# Plasmacytoid dendritic cells in cutaneous sarcoidosis

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**Abstract.** While absent from normal skin, plasmacytoid dendritic cells (pDCs) infiltrate the skin in several infectious, inflammatory, and neoplastic entities. In addition to providing anti-viral resistance, pDCs link the innate and adaptive immune responses. Sarcoidosis is an idiopathic multi-system granulomatous disease characterized by epitheliod granulomas. Its underlying immunopathogenesis involves hyperactivity of cell-mediated immune system with involvement of CD4+ T-helper cells of the Th1 subtype. Recently, pDCs have been shown to contribute to other cutaneous granulomatous disorders such as granuloma annulare (GA). Here, we intend to investigate pDC occurrence and activity in cutaneous sarcoidosis. Twenty cutaneous sarcoidosis cases and a comparable group of 20 cases of GA were retrieved from our database and were immunohistochemically tested for pDC occurrence and activity using anti-BDCA-2 and anti-MxA antibodies, respectively. Fifteen cases of cutaneous lupus erythrematosus (LE) were used as a comparison group. A semi-quantitative scoring system was used. pDCs were present in all cutaneous sarcoidosis in peri-vascular and/or peri-adnexal location admixed with lymphocytes. pDC numbers in sarcoidosis were comparable to those in GA, while pDCs were significantly more abundant in LE. MxA expression was mostly patchy in cutaneous sarcoidosis and GA cases, while LE cases showed diffuse and strong MxA expression. In conclusion, we have shown that pDCs are recruited into the skin lesions of sarcoidosis and GA. Despite the diminished type I IFN production demonstrated in our study, the consistent presence of pDCs in all cutaneous sarcoidosis cases speaks in favor of some role of these cells in the pathogenesis of granulomatous disorders.  *(Sarcoidosis Vasc Diffuse Lung Dis 2018; 35: 55-61)*

## Introduction

Several studies have recently focused on plasmacytoid dendritic cells (pDC), which are a specialized DC population (1, 2). They morphologically resemble plasma cells and express CD4, CD123, HLA-DR, blood dendritic cell antigen 2 (BDCA-2), and Toll-like receptor (TLR)7 and TLR9 within endosomal compartments. They have the ability to secrete copious amounts of type I interferons (IFNs) in response to pathogens and/or danger signals (2). In addition to anti-viral function, pDCs mediate the function of myeloid DCs, lymphocytes, and natural killer cells thus linking innate and adaptive immune responses. This is mediated by the different type I IFNs released upon pDCs activation and, to a lesser extent, the production of other pro-inflammatory cytokines such as tumor necrosis factor (TNF)-alpha and interleukin-6 (IL6). While absent from normal skin, they have been shown to participate in the pathogenesis of several cutaneous infectious (especially viral

infections such as herpetic and poxvirus infections, etc), neoplastic (melanoma, squamous cell carcinoma, lymphomas, etc), and inflammatory/autoimmune processes (1, 2). Among the latter group, pDCs and their product, type I IFNs, have been shown to play a central role in lupus erythrematosus (LE) (2).

Sarcoidosis is an idiopathic multi-system granulomatous disease that is characterized by the development of non-caseating epitheliod granulomas (3). It most commonly affects the lungs (90%), lymph nodes (90%), eyes (40%), and skin (25%). Cutaneous manifestations of sarcoidosis include specific (macules, papules, plaques, nodules, lupus pernio, and infiltration of pre-existing scars) and nonspecific lesions (such as erythema nodosum) (3). Underlying immunopathogenesis of sarcoidosis involves hyperactivity of cell-mediated immune system. Following presentation of as yet unknown antigens by monocytes bearing MHC class II molecules, CD4+ T-helper cells of the Th1 subtype become upregulated with increased production of Th1 cytokines such as IL-2 and IFN-γ leading to epithelioid granuloma formation in various tissues including skin (4). Activated T-helper cells also produce monocyte chemotactic factor which attracts circulating monocytes into peripheral tissues. Recent observations have also suggested an important role played by DCs as immunologic mediators of sarcoidosis (5). Actually, pDCs have recently been shown to contribute to other cutaneous granulomatous disorders such as granuloma annulare (GA) or granulomatous foreign-body reactions (6, 7).

