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Reduction of CD18 Promotes Expansion of Inflammatory γδ T Cells Collaborating with CD4+ T Cells in Chronic Murine Psoriasiform Dermatitis

Martina Gatzka,* Adelheid Hainzl,* Thorsten Peters,* Kamayani Singh,* Alpaslan Tasdogan,† Meinhard Wlaschek,* and Karin Scharffetter-Kochanek*

IL-17 is a critical factor in the pathogenesis of psoriasis and other inflammatory diseases. The impact of γδ T cells, accounting for an important source of IL-17 in acute murine IL-23– and imiquimod-induced skin inflammation, in human psoriasis is still unclear. Using the polygenic CD18hyp PL/J psoriasis mouse model spontaneously developing chronic psoriasiform dermatitis due to reduced CD18/β2 integrin expression to 2–16% of wild-type levels, we investigated in this study the influence of adhesion molecule expression on generation of inflammatory γδ T cells and analyzed the occurrence of IL-17–producing γδ and CD4+ T cells at different disease stages. Severity of CD18hyp PL/J psoriasiform dermatitis correlated with a loss of skin-resident VγT cells and concurrent skin infiltration with IL-17+, IL-22+, and TNF-α+ γδ TCRlow cells preceded by increases in Vγ4+ T cells in local lymph nodes. In vitro, reduced CD18 levels promoted expansion of inflammatory memory-type γδT cells in response to IL-7. Similar to IL-17 or IL-23/p19 depletion, injection of diseased CD18hyp PL/J mice with anti-γδTCR Abs significantly reduced skin inflammation and largely eliminated pathological γδ and CD4+ T cells. Moreover, CD18hyp γδ T cells induced allogeneic CD4+ T cell responses more potently than CD18+ counterparts and, upon adoptive transfer, triggered psoriasiform dermatitis in susceptible hosts. These results demonstrate a novel function of reduced CD18 levels in generation of pathological γδ T cells that was confirmed by detection of increases in CD18low γδ T cells in psoriasis patients and may also have implications for other inflammatory diseases.

Psoriasis is a common, chronic relapsing inflammatory disorder of the skin and joints with a prevalence of 2 to 3% in the worldwide population, characterized by skin infiltration with leukocytes, epidermal hyperproliferation, and the risk for development of a destructive arthritis (psoriatic arthritis) (reviewed in Refs. 1–3). Similar to other T cell–mediated autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease, Th1 and Th17 cell subsets critically contribute to the pathogenesis of psoriasis (4–7). Th17 cells represent inflammatory Th cells characterized by production of IL-17A, IL-17F, IL-21, and IL-22 and expression of the transcription factors (TFs) retinoic acid–related orphan receptor (ROR) α and γ, which expand in response to IL-23 and promote pathogenesis and autoimmunity (8–12). The critical role of IL-17 in skin inflammation could be confirmed in mouse models and human psoriasis patients and is reflected by the therapeutic efficacy of mAbs against the common p40 subunit of IL-12 and IL-23 (13, 14), such as ustekinumab, targeting IL-17–producing cells as well as Th1 cells, or selective Abs against either IL-17 or IL-23/p19 (15, 16), for the treatment of psoriasis patients with moderate to severe plaque psoriasis. Moreover, recent genome-wide association studies have identified a number of potential psoriasis susceptibility genes within the IL-23/Th17 axis, including IL-23A, IL-23R, IL-12B, as well as TNFR-associated factor 3 interacting protein 2 and tyrosine kinase 2, along with genes with skin barrier functions or HLA-C (recently reviewed in Ref. 17). Although Th17 cells were originally regarded to represent the major source of IL-17 in psoriasis, other cell types are also capable of producing IL-17 such as γδ T cells, innate lymphoid cells (ILCs), NK cells, mast cells, macrophages, and neutrophils (18). However, the significance of these other sources of IL-17 in psoriasis is still largely unclear.

As subset of atypical T cells bridging innate and adaptive immunity, γδ T cells contribute to pathological inflammation and autoimmunity (recently reviewed in Ref. 19). Challenging the established function of Th17 cells in psoriasis pathogenesis, a few independent studies recently postulated a critical role of IL-17–producing γδ T cells in two mouse models of induced acute skin inflammation (20–23). Whereas in IL-23–mediated murine skin inflammation, innate dermal γδ T cells (20) or CCR6+–positive epidermal γδ T cells (21) critically contributed to IL-17 production, an invading RORγt+ γδ T cell subset, and RORγt+ ILCs
represented the major IL-17–producing cell fractions in a model of psoriasisform dermatitis induced by TLR7 agonist imiquimod (IMQ) (22, 23). Notably, in these transient murine skin inflammation models, conventional αβ T cells only play a minor role (24), and any aspects of psoriasis induction or chronification cannot be addressed. In parallel, a proinflammatory cutaneous lymphocyte Ag (CLA)—expressing Vγ9Vδ2-positive T cell subset occurring in lesional skin of human psoriasis patients was described with potential clinical relevance in inflammatory skin diseases (25). Although in the light of these findings, the importance of γδ T cells in psoriasis pathogenesis appears obvious, the exact function and position of this immune cell type in the cascade of inflammatory events leading to disease manifestation and chronification remains unclear.

To further evaluate the function of γδ T cells in psoriasisform skin disease, in this investigation we used the CD18 hypomorphic PL/J mouse model (26–33), an established model of psoriasis on the PL/J background with reduction of CD18/Itgb2 levels to 2–16% of wild-type levels due to a hypomorphic CD18 allele. A mouse model (26–33), an established model of psoriasis on the PL/J background with reduction of CD18/Itgb2 levels to 2–16% of wild-type levels due to a hypomorphic CD18 allele. Notably, reduced or altered expression of CD18 itself or of the cally, and in its polygenic base as previously described (30–33).

