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Immunopathogenesis of psoriasis: a possible role of TGFβ/Smads pathway

Roberta Sferra[1*], Maria Concetta Fargnoli[2], Eleonora Corbelli[1], Cristina Pellegrini[2], Ketty Peris[3], Eugenio Gaudio[4], Antonella Vetuschi[1]

[1] Department of Biotechnological and Applied Clinical Sciences, Human Anatomy, University of L'Aquila, L'Aquila, Italy
[2] Department of Dermatology, Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy
[3] Department of Dermatology, Catholic University of Rome, Rome, Italy
[4] Department of Human Anatomy, University of Rome Sapienza, Rome, Italy

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Abstract

Psoriasis is a chronic immune-mediated inflammatory skin disease with both genetic and environmental factors contributing to its pathogenesis. Transforming Growth Factor beta (TGFβ) is a member of a large family of pleiotropic cytokines with three different isoforms (TGFβ1,2,3). Smads are a family of eight-related proteins that function as intracellular signaling intermediates for TGFβ once the latter is bound to its receptors (TGFbRI, II and III). The involvement of Smads in TGFβ signaling has been studied intensively in the skin in the process of wound healing. Few studies, and with controversial results, have investigated at the immunohistochemical and molecular level the role of TGFβ/Smads signaling in psoriasis.

Key words

Normal skin, psoriatic skin, cytokines, immunohistochemistry.

Psoriasis is a chronic immune-mediated inflammatory cutaneous disease affecting 2-4% of the general population. Epidemiological studies revealed that a distinct group of comorbidities are significantly associated with psoriasis, such as cardiovascular disease, diabetes, obesity, hypertension, metabolic syndrome, dyslipidemia, inflammatory bowel diseases, non-alcoholic fatty acid disease, anxiety and depression (Christophers, 2001; Gelfand et al., 2006; Azfar and Gelfand, 2008; Davidovici et al., 2010; Mehta et al., 2011; Nijsten and Stern, 2012; Skroza et al. 2013).

Clinically, psoriasis appears with well-demarcated, erythematous and scaly plaques (Fig. 1), histopathologically characterized by thickening of the epidermis, incomplete cornification with retention of nuclei in the stratum corneum, and elongated rete ridges (Fig. 2). Dilated vessels are observed in the papillary dermis and a perivascular infiltrate composed of CD4+ T cells, dendritic cells (DC) and monocytes/macrophages is present in the upper papillary dermis and one of CD8+ T cells and neutrophilic granulocytes in the epidermis.

* Corresponding author. E-mail: roberta.sferra@univaq.it.

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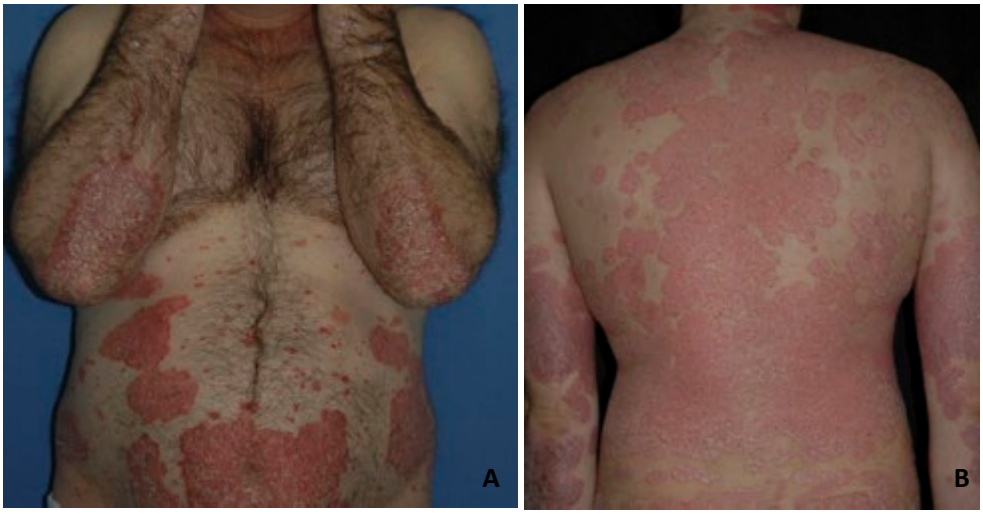

Figure 1 – Clinical manifestations of moderate to severe chronic plaque psoriasis (A, B).

