**

DCs (1×10^5 in 200 µl cultures) were stimulated with medium alone, dsRNA (poly I:C; Sigma-Aldrich, St. Louis, MO, USA), 150 µg/ml or 10 µg/ml dsRNA from Rota virus [16], or UV-inactivated HSV (4×10^5 PFU/ml; gift from Kristina Eriksson, Göteborg University) at 37°C and 5% CO₂, 95% humidity, for 24 h. IFN-α in supernatants were analyzed with ELISA as described [6]. To activate DCs before intra-articular injection, cells were stimulated as above for 4 h. After wash three times in PBS, 80,000 cells were injected intra-articularly in 20 µl PBS.

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**Figure 1. DCs accumulate in dsRNA-induced arthritis.** dsRNA (5 µg) was injected intra-articularly in the left knee in the presence of jetPEI, as described earlier [6]. Three days postinjection, the entire knee joint was removed, decalcified, embedded in Tissue-Tek, cryosectioned, and stained with rat anti-mouse CD11c (A), MHC class II (B), pDC marker 120G8 (C), and isotype control (D). Positive cells were detected with alkaline phosphatase-conjugated rabbit anti-rat IgG (Dako, Stockholm, Sweden) and developed with Liquid Permanent Red (Dako), according to the manufacturer’s instructions, followed by hematoxylin counterstaining. Representative images from one of four dsRNA-treated mice are shown. Joints from mice injected intra-articularly with vehicle only are depicted in Supplemental Fig. 1. ST, Synovial tissue; JC, joint cavity. Arrows indicate infiltration of inflammatory cells. Original magnification, 10×.
Injection protocol and arthritis evaluation

Flt3L-DCs, spleen, and Flt3L-DCs, sorted into CD11c+, PDCA-1+ (pDC), and CD11c+ PDCA-1− (cDC), were isolated and activated as described above and injected intra-articularly in a volume of 20 µl PBS in the knee. Three days after injections, knee joints were evaluated for joint inflammation on an arbitrary scale from zero through three, as depicted (see Fig. 2E and ref. [12] described earlier). The contralateral knee joints were injected with vehicle alone, and this never resulted in arthritis symptoms (data not shown).

Statistical analysis

Differences in arthritis frequency were evaluated with Fischer’s exact test. Differences in arthritis severity and differences in IFN-α production be-
RESULTS AND DISCUSSION

In many chronic inflammatory diseases, including SLE [17], Sjögrens [18], psoriasis [19], and RA [10, 20], DCs have migrated from the circulation to the site of inflammation. Here, we show that this is also valid in dsRNA-induced arthritis (Fig. 1). In this model, type I IFN signaling, activated by intra-articular deposition of viral RNA or its mimic dsRNA, induces a transient joint inflammation mediated by innate immunity [6, 16]. As shown in Fig. 1 and in Supplemental Fig. 1, the arthritic joint contains cells expressing the pan-DC marker CD11c (Fig. 1A) and the MHC class II antigen (Fig. 1B), two markers expressed by all DCs. In joints isolated from vehicle-treated mice, we could not detect DCs (Supplemental Fig. 1). The principal circulating DCs are monocyte-derived DCs (cDCs) that express CD11c and pDCs that apart from CD11c, also express 120G8 [21] and PDCA-1 [22], which is not present on cDCs. Further analysis of cells in the inflamed joint revealed that cells expressing the pDC-specific antigen 120G8 were also present in the arthritic synovium (Fig. 1C). Likewise, the corresponding human cDC and pDC are found in the inflamed synovium in RA patients [10], but their contribution to joint inflammation is unknown.

To determine if DCs present in the joint are sufficient to activate joint inflammation, we used bone marrow-derived Flt3L-DCs (phenotyped as described in Fig. 2). DCs were injected intra-articularly, and arthritis was evaluated 3 days later. Arthritis was defined as an inflammatory score of one or more (see Fig. 2E), as described in Materials and Methods. By adoptive transfer of Flt3L-DCs (Fig. 2C and D), we show that the mere presence of DCs in the joint is not sufficient to induce arthritis. A short (4-h) preactivation with dsRNA, however, turned Flt3L-DCs into potent activators of arthritis (Fig. 2C and D). Likewise, DCs loaded with necrotic cells, but not DCs alone, induce glomerulonephritis in a model of lupus-like nephritis [23]. An important proinflammatory constituent of necrotic cells is certain RNA structures that are thought to activate DCs continuously in SLE [24]. Thus, such nucleic acids or dsRNAs may trigger DCs, and if activation persists, this may result in a continuous attack on self-tissues [25, 26]. In RA patients, albeit without overt clinical viral infection, dsRNA is enriched in the synovia and in serum [5], and the presence of such dsRNA in synovia correlates to an erosive disease course [5]. Our finding that dsRNA-activated DCs induce arthritis (Fig. 2) therefore suggests that DCs found in the joint of RA patients [10, 20], concomitant with the synovial presence of dsRNA [5], could be a pathogenic force in RA.