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Materials and Methods

**Mice**

PL/J mice with a hypomorphic mutation of the CD18 gene (CD18<sup>hypo</sup>) have previously been described (27). CD18<sup>−/−</sup> littermates (CD18<sup>+</sup>) resulting from heterozygous crosses served as wild-type controls. To evaluate the function of γδ T cells in psoriasisform dermatitis induced by TLR7 agonist imiquimod (IMQ) (22, 23). Notably, in these transient murine skin inflammation models, conventional αβ T cells only play a minor role (24), and any aspects of psoriasis induction or chronification cannot be addressed. In parallel, a proinflammatory cutaneous lymphocyte Ag (CLA)—expressing Vγ9Vδ2-positive T cell subset occurring in lesional skin of human psoriasis patients was described with potential clinical relevance in inflammatory skin diseases (25). Although in the light of these findings, the importance of γδ T cells in psoriasis pathogenesis appears obvious, the exact function and position of this immune cell type in the cascade of inflammatory events leading to disease manifestation and chronification remains unclear.

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**Gene expression analysis by quantitative real-time RT-PCR**

Isolation of total cellular RNA from skin or lymph nodes (TriFast solution; Peqlab), cDNA generation (SuperScript Vilo cDNA Synthesis Kit; Invitrogen) and quantitative real-time RT-PCR (qPCR; LightCycler FastStart DNA MasterPLUS SYBR Green I SYBR Green 1Supermix; Roche) were performed according to the manufacturers’ instructions as previously described (40). Samples were analyzed in triplicate with qPCR with a LightCycler (Roche) and Gene Expression Analysis Software (Tree Star). FACS sorting was performed with an FACS Aria II system (BD Biosciences).

**Blocking Ab injection in vivo experiments**

Affected CD18<sup>hypo</sup> PL/J mice with adapted PASI scores ≥6 at an age of 3–6 mo were injected i.p. with 100 μg blocking Ab (eBioscience) against IL-17A (EBioMM17F3), IL-23 (G23-7), or the γδ TCR, Vα4, Vγ5, Vδ6, IL-17A (eBio1B7), IL-17A (clone TC1-11H18), IL-23R, and NK1.1. PhoshoAb Abs were used against Erk1/2, p38, Stat5, and Stat5pY694 (BD Biosciences). Abs against human Ags used were against CD3, CD18, CD196/CCR6, γδ TCR, Vδ6, and IL-17A. Apoptotic fractions were detected by Annexin V staining (BD Biosciences).

**Immunofluorescence staining of murine skin and lymph node samples**

Fixation, staining, and microscopy of frozen cryosections (5 μm) of mouse back skin were performed as previously described (40). To detect T cell subpopulations, sections were first incubated with Ab against mouse CD4 (clone-L3T4; eBioscience) or mouse CD8 (clone-53–6.7; eBioscience) followed by FITC-conjugated goat anti-mouse IgG. Sections were used for detection of IL-17A or Foxp3 as indicated (32, 40). All FACS analyses were performed using an FACS Canto System (BD Biosciences) and FACS Diva Software as well as FlowJo software (Tree Star). FACS sorting was performed with an FACSaria II system (BD Biosciences).
Cocultures were then stimulated with indicated amounts of anti-CD3e (2C11) and cytokines for 5 d prior to assessment of proliferation by FACS.

Adaptive transfer of γδ T cells

Freshly isolated CD3γδTCR+ cells were purified by FACS sorting and labeled with CFSE as described above. A total of 2 × 10^5 FACS-sorted CFSE-labeled CD3γδTCR+ T cells from diseased CD18<sup>hypo</sup> PL/J mice were transferred i.v. into healthy CD18<sup>hypo</sup> PL/J recipients as previously reported (32, 40).

IMQ-induced skin inflammation

Daily doses of 62.5 mg IMQ 5% cream (Aldara) were applied on shaved back skin of 8–10-wk-old BALB/c mice for 6 consecutive d and resulting clinical signs of skin inflammation monitored as previously described (41). For histological analyses, skin samples were fixed in 4% paraformaldehyde, embedded in paraffin, and subjected to H&E staining using standard methods.

Human skin and blood samples

Frozen sections of lesional skin from psoriasis patients or skin from healthy subjects were processed and analyzed as described above following immunofluorescent (IF) staining with Abs against human γδTCR, CD3, IL-17, and IL-22. Blood samples from corresponding patients and healthy controls were analyzed by FACS using human anti-CD3, γδTCR, -CD18, and -IL-17 Abs.

Statistical analysis

Where indicated, p values were calculated using the Student t test or one-way ANOVA and significance levels denoted as follows: *p < 0.05, **p < 0.01, and ***p < 0.001.

Ethical approval of clinical investigations

All clinical investigations were approved by the Ethics Committee of the University of Ulm and have been conducted according to Declaration of Helsinki principles. Written informed consent was received from participants prior to inclusion in the study.

Results

CD4+ αβ and γδTCR<sup>low</sup> γδ T cells with a memory phenotype predominated in the skin infiltrate in CD18<sup>hypo</sup> PL/J psoriasiform dermatitis