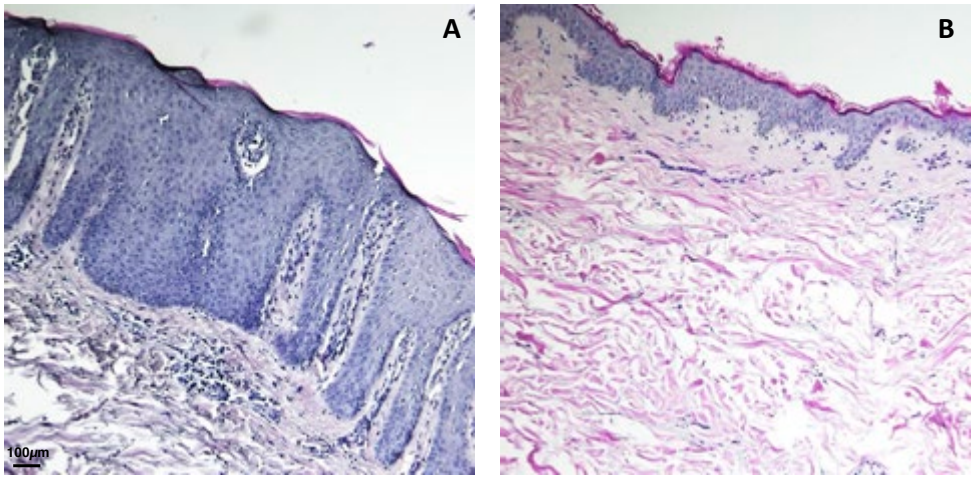

Figure 2 – Psoriatic skin (A) shows increased thickness of the epidermis and an inflammatory infiltrate in dermis, compared to control skin (B). Haematoxylin and eosin, 10x.

The exact pathogenesis of psoriasis remains to be fully determined, but both environmental and genetic factors seem to be responsible for the dysregulation of innate and adaptive immune response in psoriatic skin.

Classic genome linkage analysis has identified at least 10 chromosomal regions (termed PSORS1 to PSORS10) with statistically significant linkage to psoriasis (Perera et al., 2012). The major genetic determinant of psoriasis is PSORS1 that is located

within the major histocompatibility complex, with the HLA-Cw*060 allele being the most likely candidate as PSORS1 gene. Furthermore, genome-wide association studies have recently identified about 12 genes with various functions involved in psoriasis development (Perera et al., 2012). Notably, several of these psoriasis-associated genes belong to the interleukin (IL)-23/Th17 axis, the nuclear factor κB (NF-κB) signaling pathway and the epidermal differentiation complex, suggesting that these pathways might be critically involved in the pathogenesis of psoriasis.

In a genetically predisposed individual, all cells located in the epidermis and dermis that are involved in the maintenance of barrier integrity can determine a dysregulated response to either an environmental or a self-antigenic insult. An initial trigger such as physical trauma or bacterial products can induce a cascade of events that include the formation of complexes of the antimicrobial peptide cathelicidin LL-37 and DNA, activation of plasmacytoid dendritic cells (pDCs), and secretion of interferon (IFN)-α. By inducing pDC activation, LL-37–self-DNA complexes initiate a pathogenic cross-talk between stressed epidermal cells and recruited pDCs and induce a proinflammatory cytokine cascade including TNF-α, IL-6, and IL-1β released by keratinocytes. Activated pDCs migrate into draining lymph nodes and induce the differentiation of naive CD4+ T cells into effector cells such as Th1 and Th17 and cytotoxic T cells that produce interleukin-17 (Tc17). Immune effector cells migrate to the skin attracted by the keratinocyte-derived chemokines and infiltrate the skin. Moreover, inflammatory myeloid DCs produce TNF-α, nitric oxide radicals and IL-23. IL-23 sustains the proliferation of activated Th17 cells producing IL-17 and IL-22, while Th1 cells express IFN-γ and TNF-α (Sabat, 2007; Perera et al., 2012). The resulting cytokine milieu of IFNγ, TNF-α, IL-17 and IL-22 affects keratinocytes, increasing their proliferation and stimulating the production of pro-inflammatory cytokines and the neutrophil-recruiting chemokines that in turn sustain and amplify the chronic inflammatory disease process (Perera et al., 2012).