The ability of dsRNAs to induce arthritis is critically dependent on IFN-α [6]. In arthritis patients, production of IFN-α by DCs populating the synovia may contribute to synovial inflammation, both in juvenile idiopathic arthritis [27] and in adult RA [28]. Here, this notion is proven experimentally, where we show that IFN-α-producing DCs are highly arthritogenic, whereas DCs with impaired IFN-α production are not (Fig. 3). Arthritis induced by adoptive transfer of dsRNA-treated DCs to a healthy joint was clearly dependent on intact type I IFN signaling in the transferred DCs, illustrated by the inability of dsRNA-stimulated Flt3L-DCs from IFNAR KO mice to induce arthritis (Fig. 3A). The number and the analyzed phenotype (i.e., expression of CD11c and PDCA-1) of DCs

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generated from IFNAR KO mice were similar to that of WT (data not shown). In contrast, DCs generated from IFNAR KO mice could not produce detectable levels of IFN-α upon stimulation with dsRNA (Fig. 3C). Their inability to release IFN-α is in line with our earlier published data using spleen cells [6] and is likely a result of the lack of positive-feedback signaling in vitro [29], which contrasts with virally induced production of type I IFN in vivo [30].

The inability to produce and signal via type I IFN is likely the reason why DCs from IFNAR KO mice are not arthrito-

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**Figure 4. cDC and pDC are arthritogenic if pretreated with dsRNA.**

Flt3L-DCs (A) and freshly isolated splenocytes (B) were sorted into CD11c<sup>+</sup>, PDCA-1<sup>+</sup> (cDC) and CD11c<sup>+</sup>, PDCA-1<sup>-</sup> (pDC) and cultured for 4 h with dsRNA (poly I:C 150 μg/ml) before intra-articular injection, as described in Fig. 2. Three days postinjection, the arthritic score was determined. Mean severity (arthritic score zero through three) of arthritis is depicted in A for sorted Flt3L-DCs (n=6) and in B, for sorted splenocytes (n=4). (A) The non-DC population (double-negative) that did not induce arthritis is included; *P < 0.05 (Mann-Whitney); **P < 0.01. (C) Twenty-four-hour culture supernatants from sorted cells were analyzed for the presence of IFN-α by ELISA.
genic upon dsRNA stimulation. However, once type I IFN signaling has been triggered in the transferred cells, other pathways are critically involved. As depicted in Fig. 3B, dsRNA-activated WT DCs could still induce arthritis in IFNAR KO mice, despite lack of functional type I IFN signaling in the host (Fig. 3). It is therefore conceivable that the transferred, dsRNA-stimulated WT cells are activated further by autocrine IFN-α signaling, and this is sufficient to propagate arthritis.

Autocrine type I IFN signaling is an important maturation and activation factor for DCs, enabling expression of costimulatory molecules and adhesion molecules that facilitate recruitment of other cells [31]. Earlier studies have shown that DC cultures, using IFNAR-deficient bone marrow starter cultures, result in DCs with decreased expression of costimulatory molecules CD40, CD80, and CD86 [31] and DCs with less migratory capacity [32], which could impair the ability of DCs to propagate inflammation. Thus, apart from the direct arthritogenic effects of IFN-α [6], produced from dsRNA-treated DCs, autocrine type I IFN-mediated maturation in WT DCs could contribute to arthritis development and explain further why dsRNA-treated IFNAR KO DCs did not induce arthritis and why type I IFN signaling in donor cells was sufficient to propagate arthritis.

The arthritogenic IFN-α-producing bone marrow-derived Flt3L-DC contains cDC and pDC (cDC expressing CD11c but not PDCA-1 and pDC expressing both CD11c and PDCA-1 and a fraction of CD11c-negative non-DC cells; Fig. 2). As shown earlier by Hornung et al. [33], cDCs and pDCs release IFN-α in response to dsRNA, and both types of DCs also populated the synovia in dsRNA-induced arthritis (Fig. 1). Thus, both types of CD11c+ DCs may therefore contribute to arthritis by production of IFN-α. To resolve this, Flt3L-DCs, sorted into cDC (CD11c+, PDCA-1−) and pDC (CD11c+, PDCA-1+), were stimulated 4 h with dsRNA before intra-articular injection. Both types of dsRNA-stimulated DCs readily induced arthritis, as depicted in Fig. 4A, and produced IFN-α (Fig. 4C). In contrast, a sorted, double-negative population (non-DCs) did not induce arthritis, despite dsRNA stimulation (Fig. 4A). We also showed that CD11c+, PDCA-1− and CD11c+, PDCA-1+ cells sorted from spleen (Fig. 4B) induced arthritis if pretreated with dsRNA, also confirming that freshly isolated cell populations comprising cDCs and pDCs can induce arthritis. Taken together, the cDC and pDC populating the synovia in dsRNA-induced arthritis may both contribute to inflammation by secreting IFN-α.

In conclusion, this paper describes how DCs activated by viral nucleic acids may provoke joint inflammation by activating type I IFN signaling, which in an autocrine manner, thereafter, establishes arthritis.

AUTHORSHIP
S.C.N. contributed to the design of the experiment, performed animal and laboratory work, and took part in writing the manuscript. J.P.C. and N.H. contributed to the design of the experiment and performed animal and laboratory work. M.M. conceived of the idea, designed the experiments, and finalized the manuscript text.


**KEY WORDS:**

viral nucleic acids · cytokines · joint inflammation