CD4⁺ T Cell-Associated Pathobiology Critically Depends on CD18 Gene Dose Effects in a Murine Model of Psoriasis

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In a CD18 hypomorphic polygenic PL/J mouse model, the severe reduction of CD18 (β₂ integrin) to 2–16% of wild-type levels leads to the development of a psoriasiform skin disease. In this study, we analyzed the influence of reduced CD18 gene expression on T cell function, and its contribution to the pathogenesis of this disease. Both CD4⁺ and CD8⁺ T cells were significantly increased in the skin of affected CD18 hypomorphic mice. But only depletion of CD4⁺ T cells, and not the removal of CD8⁺ T cells, resulted in a complete clearance of the psoriasiform dermatitis. This indicates a central role of CD4⁺ T cells in the pathogenesis of this disorder, further supported by the detection of several Th1-like cytokines released predominantly by CD4⁺ T cells. In contrast to the CD18 hypomorphic mice, CD18 null mutants of the same strain did not develop the psoriasiform dermatitis. This is in part due to a lack of T cell emigration from dermal blood vessels, as experimental allergic contact dermatitis could be induced in CD18 hypomorphic and wild-type mice, but not in CD18 null mutants. Hence, 2–16% of CD18 gene expression is obviously sufficient for T cell emigration driving the inflammatory phenotype in CD18 hypomorphic mice. Our data suggest that the pathogenic involvement of CD4⁺ T cells depends on a gene dose effect with a reduced expression of the CD18 protein in PL/J mice. This murine inflammatory skin model may also have relevance for human polygenic inflammatory diseases. The Journal of Immunology, 2003, 171: 5697–5706.
Peripheral blood leukocytes derived from psoriasis patients (40, 41). Further circumstantial evidence indicating that reduced CD18 expression may causally be involved in the development of the psoriasisform dermatitis comes from the clinical observation that some LAD1 patients with only moderately reduced CD18 expression levels develop a psoriasiform dermatitis (42). Linkage analysis of psoriasis families has identified a region on chromosome 17, including, among other gene loci, ICAM-2, an important ligand of CD18 heterodimers (43). Vice versa, polymorphisms in the CD18 gene have been found predisposing to autoimmune disease either by leading to a higher ligand affinity or by increasing expression of the CD18 protein (44, 45).

Because to date only correlative evidence exists, we have set out to study the causal role of a stepwise reduction in CD18 expression on distinct T cell populations in the generation and maintenance of the psoriasiform dermatitis in PL/J mice. In contrast to CD18null mice, CD18hyp mice did not develop the psoriasiform dermatitis due to a deficient emigration of T cells into the skin. However, the residual expression of CD18 in CD18hyp mice was sufficient for T cells to extravasate. We furthermore demonstrate that the psoriasiform skin disorder in CD18hyp mice resembles human psoriasis, revealing key features such as causal involvement of CD4 lymphocytes and a prevalence of Th1 cells. Our data point to a central role of CD4 lymphocytes and Th1 cells.

Materials and Methods

Mice

Mice with a hypomorphic mutation of the CD18 gene (CD18hyp) on the PL/J inbred strain were examined (22). Additionally, mice with a CD18null mutation (CD18null) (17) were backcrossed seven generations onto the PL/J strain. CD18hypo PL/J mice were used as wild-type controls. Unless otherwise stated, all CD18hypo mice older than 6 wk. All experiments were done in compliance with the German Law for Welfare of Laboratory Animals.

Monoclonal Abs

The following mAbs were purchased from BD PharMingen or BD Biosciences (both in Heidelberg, Germany); respectively: mouse (m)CD4 (GK1.5, unconjugated, NA/LE), mCD8α (53-6.7, unconjugated, NA/LE), mIgG2a (R35-95, NA/LE), mIgG2b (A95-1, NA/LE), mCD3 (17A2), mCD8α (53-6.7, unconjugated, NA/LE), mCD25 FITC (7D4), mCD44 PE (C71/16), mCD18 PE (C71/16), mCD3 CyChrome (145-2C11), rat IgG2a FITC, PE, APC (RG7/1.30), rat IgG2b FITC, PE, APC (RG7/11.1), IL-2 PE (JES5-16E3), IL-4 PE (11B11), IL-6 PE (MP5-20F3), IL-10 PE (JES5-16E3), IL-12 PE (C15.6), IFN-γ PE (XMG 1.2), and TNF-α PE (MP6-XT22). mCD90 MACS (Thy-1.2) magnetic microbeads were from Miltenyi Biotec (Bergisch Gladbach, Germany).