Among all cytokines and growth factors, trasforming growth factor β (TGFβ) plays a role in sustaining and amplifying the inflammatory response by producing a variety of proinflammatory mediators important for the development of psoriatic lesions and then for activating the vicious cycle necessary to perpetuate the pathogenetic process. TGFβ is a member of a large family of pleiotropic cytokines, with three different isoforms (TGFβ1,2,3) found in mammals. TGFβ intracellular signal transduction pathway is mediated mainly by Smad proteins (Derynck and Zhang, 2003; Roberts et al., 2003).

Smads are a family of eight related proteins that function as signaling intermediates for the TGFβ superfamily members. Upon ligation and activation of TGFβ with its receptors (I, II and III), the phosphorylated Smad2 and Smad3 bind to the common mediator Smad4. The Smad2/Smad3-Smad4 complex translocates into the nucleus where it regulates specific TGFβ target genes. The inhibitory Smad7 antagonizes TGFβ signaling by competing with ligation of Smad2/3 to the activated receptor complex (Fig. 3).

The TGFβs have been implicated in various inflammatory diseases such as liver cirrhosis (Inagaki and Okazaki, 2007), nephritis (Inazaki et al., 2004), pulmonary fibrosis (Lawrence, 1996), inflammatory bowel diseases (Crohn's disease and ulcerative colitis) (Latella et al., 2009) and scleroderma (Lakos et al., 2004). Several experimental studies have also demonstrated that the disruption of the TGFβ/Smads signaling, owing to loss of Smad3 or an increase of Smad7 expression, confers resistance

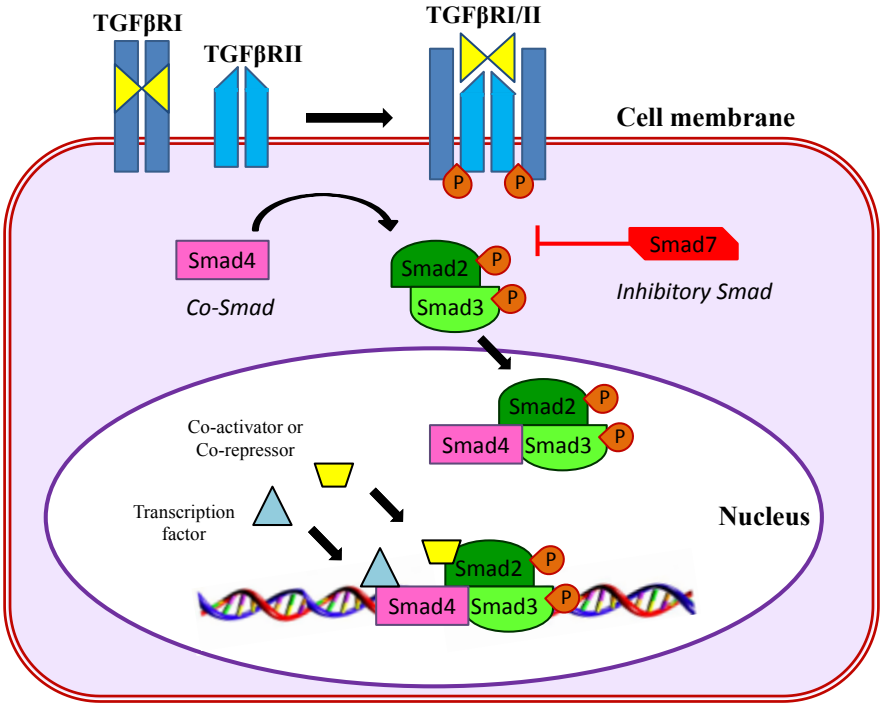

Figure 3 – TGFβ/Smad pathway.

