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Blockade of Phosphatidylinositol 3-Kinase (PI3K)δ or PI3Kγ Reduces IL-17 and Ameliorates Imiquimod-Induced Psoriasis-like Dermatitis

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Psoriasis is a chronic inflammatory skin disease triggered by interplay between immune mediators from both innate and adaptive immune systems and skin tissue, in which the IL-23/IL-17 axis is critical. PI3Kδ and PI3Kγ play important roles in various immune cell functions. We found that mice lacking functional PI3Kδ or PI3Kγ are largely protected from imiquimod (IMQ)-induced psoriasis-like dermatitis, correlating with reduced IL-17 levels in the lesions, serum, and the draining lymph nodes. TCRγδ T cells were the major IL-17-producing population in the draining lymph nodes and were significantly diminished in IMQ-treated PI3Kδ knockin and PI3Kγ knockout mice. We also show that PI3Kδ and PI3Kγ inhibitors reduced IFN-γ production by human TCRγδ T cells and IL-17 and IFN-γ production by PBMCs from psoriatic or healthy donors. In addition, inhibition of PI3Kγ, but not PI3Kδ, blocked chemotaxis of CCR6+IL-17–producing cells from IMQ-treated mice or healthy human donors. Taken together, these data indicate that PI3Kδ and/or PI3Kγ inhibitors should be considered for treating IL-17–driven diseases, such as psoriasis.

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Psoriasis is a chronic, relapsing inflammatory skin disease affecting ~2% of the population. The lesion is usually manifested as raised erythematous plaques with adherent silvery scales due to epidermal hyperplasia (acanthosis) and abnormal differentiation (parakeratosis), in combination with dermal inflammatory infiltrates and increased tortuous capillaries (angiogenesis). Although the etiology of psoriasis remains unknown, it is believed that the interplay between immune cells and mediators, and skin tissue in genetically susceptible individuals underlies psoriasis pathogenesis (1).

Accumulating evidence indicates that the IL-23/IL-17 axis is implicated in psoriasis. IL-17, the signature cytokine of Th17 cells, is also produced by innate immune cells, such as TCRγδ T cells, NKT cells, lymphoid tissue inducer cells, mast cells, and neutrophils (2). IL-23 maintains Th17 cell survival and proliferation, and promotes IL-17 production in both adaptive and innate immune cells (2–4). Polymorphisms in IL-23R and its ligand IL-12B are linked with an increased risk of developing psoriasis (5). Increased IL-17 mRNA, Th17 cells, and IL-17–producing innate cells are found in psoriatic lesions (6–8). Ultimately, clinical efficacy of both neutralizing IL-23/IL-17 p40 (9) and IL-17A confirmed the critical role of this axis in psoriasis (10).

PI3Ks are fundamental components of immune cell signaling networks. PI3Ks generate phosphatidylinositol (3,4,5)-trisphosphate, a second messenger that recruits protein kinases and other proteins to the plasma membrane, where, in turn, they initiate complex downstream signaling networks important in cell differentiation, proliferation, migration, and survival (11). The class IA PI3Ks, PI3Kα, PI3Kβ, and PI3Kδ contain Src homology 2 domains that bind tyrosines phosphorylated by receptor-associated kinases, whereas PI3Kγ, the only class IB PI3K, is activated by G protein-coupled receptors. PI3Kδ and PI3Kγ are predominantly expressed in hematopoietic cells and have been studied intensively in the context of immune-mediated diseases (12). Both isoforms are involved in the production of reactive oxygen species by neutrophils (13). PI3Kγ plays a nonredundant function in neutrophil, monocyte/macrophage, and T cell chemotaxis in vitro and in vivo (14, 15), whereas PI3Kδ mediates B cell chemotaxis (16). PI3Kδ mediates mast cell adhesion, migration, and degranulation by FcεR activation and is crucial in passive cutaneous anaphylaxis (17), whereas PI3Kγ mediates adenosine-induced degranulation in systemic anaphylaxis (18). PI3Kδ is critical for B cell development, proliferation, and differentiation, whereas PI3Kγ deficiency and PI3Kγ inhibitors offer protection in preclinical models of lupus, inflammatory arthritis, and multiple sclerosis (27–29).

