Novel Phosphoinositide 3-Kinase δ,γ Inhibitor: Potent Anti-Inflammatory Effects and Joint Protection in Models of Rheumatoid Arthritis

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ABSTRACT

Phosphoinositide 3-kinases (PI3Ks) are members of a lipid kinase signaling family that phosphorylate the 3-hydroxyl position of the phosphoinositide (PI) inositol ring. They are divided into classes I, II, and III according to structural and functional classification. These data show that PI3Kδ,γ inhibition suppresses inflammatory arthritis, as well as bone and cartilage damage, through effects on innate and adaptive immunity and that PI3Kδ,γ is a potential therapy for RA.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovitis and joint destruction. Treatment with biologic agents such as tumor necrosis factor (TNF) inhibitors has improved outcomes, but many patients have inadequate responses. Orally bioavailable small molecule inhibitors that target signal transduction have emerged as potential alternatives to expensive biologics. Recent approval of a Janus kinase inhibitor and the clinical efficacy of a spleen tyrosine kinase inhibitor for RA support the feasibility of this approach (Weinblatt et al., 2010; Fleischmann et al., 2012; Kremer et al., 2012).

Phosphoinositide 3-kinases (PI3Ks) are members of a lipid kinase signaling family that phosphorylate the 3-hydroxyl position of the phosphoinositide (PI) inositol ring. They are divided into classes I, II, and III according to structural and functional aspects (Yu et al., 1998). Class IA (PI3Kα, PI3Kδ, and PI3Kγ) isoforms and class IB (PI3Kγ isoform) are dual-specificity kinases that phosphorylate PI 4,5-diphosphate producing PI 3,4,5-triphosphate (Pirola et al., 2001). The α and β isoforms are expressed by many cell types and have been mainly targeted for oncology. The PI3K p110γ and p110δ catalytic subunits are expressed mainly in hematopoietic cells and regulate partially overlapping cell functions in adaptive and innate immunity (Okada et al., 1994; Hirsch et al., 2000; Jou et al., 2002; Ali et al., 2004). Among these, the δ isoform is especially important in B cell activation, whereas the γ isoform is required for chemokine signaling. In light of these activities and their abundant expression in RA synovium and fibroblast-like synoviocytes (FLSs) (Hayer et al., 2009; Bartok et al., 2012), p110γ and p110δ represent possible therapeutic targets for RA.

The potential benefit of targeting PI3K in RA is supported by several studies assessing the roles of PI3K p110γ and p110δ in murine arthritis models. In a collagen type II (CII)–specific antibody-induced arthritis model and in the human tissue necrosis factor transgenic model, Pik3cg−/− mice have decreased arthritis severity and cartilage damage (Pik3cg encodes p110γ). A PI3Kγ inhibitor also reduces clinical severity of arthritis and neutrophil accumulation in a collagen-induced arthritis (CIA) model (Camps et al., 2005). PI3Kγ-deficient mice or administration of PI3Kγ inhibitor in antigen-induced arthritis models using dosing regimens initiated before onset of clinical disease. Treatment of established arthritis with IPI-145 in AA, but not CIA, significantly decreased arthritis progression. In AA, histology scores, radiographic joint damage, and matrix metalloproteinase (MMP)-13 expression were reduced in IPI-145–treated rats. In CIA, joint histology scores and expression of MMP-3 and MMP-13 mRNA were lower in the IPI-145 early treatment group than in the vehicle group. The ratio of anti-CII IgG2a to total IgG in CIA was modestly reduced. Interleukin-17 production in response to CII was decreased in the IPI-145–treated group, suggesting an inhibitory effect on T-helper cell 17 differentiation. These data show that PI3Kδ,γ inhibition suppresses inflammatory arthritis, as well as bone and cartilage damage, through effects on innate and adaptive immunity and that PI-145 is a potential therapy for RA.
also decreases clinical arthritis and macrophage migration (Hayer et al., 2009; Gruen et al., 2010). In the K/BxN serum transfer model, p110δ−/− mice have reduced clinical arthritis, histology, and neutrophil infiltrates. Notably, combined p110δ and p110γ deficiency more effectively reduces disease severity than lack of either individual isoform (Randis et al., 2008).