to tissue fibrosis in many organs (Inazaki et al., 2004; Latella et al., 2009). As far as cancer is concerned, mutations of TGFβ receptors have been reported in human squamous cell carcinoma cell lines (Ichijo et al., 1990; Reiss and Stash, 1990) and decreased levels of TGFβRI or TGFβRII have been shown in a variety of neoplastic lesions including colon (Markowitz et al., 1995) and gastric cancers (Takenoshita et al., 1997).

The involvement of Smads in TGFβ signaling has been also studied intensively in the skin, especially in the process of wound healing. Here, TGFβ has been demonstrated to play a central role in inhibiting the growth of keratinocytes and in stimulating the growth of fibroblasts (Pittelkow et al., 1988; Cutroneo, 2007). Experimental studies in Smad3 knock-out mice showed that wound healing is accelerated, while local inflammatory responses are decreased (Ashcroft et al., 1999; Yu et al., 2009).

Despite this important influence of TGFβ on the homeostasis of human tissues, including normal skin, there have been only a few and controversial studies on the role of TGFβ/Smads signaling in the pathogenesis of psoriasis.

Immunohistochemical analysis of TGFβ1 showed both in normal and psoriatic skin an immunopositivity of the suprabasal keratinocytes (Kane et al., 1990). Selective association of TGFβ1 with non-proliferating keratinocytes in the suprabasal layers of the epidermis and its exclusion from the proliferating keratinocytes in the basal layer suggests that it may be a physiological regulator of keratinocyte proliferation. The intracellular localization of TGFβ1 peptide in both normal and psoriatic keratinocytes