To understand the role of PI3Kδ and PI3Kγ in psoriasis, we took advantage of the recently described imiquimod (IMQ) model (30). IMQ, a TLR7/8 agonist, is widely used topically in treatment of certain virus-associated and malignant skin diseases (31). It has been reported that IMQ can induce de novo and exacerbate pre-existing psoriasis lesions (32). In mice, daily application of IMQ...
cream induces dermatitis with many features resembling psoriasis. Clinically, IMQ-treated skin becomes red, scaly, hard, and thickened. Histologically, IMQ treatment results in keratinocyte hyperproliferation, abnormal epidermis differentiation, neoangiogenesis, and infiltration of T cells, conventional dendritic cells (DC), plasmacytoid DCs (pDC), neutrophils, and macrophages. Importantly, IMQ-induced dermatitis development is critically dependent on the IL-23/IL-17 axis (30).

We found that both PI3Kδ knockin (KI) mice, which express a catalytically inactive form of PI3Kδ, and PI3Kγ knockout (KO) mice were largely protected from IMQ-induced psoriasis-like dermatitis, correlating with reduced IL-17 in the lesion and serum and decreased IL-17–producing T cells in the draining lymph nodes (LN). PI3Kδ and PI3Kγ inhibition also reduced IL-17 production by PBMCs from psoriatic or healthy donors and measurable cytokine production by circulating TCRγδ T cells. In addition, PI3Kγ, but not PI3Kδ, inhibitors blocked chemotaxis of CCR6+ IL-17–producing cells from mice and humans. These data indicate a potential clinical usefulness of PI3Kδ and/or PI3Kγ in psoriasis and other autoimmune diseases with underlying TCRγδ T cell and IL-17–mediated pathologies.

Materials and Methods

Mice

PI3Kδ KI (p110δKO/DKO/ΔD) (19) and PI3Kγ KO (14) mice have been backcrossed to C57BL/6 >12 generations. WT littermates were used as controls for PI3Kδ KI mice (in house). C57BL/6 used as control for PI3Kγ KO mice were from Charles River Laboratory, where the PI3Kδ mice were bred. Female mice at 8–12 wk of age were used. All protocols involving live animals were approved by the Swiss Veterinary Authority (Office Vétérinaire Cantonal) in Geneva, Switzerland.

IMQ-induced dermatitis protocol

Backs of the mice were shaved with an electric clipper (B. Braun Vet Care) and then treated with depilatory cream (Nair) to remove hair. Two days later, 70–75 mg IMQ cream 5% (Aldara; 3M Pharmaceuticals) or a control cream (Vifor SA) was applied daily on the back skin for consecutive 5 d.

Mice were evaluated daily. Back redness (erythema), presence of scales (scaling), and hardness of the skin were scored using a semiquantitative scoring system from 0 to 4 based on their external physical appearance: 0 = no abnormalities; 1 = slight, 2 = moderate, 3 = marked, and 4 = severe. In addition, mice were weighed, and dorsal skin thickening was assessed by measuring double-skinfold thickness using a digital micrometer (Mitutoyo).

Histopathological and immunohistochemical analysis

Back skin samples were fixed in 4% formaldehyde and stained with H&E. Parakeratosis, acanthosis, and leukocyte infiltration were assessed to evaluate scores in a blinded way. Scores from 0 to 2 were given, as follows: 0 = no abnormalities; 1 = psoriasis-like dermatitis: epidermal acanthosis, reduction of granulose layer, and hyperkeratosis with modest leukocyte infiltration; 2 = psoriasis-like dermatitis: higher epidermal acanthosis, absence of granulose layer, and higher hyperkeratosis with leukocyte infiltration enriched in neutrophils.

