Psoriatic Inflammation Facilitates the Onset of Arthritis in a Mouse Model

Mayuko Yamamoto[1], Kimiko Nakajima[1], Mikiro Takaishi[1], Shun Kitaba[2], Yasuhiro Magata[3], Sayo Kataoka[4] and Shigetoshi Sano[1]

Psoriatic arthritis (PsA) is a seronegative, inflammatory joint disease associated with psoriasis. In most patients with PsA, skin lesions precede arthritis; however, the causality of skin inflammation for the development of arthritis remains unclear. Gp130$^{F759/F759}$ knock-in (F759) mice develop autoimmune arthritis after 1 year of age through persistent signal transducer and activator of transcription 3 (Stat3) activation due to impairment in SOCS3-dependent negative regulation. Here, we crossed F759 mice with K5.Stat3C transgenic mice, in which keratinocytes express constitutive active Stat3 (Stat3C), leading to generation of psoriasis-like skin change. F759 mice harboring the K5.Stat3C transgene not only had aggravated skin lesions but also spontaneously developed arthritis with high penetrance in adjacent paws as early as 3 weeks of age. The joint lesions included swelling of the peripheral paws and nail deformities contiguous with the skin lesions, closely resembling PsA. Histopathologic study revealed enthesitis and bone erosions, with mononuclear cell infiltrates. Quantitative reverse transcriptase–PCR (RT–PCR), immunohistochemical analyses, and flow cytometry showed upregulation of the IL-23/T helper type 17 (Th17) pathway in affected joints. Furthermore, enforced generation of psoriasis-like skin inflammation by topical treatment with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in F759 mice induced swelling of the underlying joints. This animal model renders psoriatic inflammation as the driver of arthritis and helps to further understand the pathogenesis of PsA.

Journal of Investigative Dermatology (2015) **135,** 445–453; doi:10.1038/jid.2014.426; published online 6 November 2014

INTRODUCTION

Psoriatic arthritis (PsA) is an inflammatory seronegative spondyloarthropathy associated with psoriasis. The prevalence of PsA among patients with psoriasis is 20–30% (Gladman *et al.*, 2005; Radtke *et al.*, 2009). The majority of PsA patients present with psoriasis vulgaris (PsV) before the development of arthritis. Clinical features of PsA include peripheral arthritis, axial arthritis (spondylitis), enthesitis, dactylitis, and tenosynovitis (Gottlieb *et al.*, 2008). Distal interphalangeal joints are frequently affected in PsA patients. Furthermore, nail involvement occurs in a majority of PsA patients. Recent studies demonstrated that the extended nature of the enthesis associated with distal interphalangeal joints leads to the diffuse nature of the inflammatory responses around the nail in PsA, as nails are functionally linked to the distal phalanx and enthesis (Tan *et al.*, 2007; McGonagle *et al.*, 2009). This notion explains why nail disease is frequently found in PsA patients, in particular, those with distal interphalangeal arthritis (Gottlieb *et al.*, 2008). Although PsV precedes arthritis in most patients with PsA, it remains unknown as to whether the skin and joint diseases are pathophysiologically related.

Psoriasis is a common chronic inflammatory skin disease characterized by increased proliferation, altered differentiation of the epidermis, and inflammatory cell infiltrates in the dermis. Recent studies have demonstrated that IL-23, which is essential for the development of T helper type 17 (Th17) cells, is functionally involved in the pathogenesis of psoriasis (Fitch *et al.*, 2007). The therapeutic efficacy of antibodies to IL-23, including those against either the p40 or p19 subunits, has confirmed the role of IL-23 in psoriasis (Krueger *et al.*, 2007; Nograles and Krueger, 2011). Similarly, tumor necrosis factor (TNF) inhibitors attenuate psoriasis through inhibition of the IL-23/Th17 pathway (Zaba *et al.*, 2007). More recently, direct targeting of the IL-17 pathway using monoclonal antibodies to IL-17A or the IL-17 receptor has proven to be a more potent approach to treat psoriasis (Spuls and Hooft, 2012). As most biologics for psoriatic skin disease also attenuate arthritis in PsA patients, it has been suggested that