Immunohistochemical analysis

Cyosections were prepared and stained immunohistochemically using an indirect immunoperoxidase assay, as described elsewhere (46). For detection of unbiotinylated Abs, goat F(ab′)2 anti-rat IgG and goat F(ab′)2 anti-rabbit IgG conjugated with peroxidase (Dianova, Hamburg, Germany) were used as secondary Abs, and 3-amin-9-ethyl-carbazol served as chromogen. In cases in which assay sensitivity needed to be increased, a biotinylated secondary rabbit anti-rat Ig mAb and the StreptABCComplex/AP kit were used in combination with the fast red substrate system (all DAKO, Glostrup, Denmark). Primary, secondary, and isotype control Abs were diluted in PBS with 1% BSA to maximal concentration of 1.5 μg/ml.

Microscopic evaluation was performed by counting cells in the dermis, and by relating the number of positively stained cells to the total number of cells per area, defined by a grid ocular. To quantify cells in the epidermis, the number of positively stained cells was counted at predetermined. For all measurements, the median of 30 evaluations (n = 3) is presented. The statistical significance was calculated using the Mann-Whitney U test. All countings were done by two independent observers.

In vivo depletion of T cells

To deplete T cells, mice were injected i.p. with 100–150 μg mCD4 (GK1.5) or mCD8 (53-6.7) mAb for 3 consecutive days. Subsequently, injections were performed every 3 days for a period of 45 or 33 days, respectively. Control mice suffering from similarly severe psoriasiform dermatitis were treated with isotype IgG mAb in an identical time schedule. Depletion efficiency was monitored by FACS analysis of PBMC at different time points during treatment, and by immunostainings of skin biopsies obtained at the end of the treatment period. Before and after treatment, disease severity was determined by measurement of ear thicknesses as a rough indication for skin inflammation, by assessment of the clinical picture using an adapted psoriasis activity and severity index (PSAI) score, and recorded by photography (Dental-Eye II; Yashica, Hamburg, Germany).

FACS analysis

A total of 150–400 μl peripheral blood collected from the tail vein of mice was mixed with heparin (Liquemin N 25000; Hoffmann-LaRoche, Grenz-Wyhlen, Germany) to prevent coagulation. Alternatively, cells were obtained from spleens of mice. RBC were removed using ammonium chloride potassium carbonate lysis buffer (0.15 M NaCl, 1.0 M KHCO3, 0.1 M Na2EDTA, pH 7.2). The remaining PBMC cell fraction was adjusted to 1 × 106 cells/50 μl. Subsequently, 50 μl of the cell suspension was stained with ≥ 1 μl of the fluorochrome-conjugated mAbs for 30 min at 4°C. After fixation of cells in 2% formaldehyde (Merck, West Point, PA), stained PBMC were analyzed using a FACSCalibur (BD Biosciences).

To determine cytokine production by T cells, intracellular cytokine staining was performed. CD90+ T cells were isolated from draining lymph nodes of CD18hyp and CD18null mice by magnetic cell sorting (MiniMACS columns; Miltenyi Biotec) and cultured in RPMI 1640 medium (Life Technologies, Paisley, Scotland) supplemented with 10% FCS and 4 μg/ml Ciprobay 2000 (Bayer, Leverkusen, Germany), in the presence of 3 ng/ml PMA and 300 ng/ml ionomycin (both Sigma-Aldrich, Taufkirchen, Germany), at 37°C and 5% CO2, either overnight or for 7 days. To inhibit secretion of cytokines by the cells, 1 μg/ml brefeldin A (Sigma-Aldrich) was added and cells were incubated for 4 h at 37°C, 5% CO2. A total of 1 × 106 cells was used per staining. Cells were washed twice with 1 ml PBS (1% FCS). Cells were resuspended in 50 μl PBS (1% FCS), and surface staining was performed with the indicated Abs for 30 min at 4°C. Afterward, cells were washed twice with 1 ml PBS (1% FCS), and subsequently fixed with 1% paraformaldehyde (Merck, Darmstadt, Germany) for 15 min at room temperature. Cells were washed twice and then permeabilized in 50 μl 1% saponin (Sigma-Aldrich) for 5 min at room temperature, before fixed cytokine Abs (1:10) were added to the samples (30 min, 4°C). After two more washes with 1 ml PBS (1% FCS), cells were resuspended in 500 μl PBS/1% paraformaldehyde. Subsequently, cells were analyzed by flow cytometry using a FACSCalibur.