For immunohistochemical analyses, rat mAb against CD18 (BMA, Augst, Switzerland) and biotinylated secondary Ab rabbit anti-rat IgG (DakoCytomation, Milan, Italy) were used. Immunoreactivity was detected with the streptABCComplex/HRP system (DakoCytomation) and developed with methanol 3,3 diaminobenzidine (Roche Diagnostic, Milan, Italy). Sections were analyzed on an Olympus BX41 microscope (objective Olympus Plan 4×, 10×, or 40×) equipped with an Olympus DP50 camera for images acquisition (Olympus, Milan, Italy). The quantification (number of brown pixels for CD18 staining) was performed using Image J software (National Institutes of Health) with the average value of at least five fields for each mouse.

Flow cytometry analysis and intracellular staining

Mouse TCRγδ, CD3, CD4, CD8, NK1.1, RORγt mAbs were purchased from BD Pharmingen. Mouse IL-17A and IFN-γ mAbs were purchased from BioLegend. For intracellular staining, LN cells were stimulated for 6 h with anti-CD3 at 10 μg/ml or anti-TCRγδ at 10 μg/ml before GolgiStop (BD Pharmingen) was added for the last 4 h of culture. Viability (Aquora, Invitrogen), cell surface, and intracytoplasmic cytokines were assessed by a BD LSR Fortessa and analyzed using FlowJo software (Tree Star).

Real-time quantitative PCR

Skin samples were immersed in mRNAlater solution (Applied Biosystems) and kept at −80°C, and then homogenized using the gentleMACS Dissoictor (Miltenyi Biotec), and total mRNA was purified using the Fibro Mini kit (Qiagen). Real-time quantitative PCR was performed with pre-designed primers and probes (Applied Biosystems): IL-17A (Mm00439618_m1), IL-17F (Mm00521423_m1), GAPDH (Mm99999915_g1), IFN-γ (Mm99999701_m1), IL-22 (Mm00444241_m1), IL-23 (Mm00518984_m1), and IL-6 (Mm00446190_m1). Samples were assayed on an Applied Biosystems 7500 Fast Real Time PCR machine. Quantification of relative mRNA expression was determined by the comparative cycle threshold method.

Cytokine detection

Serum IL-17 levels were measured by Quantikine kit (R&D Systems). Human Cytokine/Chemokine Luminox bead immunoassay kit (Millipore) or ELISA kit (R&D Systems) was used to measure levels of IL-17, IFN-γ, and IL-22 in the culture supernatants.

PI3K Inhibitors

IC87114 and AS605240 have been previously described (Patents WO 01/81346 and WO2004007491) (28), and AS614006 was synthesized, as described (WO2010/100144, Supplemental Fig. 3). Concentrations of compounds used in this study were chosen to be PI3Kδ or PI3Kγ isomeric selective (see Supplemental Table 1).

Human T cell purification

PBMCs were isolated from buffy coat prepared from healthy donors by Geneva Transfusion Center. CD4+CD45RA− T cells were enriched by the memory CD4+ T cell isolation kit, and TCRγδ T cells by the TCRγδ T cell isolation kit (Miltenyi Biotec). Cell purity was confirmed by flow cytometry (>90% purity). These studies were approved by Merck Serono ethics committee.

Patient samples

Venous blood was taken from five patients (three females and two males, average age of 62 y), suffering from plaque-type psoriasis with average psoriasis area and severity index 15. These patients have been diagnosed for 10 y and were subjected to systemic disease-modifying antirheumatic drug therapy. At the moment of the analysis, these patients have not taken systemic therapies for psoriasis for 2 y, and were only treated with topical therapy with calcipotriol and emollients. This study has been approved by the hospital ethics committee, and all patients have signed consent forms with age- and sex-matched healthy volunteers as controls.

Human T cell activation

CD4+CD45RA− T cells or PBMCs were stimulated with anti-CD3 at 5 μg/ml and anti-CD28 at 2 μg/ml (BD Pharmingen) in the presence or absence of IC87114, AS605240, or AS614006, and γδ T cells were stimulated with isopentenyl pyrophosphate (IPP, Sigma-Aldrich) at 5 μM in presence or absence of IC87114 and AS614006 for 3 d. Supernatants were harvested for cytokine measurement by ELISA (R&D Systems) or Luminox (Bio-Rad). Cell viability (7-aminoactinomycin D; Sigma-Aldrich) and proliferation (EdU Click-it kit; Invitrogen) were assessed by flow cytometry (FACSCalibur; BD Biosciences) and analyzed using FlowJo software (Tree Star).