Because normally, CD18 is highly expressed on αβ and γδ T cells and expression of CD18, ICAM-1, or ICAM-2 is altered in certain psoriasis patients (35–39), we set out to analyze the impact of reduced CD18 levels on γδ T cell function and inflammatory cytokine and marker expression in psoriasiform dermatitis using the CD18<sup>hypo</sup> PL/J mouse model. To characterize the skin infiltrate of affected CD18<sup>hypo</sup> PL/J mice in detail, FACS analysis of T cell subsets was performed in skin from 6–8-wk-old (young, predisease) versus 4–6-mo-old (aged, diseased) mice followed by immunohistochemical analyses and qPCR (Fig. 1). In the epidermis of CD18<sup>hypo</sup> and young CD18<sup>hypo</sup> PL/J mice, Vγ5<sup>+</sup> T cells with high expression of γδTCR (γδTCR<sup>high</sup>), representing skin resident dendritic epidermal T cells (DETcs) (8, 42), predominated and constituted up to 3% of epidermal cells (data not shown). In older diseased CD18<sup>hypo</sup> PL/J mice, however, the Vγ5<sup>+</sup> T cell fraction was greatly reduced in the epidermis compared with age-matched healthy CD18<sup>wt</sup> PL/J controls, and instead, increased percentages of γδTCR<sup>low</sup> cells were detectable in epidermal and dermal samples (Fig. 1A, top panels). Both epidermal and dermal fractions from affected CD18<sup>hypo</sup> PL/J mice also contained significantly elevated numbers of CD4<sup>+</sup> and to a lesser extent CD8<sup>+</sup> T cells (Fig. 1A, bottom panels, Supplemental Fig. 1). Detailed phenotypic analyses of γδTCR<sup>low</sup> cells revealed a heterogeneous Vγ subset composition with a predominance of Vγ4<sup>+</sup> cells and a CD3<sup>+</sup>, CCR6/CD196<sup>+</sup>, CD44<sup>+</sup>CD27<sup>+</sup> phenotype, indicating that these cells most likely represented infiltrating γδ T cells with a memory phenotype and migratory properties (Supplemental Fig. 1). The γδTCR<sup>low</sup> cells in the dermis preferentially expressed high levels of IL-17 as confirmed by ICS (Supplemental Fig. 1).

Subsequent IF analyses of frozen-skin sections confirmed the presence of large numbers of γδTCR<sup>low</sup>-IL-17<sup>+</sup> cells in the dermis of affected CD18<sup>hypo</sup> PL/J mice (Fig. 1B), constituting up to 50% of IL-17<sup>+</sup> cells in the infiltrate (Fig. 1C). In contrast, in healthy CD18<sup>wt</sup> PL/J skin, the epidermis was interspersed with multiple small and bright IL-17<sup>+</sup> γδ T cells, but only very few γδ or αβ T cells were detectable in the dermis. Moreover, the majority of dermal γδ T cells in inflamed CD18<sup>hypo</sup> PL/J skin expressed the TF RORγt critical for lineage specification of IL-17–producing cells, further substantiating the inflammatory potential of the CD18<sup>hypo</sup> γδ T cells (Fig. 1D, Supplemental Fig. 2). In addition IF analyses of the cytokine expression pattern of γδ T cells in affected CD18<sup>hypo</sup> PL/J skin, significant numbers of IL-22<sup>+</sup> and TNF-α<sup>+</sup> γδ T cells were detectable (Supplemental Fig. 2), suggesting that these atypical T cells might possess multiple functions in initiation, amplification, and maintenance of skin inflammation in this psoriasis model. Although the reduction of γδTCR<sup>high</sup> (Vγ5<sup>+</sup>) T cells in the epidermis of CD18<sup>hypo</sup> PL/J mice observed in FACS analyses in comparison with healthy controls was also detectable by IF in situ, neither IL-17 nor other inflammatory cytokines were consistently detectable in the few CD18<sup>hypo</sup> epidermal γδ T cells by IF.

In addition to qPCR analyses of total RNA from lesional skin of 4–6-mo-old affected CD18<sup>hypo</sup> PL/J mice, highly elevated levels of proinflammatory IL-17, IL-22, and IFN-γ mRNA were detectable compared with age-matched healthy controls (Fig. 1E), verifying the contribution of both Th17 and Th1 signature cytokines in this psoriasis model. This increase in proinflammatory cytokines was accompanied by a reduction of anti-inflammatory TGF-β. In addition, we found variable increases of IL-6, IL-21, IL-23, and TNF-α mRNA in CD18<sup>hypo</sup> PL/J lesional skin (Supplemental Fig. 3). The predominance of inflammatory IL-17–producing T cells belonging to either the Th17 or γδ T cell subset in lesional skin was further substantiated by detection of increased mRNA levels of markers such TF RORγt and scavenger receptor Scarb2 (Fig. 1E). In addition, expression levels of CCL20/MIP3A and CCR6, both involved in trafficking of αβ and γδ memory T cells into inflamed skin, and of general inflammatory markers like β-defensin 3 and S100A7 (psoriasin) were significantly increased in psoriatic CD18<sup>hypo</sup> PL/J skin samples (Supplemental Fig. 3).

The IL-23/IL-17 axis and γδ T cells play a key role in the development of psoriasiform dermatitis in the CD18<sup>hypo</sup> PL/J psoriasis mouse model

At first, the causal role of the IL-23/IL-17 axis in psoriasiform skin disease of CD18<sup>hypo</sup> PL/J mice was assessed in depletion experiments with anti–IL-17 and anti–IL-23/p19 blocking Abs in vivo. All four CD18<sup>hypo</sup> PL/J mice (initial PASI ≥6) injected i.p. with 100 μg blocking anti–IL-17 Ab weekly over a period of 4 wk responded with a significant reduction of adapted PASI scores by >50% already on day 14 and by up to 100% at the end of the 28-d treatment period (Fig. 2A). Similarly, in response to treatment with anti–IL-23/p19 Abs, in all four analyzed mice, clinical scores and inflammation decreased significantly by day 28 (Fig. 2B). Overall, both treatment groups, anti–IL-17 and anti–IL-23/p19, showed recovery rates that corresponded well to our historical positive controls such as treatment with the TNF-α blocker etanercept or therapeutic injection of CD18<sup>+</sup> PL/J Tregs (31, 32).