To determine cell surface expression of the IL-2Rα on T cells for assessment of cellular activation, CD90+ cells were isolated and cultured overnight, as described above. A total of 1 × 105 T cells per mouse was then analyzed by FACS using triple fluorescence staining (mCD23 FITC/mCD4 PE/mCD3 CyChrome) with ≥ 1 μl of each fluorochrome-conjugated mAb.

Cytokine release

To determine release of cytokines by T cells, CD90+ cells were isolated, as described above. Cells (2 × 105 per well) were then activated by incubation on immobilized anti-CD3 mAb and anti-CD28 mAb at the indicated concentrations in 96-well plates. After incubation for 24 h at 37°C, the supernatants were collected and frozen down at −20°C. Cytokine concentrations of IFN-γ and IL-4 were measured using Quantikine M ELISA (R&D Systems, Wiesbaden-Nordenstadt, Germany), according to the distributed protocols.

Alternatively, the indicated cytokines were determined in the supernatants of CD90+ T cells cultured in RPMI 1640 medium supplemented with 10% FCS and 4 μg/ml Ciprobay 2000, in the presence of the 3 ng/ml PMA and 300 ng/ml ionomycin, at 37°C and 5% CO2 overnight, using the Cytokine Bead Array technique (BD Mouse Inflammation CBA, BD Biosciences).
CBA; BD Biosciences). All staining and analysis were done without modification, according to the manufacturer’s instructions.

**Allergic contact dermatitis**

Allergic contact dermatitis experiments were performed, as described previously (47, 48). Briefly, mice were sensitized by painting 100 μl of 2% oxazolone (Sigma-Aldrich) in acetone/olive oil (4:1) onto the shaved abdomen. After 5 days, 10 μl of 0.5% oxazolone was applied to both sides of the ears. Ear thickness was measured before, and 3, 6, 30, and 42 h after challenge using a calibrated caliper (The Dyer Company, Lancaster, PA). Ear swelling was calculated by subtracting the ear thickness before challenge from ear thickness after challenge.

**Results**

FACS profiles of β2 integrin α subunits sharing the mutated β2 subunit (CD18<sup>hypo</sup>) differ quantitatively from those of CD18<sup>wt</sup> leukocytes

To study whether the reduced expression of the CD18<sup>hypo</sup> protein on the cell surface of leukocytes in the CD18<sup>hypo</sup> psoriasiform mouse model may result in differential capacity of CD18 to associate with the four known β2-associated α subunits, FACS analysis using CD11 (α-chain) subunit-specific mAbs was performed for CD4<sup>+</sup> T cells and overall PBMC isolated from CD18<sup>hypo</sup> and CD18<sup>wt</sup> mice. The cell surface expression of the three major α subunits, CD11a, CD11b, and CD11c, revealed a similar relative distribution as compared with the relative distribution of the α-chains on CD18<sup>wt</sup> leukocytes. However, the protein quantities of the three different α-chains were substantially reduced on the cell surfaces of CD18<sup>hypo</sup> leukocytes compared with CD18<sup>wt</sup> leukocytes. This was in parallel to the well-established reduction of CD18 molecules underlining that the β2 integrin deficiency, primarily originating from the β2 subunit (CD18), leads to a secondary reduction of the three major α-chains on CD4<sup>+</sup> T cells (Fig. 1B) as well as on overall PBMC (Fig. 1A). Evidence for a defective association between β2 integrin α- and β2-chains potentially caused by a qualitative alteration in the CD18<sup>hypo</sup> molecule was not supported by our data, as a deficiency in dimerization will normally lead to internalization of either subunit and, thereby, to a loss of cell surface expression (49, 50).