In vitro LN cell migration assay

Single-cell suspensions were prepared from axillary and inguinal LN after 4 d of IMQ application. Chemotaxis assays were performed using 24-well chemotaxis plates (Corning) with 5 μm pore-size polycarbonate filter. Murine CCL20 (PeproTech) at 30 nM with compounds at the indicated concentrations were placed in the lower well. After 3 h of chemotaxis, cells in the lower chamber were stimulated with 0.5 μM PMA and 1 μM ionomycin in presence of GolgiStop (BD Pharmingen) for 3 h. Cells were then stained for viability, surface, and intracellular Ags. Flow-count fluorescent spheres (Beckman Coulter) were added to each sample prior to flow cytometry (BD LSR Fortessa) acquisition. The number of CD3+CCR6+IL-17A+ migrated cells was analyzed using FlowJo software (Tree Star).
Human T cell chemotaxis

Chemotaxis assays were performed using chemotaxis plate (NeuroProb ChemoTx 96-well) with 5 μm pore-size membrane for 1 h. CCL20 (PeproTech) at 20 nM with serial dilution of indicated compounds was placed in the lower chamber, and isolated memory CD4+ T cell suspension was placed in the upper well. The number of migrated cells was measured using the CyQuant cell proliferation assay kit (Molecular Probes), and the plate was read in a Fluoroskan Ascent Fluorimeter.

Statistical analysis

Daily clinical score data were analyzed using two-way ANOVA with Bonferroni posttest. Other mouse data were analyzed using one-way ANOVA with Bonferroni posttest. IC50 of human T cell chemotaxis responses to PI3K inhibitors was calculated using a four-parameter log fit as given by the following formula: Y = bottom + (top − bottom)/(1 + 10^(logEC50 − X) × hill slope), where X is the logarithm of concentration and Y is the response. All data were analyzed using GraphPad Prism software.

Results

Both PI3Kδ and PI3Kγ are required for IMQ-induced skin inflammation

WT, PI3Kδ KI, or PI3Kγ KO mice were treated with IMQ or control cream on the shaved back skin for consecutive 5 d. Control cream did not elicit any changes in the skin of any of the mice (data not shown and Fig. 1C). Upon IMQ treatment, WT mice developed erythema, scaling with increased hardness, and thickness of the skin on day 1–3 and reached peak at day 4–5 (Fig. 1A–C), whereas PI3Kδ KI mice showed reduced erythema, hardness, and scaling as well as reduced thickness of the back skin in a similar kinetic compared with their WT counterparts. Interestingly, only hardening, thickening, and scaling, but not redness (erythema) of the skin were reduced in PI3Kγ KO mice (Fig. 1A–C). As previously reported, IMQ treatment led to up to 15% weight loss in C57BL/6 mice (Fig. 1D). PI3Kδ KI mice showed no difference from their WT controls despite an overall reduction of skin inflammation, whereas PI3Kγ KO mice had less weight loss over time (Fig. 1D). Histological analysis confirmed the clinical observations as the treated skin from both PI3Kδ KI and PI3Kγ KO mice showed reduced epidermal acanthosis, loss of granulose layer, hyperkeratosis, and leukocyte infiltration that was enriched in neutrophils (Fig. 2A, 2C). Leukocyte infiltration in the skin of IMQ-treated WT mice was confirmed by CD18 staining (Fig. 2B), and the cell recruitment was reduced in PI3Kδ KI and PI3Kγ KO mice (Fig. 2B, 2D). Thus, PI3Kδ and PI3Kγ are both required for IMQ-induced skin inflammation, whereas only PI3Kδ deficiency led to reduced erythema.

