CD18 in Monogenic and Polygenic Inflammatory Processes of the Skin

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The β_2 integrin family (CD11/CD18) of leukocyte adhesion molecules plays a key role in inflammation. Absence of the common β chain (CD18) leads to leukocyte adhesion deficiency-1 (LAD1) in humans. We here summarize data of two genetically defined mice models of β_2 integrin deficiency, one with a CD18 null mutation ($CD18^{-/-}$), and the other one with a hypomorphic CD18 mutation ($CD18^{hypo}$). Firstly, we focus on the underlying mechanism of a severely impaired wound healing in $CD18^{-/-}$ mice, outlining a scenario in which a defective extravasation and phagocytosis of $CD18^{-/-}$ neutrophils results in delayed myofibroblast-dependent wound contraction owing to a deficient transforming growth factor-β_1 release. Based on this, we have identified a potential therapy that fully rescued the impaired wound healing in $CD18^{-/-}$ mice. Secondly, we expand on a $CD18^{hypo}$ PL/J mouse model closely resembling human psoriasis. Apart from common clinical and pathophysiological features, this psoriasiform dermatitis also depends on the presence of activated $CD4^+$ T cells. We here recapitulate the influence of a reduced CD18 gene expression on T-cell function, also with regard to CD18 gene–dose effects, and its contribution to the pathogenesis of this disease. Taken together, these unique features make this model a valuable tool for investigations into the pathogenesis of human psoriasis – including its polygenic base – and future preclinical studies.

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β_2 INTEGRINS IN INFLAMMATION

During the last years, the genetic approach for studies on leukocyte and endothelial cell adhesion molecules has been successfully implemented (Mayadas *et al.*, 1993; Sligh *et al.*, 1993; Wilson *et al.*, 1993; Carlos and Harlan, 1994; Bullard *et al.*, 1995; Coxon *et al.*, 1996; Frenette *et al.*, 1996; Lu *et al.*, 1997; Jung *et al.*, 1998; Stark *et al.*, 2005). Genetically defined mice represent models of human diseases, which allow the functional analysis of genes and of the polygenic susceptibility for inflammatory disorders such as autoimmune diseases, arthritis, psoriasis, atopic dermatitis, asthma, and atherosclerosis (Leiter, 1989; Garchon *et al.*, 1991; Rubin and Smith, 1994; Shih *et al.*, 1995; Bullard *et al.*, 1996; Lam-Tse *et al.*, 2002; Olofsson *et al.*, 2003; Laitinen *et al.*, 2004; Andersson and Holmdahl, 2005; Kere, 2005; Liston and Goodnow, 2005; Mazon Pelaez *et al.*, 2005). Our group has a long-standing interest in further defining the role of β_2 integrins in complex skin diseases. β_2 integrins are leukocyte adhesion molecules exclusively expressed on hematopoietic cells being responsible for cell–cell contacts in a variety of inflammatory interactions, and in particular, they participate crucially in the extravasation process of leukocytes (Figure 1) (Arnaout, 1990; Harris *et al.*, 2000). After tethering and rolling, they play a unique role in firm adhesion and transmigration through endothelia (Springer and Anderson, 1986). The common β chain (CD18) associates with four α subunits (α_L, α_M, α_X, and α_D) forming distinct functional heterodimers termed LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18), gp150,95 (CD11c/CD18), and CD11d/CD18 (Arnaout, 1990). These receptors interact with more than 20 ligands, of which the most prominent belong to the family of intercellular adhesion molecule (Dustin and Springer, 1988; Staunton *et al.*, 1989; Diamond *et al.*, 1990; Vazeux *et al.*, 1992; de Fougerolles *et al.*, 1994).

Lack of CD18 leads to leukocyte-adhesion deficiency type-1 (LAD1), a recessively inherited disorder in which deletions, truncations, substitutions, or frameshift mutations impair β_2-integrin function (Kishimoto *et al.*, 1987; Arnaout, 1990). The severity of this disease correlates with the degree of loss of CD18 (Anderson *et al.*, 1985). In the absence of functional CD18, severe defects in cell–cell cooperation occur leading to a lack of homotypic lymphocyte adhesion (Mentzer *et al.*, 1985; Rothlein and Springer, 1986; Koopman *et al.*, 1992) and hampered T-cell activation (Davignon *et al.*,

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Abbreviations: EPC, endothelial progenitor cell; hypo, hypomorphic; LAD1, leukocyte-adhesion deficiency type-1; PASI, psoriasis area and severity index; SMA, smooth muscle actin; TGF, transforming growth factor; WT, wild type

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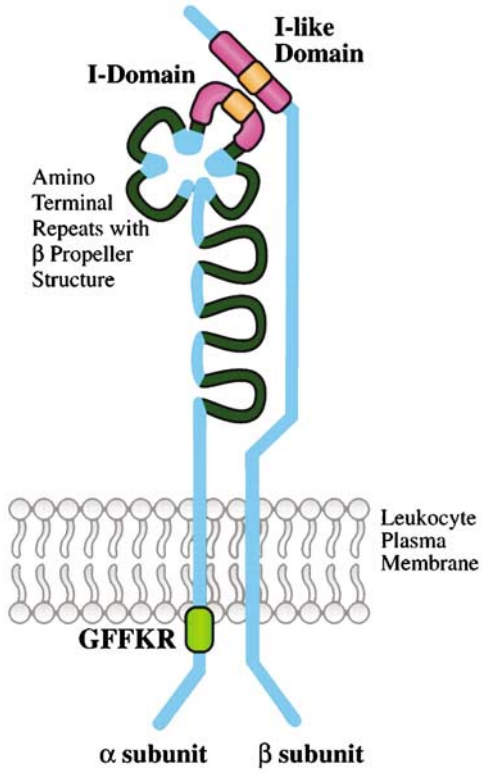