**T cells are highly increased in psoriatic lesions of CD18<sup>hypo</sup> mice**

To study the composition of the inflammatory infiltrate of the psoriasiform dermatitis, 30 skin sections taken from CD18<sup>hypo</sup> and CD18<sup>wt</sup> mice (n = 3) were immunostained with anti-CD4 and anti-CD8 mAbs (Fig. 2). Compared with CD18<sup>wt</sup> skin with only a few CD4<sup>+</sup> T cells in the dermis (Fig. 2A), the number of CD4<sup>+</sup> and CD8<sup>+</sup> T cells was highly increased, both in the epidermis and dermis of CD18<sup>hypo</sup> mice (Fig. 2B). Statistic evaluation revealed that this increase was highly significant for both cell types (p < 0.0001, Fig. 2C).

**Depletion of CD4<sup>+</sup> T cells results in resolution of psoriatic lesions**

To analyze the potential role of T cells in the pathogenesis of the psoriasiform dermatitis, both CD4<sup>+</sup> and CD8<sup>+</sup> T cells were removed in vivo in CD18<sup>hypo</sup> mice using depleting mAbs. Depletion of CD4<sup>+</sup> T cells in CD18<sup>hypo</sup> mice with a severe psoriasiform phenotype including extensive scaling and alopecia (n = 3, Fig. 3A) led to a complete resolution of the psoriasiform dermatitis after 6 wk of treatment (Fig. 3B), while skin lesions in mice treated with the isotype control mAb remained unchanged (data not shown). Mice treated with an isotype control mAb showed a prominent CD4<sup>+</sup> T cell population (Fig. 3C). By contrast, i.p. administration of the anti-CD4 mAb resulted in an almost complete removal of CD4<sup>+</sup> T cells from the blood circulation (Fig. 3D). Depletion of CD4<sup>+</sup> T cells was further confirmed by immunohistochemistry of the skin. Administration of an isotype-matched control mAb did not reveal any effect on numbers of CD4<sup>+</sup> T cells in the psoriasiform skin lesions (Fig. 3E). By contrast, removal of CD4<sup>+</sup> T cells from the skin was almost complete when anti-CD4 mAbs were injected (Fig. 3F).

The effect of the CD4<sup>+</sup> T cell-depleting mAbs was evaluated by the severity of clinical symptoms using an adapted PASI score as used for assessment of the severity of human psoriasis elsewhere. For CD18<sup>hypo</sup> mice, the PASI score was modified accordingly: 0 =
no symptoms; 1 = slight erythema of the ears; 2 = strong erythema of the ears; 3 = slight hair loss at the head; 4 = extensive hair loss including the trunk; 5 = slight hair loss, isolated scaling; 6 = extensive hair loss, widespread slight scaling; 7 = extensive hair loss, widespread hair loss, strong scaling at few, smaller areas; 10 = extensive hair loss, extensive scaling at a large area of the body. A complete resolution of the psoriasiform dermatitis was observed in two (10 to 1), and an almost complete resolution in one animal (8 to 2) treated with anti-CD4 mAbs. This clearance of the dermatitis persisted for an observation period of 6 mo after the last anti-CD4 mAb injection. For the three CD18hypo mice treated with isotype IgG mAbs, no significant changes in the PASI scores were evident (Fig. 4A).

Similar results were obtained by the determination of ear thicknesses before and after treatment as a clinical assessment of the severity of skin inflammation. In mice treated with anti-CD4 mAbs, ear thicknesses substantially decreased, suggesting a reduced inflammation, while ear thicknesses remained unchanged in the control mice (data not shown).