FIGURE 1. IMQ-induced skin inflammation is clinically reduced in PI3Kδ KI and PI3Kγ KO mice. (A) Representative images of IMQ-induced psoriasis-like lesions in WT mice (left panels) compared with PI3Kδ KI (top right) or PI3Kγ KO mice (bottom right) were taken after 5-d IMQ treatment. (B) Daily mean disease severity is depicted as back skin erythema, hardening, and scaling scores in IMQ-treated mice. Symbols represent mean score ± SEM of six mice per group. Data shown are representative of three experiments for PI3Kδ KI mice and two experiments for PI3Kγ KO mice. (C) Skin thickening is depicted as back skin double-skinfold thickness. Symbols represent mean score ± SEM of at least four mice in control groups and six mice in IMQ-treated groups. (D) Daily body weight (BW) loss is depicted as a percentage compared with day 0. Symbols represent mean score ± SEM of at least four mice in control groups and six mice in IMQ-treated groups. Data shown are representative of three experiments for PI3Kδ KI mice and two experiments for PI3Kγ KO mice. *p < 0.05, **p < 0.01, ***p < 0.001.
Both PI3K<sub>d</sub> and PI3K<sub>g</sub> are required for IL-17 production in IMQ-induced skin inflammation

We next examined cytokine expression in the skin lesions and serum of the mice after 5-d treatment. In line with previously reported data (30), Il17a, Il17f, Il23a, and Il6 mRNA expression was upregulated in the skin lesions of IMQ-treated WT mice (Fig. 3A). By contrast, Ifng, Il22, or Il21 mRNA was undetectable in all groups (data not shown). Il17a and Il17f transcripts were reduced in both PI3K<sub>d</sub> KI and PI3K<sub>g</sub> KO mice, whereas Il6 transcripts were not changed. Il23a was reduced in PI3K<sub>g</sub> KO, but not PI3K<sub>d</sub> KI mice (Fig. 3A). Consistent with mRNA levels in the skin, serum IL-17A was increased in IMQ-treated WT mice, but not in PI3K<sub>d</sub> KI or PI3K<sub>g</sub> KO mice (Fig. 3B). These data indicate that either PI3K<sub>d</sub> or PI3K<sub>g</sub> deficiency resulted in diminished amounts of IL-17 in the skin lesions and serum following IMQ treatment.

Both PI3K<sub>d</sub> and PI3K<sub>g</sub> are required for IMQ-induced IL-17 production from CD4<sup>+</sup> and TCR<sup>γδ</sup> T cells in the draining LN

We next analyzed T cell subsets in the draining LN using flow cytometry at the end of 5-d IMQ treatment. A ROR<sup>γt</sup> population from IMQ-treated WT mice was CD3 positive, but CD4 and CD8 negative (Fig. 4A). After 6 h of restimulation with anti-CD3, we found that in the IMQ-treated WT mice, 20–60% of TCR γδ T cells, but <0.5% of the CD4<sup>+</sup> T cells were IL-17A<sup>+</sup> (Fig. 4B),
which corresponded to ~10 times as many IL-17–producing TCRγδ T cells as CD4+ T cells in the LN (Fig. 4C, 4D). The numbers of both IL-17+CD4+ T cells and IL-17+ TCRγδ T cells were diminished in PI3Kδ KI and PI3Kγ KO mice (Fig. 4B–D). No IL-17A was detected in CD8+ or NKT cells, whereas IFN-γ was mainly found in CD8+ T cells, but not TCRγδ T cells (data not shown and Supplemental Fig. 1). The percentage of IFN-γ–producing CD8+ T cells was reduced in PI3Kδ KI mice, although unchanged in PI3Kγ KO mice (data not shown and Supplemental Fig. 1). The percentage of IFN-γ–producing CD8+ T cells was reduced in PI3Kδ KI mice, although unchanged in PI3Kγ KO mice (Supplemental Fig. 1). Thus, TCRγδ T cells were the main immediate source of IL-17A in the draining LN of IMQ-treated mice, and both PI3Kδ and PI3Kγ deficiency led to reduced number of IL-17A+γδ T cells. These results suggest that both PI3Kδ and PI3Kγ are required for pathways that lead to IL-17 production by TCRγδ T cells following IMQ treatment.