This prompted us to investigate the possible role of pDCs in the pathogenesis of cutaneous sarcoidosis as the prototype of granulomatous disorders in comparison to GA and LE, which has not been well-explored yet.

## Materials and methods

The study was approved by the institutional review board of the American University of Beirut Medical Center. Archival materials with a diagnosis of sarcoidosis and GA were retrieved from the dermatopathology database at the dermatology department at the American University of Beirut-Medical Center. A total of 20 cases of sarcoidosis and 20 cases of GA fit criteria for inclusion in the study. Fifteen

cases of cutaneous LE were used as a comparison group. The histologic sections of all cases were re-reviewed and the diagnoses were confirmed by the dermatopathologist (OA). Only straightforward cases that fit the clinicopathological features of sarcoidosis and GA were chosen for the study. Cases in which the diagnosis was not certain were excluded. Clinical information was extracted and all patient data were deidentified.

### Immunohistochemical analysis

Immunohistochemical analysis was performed on sections obtained from formalin-fixed, paraffin-embedded tissue using antibodies (Abs) to BDCA-2 (Mouse IgG1, Clone 124B3.13, Dendritics, France) and myxovirus protein A (MxA) (M143, University of Freiburg, Germany; Professor Haller). While anti-BDCA2 Ab is a specific pDC marker (1, 2), anti-MxA Ab indirectly assesses pDC activity in that MxA is well established as a surrogate marker for local type 1 IFN production (8). pDC content was scored on formalin fixed paraffin-embedded tissue sections stained for BDCA2 and reported as percentage of the total mononuclear infiltrate: 0 (very rare positive cells), 1 (1-10% of positive cells), 2 (10-50% of positive cells), 3 (>50% of positive cells). The MxA staining was scored as: 0=negative, 1=patchy staining, and 2=diffuse staining. Appropriate positive and negative controls were used.

### Statistical analysis

Statistical analysis was performed by using the Mann-Whitney test to analyze statistical differences in pDC and MxA scores between the different entities. A two-tailed P-value of <0.05 was considered statistically significant.