The observed resolution of psoriasiform skin disease upon selective blockade of components of the IL-23/IL-17 axis not directly affecting Th1 cells distinctively suggests a trigger role for IL-23...
FIGURE 1. Loss of skin-resident $\gamma \delta$ T cells and infiltration with $\gamma \delta$TCR$^{low}$ and Th17 T cells in CD18$^{hypo}$ PL/J lesional skin. (A) Shift of the $\gamma \delta$T cell fraction from $\gamma \delta$TCR$^{high}$ (γδH) to $\gamma \delta$TCR$^{low}$ (γδL) cells in epidermis (right panel) and dermis (left panel) of lesional CD18$^{hypo}$ compared with CD18$^{wt}$ PL/J skin ($n \geq 10$, representative plots shown). Fractions of $\gamma \delta$TCR$^{low}$ CD3$^+$ CD4$^+$ and CD8$^+$ T cells were also increased in inflamed CD18$^{hypo}$ PL/J skin. (B) Significant increase of dermal IL-17$^+$ γδ T cells in affected CD18$^{hypo}$ PL/J mice in IF analysis of frozen skin sections compared with healthy CD18$^{wt}$ PL/J skin (γδTCR$^+$ cells, green; IL-17$^+$ cells, red; nuclei, blue). Original magnification ×40; $n \geq 4$, representative example shown. White arrows point to epidermal γδ T cells. The white dotted line separates epidermis (e) and dermis (d). (C) To quantify γδTCR$^+$IL-17$^+$, γδTCR$^+$IL-17$^-$$^2$, and γδTCR$^{-}$$^2$IL17$^+$ cells in the skin CD18$^{hypo}$ and CD18$^{wt}$ PL/J mice, positively stained cells were counted. (D) In parallel, γδTCR$^+$ROR$\gamma$$^+$, γδTCR$^+$ROR$\gamma^-$$^2$, and γδTCR$^{-}$$^2$ROR$\gamma^+$ cells were quantified in skin samples from the same mice. For all measurements, the median of specifically stained T cells counted in 10–15 high-power fields is presented (n ≥ 3). (E) Increased mRNA expression of inflammatory cytokines and Th17/γδ T cell markers in lesional skin of CD18$^{hypo}$ PL/J mice. mRNA profiling for indicated genes was performed with skin samples from 4–6-mo-old diseased CD18$^{hypo}$ PL/J mice with PASI ≥6 or age-matched healthy CD18$^{wt}$ PL/J control mice (n = 4 each genotype and each gene). **p < 0.05, ***p < 0.01.
and IL-17 in psoriasiform dermatitis in the CD18<sup>hypo</sup> PL/J mouse model. Although slight fluctuations in PASI scores generally occurred in all diseased mice over a 28-d period, no significant disease resolution in mice receiving isotype control IgG was observed. In some control mice, aggravation of disease was detected.

To verify the clinical significance of the changes in gd T cell populations observed in skin and lymph nodes of affected CD18<sup>hypo</sup> PL/J mice, subsequent depletion experiments with Abs against the gd TCR (GL3) were conducted as described above. Notably, in all four mice injected with anti-gd TCR Abs, clinical psoriasis scores significantly improved after 4 wk of treatment (Fig. 2C). Although one half of the mice responded very well to anti-gd TCR treatment, in two mice, despite overall improvement, a small hyperkeratotic plaque persisted on the back, showing a diminished response to anti-gd TCR Abs in comparison with treatment with anti-IL-17 or anti-p19 Abs. Subsequently, we therefore also applied an Ab specifically targeting the inflammatory V<sub>γ</sub>4<sup>+</sup> T cell subset (UC3) that similarly caused partial disease remission (data not shown).

Analysis of cytokine expression in back skin of CD18<sup>hypo</sup> PL/J mice posttreatment of all three treatment groups after 28–35 d moreover revealed significant reduction of elevated mRNA levels of IL-17 and IFN-γ as well as of β-defensin. Upon treatment with anti-IL-17 and anti-IL-23/p19, almost complete normalization of elevated mRNA levels of IL-17, IL-22, and IFN-γ as well as of β-defensin and S100A7 had occurred, again pointing to a trigger role of Th17 signature cytokines in CD18<sup>hypo</sup> PL/J psoriasiform dermatitis (Fig. 2D, gray bars). In anti-gd TCR Ab–treated skin, a significant decrease of pathological mRNA expression of inflammatory cytokines IL-17 and IFN-γ was detectable, whereas IL-22 expression remained elevated (Fig. 2D, black bars). In addition, a significant reduction of the T cell skin infiltrate upon anti-IL-17, anti-IL-23, anti-gd TCR, and anti-TNF-α Abs (n ≥ 3, representative samples shown). *p < 0.1, **p < 0.05.
IL-23R<sup>+</sup> memory γδ T cells accumulated in lymph nodes of CD18<sup>hypo</sup> PL/J mice prior to the onset of skin disease and produced high levels of IL-17 in vitro upon IL-23 stimulation

To unravel the origin of the γδ T cells found in lesional skin of CD18<sup>hypo</sup> PL/J mice, thymi, and local skin-draining lymph nodes of young and old mice were subsequently subjected to analysis. Although no obvious increases in γδ T cells were detectable in CD18<sup>hypo</sup> PL/J thymi, already in young CD18<sup>hypo</sup> PL/J mice, we consistently observed significant increases in γδ T cell fractions in cervical, axillary, and inguinal lymph nodes prior to the onset of skin disease (Fig. 3A). Upon disease manifestation, γδ T cells mainly expressing the Vγ<sub>4</sub>-TCR successively accumulated in local lymph nodes as shown by FACS analysis (Fig. 3B). The majority of these γδ T cells expressed IL-23R and CCR6 and had a CD44<sub>high</sub>CD27<sub>low</sub> memory phenotype (Fig. 3B, Supplementary Fig. 4). According to IF and ICS analyses, a fraction of these γδ T cells expressed elevated levels of IL-17 (Fig. 3C–E). In parallel, a concomitant increase in CD4<sup>+</sup> T cells with a CD44<sup>high</sup>/CD27<sup>−</sup>CD62L<sup>low</sup> memory phenotype was detectable in CD18<sup>hypo</sup> PL/J lymph nodes, and a subset of CD18<sup>hypo</sup> PL/J mice generally developed lymphadenopathy with age as previously reported (31). Moderate increases in γδ T cells and Vγ<sub>4</sub>-T cells were also observed in blood samples and spleens of selected affected CD18<sup>hypo</sup> PL/J mice (data not shown).