Figure 1. **Features of β_2 integrins.** The $\alpha\beta$ heterodimeric structure is common to all integrins. The α chain includes seven extracellular N-terminal homologous repeats organized into a β propeller structure. The α chain I domain is shown in *pink* with the embedded metal ion-dependent adhesion site motif in *orange*, and the β chain I-like domain with metal ion-dependent adhesion site motif is shown in a corresponding manner. The GFFKR sequence in the cytoplasmic tail of the α subunit is involved in heterodimer assembly and regulation of ligand recognition. The heterodimer is illustrated in the ''closed'' or inactive state that undergoes tertiary and quaternary changes in response to inside-out signals. See ''Structure and distribution,'' ''Ligand Recognition,'' and ''Inside-out Signaling'' for details. (This figure was reproduced from Harris *et al.* (2000), with permission from The American Society for Biochemistry and Molecular Biology.)

1981; Bachmann *et al.*, 1997; Scharffetter-Kochanek *et al.*, 1998; Schönlau *et al.*, 2000) accompanied by a reduced IL-2 release (Shier *et al.*, 1996; Zuckerman *et al.*, 1998; Kim *et al.*, 1999; Schönlau *et al.*, 2000). Patients suffering from LAD1 are typically subject to recurrent bacterial or fungal infection, peripheral leukocytosis, impaired wound healing, weak or absent vaccine response (Fischer *et al.*, 1986; Ochs *et al.*, 1993; Anderson and Smith, 2001), and, in case of moderate β_2 integrin-expression deficiency, also psoriasiform dermatitis (van de Kerkhof and Weemaes, 1990).

In the past years, we have established and/or studied two genetically defined mice models of β_2 integrin deficiency, following two different mutation strategies for the CD18 locus: (1) Introduction of the neomycin resistance cassette into the splice acceptor site of exon 3 resulted in an out-of-frame mutation yielding no functional CD18 protein at all (Scharffetter-Kochanek *et al.*, 1998). (2) Duplication of exons 2 and 3 by introduction of an insertion mutation in the murine CD18 gene yielded a hypomorphic mouse model of CD18 with a severe reduction in CD18 expression with only 2–16% of wild-type (WT) levels of CD18 (Wilson *et al.*, 1993; Bullard *et al.*, 1996).

WOUND-HEALING DEFECT OF CD18$^{-/-}$ MICE OWING TO A DECREASE IN TGF-β_1 AND MYOFIBROBLAST DIFFERENTIATION

Herein, we will first focus on the underlying mechanism of severely impaired wound healing in LAD1 (Peters *et al.*, 2005). For this purpose, we have used the previously generated full knockout mouse model (CD18$^{-/-}$), which closely reflects the clinical phenotype of patients suffering from severe-type LAD1 with spontaneously occurring erosions and wounds in mechanically stressed facial areas (Scharffetter-Kochanek *et al.*, 1998). Firstly, it is of importance to define precisely the parameter and wound model (primary *versus* secondary wound healing, re-epithelialization *versus* wound contraction) employed for studies on tissue and wound repair (Singer and Clark, 1999; Werner and Grose, 2003). Therefore, to analyze secondary wound healing under standardized conditions, full-thickness 5-mm punch biopsies were produced on the back of CD18$^{-/-}$ and WT control mice. Monitoring of wound sizes revealed a significant delay in wound healing from days 5 to 14 in CD18$^{-/-}$ mice compared to WT mice (Figure 2). As differences in wound sizes between WT control mice and CD18$^{-/-}$ mice were most pronounced from days 5 to 10 postwounding, which represents the phase of myofibroblast-mediated wound contraction, expression of myofibroblast markers was studied in granulation tissue at different stages of tissue repair by immunohistochemistry and Western blot analysis. Myofibroblasts are characterized by the expression of α-smooth muscle actin (α-SMA) (Tomasek *et al.*, 2002), the actin isoform characteristic of vascular smooth muscle cells (Skalli *et al.*, 1986; Serini and Gabbiani, 1999), which confers a high contractile activity to these cells, *in vivo* (Serini and Gabbiani, 1999) and *in vitro* (Arora and McCulloch, 1994; Hinz *et al.*, 2001, 2003). In CD18$^{-/-}$ mice, α-SMA expression was substantially reduced at days 5 and 7, compared to the WT controls. Besides, ED-A splice variant of fibronectin, another myofibroblast differentiation marker (Serini *et al.*, 1998), was significantly decreased in CD18$^{-/-}$ mice as compared to the WT. Vimentin served as a housekeeping protein to equilibrate for equal numbers of fibroblastic cells, and did not differ in CD18$^{-/-}$ mice *versus* WT mice at these time points. Consequently, the detected reduction in myofibroblast differentiation markers reflected a decrease in the number of myofibroblastic cells in wounds of CD18$^{-/-}$ mice.