## Results

Patients (8 men and 12 woman) with sarcoidosis ranged in age from 33 to 64 years (mean of 46 years). Most (15 of 20 cases; 75%) were localized, while 5 (25%) patients had generalized lesions. Among localized cases, lesions were located on face in 10 cases, extremities in 3 cases, and trunk in 2 cases. Patients (4 men and 16 woman) with GA ranged in age from
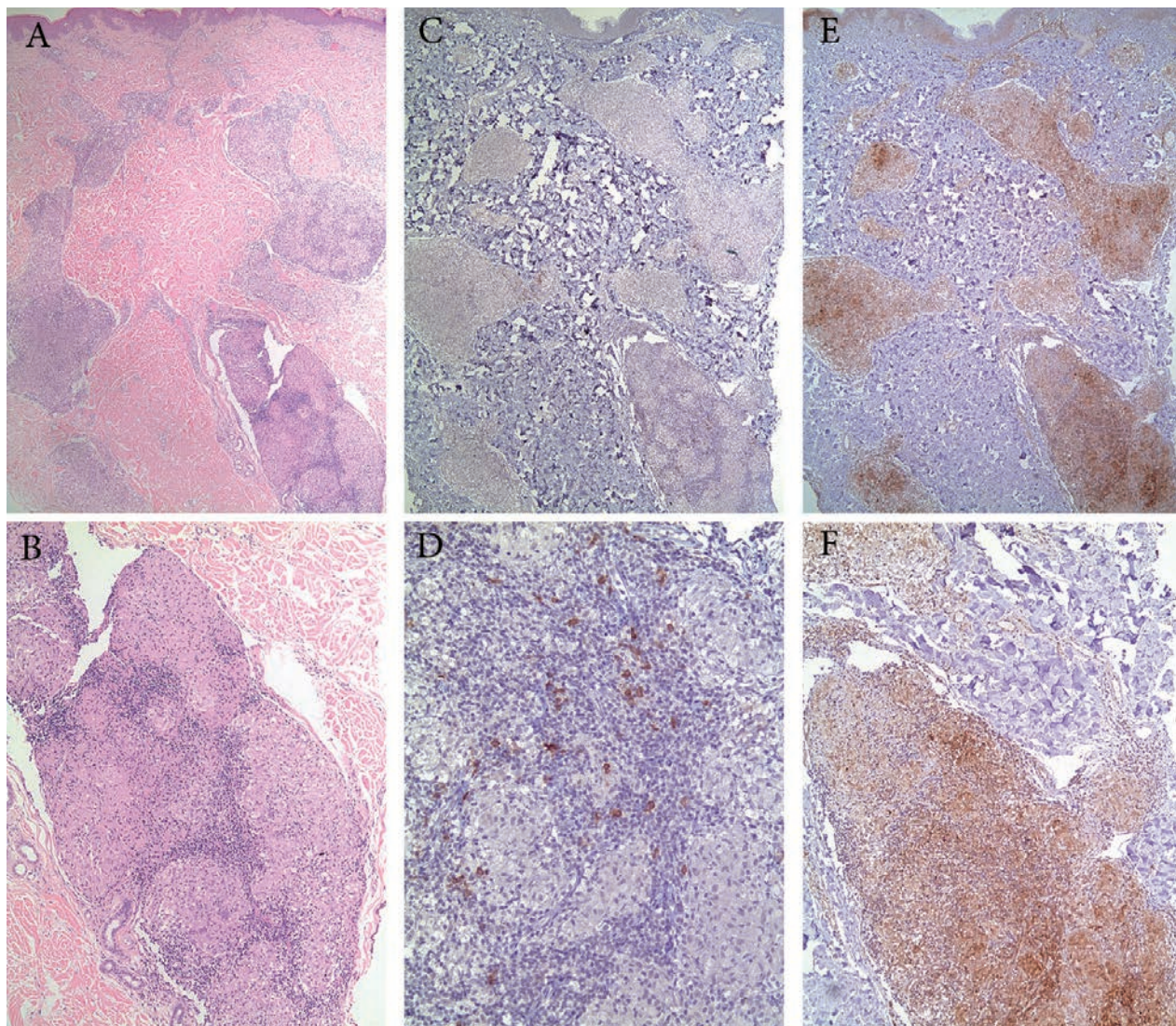
**Fig. 1.** Cutaneous sarcoidosis: A,B. Representative case showing dermal epithelioid granulomas (Hematoxylin-eosin stain; original magnification: Ax40, Bx200). C,D. BDCA-2 immunostaining highlighted pDCs in a perivascular and periadnexal distribution (original magnifications: Cx40; Dx200). E,F. MxA immunostaining of both the epithelium and inflammatory cells (original magnifications: Ex40, F x200)

4 to 75 years (mean of 39 years). Most (17 of 20 cases; 85%) were localized, while 3 (15%) patients had generalized GA. Among localized cases, lesions were located on upper extremities in 10 cases, lower extremities in 5 cases and trunk in 2 cases.

*Plasmacytoid dendritic cells in sarcoidosis and GA compared to LE*

This was assessed using antibodies against BDCA-2, which is a specific pDC marker expressed on the surface of pDCs (1, 2), pDCs were identified as medium-sized round cells. pDCs were present in all 20 sarcoidosis (Fig. 1C,D) and 20 GA (Fig. 2B,E) cases mainly in close proximity to small blood vessels and/or adnexal structures admixed with lymphocytes (Table 1). There was no significant difference in pDC scores between sarcoidosis and GA. Compared to sarcoidosis and GA cases, pDCs were significantly more abundant in LE (Figure 3B) with a significantly higher pDC score (p<0.05).

**Table 1.** Frequency and activity of pDCs in cutaneous sarcoidosis, GA, and LE (%)

| Entity (number of cases) | Frequency of pDC infiltration | pDC score* | | | | MxA score** | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 0 | 1 | 2 |
| Sarcoidosis (20) | 20 | 0 | 6 | 13 | 1 | 0 | 19 | 1 |
| GA (20) | 20 | 0 | 4 | 14 | 2 | 0 | 18 | 2 |
| P value# | 1 | >0.05 | | | | >0.05 | | |
| LE cases (15) | 15 | 0 | 0 | 1 | 14 | 0 | 0 | 15 |
| P value## | 1 | <0.05 | | | | <0.05 | | |
| P value### | 1 | <0.05 | | | | <0.05 | | |