Both PI3Kδ and PI3Kγ are required for cytokine production by human memory and γδ T cells

The IL-23/IL-17 axis is pivotal in psoriasis pathogenesis, as shown by impressive clinical efficacy of neutralizing Abs against the IL-23/IL-12 common subunit p40 (Ustekinumab) and IL-17A (9, 10). In chronic inflammatory conditions, including psoriasis, CD4+ memory T cells are believed to be the major source of IL-17A (1). We therefore assessed the effects of PI3Kδ (IC87114) and PI3Kγ (AS614006 and AS605240) inhibitors (Supplemental Fig. 3, Supplemental Table I) on human memory T cell IL-17 production. None of the compounds showed any effect on T cell viability up to 10 μM (data not shown), and only PI3Kγ inhibitors at the concentrations above 2 μM showed modest inhibition of T cell proliferation. Consistent with published data, IC87114 inhibited
IL-17A production in a dose-dependent manner with an IC₅₀ of 4 μM (Supplemental Fig. 2) and, at 10 μM, it almost completely abrogated IL-17A production (Fig. 5A). Interestingly, both PI3Kγ compounds AS614006 and AS605240 inhibited IL-17A production dose dependently with IC₅₀ of 0.3 and 1.8 μM (Supplemental Fig. 2), with complete inhibition at 3 and 10 μM, respectively (Fig. 5A). In addition, both PI3Kδ and PI3Kγ inhibitors reduced IFN-γ, IL-22, GM-CSF, IL-6, IL-4, and IL-13 (Fig. 5A and data not shown). Of note, IL-22 and IFN-γ are also important contributors to the pathology associated with psoriasis (33, 34). Importantly, IC87114 and AS614006 also inhibited IL-17 and IFN-γ production by PBMCs isolated from psoriasis patients and healthy donors with similar potency (Fig. 5B).

The majority of human TCRγδ T cells recognize IPP, an essential intermediate for isoprenoid synthesis that is common to both microbes and humans (35). We isolated human γδ T cells from PBMCs of healthy donors and stimulated them for 3 d with IPP in presence of IC87114 or AS614006. In line with published data, only IFN-γ, but not IL-17, was detected under this condition (36). Both PI3Kδ and PI3Kγ inhibitors inhibited IFN-γ production by

**FIGURE 5.** PI3Kδ and PI3Kγ inhibitors reduce cytokine production by human memory CD4⁺ T cells, by PBMCs from healthy donors and psoriasis patients, and IFN-γ production by human γδ T cells from healthy donors. (A) Human CD4⁺CD45RA⁻ T cells were isolated from PBMCs of healthy donors. Cells were treated with anti-CD3/CD28 in presence of PI3Kδ or PI3Kγ inhibitors at the indicated concentrations for 3 d. IL-17A, IFN-γ, and IL-22 levels in the supernatant were determined by Luminex. Error bars represent mean ± SD of cell culture duplicates and are representative of five donors from individual experiments for IL-17A and two for IFN-γ and IL-22. (B) PBMCs from healthy donors and psoriasis patients were stimulated with anti-CD3/CD28 in the presence of PI3Kδ or PI3Kγ inhibitors at the indicated concentrations for 3 d. IL-17A and IFN-γ levels in the supernatant were determined by Luminex. Error bars represent mean ± SD of cell culture duplicates. Representative data from two of five healthy donors and two of the five psoriasis patients are shown. (C) Human γδ T cells were isolated from PBMCs of healthy donors. Cells were stimulated with IPP in the presence of PI3Kδ or PI3Kγ inhibitors at indicated concentrations for 3 d. Supernatant IFN-γ levels were determined by ELISA. Error bars represent mean ± SD of cell culture duplicates, and data are representative of two individual experiments.
human TCRγδ T (Fig. 5C) without affecting cell viability. Therefore, human TCRγδ T cells, like mouse TCRγδ T cells, appear to require both PI3Kδ and PI3Kγ activities for optimal cytokine production.

PI3Kγ, and not PI3Kδ, is required for CCR6+ T cell migration toward CCL20

CCL20 has been found in psoriatic skin lesions along with CCR6 and IL-17A, suggesting that CCL20 can attract CCR6+ IL-17A–producing cells to inflamed skin (37). To determine whether PI3Kδ or PI3Kγ mediates CCR6+ T cell migration, we preincubated cells isolated from the LN of IMQ-treated WT mice with IC87114 or AS614006 and subjected the cells to in vitro transwell chemotaxis assay toward CCL20. AS614006, but not IC87114, inhibited the migration of CD3+CCR6+IL-17+ in a dose-dependent manner (Fig. 6A).