Besides, transforming growth factor (TGF)-β signalling seemed to be involved as TGFβRII protein levels were also decreased in CD18$^{-/-}$ granulation tissue at day 5 after wounding. Interestingly, TGF-β_1 is one major cytokine inducing α-SMA expression, myofibroblast differentiation (Desmoulière *et al.*, 1993), and subsequent wound contraction (Montesano and Orci, 1988). This action of TGF-β_1 depends on the presence of ED-A fibronectin in the extracellular matrix (Serini *et al.*, 1998). To assess TGF-β_1

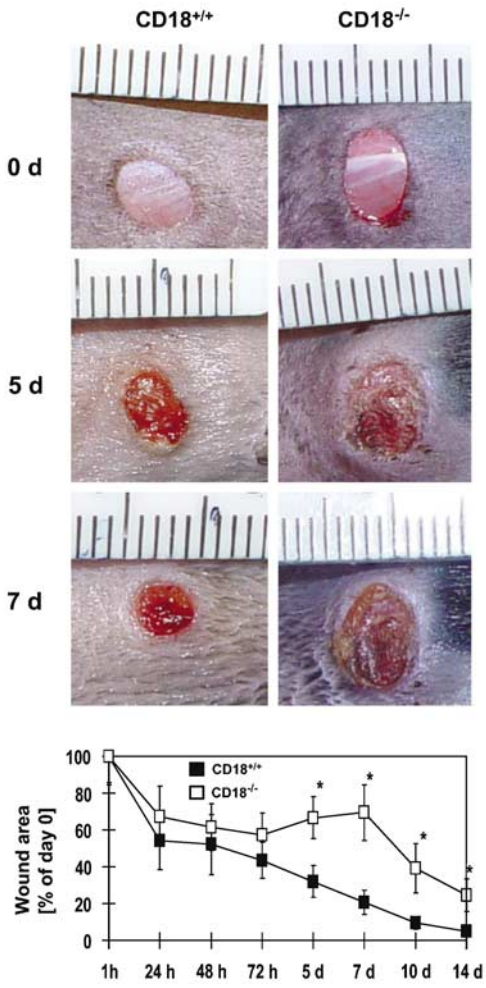

Figure 2. **Wound closure of full-thickness wounds is delayed in CD18$^{-/-}$ mice.** Full-thickness (including the *panniculus carnosus*) excisional wounds were punched at two sites in the middle of the dorsum using 5-mm biopsy round knives. Each wound region was digitally photographed at indicated time points, and wounds areas were calculated using Adobe Photoshop software. (**a**) Macroscopic observation of wounds in CD18$^{-/-}$ and WT mice. Representative results of six wounds in each cohort are shown. (**b**) Wound sizes at any given time point after wounding were expressed as the percentage of initial (day 0) wound area for CD18$^{-/-}$ and WT mice. Results are expressed as the mean $\pm$ SD ($n = 6$). * $P < 0.05$. (This figure was reproduced from Peters *et al.* (2005), with permission from the Nature Publishing Group.)

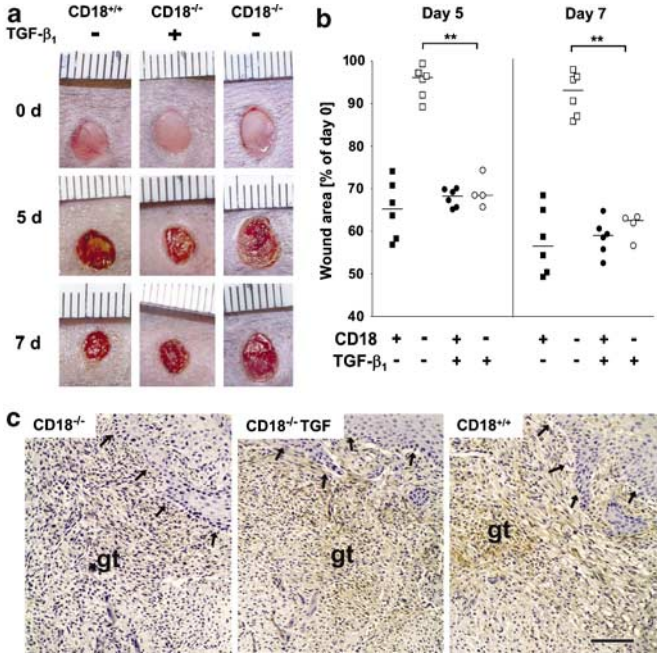

Figure 3. **Injection of TGF-β_1 in wound margins rescues wound closure and myofibroblast differentiation in CD18$^{-/-}$ mice.** Recombinant human TGF-β_1 (''TGF'') was injected subcutaneously at four sites around the wound, allowing to infiltrate the wound margins, at a total dose of 0.45 μg per wound. Mock injections were made using only the solvent NaCl 0.9%. First, injections were carried out on day 1 after wounding, followed by further injections every second day until wounds were harvested. (**a**) Macroscopic observation of wounds in CD18$^{-/-}$, WT mice with or without injection of TGF-β_1. (**b**) Wound sizes were assessed at the indicated time points after wounding as described previously. Bars depict the median of each cohort. **$P < 0.005$. (**c**) Paraffin-embedded granulation tissue of CD18$^{-/-}$ and WT mice was stained immunohistochemically for α-SMA 5 days after wounding. The bar indicates 100 μm; de, adjacent dermis; gt, granulation tissue; arrows indicate the newly formed epidermal leading edges. (This figure was reproduced from Peters *et al.* (2005), with permission from the Nature Publishing Group.)