Granuloma annulare (GA), lupus erythrematosus (LE), myxovirus resistance gene A (MxA), plasmacytoid dendritic cell (pDC)
* BDCA2+ pDC content was scored as percentage of total mononuclear infiltrate: 0 (no positive cells), 1 (1-10% positive cells), 2 (10-50% positive cells), 3 (>50% positive cells).
** MxA staining was scored as: 0=negative, 1=patchy, and 2=diffuse.
Statistical analysis was performed using Mann-Whitney test with two-tailed P-value of <0.05 considered statistically significant. P values in the table relate to comparisons between sarcoidosis versus GA (#), sarcoidosis versus LE (##) and GA versus LE (###).
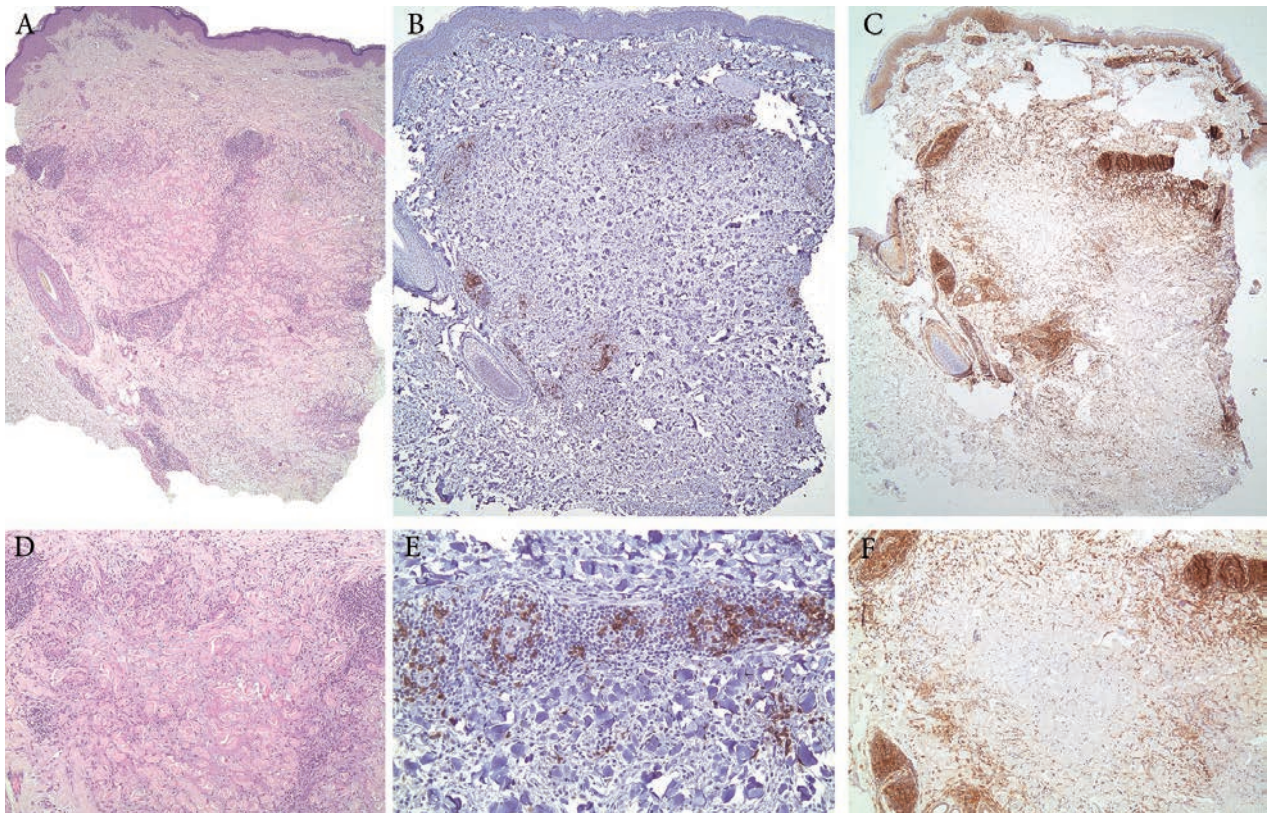