To evaluate the role of PI3Kδ and PI3Kγ in human memory T cell migration, we assessed CD4+CD45RA- cell directional movement toward CCL20 in presence of IC87114, AS614006, or AS605240 in an in vitro transwell assay. We found that 35–50% of CD4+CD45RA- cells expressed CCR6 (data not shown). At 20 nM, CCL20 induced an optimal migration of freshly isolated human memory CD4+CD45RA- cells from the typical bell-shaped curve (Supplemental Fig. 2C). Under this condition, IC87114 did not show significant effect up to 30 μM (Fig. 6B). In contrast, both PI3Kγ compounds, AS614006 and AS605240, inhibited CCL20-induced human memory T cell migration with IC50 of 1.7 and 1.3 μM, respectively (Fig. 6B). These data indicate that PI3Kγ is required for CCL20-mediated migration of Th17 cells, but PI3Kδ is not.

Discussion

Psoriasis research has been hampered by a lack of appropriate animal models that mimic the complex phenotype and pathogenesis of human psoriasis. This is thought to be due in part to differences between human and mouse skin morphology as well as species-specific immune cell functions. The recently described IMQ mouse model recapitulates key features of psoriasis, such as the appearance of skin lesions and the involvement of the immune system and IL-23/IL-17 axis (30). In both humans and mice, the application of IMQ to the skin leads to rapid infiltration of pDC and type I IFN response, and subsequent erythematosis, epidermal thickening, and scaling, indicating that TLR7/8 induce common early events leading ultimately to psoriatic lesions. Indeed, TLR7 ligand LL37-self RNA complexes are present in psoriasis lesions and associated with DC activation (38). Thus, IMQ-induced dermatitis can serve as a useful model to explore the function of downstream inflammatory mediators leading to psoriasis.

In this study, the IMQ-induced psoriasis-like skin inflammation model was used to assess the potential roles of PI3Kδ and PI3Kγ in psoriasis. We found that both PI3Kδ and PI3Kγ mutant mice are largely protected from IMQ-induced dermatitis, correlating with reduced IL-17 levels in serum, skin lesion, and draining LN. In addition, we found that IMQ-induced IL-17 was mainly produced by TCRγδ T cells and, to a lesser extent, by CD4+ T cells in the draining LN, consistent with recent publications (39, 40). The number of IL-17–producing TCRγδ T cells was dramatically reduced in PI3Kδ- and PI3Kγ-deficient mice.

Although CD4+ T cells are considered to be a major source of IL-17, especially in autoimmune diseases, other cells, including TCRγδ, NK, CD8+ T cells, and lymphoid tissue inducer cells, as well as mast cells and neutrophils, can also produce IL-17 and contribute to disease development (2). These cells express the IL-23R and RORγt and often do not require TCR cross-linking for IL-17 induction (4), thus providing an immediate source of IL-17 before the adaptive immune cells respond. Our data indicate PI3Kδ and PI3Kγ contribute to IL-17 production not only by CD4+ T cells, but also by TCRγδ T cells that might play important roles in initiation of pathogenic process in psoriasis.
IL-17–precommitted TCRγδ T cells have been described recently to be located in mouse dermis (41, 42), and dermal TCRγδ T cells can rapidly produce IL-17 following IL-23 and/or IL-1β stimulation (42). IL-23 upregulated in IMQ-treated skin thereby can induce IL-17 expression by dermal TCRγδ T cells. Interestingly, despite a common reduction of IL-17A and IL-17F, IL-23 and IL-6 transcripts were only reduced in the lesions of PI3Kδ KO, but remained normal in PI3KδΔI KI mice. Given the central role of PI3Kδ in leukocyte migration (14, 43) and that PI3Kδ is not involved in IMQ signaling in pDC (44), it is plausible that, following IMQ treatment, PI3Kδ mediates DC and macrophage recruitment to the lesion that produces IL-23/IL-6 to induce IL-17A/IL-17F by dermal TCRγδ T cells and/or other innate IL-17–producing cells.