[1]*Department of Dermatology, Kochi Medical School, Kochi University, Nankoku, Japan;* [2]*Department of Dermatology, Graduate School of Medicine, Osaka University, Suita, Japan;* [3]*Department of Molecular Imaging, Applied Medical Photonics Laboratory, Medical Photonics Research Center, Hamamatsu University School of Medicine, Hamamatsu, Japan and* [4]*Science Research Center, Kochi University, Nankoku, Japan*

Correspondence: Shigetoshi Sano, Department of Dermatology, Kochi Medical School, Kochi University, Kohasu, Okocho, Nankoku, Kochi 783-8505, Japan. E-mail: sano.derma@kochi-u.ac.jp

Abbreviations: FDG-PET, fluorodeoxyglucose positron emisson tomography; gp130$^{F759/F759}$, knock-in mice, F759; PsA, psoriatic arthritis; PsV, psoriasis vulgaris; RT–PCR, reverse transcriptase–PCR; Stat3, signal transducer and activator of transcription 3; Th17, T helper type 17; TNF, tumor necrosis factor; TPA, 12-O-tetradecanoylphorbol-13-acetate

Received 7 February 2014; revised 8 September 2014; accepted 16 September 2014; accepted article preview online 30 September 2014; published online 6 November 2014

an immunological pathomechanism is shared by those joint and skin diseases (Raychaudhuri *et al.*, 2012).

Recent genetic analyses demonstrated that PsV and PsA harbor common susceptibility genes, including those associated with HLA class I, NF-κB and IFN signaling, and the IL-23/Th17 pathway (Nograles *et al.*, 2009; Elder *et al.*, 2010; Rahman and Elder, 2012). Some genes are more strongly associated with PsA than with PsV (Lloyd *et al.*, 2012). However, the underlying mechanism for the ''PsV to PsA transition'' still remains unclear. Traumatic injury in joints has been recognized to be a trigger for the subsequent development of PsA, known as joint Koebner or the deep Koebnerization (Punzi *et al.*, 1998; McGonagle *et al.*, 2011).

The elucidation of the pathogenesis of PsA has been hampered by the relative paucity of biopsy specimens from joint lesions. Furthermore, relevant animal models of PsA are not available, although the generation of arthritis has been described in some murine models of psoriasis (Zenz *et al.*, 2005; Hwang *et al.*, 2011). We have recently established a mouse model of psoriasis, K5.Stat3C transgenic mice, in which a constitutively active signal transducer and activator of transcription 3 (Stat3) transgene (Stat3C) is expressed in the epidermis. These mice develop psoriasis-like lesions either spontaneously or induced by wounding stimuli or topical treatment with 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) (Sano *et al.*, 2005, 2008). Their skin lesions resemble psoriasis regarding the clinical appearance, histopathology, immunological conditions including the activation of IL-23/Th17 signaling, and sensitivities to biological agents such as monoclonal antibodies to IL-17A, IL-12/23p40, and IL-23p19 (Nakajima *et al.*, 2011). However, K5.Stat3C mice do not develop arthritis even if the skin lesions become severe. $Gp130^{F759}$ homozygous knock-in (referred to as F759) mice harbor a mutant variant of gp130 where Y759 is substituted for phenylalanine (F), leading to Stat3 activation due to the absence of SOCS3-mediated suppression (Ohtani *et al.*, 2000). The IL-6/gp130/Stat3 pathway in F759 mice induces Th17 cell activation with age, leading to the development of rheumatoid arthritis–like disease in the limbs at 12–18 months after birth (Atsumi *et al.*, 2002). In this study, we crossed F759 mice with K5.Stat3C mice and found that K5.Stat3C:F759 mice spontaneously develop severe psoriasis-like lesions and joint diseases in their paws as early as 3 weeks of age. In addition, the potential involvement of the IL-23/Th17 pathway in the development of arthritis suggests that K5.Stat3C:F759 mice represent a relevant model of PsA. Similar to the effect of introduction of Stat3C transgene, enforced development of psoriasis-like lesions in F759 mice by topical application of TPA led to swelling of the underlying joints, suggesting that psoriatic inflammation facilitated arthritis.