**Fig. 2.** Granuloma annulare: A,D. Representative case showing palisading granulomatous dermatitis in the dermis (Hematoxylin-eosin stain; original magnification: Ax40, Dx200). B,E. BDCA-2 immunostaining highlighted pDCs in a perivascular and periadnexal distribution (original magnifications: Bx40; Ex200). C,F. MxA immunostaining of both the epithelium and inflammatory cells (original magnifications: Cx40, F x200)
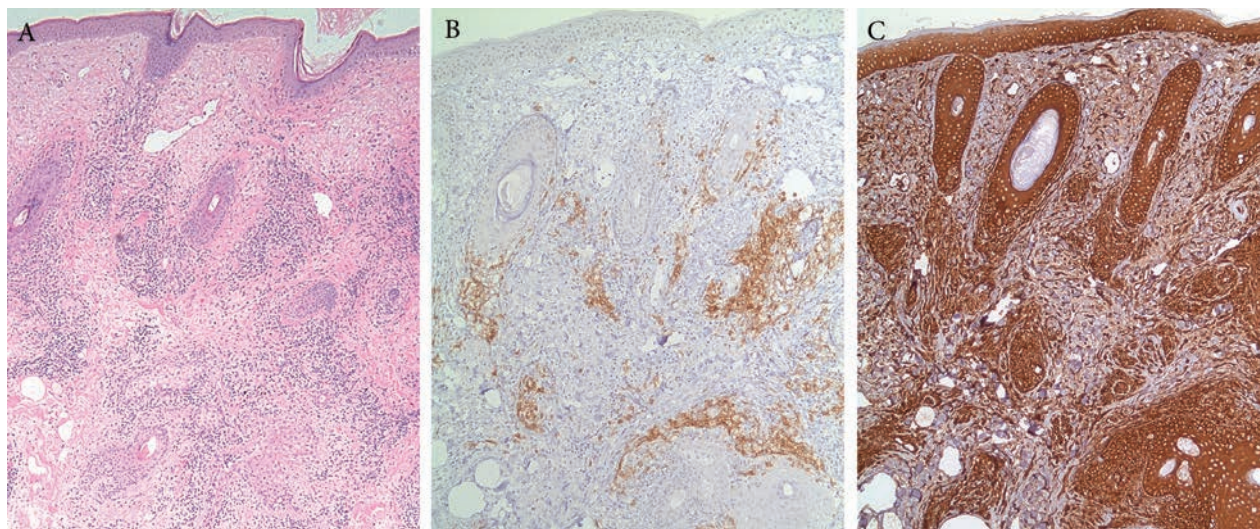
**Fig. 3.** Lupus erythrematosus: A. Representative case (Hematoxylin-eosin stain; original magnification: x40). B. BDCA-2 immunostaining highlighted numerous pDCs in a perivascular and periadnexal distribution (original magnifications: x100). C. Intense MxA immunostaining of both the epithelium and inflammatory cells (original magnifications: x40).

## MxA expression

MxA, a protein induced by type 1 IFNs, is a surrogate marker of local tissue type 1 IFN production. Thus, MxA expression represents an indirect assessment of pDC activity given that pDCs are the major source and the most potent producers of type I IFNs (2, 8). In most sarcoidosis (19/20 cases, 95%) and GA (18/20 cases, 90%) cases (Fig. 1E,F; Fig. 2C,F), MxA expression was focal/patchy with no significant differences between the two entities (Table 1), while all LE cases showed diffuse and strong cytoplasmic MxA expression in keratinocytes and inflammatory cells (Fig. 3C).

## Discussion

Studies investigating the role of pDCs in sarcoidosis are limited. pDC number was reported to be reduced in peripheral blood of sarcoidosis patients (9). pDCs were shown to be increased in bronchoalveolar lavage (BAL) and muscular lesions of sarcoidosis patients (10, 11). Recently, numerous pDCs were demonstrated as part of the dermal infiltrate surrounding sarcoidal granulomas in a case of IFN-α-induced sarcoidosis koebnerized along venous drainage lines (12).