In vitro restimulation assays of single-cell suspension prepared from regional lymph nodes of either CD18<sup>hypo</sup> or CD18<sup>wt</sup> PL/J mice with IL-2, IL-23, or a combination of both for 4 h, high amounts of IL-17 mRNA were inducible in CD18<sup>hypo</sup> total lymphocytes compared with CD18<sup>wt</sup> PL/J lymphocytes as detected by qPCR (Fig. 3D). In FACS analyses, we confirmed that increased fractions of CD18<sup>hypo</sup> PL/J γδ T cells produced IL-17 upon stimulation with IL-23/IL-2 in the absence of TCR stimulation. In addition, the subfraction of CD4<sup>+</sup> capable of IL-17 production upon stimulation with a combination of anti-CD3 and IL-23 showed high levels of IL-17R<sup>+</sup> (Fig. 4). According to IF and ICS analyses, a fraction of these γδ T cells expressed elevated levels of IL-17 (Fig. 3C–E). In parallel, a concomitant increase in CD4<sup>+</sup> T cells with a CD44<sup>high</sup>/CD27<sup>−</sup>CD62L<sup>low</sup> memory phenotype was detectable in CD18<sup>hypo</sup> PL/J lymph nodes, and a subset of CD18<sup>hypo</sup> PL/J mice generally developed lymphadenopathy with age as previously reported (31). Moderate increases in γδ T cells and Vγ<sub>4</sub>-T cells were also observed in blood samples and spleens of selected affected CD18<sup>hypo</sup> PL/J mice (data not shown).

**Lymph node CD18<sup>hypo</sup> PL/J γδ T cells expressed increased levels of IL-7Ra/CD127 and expanded more rapidly upon IL-7 stimulation in vitro compared with wild-type counterparts**

To further investigate the mechanism of γδ T cell expansion in lymph nodes of CD18<sup>hypo</sup> PL/J mice, detailed analyses of cytokine receptor expression and in vitro stimulation assays were then performed using lymph node T cells from 4-wk-old CD18<sup>hypo</sup> PL/J mice prior to disease manifestation. The predesease CD18<sup>hypo</sup> PL/J γδ T cell pool did not contain elevated numbers of activated or memory-type CD44<sup>high</sup>/CD27<sup>−</sup> T cells compared with wild-type controls (Fig 4A). Notably, greater fractions of predesease CD18<sup>hypo</sup> PL/J lymph node γδ T cells expressed significantly increased levels of IL-7Ra/CD127, a cytokine receptor implicated in mature T cell homeostasis (43) (Fig. 4B). Upon in vitro stimulation with IL-7 for 5 d, CD18<sup>hypo</sup> PL/J γδ T cells expanded at increased rates compared with CD18<sup>wt</sup> PL/J T cells. In addition, after IL-7 stimulation, greater fractions of CD18<sup>hypo</sup> PL/J γδ T cells had a CD44<sub>high</sub>/CD27<sub>−</sub> memory phenotype and expressed IL-23R (Fig. 4C, 4D). Subsequent in vitro stimulation with either IL-23 or TCR agonists (ionomycin and PMA) revealed that increased numbers of CD18<sup>hypo</sup> PL/J γδ T cells were capable of producing IL-17 (Fig. 4E). Reduced CD18 levels on γδ T cells therefore promoted expansion of inflammatory IL-17–producing CD44<sub>high</sub>/CD27<sub>−</sub> γδ T cells in response to IL-7 stimulation in vitro.

**Stimulatory function of inflammatory CD18<sup>hypo</sup> PL/J γδ T cells on CD4<sup>+</sup> T cell proliferation in vitro and on psoriasiform dermatitis upon adoptive transfer in CD18<sup>hypo</sup> PL/J mice**

To test the hypothesis that pathological γδ T cells may trigger and enhance the inflammatory cascade leading to psoriasis outbreaks, we first analyzed the effect of different γδ T cell subsets on CD4<sup>+</sup> effector T cell (Teff) proliferation in vitro and in vitro coculture assays performed as MLRs. To this end, freshly isolated FACS-purified γδ TCR<sup>+</sup> or CD4<sup>+</sup> T cells from affected CD18<sup>hypo</sup> PL/J mice or healthy controls and purified CFSE-labeled CD4<sup>+</sup> T cells from BALB/c mice were cocultured for 5 d prior to performing proliferation analysis by FACS (Fig. 5A). Purity of the isolated γδ T cell population used for coculture assays was generally >95% as confirmed by their CD3<sup>+</sup>/γδ TCR<sup>+</sup> status in FACS analyses. Whereas CD4<sup>+</sup> CD18<sup>hypo</sup> PL/J T cells induced only a marginal proliferative response of allogeneic CD4<sup>+</sup> BALB/c T cells, γδ T cells from either skin or lymph nodes of CD18<sup>hypo</sup> PL/J mice significantly increased the proliferation of allogeneic BALB/c T cells and reduced apoptotic rates of proliferating cells (Fig. 5A).