cytokine levels in wound tissue of CD18$^{-/-}$ mice *versus* WT mice, we performed immunohistochemistry for TGF-β_1 on sections of 5- and 7-day-old wounds as well as ELISA measurements for quantification of active TGF-β_1 from wound tissue lysates. As a result, there was distinctly less TGF-β_1 detectable in the newly formed granulation tissue of CD18$^{-/-}$ mice at both of these time points after wounding, as compared to the WT controls. These data suggested that reduced amounts of TGF-β_1 had accounted for the decreased numbers of α-SMA-positive myofibroblasts and the impaired wound contraction in CD18$^{-/-}$ mice.

In order to prove a causal role for the TGF-β_1 deficiency in the reduced wound closure of CD18$^{-/-}$ mice, we substituted the lacking endogenous TGF-β_1 by injecting recombinant human TGF-β_1 subcutaneously at four sites around the wound (Ashcroft *et al.*, 2003). Mock injections were made using NaCl 0.9%. Gross evaluation and digital measurements of wound sizes revealed that the previously observed wound healing defect was fully restored in CD18$^{-/-}$ mice treated with recombinant TGF-β_1, a finding further confirmed by the detection of α-SMA-positive myofibroblasts at wild-type levels in these mice (Figure 3). These data strongly suggested that TGF-β_1 deficiency was the major cause of an impaired tissue repair in CD18$^{-/-}$ mice. However, the mechanism underlying this TGF-β_1 deficiency needed clarification.

To tackle the question which cells may have contributed to the observed TGF-β_1 deficiency in wounds of CD18$^{-/-}$ mice, one has to consider that normal tissue repair follows a sequence of events involving clotting, inflammation, remodelling, and re-epithelialization (reviewed by: Martin, 1997; Singer and Clark, 1999). Shortly after injury, first neutrophils and later macrophages invade the wound site. Although such inflammatory cells are an important source of growth factors

and cytokines (Werner and Grose, 2003), adhesion molecules, and in particular integrins, are required for their extravasation. We and others have previously shown that in mouse models for toxic dermatitis and thioglycolate-induced peritonitis, extravasation of neutrophils depended highly on β_2 integrins (Mizgerd *et al.*, 1997; Scharffetter-Kochanek *et al.*, 1998; Walzog *et al.*, 1999; Grabbe *et al.*, 2002). To investigate whether egress of leukocytes from the blood vessels may, in analogy to dermatitis, be dependent on β_2 integrins also during wound healing, we set out to detect inflammatory cells infiltrating the wound area during the healing process. In a time-course analysis, wounds were harvested and stained with GR1 monoclonal antibodies for neutrophils or with F4/80 for macrophages. Subsequently, neutrophils and macrophages, which had egressed from the vessels into the interstitial tissue of the wound bed, were counted. Almost no neutrophils were detected in the granulation tissue of $CD18^{-/-}$ wounds, compared to high numbers of egressed neutrophils in WT wounds at 24 and 48 hours. However, similar numbers of egressed macrophages were observed at the wound sites of WT and $CD18^{-/-}$ mice. These data demonstrated that wounds of $CD18^{-/-}$ mice remained almost devoid of neutrophils, whereas infiltration by macrophages was normal (Peters *et al.*, 2005).

In secondary wound healing, TGF-β_1, crucially required for normal wound closure, is released by macrophages during the engulfment of apoptotic polymorphonuclear neutrophils at the wound site (Fadok *et al.*, 1998; McDonald *et al.*, 1999; Taylor *et al.*, 2000). As we demonstrated, defective migration leads to a severe reduction of neutrophils in wounds of $CD18^{-/-}$ mice. Hence, we hypothesized that infiltrating macrophages might not sufficiently phagocytose apoptotic neutrophils in wounds of $CD18^{-/-}$ mice. Macrophages would thus be lacking their main stimulus to secrete TGF-β_1. Consequently, the effect of phagocytosis of viable and apoptotic neutrophils by $CD18^{-/-}$ and WT macrophages was studied. We found that only apoptotic but not viable neutrophils, after being engulfed by macrophages, caused a dramatic induction in the release of TGF-β_1, in line with earlier reports (Fadok *et al.*, 1998; McDonald *et al.*, 1999). In this context, it was an astounding finding that $CD18^{-/-}$ neutrophils had a higher resistance toward spontaneous apoptosis, as absent/reduced DNA laddering and annexin-V staining showed in $CD18^{-/-}$ compared to WT neutrophils (Weinmann *et al.*, 2003). To study whether, apart from the reduced extravasation of neutrophils into wound beds, release of TGF-β_1 occurring during phagocytosis of neutrophils by macrophages may also be dependent on CD18, we *in vitro* co-cultured apoptotic $CD18^{-/-}$ and WT neutrophils with $CD18^{-/-}$ or WT macrophages. Supernatants were subjected to ELISA for TGF-β_1. As a result, a time-dependent, up to 20-fold increase in active TGF-β_1 release with a maximum at 24 hours was observed in co-cultures of WT apoptotic neutrophils and macrophages (Peters *et al.*, 2005). Interestingly, TGF-β_1 release did not exceed control levels after 24 hours in co-cultures of $CD18^{-/-}$ neutrophils with either WT or $CD18^{-/-}$ macrophages. In summary, these data indicated that a maximal release of TGF-β_1 required CD18 expression on both neutrophils and macrophages, but CD18 expression on apoptotic neutrophils in particular seems to be of great importance for sustained TGF-β_1 release by macrophages. With regard to the underlying pathophysiological mechanisms, the observed deficiency in TGF-β_1 release in the absence of CD18 was caused indeed by a reduced phagocytotic efficacy of macrophages to engulf neutrophils owing to an impaired adhesion between phagocytotic effector and target cells (also see van Spriel *et al.*, 2001), thus attributable mainly to an impaired ''physical attachment'' in case adhesion molecules were absent (Peters *et al.*, 2005). Potential contributions of the β_2 integrin family to downstream signalling (Piccardoni *et al.*, 2004) during phagocytosis are currently being investigated.

In summary, an impaired recruitment of neutrophils into the wound bed, an enhanced apoptotic resistance of neutrophils, and a reduced release of TGF-β_1 from macrophages upon defective engulfment of apoptotic neutrophils collectively contribute to the observed deficiency of TGF-β_1 in $CD18^{-/-}$ mice. Hence, CD18 deficiency has multiple effects on TGF-β_1 availability.

As TGF-β_1 enhances angiogenesis, we also studied vessel formation in wound granulation tissues. A decrease in platelet/endothelial cell adhesion molecule-1 (CD31) expressing blood vessels in $CD18^{-/-}$ as compared to WT mice was observed by immunofluorescence histology of wounds in a time-course analysis (Schruefer *et al.*, 2006). This difference in angiogenesis could only be partly rescued by TGF-β_1 injection, providing evidence that in addition to the TGF-β_1 deficiency, other mechanisms may contribute to the impaired vessel formation. Because β_2 integrins are strongly expressed on endothelial progenitor cells (EPCs), the role of β_2 integrins for *in vivo* homing of EPCs in neovascularization was studied (Chavakis *et al.*, 2005). Firstly, our results showed that β_2 integrins mediated the adhesive interactions of EPCs to mature endothelial cells and to extracellular matrix proteins, and were critical for chemokine-induced transendothelial migration of EPCs *in vitro*. Secondly, using a murine *in vivo* hind limb ischemia model, proximal femoral arteries were ligated and, by consequence, distal tissue nutrition and regeneration depended on new vessel formation. By transfusion of murine Sca-1^{+}Lin^{-} hematopoietic progenitor cells from $CD18^{-/-}$ mice, it could be demonstrated that β_2 integrins were involved in the homing of hematopoietic progenitor cells to sites of ischemia and were critical for their neovascularization capacity. This was shown quantitatively by assessing the reconstitution of vessel density and functionally by determining distal blood flow at given time points after transfusion of EPCs into recipients using color-encoded Doppler laser analysis. Interestingly, pre-activation of β_2 integrins on the transfused EPC further augmented the *in vivo* neovascularization capacity of EPC, indicating a novel therapeutic strategy to promote homing of EPC (Chavakis *et al.*, 2005).

In summary, CD18 is central for the emigration of neutrophils into the wound bed. Murine $CD18^{-/-}$ macrophages release significantly less TGF-β_1 after phagocytosis of apoptotic neutrophils compared to WT macrophages. This

reduced release of TGF-β_1 is owing to an impaired adhesion and subsequently impaired phagocytosis of CD18$^{-/-}$ neutrophils by macrophages. Exclusively apoptotic neutrophils can induce TGF-β_1 release from macrophages, whereas apoptosis is significantly reduced in CD18$^{-/-}$ neutrophils compared to WT neutrophils, owing to an enhanced bcl-x/bax-α ratio. All of these mechanisms likely contribute to the observed TGF-β_1 deficiency and defective healing of acute wounds, which can be widely rescued by the local injection of recombinant TGF-β_1 in CD18$^{-/-}$ mice. In addition, β_2 integrins play a non-redundant role for homing and neovascularization capacity of EPCs.

The herein summarized data thus demonstrates that the paracrine secretion of growth factors induced by wound-infiltrating neutrophils is essential for myofibroblast differentiation in tissue repair of cutaneous full-thickness wounds, implying a therapeutic value for growth factor substitution during specific wound healing deficiency. Furthermore, the pre-activation of β_2 integrins appears to be a feasible tool to improve the efficacy of EPC-induced neovascularization, possibly relevant for patients with ischemic disease and distinct tissue repair disturbances. Regarding the importance of β_2 integrins in the mobilization and migration of hematopoietic cells during tissue repair, this endorses an analogous conception as outlined also for other adhesion molecules in (a) double P- and E-selectin-deficient mice (Subramaniam *et al.*, 1997), (b) functionally P- and E-selectin-deficient mice (mice deficient in β-1,4-galactosyltransferase, which glycolsylates the P- and E-selectin ligands (Mori *et al.*, 2004)), and (c) LAD2 patients suffering from a glycosylation deficiency owing to a gene defect in the GDP-fucose transporter (Luhn *et al.*, 2001). These all have in common that affected individuals show an altered emigration of neutrophils and/or macrophages, combined with an impaired wound healing. Interestingly, intercellular adhesion molecule-1$^{-/-}$ mice lacking one of the multiple counter receptors of the β_2 integrins only reveal a subtle wound-healing defect (Nagaoka *et al.*, 2000). However, none of these studies have directly analyzed wound contraction.

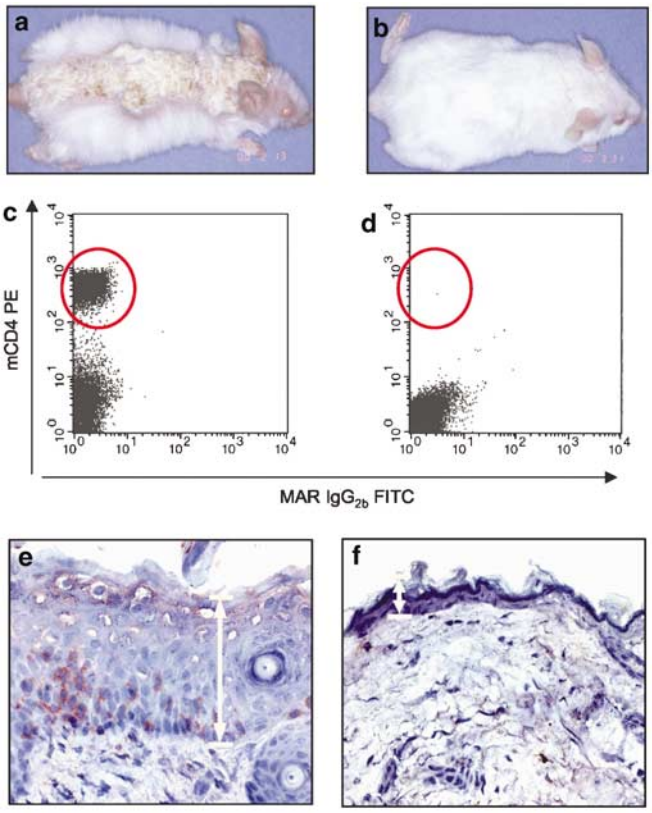

Figure 4. ***In vivo*** **depletion of CD4$^+$ T cells in CD18^{hypo} mice.** To monitor the clinical effect of CD4$^+$ T cell depletion, neutralizing antibodies were injected intraperitaneously at a dose of 100–150 μg twice weekly. (**a**) A CD18^{hypo} mouse with a severe psoriasiform dermatitis. (**b**) The same mouse 6 weeks after treatment with CD4$^+$ T-cell-depleting mAbs. Depletion efficiency was evaluated by FACS analysis of peripheral blood cells from CD18^{hypo} mice treated with the (**c**) isotype control mAbs or with (**d**) CD4$^+$ T cell-depleting mAbs. . Mouse anti-rat (MAR) IgG2b FITC mAbs were applied for the detection of residual rat anti-mCD4 mAbs, which had previously been used for depletion of CD4$^+$ T cells. The red circle highlights the CD4$^+$ T-cell population. Skin sections from CD18^{hypo} mice treated with (**e**) isotype control mAbs or with (**f**) CD4$^+$ T-cell-depleting mAbs were immunostained with mCD4 mAbs (original magnification $\times$ 400). Arrows indicate the murine full-thickness epidermis from cornified to basal layer. (This figure was reproduced from Kess *et al.* (2003), with permission from The American Association of Immunologists.)

THE ROLE OF CD18 IN A MURINE T-CELL-DEPENDENT PSORIASIS MODEL

Another genetically defined mouse model with a specific gene defect in the CD18 locus, currently the only available polygenic mouse model for a psoriasiform skin disease, helped us to explore the involvement of distinct T-cell populations in this inflammatory disease. In addition, it will allow for identification of modifier genes and their interaction in the precipitation of this disease.

Owing to a hypomorphic CD18 mutation, a skin disease developed in PL/J mice, which strongly resembled human psoriasis clinically, histologically, and in response to therapy (Wilson *et al.*, 1993; Bullard *et al.*, 1996). Affected mice presented with erythema, crusts, scaling, and alopecia starting at the face and involving the trunk (Figure 4). Compared to the two-layered epidermis in WT mice, epidermal thickening occurred in CD18^{hypo} mice, with subcorneal micro-abscesses and hyperkeratosis as well as a severe T-cell-dominated infiltrate in the dermis, all of which characteristic hallmarks of human psoriasis. Only mice homozygous for the CD18-hypomorphic (CD18^{hypo}) mutation but not heterozygous littermate controls developed this phenotype, suggesting that the disease was recessively inherited. Interestingly, the psoriasiform skin inflammation only occurred on PL/J mice carrying the CD18^{hypo} mutation but not on the 129Sv or C57BL/6J genetic background with the same mutation (Bullard *et al.*, 1996). Hence, apart from the CD18 mutation, a small number of genes were necessary to precipitate the phenotype. No infectious agent was detected, whereas corticosteroids suppressed the psoriasiform phenotype, suggesting the involvement of an autoimmune or otherwise (sterile) inflammatory process.

The pathogenic role of β_2 integrins in human psoriasis is poorly understood. CD11b expression has been reported to be reduced on peripheral blood leukocytes derived from psoriasis patients (van Pelt *et al.*, 1998). Further experimental evidence comes from LAD1 patients with a moderately reduced CD18 expression suffering from psoriasiform skin disease (van de Kerkhof and Weemaes, 1990). Linkage analyses of psoriasis families identified a region on chromosome 17 (Tomfohrde *et al.*, 1994), including among other gene loci, intercellular adhesion molecule-2, an important ligand of CD11/CD18 heterodimers. *Vice versa*, polymorphisms in the CD18 gene were found predisposing to autoimmune disease either by leading to a higher ligand affinity or by increasing expression of the CD18 protein (Gencik *et al.*, 2000; Meller *et al.*, 2001). Different cell types have been suspected to be the primary inducer in the pathogenesis of psoriasis (van den Oord and de Wolf-Peeters, 1994; Nickoloff, 1999; Nickoloff and Nestle, 2004; Ozawa and Aiba, 2004). Increasing evidence led to the current view that T cells were the main suspects responsible for its initiation (Nickoloff *et al.*, 2000; Krueger, 2002). Therefore, we first studied different T-cell subpopulations in the skin of $CD18^{hypo}$ psoriasis mice (Kess *et al.*, 2003). Compared to WT mice, a significant increase in $CD4^+$ T cells was found in the epidermis and the dermis of $CD18^{hypo}$ mice compared to WT mice. Most interestingly, effective depletion of $CD4^+$ T cells, as proven by FACS analysis, revealed a complete resolution of the psoriasiform skin disease within 6 weeks of treatment (Figure 4). The effect of the $CD4^+$ T-cell-depleting monoclonal antibodies was evaluated by the severity of clinical symptoms using an adapted murine psoriasis area and severity index (PASI) score (Kess *et al.*, 2003), as commonly used for the assessment of severity in human psoriasis. A complete resolution of the psoriasiform skin disease was observed in two mice (PASI 10 down to 1) and an almost complete resolution in one mouse (PASI 8 down to 2) treated with the anti-CD4 monoclonal antibodies. This clearance of the skin inflammation persisted for an observation period of 6 months after the last administration of depleting antibody, whereas for three further $CD18^{hypo}$ mice mock-treated with IgG isotype antibodies, no significant changes in the PASI scores were evident. As $CD4^+$ T cells can exert their effects via activation of cytotoxic effector functions of $CD8^+$ T cells, $CD18^{hypo}$ mice were also treated with $CD8^+$ T-cell-depleting mAb in an analogous experimental setting (Kess *et al.*, 2003). As monitored by the adapted PASI score, treatment with neither $CD8^+$ T-cell-depleting monoclonal antibodies nor isotype-matched control antibodies resulted in any improvement of the psoriasiform skin disease. These results suggested that $CD8^+$ T cells were not crucial for the pathogenesis of the inflammatory skin disease of this psoriasis-like mouse model. However, apart from $CD4^+$ T cells, a distinct role in the pathogenesis of this skin disease was also attributed to $CD8^+$ T cells by other investigators (Barlow *et al.*, 2004), possibly on the basis of a slightly different segregation of genes during the maintenance/backcrossing of the respective mouse strain (Barlow *et al.*, 2003).

To determine the activation state of T cells in affected mice, $CD90^+$ T cells were isolated from draining lymph nodes of diseased $CD18^{hypo}$ and healthy WT mice (Kess *et al.*, 2003). A significant increase in the expression of CD25 (IL-2Rα chain) as a measure of T cellular activation was detected on $CD4^+$ but not on $CD8^+$ T cells obtained from diseased $CD18^{hypo}$ mice. Because the mean expression of CD25 on $CD4^+$ T cells was twice as high in $CD18^{hypo}$ as in WT mice, this suggested an increased state of activation in $CD4^+$ T cells from $CD18^{hypo}$ mice. To further determine whether T cells obtained from draining lymph nodes had been primed to secrete either Th1- or Th2-type cytokines, we measured the release of IFN-γ or IL-4 after culturing with different concentrations of immobilized anti-CD3 and anti-CD28 monoclonal antibodies. $CD90^+$ T cells from affected $CD18^{hypo}$ mice released up to 40-fold higher amounts of IFN-γ compared to WT mice, whereas no IL-4 could be detected in the supernatants of activated T cells of either genotype. These data pointing at a prevalence of Th1-type cytokines in $CD18^{hypo}$ mice was supported by studies using an extended Th1/Th2 panel for cytometric bead array detection of cytokines (Kess *et al.*, 2003). Apart from IFN-γ, very high levels of the Th1 key cytokine IL-2 were also detected in the supernatants of $CD18^{hypo}$ T cells. However, the Th2 cytokine IL-10 was slightly increased. Still, these data clearly demonstrated the prevalence of Th1 cytokines in T cells isolated from the skin lesion draining lymph nodes of $CD18^{hypo}$ mice.

To address the question whether total absence of CD18 may equally lead to the development of a psoriasiform phenotype in mice of the PL/J strain, a PL/J mouse line with a complete deficiency in CD18 ($CD18^{-/-}$) was generated (Kess *et al.*, 2003). Remarkably, during an observation period of more than 2 years, PL/J $CD18^{-/-}$ mutants ($n = 200$) did not develop any psoriasiform skin disease. Consequently, we hypothesized that the T cells required for the psoriasiform dermatitis of $CD18^{hypo}$ mice may not be able to enter the skin in complete absence of CD18 adhesion molecules, thus being hampered to exert their inflammatory effector functions. As antigen-specific T cells are most likely to play a central role in the pathogenesis of psoriasis, but the responsible antigen could so far not be identified, it is difficult to study the emigration kinetics of antigen-specific T cells. Therefore, we used a model of T-cell-mediated type-IV hypersensitivity, allowing the induction of an allergic contact dermatitis under standardized conditions with oxazolone as a defined antigen (Kess *et al.*, 2003). To differentiate clearly between the deliberately induced allergic contact dermatitis and the spontaneously developing psoriasiform skin inflammation in $CD18^{hypo}$ PL/J mice, we only analyzed clinically healthy $CD18^{hypo}$ mice, comparing these mice with equally treated $CD18^{-/-}$ and WT mice as controls. As a result, allergic contact dermatitis could only be induced in $CD18^{hypo}$ and WT mice but not in $CD18^{-/-}$ mice upon repeated oxazolone challenge. As hematoxylin and eosin and immunostainings of sections from oxazolone-challenged ears revealed, this was owing to a failure of $CD18^{-/-}$ T cells, both $CD4^+$ and $CD8^+$, to emigrate from the vessels into the tissue, thus causally contributing to the unresponsiveness of

$CD18^{-/-}$ mutants to oxazolone. Interestingly, T cells isolated from the draining lymph nodes of sensitized $CD18^{-/-}$ mice could rescue the ear swelling phenotype in response to oxazolone challenge when directly injected in the ears of $CD18^{-/-}$ mice before the re-challenge with oxazolone, suggesting that CD18 was not absolutely required for priming of naïve T cells but was indispensable for T-cell extravasation (Grabbe *et al.*, 2002). Hence, 2–16% of CD18 gene expression proved to be sufficient for oxazolone-specific T cells to emigrate from blood vessels during allergic contact dermatitis (Kess *et al.*, 2003). Given that in psoriasis vulgaris specific T cells are most likely directed against an, to date, undefined epidermal antigen, our finding that T cells cannot efficiently extravasate into the skin in $CD18^{-/-}$ mutants may explain the absence of a psoriasiform phenotype in these mice. These findings support the conclusion that the pathogenic involvement of $CD4^+$ T cells in the skin disorder of the $CD18^{hypo}$ PL/J mice depends on a gene-dose effect of CD18 expression.

C57BL/6 or 129Sv mice with the identical $CD18^{hypo}$ mutation leading to the psoriasiform dermatitis in PL/J mice do not develop an inflammatory skin disease (Bullard *et al.*, 1996; Barlow *et al.*, 2003; Kess *et al.*, 2003). These data indicate that apart from the $CD18^{hypo}$ mutation, other gene loci are responsible for the development and the onset of this inflammatory skin disease. In order to further identify involved chromosome fragments, backcross analysis between the susceptible PL/J strain and the resistant C57BL/6 strain was performed. This cross resulted in an F1 generation with no obvious phenotype. When crossing back mice of the F1 generation to the susceptible $CD18^{hypo}$ PL/J mice, 30% of the resulting N2 generation revealed a psoriasiform phenotype, suggesting that apart from the $CD18^{hypo}$ mutation other modifier genes were required for the development of this phenotype (Bullard *et al.*, 1996; Barlow *et al.*, 2003). In order to analyze the critical chromosome fragments of the two mouse strains involved in the precipitation of the phenotype, a genome-wide microsatellite analysis was performed from genomic DNA of the N2 backcross generation. Collectively, the assessment of the LOD scores for the linkage of specific chromosome regions with the disease revealed that, in addition to the CD18 mutation, two genes were found to determine the susceptibility and four or more genes determine the severity of the psoriasiform dermatitis in $CD18^{hypo}$ PL/J mice. Most prominent linkage (LOD score >5) was found for gene loci on chromosome 10, revealing a strong linkage for the incidence of the psoriasiform dermatitis, and on chromosome 6 for the time point of onset of the disease. In addition, female mice were observed to be more susceptible for the psoriasiform skin inflammation than male mice (data submitted for publication). Currently, the generation of congenic strains and positional cloning is now underway to finally identify the genes involved in the psoriasiform skin disease and their potential interaction with CD18.

The herein summarized data on the role of β_2 integrins in a murine model of psoriasis in $CD18^{hypo}$ PL/J mice may further support the conception of T cell-dependent skin inflammation and have relevance also for the understanding of polygenic human inflammatory skin diseases.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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