In addition to these studies, our hypothesis in this study concerning a pDC role in cutaneous sarcoidosis pathogenesis was based on several observations. First, pDCs are known to enhance a Th1-biased cellular immune response, which is thought to be involved in sarcoidosis (4). Second, cutaneous sarcoidosis has been reported to occur at site of herpetic infections or in association with systemic viral infections such as hepatitis C and EBV (12-14). Since pDCs mainly function in anti-viral resistance, their involvement in sarcoidosis would not be surprising (1, 2). Third, sarcoidosis has been associated with several inflammatory disorders such as lupus erythematosus, psoriasis and vitiligo (15-17), in which evidence suggests significant pDC role in their underlying pathogenesis (2). Fourth, several reports have described induction of cutaneous and/or systemic sarcoidosis following administration of IFN-α, the endogenous local counterpart of which is mainly produced by pDCs (12, 18). Fifth, evidence from genetic studies also suggests a role of pDCs and their product in sarcoidosis. One study demonstrated that the possession of the IFNA allele was associated with higher levels of IFN-α and significantly increased risk for sarcoidosis (19). Carriage of two functional single-nucleotide polymorphisms, rs10954213A and rs2280714A, of IFN regulatory factor 5, which is a downstream signaling pathway

that is involved in pDC activation leading to production of pro-inflammatory cytokines and co-stimulatory molecules (1), were shown to be associated with conferred significant risks for sarcoidosis (20). Sixth, TNF inhibitors, known to be secondary inducers of IFN-α which is the main product of pDCs, have previously been reported to induce cutaneous and/or systemic sarcoidosis (21). Finally, pDCs have been shown to be present in other cutaneous granulomatous disorders such as GA or granulomatous foreign-body reactions, probably also contributing to their pathogenesis (6, 7).

Our study confirmed our hypothesis. pDCs were present consistently in all sarcoidosis and GA cases in a perivascular and/or peri-adnexal distribution usually accompanying the lymphocytic infiltrate surrounding the granulomas. The lymphocyte rich areas surrounding granulomas are believed to be a transit zone where newly recruited monocytes enter and then differentiate into epithelioid cells (22, 23). In addition, these areas might be places where immunologic reactions occur as various cell types such as CD8+ cells, CD4+ cells, B cells, and macrophages are present (4, 5, 24). pDCs distributed in these areas may thus interact with these different cells contributing to granuloma formation.

As indirectly assessed by MxA patchy staining in most sarcoidosis cases, local type I IFN production was diminished. Interestingly and in contrast to inflammatory cutaneous conditions such as LE, this finding suggests a role for pDCs reminiscent of their role in contributing to immunologic tolerance of neoplastic conditions (1). Such a role in sarcoidosis is supported by newly emerging evidence. Foxp3-positive T-regulatory cells (Tregs) have been shown to be present in peripheral blood of patients with sarcoidosis, BAL, and cutaneous sarcoidal granulomas (25, 26). In addition, high-level Inducible Co-stimulator (ICOS) expression on lung regulatory T cells has been shown to be associated with pulmonary sarcoidosis possibly implicating the ICOS/ICOS-L axis in disease pathogenesis (27). Such mechanisms have been shown to be employed by pDCs in inducing an immunosuppressive state in malignancies such as melanomas (28). This thus raises the possibility that pDCs may be contributing to the anergic state in sarcoidosis.

Our study also confirmed the presence of pDCs in GA lesions which was demonstrated previously in a study by Chavan et al (6). Similar to cutaneous sarcoidosis, GA has several characteristics which make the presence of pDCs not surprising: the main mechanism underlying GA is a Th1-biased cellular immune response (29); GA has been reported to occur at site of herpetic infections or in association with systemic viral infections such as hepatitis B or C (29); Imiquimod has previously been reported to be effective in GA treatment (30).

In summary, we have shown that pDCs are recruited into the skin lesions of sarcoidosis and GA. Their consistent presence in all sarcoidosis and GA cases speaks in favor of a significant role of these cells in their pathogenesis. The diminished ability to produce type I IFNs demonstrated in our study may also raise the possibility of pDCs contribution to the immune anergic state of sarcoidosis. Future studies should try to focus on uncovering the exact mechanisms of pDC involvement in sarcoidosis, GA, and other granulomatous disorders of the skin, which may have important therapeutic implications.

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