IL-17A levels in serum were largely reduced in both PI3Kδ KO and PI3Kγ KO mice upon IMQ treatment, which correlated with the skin mRNA levels and LN cytokine profiles, suggesting that TCRγδ T cells, and possibly other innate IL-17–producing lymphoid cells as well (39), are the major source of serum IL-17 upon IMQ treatment.

Some differences were noted between the PI3Kδ- and PI3Kγ-deficient mice, as follows: PI3Kδ KO mice, but not the PI3Kγ KO mice, were protected against erythema. IFN-γ increases vascular permeability in vivo (45). Therefore, a reduction of IFN-γ in PI3Kδ KO, but not PI3Kγ KO mice (Supplemental Fig. 1), may explain this difference.

IMQ induces skin inflammation-independent severe systemic effects, with weight loss up to 15% in C57BL/6, which has been correlated to transiently induced high level of circulating IL-6 (30). We observed that PI3Kδ but not PI3Kδ deficiency partially protected mice from body weight loss. We also found that only PI3Kγ KO mice had reduced IL6 expression in the skin lesions, and previous studies have shown that PI3Kδ regulates IL-6 production from innate cells such as macrophages (46). It is therefore possible that PI3Kδ-deficient mice were protected from weight loss by their reduced IL-6 levels following IMQ treatment.

In humans, CD4+IL-17+ cells have been isolated from psoriasis plaques, and CCL20, which recruits CCR6-expressing Th17 cells, is abundant in psoriatic lesions (37). Therefore, although TCRγδ T cells are the predominant population of IL-17–producing cells in the IMQ model, other IL-17–producing cells from the adaptive immune system, especially TH17 cells, might become potent key players in the chronic disease observed in humans. Our data confirmed that PI3Kδ is important for human memory CD4+ T cell IL-17A production, and demonstrated that PI3Kδ contributes to IL-17 production by TH17 cells, likely via downregulation of RORγ, as recently reported (47). Importantly, both PI3Kδ and PI3Kγ inhibitors reduced IL-17 and IFN-γ production from PBMCs of psoriasis patients as from healthy donors, indicating that similar signaling pathways function under disease condition.

In addition, PI3Kγ inhibitors reduced memory CD4+ T cell migration toward CCL20, suggesting an additional mode of action of PI3Kγ in limiting CCR6+ IL-17–producing CD4+ T cell recruitment to psoriasis lesion. Notably, skin-homing IL-17+ Vγ9Vδ2 T cells have been found in psoriasis lesion and circulation, and some Vγ9Vδ2 T cells are also CCR6 positive (48). These TCRγδ T cells can be recruited from blood immediately after skin disruption, thus constituting the first wave of T cell skin infiltration that produces proinflammatory cytokines and chemokines to initiate inflammation cascade (48). In peripheral blood, ~1% of the Vγ2Vδ2 (Vγ2 is also termed Vγ9 [TRG9]) (49) T cells can be detected by intracellular staining after PMA/ionomycin stimulation (50). The paucity of these cells in peripheral blood may explain why IL-17A was not detectable upon IPP stimulation of PBMCs. Nevertheless, both PI3Kδ and PI3Kγ inhibitors reduced IFN-γ production, revealing a requirement for both PI3Kδ and PI3Kγ in human TCRγδ T cell function. It remains possible that IL-17–producing TCR γδ cells are more prominent in humans at the peak of psoriatic skin inflammation, as was observed in the mice.

In conclusion, we show that TCRγδ T cells are the predominant IL-17–producing cells following IMQ treatment in mice. PI3Kδ or PI3Kγ blockade ameliorates IMQ-induced psoriasis-like disease correlating with decreased IL-17–producing γδ T cells in mice and inhibits IL-17 and IFN-γ production by T cells from healthy donors and psoriasis patients. PI3Kγ inhibitors, in addition, limit chemotaxis of human TH17 cells. These data support further investigations into developing new treatment strategies for psoriasis and other autoimmune diseases with underlying TCRγδ T cell- and IL-17–mediated pathologies.

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