RESULTS

K5.Stat3C:F759 mice spontaneously develop severe psoriatic skin lesions and swelling of the paws

We crossed K5.Stat3C mice with F795 mice, and the phenotypes were compared among the pups harboring genotypes of F759/0 (heterozygous), F759 (homozygous), K5.Stat3C:F759/0, and K5.Stat3C:F759. Strikingly, K5.Stat3C:F759 mice showed swelling in the paws from 3 weeks of age, and most of them became worse as they aged (Figure 1a). Mice with the other three genotypes did not develop any joint disease during the study. In addition, K5.Stat3C:F759 mice simultaneously developed scaly skin lesions in the paws, whereas the skin of K5.Stat3C:F759/0 remained intact. This observation suggested that Stat3 activation in epidermal keratinocytes of K5.Stat3C mice was reinforced by SOCS3 inhibition due to the F759 mutation in the gp130 gene (Ohtani *et al.*, 2000). The ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) imaging, by which inflamed joints of PsA are detected (Tan *et al.*, 2006; Takata *et al.*, 2011), revealed a distinct accumulation of ^{18}F-FDG in the hindlimb paws of K5.Stat3C:F759 mice (Figure 1e), suggesting inflammation in the joints, whereas the paws of F759 mice showed accumulation at the background level (Figure 1d). Histopathology of the paws revealed that K5.Stat3C:F759 mice showed psoriasis-like changes, including acanthosis and dermal cell infiltrates (Figure 1g), whereas mice with the other three genotypes were devoid of dermal inflammation (Figure 1f). The joint involvements in the paws were evaluated according to the clinical severity from 0 to 4, as described in the Materials and Methods. The cumulative incidence of joint disease during the first 9 weeks after birth in mice with different genotypes clearly showed that K5.Stat3C:F759 mice exclusively developed paw joint involvements (Table 1). The penetrance of joint disease found in K5.Stat3C:F759 mice was as high as 75% (27 out of 36 mice).

Nail lesions of K5.Stat3C:F759 mice

Mice affected with the most severe disease (clinical score 4) showed the contracture of paw joints and nail involvement in addition to scaly, keratotic skin lesions (Figure 2b) compared with wild-type mice (Figure 2a). The nail lesions in K5.Stat3C:F759 mice were thickened and hyperkeratotic. They closely resembled the nail deformities in psoriatic patients—that is, pitting of nails, oncholysis, and subungual hyperkeratosis (Tan *et al.*, 2012). Histopathology of the nail lesions in K5.Stat3C:F759 mice demonstrated a marked hyperkeratosis in the nail plate, nail matrix, and nail bed leading to nail deformity and oncholysis (Figure 2d), sharply contrasting with wild-type mice, in which the nail bed was attached tightly to the nail plate (Figure 2c). The nail plate deformity in K5.Stat3C:F759 mice might be due to a hyperkeratotic abnormality in the nail matrix with underlying inflammatory cell infiltrates, suggesting carpal enthesitis. Intracorneal infiltration of neutrophils was also found. Taken collectively, the nail lesions in K5.Stat3C:F759 mice closely resemble nail psoriasis clinically and histopathologically in human PsA.

Severe skin and joint lesions in older K5.Stat3C:F759 mice

As K5.Stat3C:F759 mice aged, the skin lesions and arthritis tended to become worse. A representative mouse at 31 weeks of age is shown. They developed progressive skin lesions on the limbs, tails, and back, in which a very thickly scaled ''ostraceous psoriasis-like'' feature was noted (Figure 3a, b,