Because CD4+ T cells can exert their effects via activation of cytotoxic effector functions of CD8+ T cells, CD18hypo mice were also treated with CD8+ T cell-depleting mAbs in an analogous experimental setting. As monitored by the PASI score, treatment with CD8+ T cell-depleting mAbs, or isotype-matched control mAbs, did not result in any improvement of the psoriasiform dermatitis (Fig. 4B). This was accompanied by an ongoing increase in ear thicknesses before and after treatment, indicating the persistence of skin inflammation (data not shown). Even though no clinical improvements were observed, immunohistochemistry showed that CD8+ T cells had been successfully depleted from the skin of mice, confirming that sufficient amounts of CD8-depleting mAbs had been administered during the time of treatment. This was further supported by FACS analysis, showing that CD8+ T cells were drastically reduced in the peripheral blood of these mice as compared with mice treated with isotype-matched mAbs. The latter still had a prominent CD8+ T cell population (data not shown). These results suggest that CD8+ T cells are not crucial for the pathogenesis of the dermatitis of this psoriatic mouse model.

Activated T cells with a bias toward Th1 cytokines prevail in CD18hypo mice

To determine the ex vivo activation state of T cells, CD90+ T cells were isolated from draining lymph nodes of CD18hypo and CD18wt mice. A significant increase in the expression of CD25 (IL-2Rα) as a measure of T cellular activation was detected on CD4+, but not on CD8+ T cells obtained from CD18hypo mice (Fig. 5). Because the mean expression of CD25 on CD4+ T cells was twice as high in CD18hypo as in CD18wt mice, this suggested an increased state of activation in CD4+ T cells from CD18hypo mice.
To 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 mAbs. CD90+/H11001 T cells from CD18hypo mice released up to 40-fold higher concentrations of IFN-γ compared with CD18wt mice (Fig. 6A), while no IL-4 could be detected in the supernatants of activated T cells of either group (data not shown).

Because these initial results pointed at a prevalence of Th1 cells in CD18hypo mice, a wider range of Th1/Th2 key cytokines released was screened. Cytometric bead arrays (CBA) were used to detect cytokines in the supernatants of overnight-cultured T cells, and flow cytometry was used to measure intracellular cytokines. As shown in Fig. 6B, highly increased concentrations of the Th1-type cytokine IFN-γ were measured in the supernatants of CD18hypo T cells using the CBA, whereas also with this method, the Th2 cytokine IL-4 was near the lower detection limit, thereby confirming our previous results obtained by ELISA. Besides IFN-γ, very high levels of the Th1 key cytokine IL-2 also were detected in the supernatants of CD18hypo T cells. However, the Th2 cytokine IL-10 also was slightly increased. Still, these data clearly demonstrate the prevalence of Th1 cytokines in T cells isolated from the skin lesion-draining lymph nodes of CD18hypo mice. In comparison, supernatants of CD18wt T cells neither showed increased amounts of the Th1- nor of the Th2-type cytokines tested.

These results were further supported by intracellular cytokine staining of the isolated T cells. For all Th1-type cytokines measured, the increase in cytokine-producing CD4+ T cells from CD18hypo mice was >2-fold, when compared with the wild type (Fig. 7A). In contrast to CBA analyses of the supernatants, a pronounced increase in intracellular IL-12 was found. T cells producing Th2-like cytokines were only slightly increased (Fig. 7B).

CD18null mice do not reveal any psoriasiform phenotype

To investigate 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 complete CD18 deficiency (CD18null) was generated. Interestingly, PL/J CD18null mutants (n = 200) did not develop any psoriasiform skin disease during an observation period of more than 2 years.

An allergic contact dermatitis can be induced in CD18wt and CD18hypo, but not in CD18null mice