On the basis of these data and the partially overlapping functions of PI3Kγ and PI3Kδ, we evaluated the effect of a potent PI3Kδ,γ inhibitor, IPI-145 (Winkler et al., 2013), on the development and progression of chronic inflammatory arthritis in rat adjuvant-induced arthritis (AA) and mouse CIA. Two separate models in two species were used to profile the pharmacology to assure that the effects are not species or model specific. In mouse CIA, antibody to CII and activation of complement are dominant and genetic background permits model specific. In mouse CIA, antibody to CII and activation of complement are critical. In AA, the activation of T cells and neutrophils are much less important. The novel inhibitor demonstrated striking benefit, ameliorating initiation and progression of arthritis, reducing synovial inflammation, and decreasing joint damage. These data support development of a PI3Kδ,γ inhibitor such as IPI-145 in RA.

**Materials and Methods**

**PI3Kδ,γ Inhibitor**

IPI-145 is a potent PI3Kδ,γ inhibitor, which was provided by Infinity Pharmaceuticals, Inc. (Cambridge, MA). The \( K_D \) values for IPI-145 are 0.023 nM for PI3Kδ, 0.24 nM for PI3Kγ, 1.56 nM for PI3Kδ, and 25.9 nM for PI3Kα. The four PI3K isoforms were also evaluated using an enzymatic assay that measured \([32P]\)ATP hydrolysis, demonstrating IC50 values of 2.5 and 27 nM for PI3Kδ and PI3Kγ, respectively, and 1602 and 85 nM for PI3Kα and PI3Kβ, respectively (Winkler et al., 2013). Figure 1 shows the chemical structure of IPI-145.

**Rat AA**

Male Lewis rats (180–200 g body weight; Charles River Laboratories, Wilmington, MA) were immunized with 0.1 ml complete Freund’s adjuvant supplemented with heat-killed *Mycobacterium tuberculosis* at the base of the tail on day 0 as previously described (Boyle et al., 2001). Paw swelling was determined using water displacement plethysmometry. On day 8, the rats were randomized into the following oral gavage treatment groups: 1) early treatment group (50 mg/kg IPI-145 beginning on day 8, \( n = 17 \); 2) late treatment group (vehicle from days 8–12 and then IPI-145 50 mg/kg beginning on day 13, \( n = 15 \)); or 3) vehicle (\( n = 16 \)). The drug dosages were determined by body weight on day 0. Radiographs obtained of the hind paws were assessed using the following semiquantitative scoring system: demineralization (0–2), ankle and midfoot erosions (0–2), calcaneal erosion (0–1), and heterotopic bone formation (0–1) (Boyle et al., 2001). All animal experiment protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California San Diego.

**Mouse CIA**

Male DBA/1 mice (aged 6–8 weeks; Harlan Laboratories, Indianapolis, IN) were injected at the base of the tail with 100 μg of bovine CII (Chondrex Inc, Redmond, WA) in 0.05 M acetic acid mixed with complete Freund’s adjuvant as previously described (Han et al., 1998). On day 21, 100 μg of bovine CII in phosphate-buffered saline was injected intraperitoneally. On day 28, 5 μg of lipopolysaccharide was administered intraperitoneally to synchronize the disease. Clinical arthritis scores were measured using a semiquantitative scoring system with a maximum score of 16 per each mouse, as previously described (Luo et al., 2011). On day 20, the mice were divided into four groups: 1) vehicle treatment group (\( n = 7 \); 2) 10 mg/kg IPI-145 treatment group (\( n = 8 \)); 3) IPI-145 50 mg/kg early treatment group (\( n = 8 \)); and 4) IPI-145 50 mg/kg late treatment group (vehicle until day 28, then IPI-145 beginning on day 30; \( n = 8 \)). The drug dosages were determined by average body weight on day 0. For the first three groups, the vehicle and two doses of drug were injected intraperitoneally once a day from day 20 to day 42. The last group was injected with vehicle from day 20 to day 29, and then 50 mg/kg IPI-145 was administered from day 30 to day 42.

**Microcomputed Tomography**

Ankle joints of rats were imaged using a SkyScan 1076 micro-CT 40 system (Bruker-MicroCT, Kontich, Belgium) with the assistance of Dr. Robert Sah (University of California, San Diego, CA) as previously described (Hammaker et al., 2012). The images were assessed using a semiquantitative visual scoring scale of 0–4 for calcaneal erosion, midfoot osteopenia, midfoot erosion, midfoot cartilage damage, metatarsophalangeal joint osteopenia, metatarsophalangeal erosion, and metatarsophalangeal cartilage damage, for a maximum score of 28.

**Histopathologic Assessment of AA and CIA**

For AA, the hind paws were removed on day 21, fixed in 10% formalin, and decalcified for 2–3 days. Paraffin-embedded tissue sections were stained with hematoxylin and eosin. A histological score was determined using a semiquantitative scale that measures synovial inflammation, cartilage integrity, bone erosions, marrow infiltration, proteoglycan loss (in safranin O–stained sections), and extra-articular inflammation, as previously described (Boyle et al., 2001). For CIA, hind paws were removed on day 42, fixed in 10% formalin, decalcified in 10% EDTA, and embedded in paraffin. The paraffin sections were stained with hematoxylin and eosin. Histopathological change was assessed using a semiquantitative scoring system as follows: synovial inflammation, extra-articular inflammation, and bone erosion with a 0–4 scale for each feature and maximum score of 12 per mouse, as previously described (Luo et al., 2011).

**Quantitative Real-Time Polymerase Chain Reaction**

Tissue RNA was isolated from snap-frozen ankle joints using RNA Stat and the RNeasy Lipid Tissue Mini Kit according to the manufacturer’s protocol (Qiagen, Valencia, CA). Using cDNA, mRNA...
expression of interleukin (IL)-6 and matrix metalloproteinases (MMPs) was measured by the TaqMan Gene Expression Assay (Applied Biosystems, Carlsbad, CA). The threshold cycle (Ct) values were used to calculate the number of cell equivalents using a standard complementary DNA curve as previously described (Boyle et al., 2003). The data were normalized to hypoxanthine guanine phosphoribosyltransferase expression and the results are expressed as relative expression units.

Western Blot Analysis

Ankle joints were snap-frozen and protein was extracted using a modified radioimmunoprecipitation assay buffer as previously described (Hammaker et al., 2007). Whole-tissue lysates (15 μg of total protein) were fractionated by NuPAGE 4%–12% Bis-Tris gel electrophoresis (Invitrogen, Carlsbad, CA) and then transferred to nitrocellulose membrane (Bio-Rad, Hercules, CA). Nonspecific binding was blocked with 5% nonfat milk in 0.05% Tween 20/Tris-buffered saline (TBS). The membranes were incubated with horseradish peroxidase conjugated anti-rabbit secondary antibody (diluted 1:1000; Cell Signaling Technology, Danvers, MA). Anti-β-actin (1:2000; Sigma-Aldrich, St. Louis, MO) was used as a loading control. After washing, the membranes were incubated with horseradish peroxidase–conjugated anti-rabbit secondary antibody (1:2000) for 2 hours at room temperature. Immunoreactive proteins were detected with an Immun-Star anti-rabbit secondary antibody (1:2000) for 2 hours at room temperature. Densitometric analysis was performed using Quality One 1-D analysis software (Bio-Rad) and normalized relative to the bands of β-actin.

Serum Antibody Assays

On day 42, sera of CIA mice were obtained with cardiac punctures. Serum anti-CII total IgG, IgG1, and IgG2a levels were measured using enzyme-linked immunosorbent assay kits according to the manufacturer’s protocols (Chondrex). IgE concentration was measured in 1:50 diluted sera according to the manufacturer’s protocol (BD Biosciences, San Jose, CA).

T-Cell Proliferation and Cytokines

On day 42 in CIA, spleens were removed from the 50 mg/kg IPI-145 early treatment mice and vehicle-treated mice (n = 3 each). 5×10⁵ splenocytes were stimulated with 50 μg/ml CII CB11 fragment (Chondrex) as previously described (Mohammed et al., 2003; