Montagna Symposium on the Biology of Skin

Key Role of Macrophages in the Pathogenesis of CD18 Hypomorphic Murine Model of Psoriasis

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Psoriasis is a chronic skin disorder of unsolved pathogenesis affecting skin in 2–3% of the general population. Research into the pathogenesis of psoriasis has profited from suitable animal models. Previously, we reported on the CD18 hypomorphic ($CD18^{hypo}$) PL/J mouse model clinically resembling human psoriasis, which is characterized by reduced expression of the common chain of β_2-integrins (CD11/CD18) to only 2–16% of wild-type levels. Aside from common clinical and pathophysiological features shared with human psoriasis, the psoriasiform skin disease in $CD18^{hypo}$ PL/J mice also depends on the presence of $CD4^+$ T-cells. This review focuses on the role of activated macrophages in the pathogenesis of $CD18^{hypo}$ T-cell-mediated mouse model of psoriasis, and extends our understanding in unrestrained pathogenic T-cells whose activation may be crucial for the recruitment and activation of macrophages within skin. The findings in the $CD18^{hypo}$ PL/J model are discussed in the context of current literatures of human and other autoimmune disorders.

Journal of Investigative Dermatology (2009) **129,** 1100–1114;
doi:10.1038/jid.2009.43; published online 26 February 2009

INTRODUCTION

Skin from psoriatic patients is characterized by a dense dermal infiltrate that predominantly consists of T-cells, dendritic cells (DCs), natural killer T-cells, and macrophages (Clark and Kupper, 2006). The epidermis of psoriatic skin is hyperproliferative and fails to undergo normal differentiation, resulting in a marked thickening of the epidermis and increased scale formation. The dispute in the field is whether psoriasis is a disease of autoreactive T-cells or whether it reflects an intrinsic defect within the epidermis, or both. There is more recent evidence from genetic mouse models that macrophages can contribute to T-cell-mediated and epidermis-mediated psoriasiform skin inflammation (Stratis *et al.*, 2006; Wang *et al.*, 2006).

Activated macrophages are major producers of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-1β, and IL-6 (Salkowski *et al.*, 1995; Marble *et al.*, 2007), and play a crucial role in modulating immune responses (Holt *et al.*, 1993; Thepen *et al.*, 1994; Gordon, 1995). Macrophages are heterogeneous and versatile bone marrow-derived cells that produce a wide range of mediators and exert a multitude of biological functions (Ganz, 1993). They can not only serve as antigen-presenting cells, but also directly inhibit antigen presentation by DCs (Holt *et al.*, 1993). T-cell proliferation, phenotype, and ultimately the type of immune response induced, can distinctly be influenced by macrophages (Thepen *et al.*, 1994, 1996; Strickland *et al.*, 1996; Grewe *et al.*, 1998). Macrophages are specifically polarized by the microenvironment to mount different functional programs. Initial signals from microbes, through their pathogen-associated molecular patterns (Schnare *et al.*, 2000), followed by a second signal, such as IFN-γ, gives rise to ''classically activated macrophages''. In early immune responses, IFN-γ is produced by natural killer and natural killer T-cells; later the main source of IFN-γ are antigen-specific T-helper-1 (Th1) cells (Young, 2006). These stimuli generally activate macrophages to produce TNF-α, monocyte chemotactic protein-1 (MCP-1), inducible nitric oxide synthase (iNOS), IFN-γ, and to promote strong IL-12-mediated Th1 responses. When macrophages are activated in the presence of IL-4, IL-10, transforming growth factor-β, or glucocorticoids, they become ''alternatively activated macrophages'' being characterized by dectin-1 expression and supporting Th2-associated immune responses. However, activation of dectin-1 with the fungal β-glucan triggers a severe autoimmune arthritis in genetically susceptible mice (Yoshitomi *et al.*, 2005). Thus, in inflammatory processes macrophages contribute to both initiation of inflammation and to its resolution. However, during chronic inflammatory responses in the skin and most likely other tissues, interaction between macrophages and T-cells may lead to a vicious cycle, which, by itself, is capable of maintaining local inflammation without the necessity of external stimuli (Avice *et al.*, 1998).

To characterize mouse macrophages in various biological processes, a panel of antibody-defined markers, expressed during different stages of mouse macrophage development,

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Abbreviations: $CD18^{hypo}$, CD18 hypomorphic mutation; DC, dendritic cell; DLN, draining lymph node; iNOS, inducible nitric oxide synthase; LT, lymphotoxin; MCP, monocyte chemotactic protein; PASI, psoriasis activity and severity index; Th, T-helper cell; TipDC, TNF-iNOS-producing DC; TNF-α, tumor necrosis factor-α; TNFR, TNF receptor; T_{reg}-cells, regulatory T-cells

Received 31 January 2008; revised 20 March 2008; accepted 13 April 2008; published online 26 February 2009

was previously reported. These markers included (1) macrophage precursors and immature macrophages (ER-MP12, ER-MP20, ER-MP54, ER-MP58); (2) mature macrophages in general (F4/80, BM8, Mac-1, Mac-2, ER-BMDM1); (3) macrophage subsets (ER-HR3, ER-MP23, ER-TR9, Forssman antigen, MOMA-1, MOMA-2, Monts-4, SER-4), and (4) IFN-γ-stimulated macrophages (H-2Ia, LFA-1, intercellular adhesion molecule-1, 158.2, MBR-2, TM-2, TM-4, TM-5) (Leenen *et al.*, 1994).

In the past, some surface markers, including CD14, CD68, HLA-DR, RM3/1, and CD11c, have been used to describe the phenotypic diversity of the dermal macrophage population in humans (Weber-Matthiesen and Sterry, 1990; Djemadji-Oudjiel *et al.*, 1996). Besides these markers, a recent study reported that a population of dermal macrophage/macrophage-like cells expresses CD163 and factor XIIIa in normal human skin (Nestle and Nickoloff, 2007; Zaba *et al.*, 2007b). $CD163^+$ cells phagocytose large particles in a tattoo and have the structural features of macrophages (Zaba *et al.*, 2007b). CD163 is a hemoglobin/haptoglobin complex-binding macrophage scavenger receptor expressed on the majority of tissue macrophages (Fabriek *et al.*, 2005). Expression of factor XIIIa is related to cell activation and is inducible via IL-4 in alternatively activated macrophages (Torocsik *et al.*, 2005).

In human psoriasis, the number of epithelium-lining macrophages was reported to increase in lesional skin. These macrophages, which line dermal–epidermal junctions, may play a role in the regulation of epidermal proliferation and differentiation (van den Oord and de Wolf-Peeters, 1994; Djemadji-Oudjiel *et al.*, 1996) or vigorous interactions between macrophages and keratinocytes (Djemadji-Oudjiel *et al.*, 1996), and may be involved in the pathogenesis of psoriasis (van den Oord and de Wolf-Peeters, 1994; Djemadji-Oudjiel *et al.*, 1996). Macrophages secrete a variety of proinflammatory cytokines, such as TNF-α, IL-1β, IFN α/β, IL-6, IL-10, IL-12, and IL-18 cytokines, under different conditions (Willment *et al.*, 2003).

Research into the pathogenesis of human psoriasis has profited, at least in part, from suitable animal models. Most of these, however, reveal only a single or a few aspects resembling human psoriasis (Carroll *et al.*, 1995; Schon *et al.*, 1997; Pasparakis *et al.*, 2002; Sano *et al.*, 2005; Zenz *et al.*, 2005).