Because Ag-specific T cells are most likely to play a central role in the pathogenesis of psoriasis, but the responsible Ag could not yet be identified, it is difficult to study the emigration kinetics of Ag-specific T cells. Therefore, we have used a model, which allows inducing of an allergic contact dermatitis, a T cell-mediated
hypersensitivity reaction of type IV, as classified by Coombs and Gell, under standardized conditions using oxazolone as a defined Ag. Because the absence of a psoriasiform phenotype in CD18null mutants may be due to a lack of T cell emigration from blood vessels (20), we compared the T cell-dependent inflammatory response of CD18wt, CD18hypo, and CD18null mice on the PL/J strain after induction of an allergic contact dermatitis. To clearly differentiate between the induced allergic contact dermatitis and the spontaneously developing psoriasiform dermatitis in CD18hypo PL/J mice, we only analyzed clinically healthy CD18hypo mice in which the lateron evolving psoriasiform dermatitis was not yet present, and compared these mice with equally treated CD18wt and fully CD18-deficient (CD18null) mice as controls. Upon repeated oxazolone challenge, allergic contact dermatitis could be induced in CD18hypo and CD18wt mice, but not in CD18null mutants (Fig. 8). No significant difference in ear swelling was detectable between CD18hypo and CD18wt mice after 30 h (p = 0.132) or 42 h (p = 0.1, Mann-Whitney U test). However, onset of ear swelling was delayed in CD18hypo mice, and ear thicknesses were further increased after 42 h. H&E stainings (Fig. 9A) displayed a prominent perivascular and diffuse infiltration of inflammatory cells as well as spongiosis of the epidermis in CD18hypo and CD18wt mice, indicating a strong allergic response to oxazolone. By contrast, only a slight edema without any inflammatory cells was detected in CD18null mutants. To determine whether failure of T cells to emigrate from the vessels into the tissue causally contributed to the unresponsiveness of CD18null mutants to oxazolone challenge, immunostainings with mAbs against CD4 (Fig. 9B) and CD8 (Fig. 9C) were performed of sections derived from oxazolone-challenged ears. Both CD18wt and CD18hypo mice showed a clear increase in CD4+ and CD8+ T cells in the tissue, while virtually no

T cells were observed in the oxazolone-challenged ears of CD18null mice. Hence, 2–16% of CD18 gene expression apparently was sufficient for oxazolone-specific T cells to emigrate from blood vessels during the allergic contact dermatitis. Given the fact that in psoriasis vulgaris specific T cells also are directed against a to date unidentified epidermal Ag (51, 52), our finding that T cells cannot efficiently extravasate into the skin in CD18null mutants may explain the absence of a psoriasiform phenotype in these mice. Our findings support the conclusion that the pathogenic involvement of CD4+ T cells in the skin disorder of the CD18hypo PL/J mice depends on a gene dose effect of CD18 expression.

Discussion

In this study, we demonstrate that for the murine psoriasiform dermatitis found in CD18hypo PL/J mice CD4+ T cells, but not CD8+ T cells, are mandatory. We further reveal that: 1) CD4+ T cells from skin-draining lymph nodes reveal an altered activation pattern and an increased production of the Th1 key cytokines IL-2, IL-12, and IFN-γ; 2) the potential of CD4+ T cells to elicit murine psoriasis needs to be linked to the hypomorphic expression of the CD18 gene between 2 and 16% of normal expression levels; and 3) at
least one other allele present in PL/J mice, but not in C57BL/6 mice, is required in addition to the CD18 hypomorphic mutation (22).

We thus evolved two major principles for murine psoriasis that have direct relevance for human psoriasis: first, the necessity of CD4\(^+\) cells, but not CD8\(^+\) T cells is consistent with those concepts on human psoriasis, which assign a mandatory role to CD4\(^+\) cells (53, 54) and Th1 cells (55, 56), while our findings do not support concepts, which include CD8\(^+\) T cells among the decisive constituents for psoriasis (57, 58). Second, the polygenic nature of murine psoriasis including reduced gene dose for CD18 is consistent with observations that reduced CD18 expression is one feature in human psoriasis (40–42). Thus, our murine psoriasis model is the first polygenic model for psoriasis. It has already partially unraveled gene dose effects of CD18 and it will allow identification of further involved genes.

In accordance with the current view that psoriasis is a T cell-mediated immunological disease (27), T cells are crucial for the generation and maintenance of the skin disease in this mouse model. We show that highly increased numbers of CD4\(^+\) and CD8\(^+\) T cells reside in the skin of CD18\(^{hypo}\) PL/J mice, a hallmark also in human psoriasis (53, 59). The higher number of CD4\(^+\) compared with CD8\(^+\) T cells in the skin of CD18\(^{hypo}\) mice with severe psoriasiform phenotype provides first evidence that CD4\(^+\) T cells were prepared as described for the CBA cytokine detection (n = 2). This time, T cells were cultured in the presence of 3 ng/ml PMA and 300 ng/ml ionomycin overnight before supernatants were obtained for CBA.