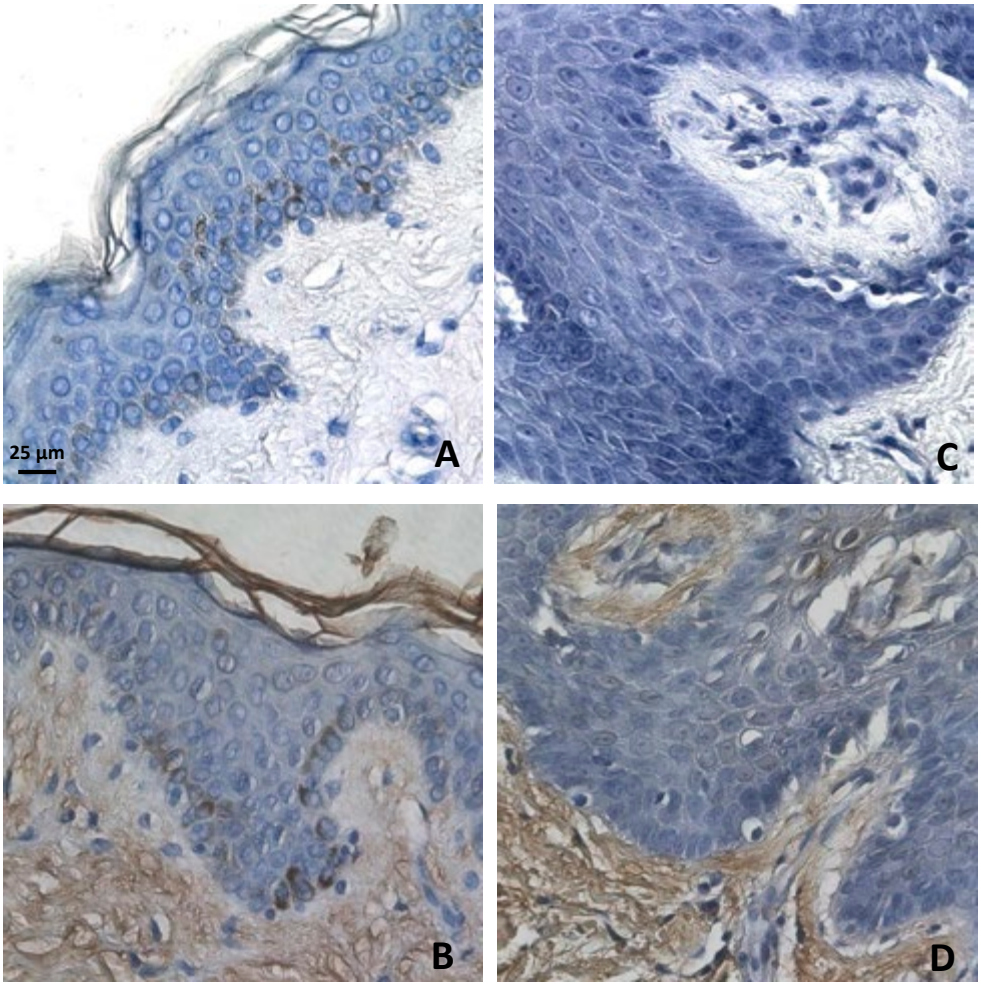

Figure 4 – TGFβ1 is expressed in the basal layer of the epidermis in normal skin (A) while there is no evidence of immunoexpression in the epidermis of psoriatic skin (C). TGFβRI is present both in the basal layer of the epidermis and widely spread in connective tissue of dermis of normal skin (B). In psoriatic skin TGFβRI is only present in connective tissue of dermis (D). Immunohistochemistry, 40x.

suggests that it is constitutively synthesized by epidermal keratinocytes *in vivo* (Kane et al., 1990). Since TGFβ1 was detected in the dermis as well as in the epidermis in several sections of both plaque and non-plaque skin from psoriatic patients, there may be additional sites of TGFβ1 synthesis in the psoriatic dermis or the distribution of keratinocyte-derived TGFβ1 may be altered in psoriatic skin (Kane et al, 1990).
Conversely, other Authors reported that TGFβ1 was detected in neither epidermis nor dermis in both normal and psoriatic skin while TGFβ2 was found in the intercellular spaces of all epidermal layers in normal skin and was decreased in psoriatic epider-

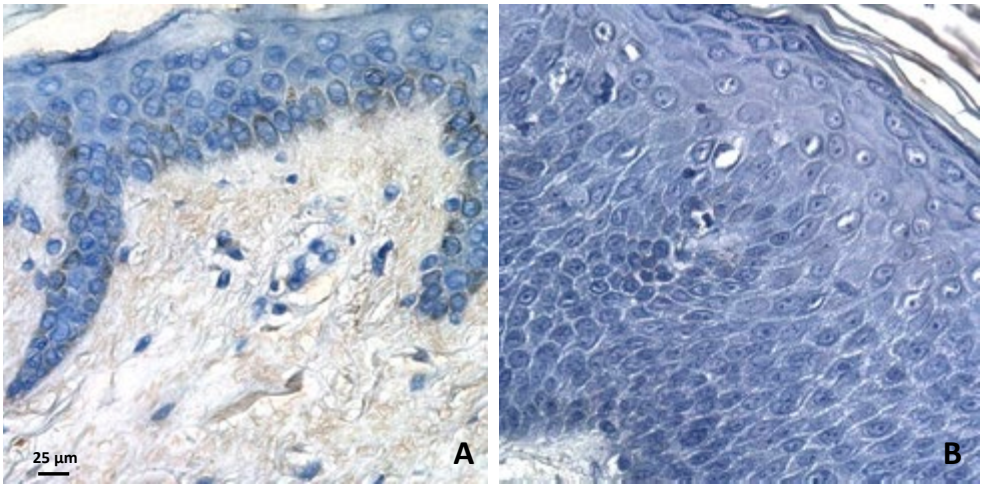

Figure 5 – Positive staining for Smad3 is located in basal keratinocytes and is widely spread in the connective tissue of dermis of normal skin (A); no evidence of immunoreaction for Smad3 in psoriatic skin (B). Immunohistochemistry, 40x.

	MARKER EXPRESSIONS IN PSORIATIC SKIN VS NORMAL SKIN					
	TGFβ1	TGFβ2	TGFβRI	TGFβRII	SMAD 2, 4, 6	SMAD 3
KANE et al., 1990	=					
WATAYA-KANEDA et al., 1996	=	↓				
YU et al., 2009	=	=	↓	↑	↓	
GAMBICHLER et al., 2013	↑		↓			↑

Figure 6 – Expression of different markers in psoriasis vs. normal healthy skin.

mis; in contrast, TGFβ3 was present in the subepidermal area of the psoriatic skin as in the normal human skin (Wataya-Kaneda et al., 1996). Since TGFβ is a potent growth inhibitor of human keratinocytes, the decrease of TGFβ2 in the epidermis of psoriatic skin may contribute to epidermal hyperplasia, a hallmark of psoriasis.

As TGFβ has an antiproliferative role on the epidermis, TGFβ receptors (TGFβR) are needed to mediate this effect. Leivo et al. (1998) observed intense immunohistochemical staining for both receptors in basal and suprabasal epidermal keratinocytes in skin from healthy subjects and non-lesional psoriatic skin, but no staining in the epidermis of lesional psoriatic skin. A significant reduction of TGFβ receptors in psoriatic epider-

mis and the decreased expression of both TGFβ receptors (TGFβRI and TGFβRII) could be a reason for diminished perception of TGFβ1 signaling (Leivo et al., 1998).

By investigating the expression of TGFβ1, 2 and 3 and their receptors both in lesional and non lesional psoriatic skin compared to skin from healthy subjects by real time PCR, no differences in TGFβ1 and 2 mRNA levels were found between psoriatic and normal skin; upregulation of TGFβ3 mRNA only in non-lesional psoriatic skin may be responsible for inhibiting epidermal hyperproliferation. In the same cases TGFβRI was downregulated together with Smad 2, 4 and 6 mRNA expression, confirming the crucial role of these receptors in the pathogenesis of the disease (Yu et al.,2009). On the other hand, Gambichler et al. (2013) observed increased mRNA levels for TGFβ1 as well as Smad3, while confirming decreased mRNA levels for TGFβRI in psoriatic skin (Tab. 1)

The role of TGFβ/Smads signaling in the pathogenesis of psoriasis is supported by the efficacy of current therapeutic strategies, known to impact this pathway. The beneficial effect of 1α, 25-dihydroxyvitamin D_3 (active derivative of vitamin D_3) in psoriasis seems to be mediated at least in part by TGFβ and Smad signaling (Yanagi et al., 1999; Yanagisawa et al., 1999; Oyama et al., 2000; Yu et al., 2009). Other Authors have shown that UVB blocks *in vitro* cellular responsiveness to TGFβ through downregulation of TGFβRII receptor and induction of Smad 7 (Quan et al., 2002).

We recently performed an immunohistochemical study on the role of TGFβ/Smads signaling in psoriasis in a limited number of samples. Our results demonstrated a downregulation of TGFβ1 and TGFβRI in the epidermis of all psoriatic specimens compared to control skin, which showed a marked immunopositivity in the basal keratinocytes and in the connective tissue of the dermis (Fig. 4). Our data also revealed a decreased expression of Smad 3 in basal keratinocytes of psoriatic skin compared to control skin (Fig. 5).

Further studies are needed to better clarify the role of TGFβ/Smads signaling in the pathogenesis of psoriasis, in order to highlight whether it is directly involved in the development of disease and, therefore, might be a promising therapeutic target of antipsoriatic drugs in the future.

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