Previously, introduction of an insertion mutation in the murine CD18 gene, resulting in duplication of exons 2 and 3, yielded a mouse model with severe reduction of CD18 expression, with only 2–16% of wild-type (wt) levels (Wilson *et al.*, 1993). Due to this hypomorphic CD18 mutation ($CD18^{hypo}$), a chronic inflammatory skin disease develops in PL/J mice, which closely resembles human psoriasis clinically, histologically, in its polygenic nature and in its response to therapy (Bullard *et al.*, 1996; Kess *et al.*, 2003; Wang *et al.*, 2006). Affected mice present with erythema, crusts, and scaling, as well as abnormal keratinocyte proliferation/differentiation, subcorneal microabscesses, and increased inflammatory infiltrate. In severely affected mice, reversible alopecia was observed, a feature, which may only rarely, if at all, occur in human psoriasis (Shuster, 1972). The psoriasiform skin disease was only observed when the $CD18^{hypo}$ mutation was backcrossed on the PL/J, but not on the C57BL/6J or 129/SvEv inbred mouse strains. Homozygous mutant mice on a PL/J × C57BL/6J F1 background did not develop the disease, despite the $CD18^{hypo}$ mutation. Backcross analysis suggests that, in addition to the $CD18^{hypo}$ mutation, a small number of other genes determine susceptibility to the disease (Bullard *et al.*, 1996; Kess *et al.*, 2006). Hence, the $CD18^{hypo}$ PL/J model qualifies as a polygenic model for chronic skin inflammation. Such a polygenic nature has been claimed for human psoriasis (Schon and Boehncke, 2005).

As in patients treated for severe psoriasis (van de Kerkhof and Weemaes, 1990; Feldman *et al.*, 2001), this chronic psoriasiform skin disease of the $CD18^{hypo}$ PL/J mouse model can be suppressed by corticosteroids (dexamethasone), suggesting involvement of an inflammatory process. T-cells are important in the generation of the inflammatory skin disease in this $CD18^{hypo}$ PL/J model (Kess *et al.*, 2003; Barlow *et al.*, 2004). This is analogous to the affected skin of psoriatic patients in which $CD4^+$ T-cells prevail (Morel *et al.*, 1992; Wrone-Smith and Nickoloff, 1996; Schon *et al.*, 1997; Sugiyama *et al.*, 2005; Conrad *et al.*, 2007).

CD18 represents the common β_2-chain of the β_2-integrin family. β_2-Integrins (CD11/CD18) are leukocyte adhesion molecules exclusively expressed on hemopoietic cells and are responsible for cell–cell contacts in a variety of inflammatory interactions (Hynes, 1987). At present, four different β_2-integrins have been characterized, all of which are heterodimeric cell-surface molecules consisting of the CD18 molecule and one of the CD11 molecules: CD11a, CD11b, CD11c, or CD11d. These heterodimeric molecules interact with more than 20 known ligands, of which the most prominent belong to the intercellular adhesion molecule family (Carlos and Harlan, 1994).

The pathogenic role of β_2-integrins in human psoriasis and other inflammatory skin diseases is less well understood. Circumstantial evidence indicating that reduced CD18 expression may causally be involved in the development of this psoriasiform dermatitis, comes from the clinical observation that some patients suffering from leukocyte adhesion deficiency syndrome-1, with only moderately reduced CD18 expression levels, develop a psoriasiform skin disease (van Pelt *et al.*, 1998). Linkage analysis of psoriasis families has identified a region on chromosome-17, including among other loci the intercellular adhesion molecule-2 locus, an important ligand of the CD11/CD18 heterodimers (Tomfohrde *et al.*, 1994). Furthermore, polymorphisms in the CD18 gene have been found that predispose to autoimmune diseases (Gencik *et al.*, 2000; Meller *et al.*, 2001).

Until today, the cause of psoriasis remains unknown. Here, we describe the causal contribution of activated macrophages in the initiation and maintenance of the psoriasiform skin disease, and summarize recent data on the impaired function of regulatory T-cells (T_{reg}-cells) being responsible for the accelerated proliferation of pathogenic T-cells, which contribute to the recruitment and activation of