FIGURE 7. Detection of intracellular Th1/Th2 cytokine expression patterns in T cells from CD18\(^{hypo}\) (filled bars) and CD18\(^{wt}\) (open bars) mice. A, Concentration of IFN-\(\gamma\) in supernatants of CD90\(^+\) T cells isolated from skin-draining lymph nodes of CD18\(^{hypo}\) and CD18\(^{wt}\) mice 24 h after stimulation with different concentrations of mCD3 and mCD28 mAbs (n = 3), as detected by ELISA. *, p < 0.05 for CD18\(^{hypo}\) vs CD18\(^{wt}\) T cells. B, Simultaneous measurement of the indicated cytokines in supernatant CD90\(^+\) T cells by CBA (n = 2). T cells were cultured in the presence of 3 ng/ml PMA and 300 ng/ml ionomycin overnight before supernatants were obtained for CBA.
T cells are important in the generation and maintenance of the skin disease in this psoriasis model. This is in analogy to affected skin of psoriasis patients in which CD4^+ T cells prevail (29, 54, 59). In CD18^hypo^ PL/J mice, depletion of CD4^+ T cells, but not CD8^+ T cells, results in the complete resolution of the skin disease. This is also consistent with human psoriasis, as treatment with Abs directed against CD4 cures psoriatic lesions or significantly decreases the PASI score (29–31). In psoriatic CD18^hypo^ PL/J mice, only CD4^+^ T cells, but not CD8^+^ T cells from skin-draining lymph nodes present signs of activation, such as enhanced IL-2Rα expression in CD4^+^, but not in CD8^+^ T cells. Thus, our results point out at a central role of CD4^+^ T cells in the pathogenesis of the psoriasiform skin disorder of CD18^hypo^ PL/J mice.

This is in line with the hypotheses of Thivolet and Nicolas (54) and Valdimarsson et al. (53), who consider CD4^+^ T cells to be the key players in the pathogenesis of psoriasis, while Prinz (57) and Nickoloff (58) favor a cooperation between CD4^+^ and CD8^+^ T cells responsible for the development of psoriatic plaques. Our findings show that only CD4^+^ and not a cooperation between CD4^+^ and CD8^+^ T cells is required to sustain the psoriasiform disease.

The type of T cell effectors identified in the murine CD18^hypo^ PL/J model corresponds to that of human psoriasis, which is a Th1 disease (55, 56). Compared with CD18^wt^ mice, stimulated T cells from affected CD18^hypo^ mice released up to 40-fold higher concentrations of the Th1 cytokine IFN-γ, and also produced considerable amounts of IL-2 and IL-12, whereas characteristic Th2 cytokines were not detected, apart from low levels of IL-10. This points to a prevalence of Th1 cells in the psoriasiform dermatitis of CD18^hypo^ mice. The skewing of the cytokine pattern from the Th1 to the Th2 type recently proved to be an effective therapeutic strategy in psoriasis patients. In fact, treatment with Th2 cytokines such as IL-4, IL-10, or IL-11 or blocking of Th1 cytokines by anti-IFN-γ mAbs has been successfully tested in clinical trials (60–63).

Our major finding, however, is that this T cell-mediated psoriatic skin disease in PL/J mice most likely depends on the CD18 gene dose and subsequent expression levels of CD18 protein, with no inflammatory disease in CD18null and CD18^wt^ mice. This conclusion is at least partly supported by our findings that the expression levels of all four possible α subunits differed only in quantity, according to the reduction in total levels of β2 integrin heterodimers, when compared with CD18 wild-type leukocytes; and also by previously published results derived from studies on patients suffering from LAD1 (49, 50, 64, 65). These authors stated that deficiency in β2-integrin heterodimers appears to be quantitative rather than qualitative, with two patients expressing ~0.5% and one patient 5% of normal amounts of CD18. The latter patients had α/β complexes on the cell surface of their leukocytes, as detectable by immunoprecipitation with reduced absolute numbers, but similar ratios compared with healthy controls. However, our data do not allow us to completely exclude the possibility that the CD18^hypo^ gene product may exhibit qualitative differences to CD18 wild-type proteins.

Results from our studies of allergic contact dermatitis show that residual CD18 expression is distinctly required for the extravasation of reactive T cells, while in CD18null PL/J mutants, Ag-specific T cells cannot emigrate from the blood vessels. Psoriasis is regarded to be an autoimmune disease (53, 57, 59). This implicates that T cells recognize self Ags in great numbers, and may subsequently fail to initiate a relevant inflammatory response. If T cells are severely impaired to enter the skin, as is the case in the CD18null mutation, they do not have access to specific self Ags in great numbers, and may subsequently fail to initiate a relevant inflammatory response. In fact, after induction of an allergic contact dermatitis, no T cells were observed in the ears of CD18null mice, pointing to a lack of T cell emigration from blood vessels. This has already been shown in CD18null mutants of a different genetic background (129/Sv × C57BL/6J), suggesting that the failure of T cell emigration rather depends on the CD18 deficiency and not on the genetic background (20). This may explain at least in part the absence of a psoriasiform phenotype in PL/J CD18null mutants. By contrast, in CD18^hypo^ PL/J mice, highly increased numbers of CD4^+^ and CD8^+^ T cells, comparable to CD18^wt^ littermates, were present in the skin after induction of an allergic contact dermatitis indicating that a CD18 rest expression of 2–16% is sufficient for the emigration of T cells from skin vessels into the tissue. Our findings are in line with results of a study on 31 psoriasis patients who had been
treated with different doses of a humanized mAb against CD11a, a part of the heterodimeric receptor LFA-1 (CD11a/CD18). Clinical resolution of psoriasis could only be achieved when saturating concentrations of the mAb against CD11a were applied, while nonsaturating concentrations were ineffective (39). The authors did not give any explanation for the observed dose-dependent effect. In this study, we provide direct evidence that the complete prevention of T cell emigration requires a complete structural or functional absence of CD18 heterodimers.

Interestingly, in CD18null mice with unaffected T cell emigration, no psoriasiform dermatitis develops, suggesting that apart from the ability of proper emigration of T cells, additional pathogenic factors depending on reduced CD18 expression are mandatory for the development of the murine psoriasis. Among several possibilities, reduced CD18 expression may cause the generation and persistence of autoreactive T cells, e.g., by impairing normal deletion in the thymus.

In fact, β2 integrins have been reported to be involved in thymic T cell development and selection. LFA-1 (CD11a/CD18) mediates differentiation from CD4+CD8+ to CD4+CD8+ thymocytes (66) and plays a central role in the regulation of apoptosis during negative selection of autoreactive thymocytes (67, 68). The role of CD18 in lymphocyte activation has been characterized in secondary lymphoid organs. During immune responses, β2 integrins are crucial for the adjustment of Ag-dependent activation thresholds. In the absence of CD18, a 100-fold increase in Ag concentrations is required for efficient T cell activation (16). This may in part be caused by defects in structures, such as immunologic synapses in CD18null mice.4 Similar structural defects may also account for functional deficiencies in primary lymphoid organs such as the thymus, if CD18 is reduced or absent.

Other animal models with similarity to psoriasis have been described and include the mouse mutations flaky skin, chronic proliferative dermatitis, transgenic ILA-B27 rats (69), graft-vs-host disease due to differences in the minor histocompatibility Ags (70), epidermal dysregulation of NF-kB-mediated signaling (71), transgenic β2 integrin overexpression in the murine suprabasal epidermis (72), and transplantation of human psoriasis-affected skin onto SCID mice (73). All these models reveal some similarities to human psoriasis both in terms of the clinical and histological picture and various aspects of its pathogenesis. Two models even mimic the autoreactive nature of T cells in psoriasis (70, 73). By contrast to all models, the disease model described in this work is of particular interest in that one relevant mutation resulting in reduced CD18 expression is known, and it is feasible to identify major modifier genes and their impact on thymic selection or other tolerance-maintaining processes. In this study, we provide first direct evidence that reduced CD18 expression is distinctly involved in the pathogenesis of a psoriasiform skin disease. Gene dose effects in other cell surface receptors crucial in the control of immune cell interaction such as CD40 (74) and CD19 (75) have recently been described to promote the development of autoimmune disorders. In conclusion, the CD18−/− PL/J mouse model represents a valuable tool for future investigations in the pathogenesis of psoriasis, and should help to clarify the role of a CD18 rest expression of 2–16% in the development and maintenance of the psoriasiform phenotype.

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