To causally confirm a potential trigger function of γδ T cells in psoriatic inflammation, in subsequent adoptive transfer experiments, ~2 × 10<sup>5</sup> FACS-sorted and CFSE-labeled γδ T cells purified from lymph nodes of affected CD18<sup>hypo</sup> PL/J mice were adoptively transferred into young healthy CD18<sup>hypo</sup> PL/J hosts via tail vein injections. The control group received injections of equal volumes of PBS to evaluate the contribution of the Koebner effect to potential psoriasis outbreaks. All four healthy recipient CD18<sup>hypo</sup> PL/J mice injected with γδ T cells from affected mice, but not the PBS-injected mice, developed mild clinical signs of psoriasiform skin disease, showing PASI scores from 2–5 after 14 d postinjection (Fig. 5B). Two mice developed red ears or mild facial dermatitis. One mouse in addition developed a psoriatic plaque on the back skin or behind the ear. The clinical data correlated with presence of CFSE-positive γδ T cells in the skin and local lymph nodes as detected by FACS analysis (Fig. 5C). Similar to topical IMQ application, which locally induces γδ T cell–mediated skin inflammation, adoptive transfer of γδ T cells into young CD18<sup>hypo</sup> PL/J mice led to a mononuclear skin infiltrate, epidermal hyperproliferation, and hyperkeratosis as shown by H&E staining (Fig. 5D). In contrast to the local restriction to the application area in response to IMQ, adoptive γδ T cell transfer induced skin inflammation at the typical predilection sites, such as facial area, neck, and back of injected CD18<sup>hypo</sup> PL/J mice. Our data thus confirmed that inflammatory CD18<sup>hypo</sup> PL/J γδ T cells are capable of trafficking from peripheral blood into lymph nodes and skin and of initiating skin inflammation in healthy CD18<sup>hypo</sup> PL/J mice.

**Inflammatory γδ T cells with altered CD18 expression are present in patient blood in acute psoriasis and lesional skin**

To assess the occurrence of inflammatory γδ T cells with immunostimulatory potential at different stages of human psoriasis, biopsies from lesional skin of patients with acute or chronic psoriasis prior to application of any systemic treatments were analyzed histologically by IF staining using Abs against human γδ TCR and IL-17. In eight out of nine psoriasis skin samples, increased fractions of IL-17–produced γδ T cells could be detected in the dermis, whereas healthy control skin harbored no or only very few γδ T cells (Fig. 6A). Generally, γδ T cells did not present the majority of IL-17–positive T cells in the skin infiltrate, but were more abundant in acute phases of psoriasis and restricted to distinct areas of the psoriatic plaques. Subfractions of inflammatory γδ T cells in psoriatic skin also expressed TNF-α (not shown).
In addition, percentages of γδ and αβ T cells as well as CD18 levels on γδ T cells were analyzed in blood samples from the same psoriasis patients and age-matched healthy controls by FACS analysis. In patient blood during acute psoriasis outbreaks,
a general increase in the \( \gamma \delta \) TCR/CD3 ratio was detectable (Fig. 6B), whereas CD18 levels on \( \gamma \delta \) T cells were decreased (Fig. 6C). However, in blood from patients with chronic psoriatic plaques, the \( \gamma \delta \) TCR/CD3 ratio was normal or even slightly decreased. Although these results point to a potential role for CD18low \( \gamma \delta \) T cells in human skin inflammation, conventional T cells represented the predominant T cell subset in chronic psoriatic plaques and are likely critically required for psoriasis chronification in humans and mice.

**Discussion**

The importance of \( \gamma \delta \) T cells in the pathogenesis of psoriasis as part of the IL-17/IL-23 axis has started to be recognized relatively recently (20–23, 25). However, most data supporting a role for inflammatory IL-17–producing \( \gamma \delta \) T cells come from models of acute IL-23– or IMQ-induced murine skin inflammation (20–23),...
In blood samples from patients with acute psoriasis outbreaks, increased stained T cells counted in 10–15 high-power fields is presented (black dots) and healthy controls (white dots); positively stained cells were (T cells of psoriasis patients had significantly reduced CD18 levels

Presence of inflammatory γδ T cells in skin and blood samples from psoriasis patients. (A) Lesional skin of psoriasis patients contains significantly elevated numbers of IL-17+ γδ T cells as detected by IF staining of cryosections (n = 9; eight out of nine samples had enhanced γδTCR±IL17+ cell counts), whereas healthy human skin is devoid of this T cell type or contains only minimal γδ T cell numbers (γδTCR+ cells, green; IL-17+ cells, red; nuclei, blue). Original magnification ×40; n ≥ 3, representative example shown. (B) Quantification of γδTCR±IL-17+, γδTCR±IL-17+, and γδTCR±IL7+ cells in the skin of psoriasis patients (black dots) and healthy controls (white dots); positively stained cells were counted as indicated. For all measurements, the median of specifically stained T cells counted in 10–15 high-power fields is presented (n ≥ 3). (C) In blood samples from patients with acute psoriasis outbreaks, increased γδTCR/CD3 ratios were detectable by FACS analyses compared with age-matched healthy subjects (n ≥ 5, representative dot plots shown). (D) Blood γδ T cells of psoriasis patients had significantly reduced CD18 levels (n ≥ 5, representative examples are shown). **p < 0.05, ***p < 0.01.

where the mode of disease induction might bypass events critical and sufficient for disease initiation and progression of infect- or stress-triggered type 1 or type 2 psoriasis. Importantly, unlike human psoriasis, IMQ-induced murine dermatitis does not progress to a chronic stage. According to our data presented in this study and previous reports, reduced CD18 expression levels are found on blood leukocytes in psoriasis patients (35–39), and in a recent independent study, an inflammatory CLA+ Vγ9Vδ2 T cell subset in human blood capable of infiltrating the skin has been identified in psoriasis patients (25). To enhance these in part rather correlative data, we provide direct evidence for a causal relationship between reduced CD18 expression levels on immune cells and expansion of inflammatory skin-tropic γδ T cells potentiating psoriasisform dermatitis in the chronic CD18<sup>b<sub>hypo</sub></sup> PL/J psoriasis mouse model that closely mimics human psoriasis.

In the first part of this investigation, we showed that inflammatory γδTCR<sup>low</sup> γδ T cells are increased in lesional skin of CD18<sup>b<sub>hypo</sub></sup> PL/J mice and that expression of Th1 cytokine mRNAs, like IFN-γ and TNF-α, as well as of IL-17 and IL-22 mRNAs, produced by either Th1, Th2, or γδ T cells, are all detectable at elevated levels. This cytokine expression pattern is similar to the inflammatory response in human psoriatic plaques and therefore validates the CD18<sup>b<sub>hypo</sub></sup> PL/J psoriasis model (44, 45). Although skin γδ T cells have previously been reported to primarily produce IL-17, our IF analyses of CD18<sup>b<sub>hypo</sub></sup> PL/J skin sections convincingly demonstrated that dermal γδ T cells significantly contributed to IL-17, IL-22, and TNF-α production. Notably, the selective blockade of the IL-23/IL-17 axis of T cell autoimmunity using either anti–IL-17 or anti–IL-23/p19 Abs was therapeutically effective in the CD18<sup>b<sub>hypo</sub></sup> PL/J mouse model and sufficient to reduce PASI scores by up to 100% in selected mice. Because anti–IL-17 and anti-p19 Abs target two important IL-17-producing T cell subsets, namely Th17 as well as inflammatory γδ T cells, these T cell subsets likely possess key functions in psoriasis, as their blockade also causes successive reduction of Th1 cytokines, such as IFN-γ and TNF-α. Consistently, a stimulating effect of IL-17 on IFN-γ–producing cells has previously been described in other inflammation models (46, 47). The slightly lower efficiency of anti-γδTCR treatment in our depletion experiments may either reflect an insufficient effect of anti-γδTCR on an established Th1 and Th17 cell infiltrate or on other innate immune cells such as IL-22<sup>+</sup>Ror<sup>+</sup> ILCs (22), a partially activating effect of the Abs used (48) or a simultaneous elimination of beneficial immunosuppressive γδ T cells (49). The persistence of Ror<sup>+</sup> ILCs likely also accounts for lacking reduction in IL-22 levels after anti-γδTCR Ab treatment, as Ror<sup>+</sup> ILCs were also shown to be major IL-22 producers in other skin inflammation models (22). Confirming the crucial role γδ T cells in CD18<sup>b<sub>hypo</sub></sup> PL/J psoriasiform dermatitis with a genetic approach using δTCR-deficient mice (e.g., B6.129P2-T<sub>crd</sub><sup>+</sup>Mom<sup>−/−</sup>; The Jackson Laboratory) is complicated by the need for a complete transfer of potential susceptibility genes present in the PL/J background though. In addition, it has to be noted that δTCR<sup>−/−</sup> mice develop a spontaneous dermatitis depending on the background (48), likely due to simultaneous elimination of protective subsets like DETC.

A potential trigger role of γδ T cells in psoriasiform skin disease is also suggested by the early increase of γδ T cells observed in regional lymph nodes of young CD18<sup>b<sub>hypo</sub></sup> PL/J mice that preceded any detectable clinical signs of skin inflammation or changes in skin γδ T cell populations. Our detailed marker and expression analysis of T cell subpopulations in CD18<sup>b<sub>hypo</sub></sup> PL/J skin and lymph nodes classified these accumulating γδ T cells to be positive for scavenger receptor Scar2 and to have an inflammatory, activated memory-like phenotype with an IL-23R<sup>+</sup>CCR6<sup>+</sup>CD44<sup>+</sup>CD27<sup>−</sup> surface profile. In addition, inflammatory γδ T cells in CD18<sup>b<sub>hypo</sub></sup> PL/J lymph nodes were capable of producing large
amounts of IL-17 in response to IL-23 stimulation in vitro independent from TCR agonists and may therefore be easily recruit-able in an inflammatory environment, underlining their role in psoriatic pathogenesis. Scart2-gα T cells were previously described as potent IL-17 producers even under noninflamed conditions and capable of recirculating between lymph nodes and dermis (50).

According to our in vitro stimulation assays, inflammatory memory-type CD18hypo PL/J γδ T cells expanded at increased rates in response to IL-7, confirming the recently defined role of IL-7 in memory γδ T cell differentiation (51). Although additional events may account for peripheral activation and accumulation of inflammatory γδ T cells in CD18hypo PL/J psoriasiform dermatitis (including environmental Ags or stress stimuli), reduced CD18 levels, potentially via upregulation of IL-7Rα, likely confer an intrinsic bias toward differentiation into IL-17-producing inflammatory γδ T cells. Because no obvious changes in thymic γδ T cell development were evident in our previous analysis of young CD18hypo compared with CD18hi PL/J mice, in particular no increases in thymic γδ T cell numbers or expression of γδ T cell marker Scat2, we conclude that the accumulation of Vγ4+ T cells results from peripheral activation or altered maintenance rather than from an early development defect. However, it is possible that CD18hypo PL/J γδ T cells are already differentially imprinted during fetal thymic development and therefore more prone to become inflammatory skin-infiltrating cells upon IL-7, stress signals, or contact with an inflammatory environment. Consistently, Scat2-gα γδ T cells were reported to undergo lineage specification in the thymus upon intermediate TCR stimuli and to then preferentially home to skin-draining lymph nodes and skin (50).

The results of the adoptive transfer experiments suggest that IL-23R+CCR6+Vγ4+ T cells accumulating in the lymph nodes are able to infiltrate the skin and may later accumulate or expand in CD18hypo PL/J dermis. Therefore, we propose that pathological γδ T cells first get activated in local lymph nodes (e.g., either by direct contact with pathogen or unspecific stimuli or via activated dendritic cells [DCs]) and then migrate to the skin to induce the inflammatory response. Consistently, recirculation of Vγ4+ T cells upon activation between local lymph nodes and sites of inflammation has been reported in other autoimmune models (21). However, we cannot exclude that apart from lymph node γδ T cells, resident dermal γδ T cells in CD18hypo PL/J mice also get activated upon unspecific stimuli or via IL-23 produced by DC and then proliferate locally to enhance skin inflammation. Enhanced activation of inflammatory T cells in the skin or in local lymph nodes with subsequent skin infiltration was also observed in other models of trafficking receptor deficiencies such as CCR7- or CCR4-deficient mice, respectively (52, 53), that might have common pathomechanisms with CD18 deficiency. Interestingly, targeting CCR6 expressed on the Th17 subset of CD4+ T cells and on γδ T cells, which both contribute to production of IL-17A/F, IL-22, TNF-α, and other cytokines, has recently been suggested as promising therapeutic strategy for psoriasis (54).

Using an MLR model, we moreover demonstrated that inflammatory CD18hypo PL/J γδ T cells effectively enhanced CD4+ Teff proliferation in vitro. Consequently, activated γδ T cells in local lymph nodes and skin of CD18hypo PL/J mice likely also have stimulatory effects on CD4+ T cells and potentially facilitate inflammatory Th17 differentiation, either through direct contact with CD4+ Teffs or via an influence on either DCs or Tregs. These data underscore the causal stimulatory effect of CD18hypo γδ T cells on CD4+ Teff proliferation enhancing or sustaining the psoriasiform skin inflammation in both the murine CD18hypo PL/J psoriasis model and in human psoriasis. Consistently, in mouse models of other autoimmune diseases, such as neuroinflammation (55, 56), uveitis (57, 58), arthritis (59–62), and inflammatory bowel disease (63), stimulating functions of γδ T cells on CD4+ T cells and inflammation have been reported. γδ T cells functioned as APCs, enhancing CD4+ T cell activation (55, 56), or promoted differentiation of naive T cells into autoreactive Th17 cells while inhibiting peripheral Tregs (57, 58). Similarly, activated circulating human Vγ9Vδ2 T cells, in addition to their functions in innate immunity, have been attributed with the capability to serve as professional APCs to conventional αβ TCR T cells (64, 65). In fact, whereas resting γδ T cells do not present Ag, activated γδ T cells were shown to be at least as potent as mature DCs in priming naive CD4+ and CD8+ T cells as early as 18 h postactivation (61).

Moreover, γδ T cells can stimulate the maturation of immature DC (66–68), thereby potentially leading to increased in activation of inflammatory αβ T cells. Consistently, we observed enhanced αβ TCR CD4+ T cell proliferation and increased induction of IL-17 upon in vitro coculture with CD18hypo PL/J γδ T cells. Therefore, it is interesting to speculate that altered interaction of γδ and CD4+ T cells might enhance inflammatory immune responses in skin and lymph nodes of CD18hypo PL/J mice.

Due to the broad expression of CD18 on hematopoietic cells, the alterations of γδ T cells in the CD18hypo PL/J model act in conjunction with altered development and function of other T cell and immune cell types resulting in a predominance of inflammatory cells and signals that in collaboration cause autoimmunity and skin inflammation. Importantly, we could previously demonstrate that numbers of natural Tregs progressively decline in CD18hypo PL/J mice and that suppressive activity of Tregs is diminished as a consequence of impaired TGF-β production (32). More recently, enhanced conversion of CD18hypo PL/J Tregs into Th17 in psoriasiform skin and lymph nodes has been identified as additional pathomechanism (40). From these data, we conclude that suboptimal CD18 levels on T cells might generally increase plasticity and favor inflammatory properties via expression of RORyt and IL-17 secretion while suppressing regulatory properties mediated by Foxp3 and TGF-β and thereby contribute to manifestation of autoimmune diseases (69, 70). In this context, another surveillance mechanism with potential regulatory function in murine skin inflammation might be defective in CD18hypo PL/J mice, as we observed that potentially beneficial skin-resident Vγ5+ DETCs (49) progressively disappeared from affected epidermis in CD18hypo PL/J mice upon disease chronication. Consequently, in CD18hypo PL/J psoriasiform skin, an imbalance between skin-protective Vγ5+ and inflammatory Vγ5− γδ T cells occurred either as a consequence of impaired CD18 function, altered γδ T cell maintenance or clearance in an inflamed environment.

In the sequence of events in psoriasis pathogenesis, starting with activation of DCs in the skin, followed by recruitment of αβ T cells and subsequently macrophages and neutrophils, our results from the CD18hypo PL/J mouse model now confirmed that inflammatory γδ T cells, once activated, likely act as sensitive and easily recruitable triggers at an early stage of disease ignition or relapse. γδ T cells might get activated in response to IL-23 or unspecific stress stimuli, expand in dermis and/or local lymph nodes, and, through secretion of inflammatory cytokines IL-17, IL-22, and TNF-α as well as direct stimulatory mechanisms, recruit additional conventional T cells to facilitate progression of skin inflammation (71). Important elements in chronication of psoriasiform dermatitis in the CD18hypo PL/J mouse model are additional defects in other immune cells, in particular, defects in CD4+CD25+ Tregs, that possess an increased tendency to convert into Th17 cells (31, 32, 40) and alterations in macrophages (30).

In conclusion, our results suggest that the therapeutic effective-ness of targeting the IL-17/IL-23 axis in psoriasis results from
a dual effect on $\alpha$β and $\gamma\delta$ T cells, and possibly other immune cells, and, depending on the cellular composition of the skin infiltrate, can be as potent as TNF-α blockade. Moreover, our data strongly confirm the central role of CD18 in regulation of T cell development, activation, and autoimmunity via its effects on $\gamma\delta$ and $\alpha$β T cell biology. The significance of inflammatory $\gamma\delta$ T cells in human psoriasis and the effect of therapeutic cytokine neutralization, such as anti-TNF-α, anti-IL-23, and anti-IL-17 strategies, on $\gamma\delta$ T cells will need to be explored in larger patient cohorts in the future.

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Disclosures

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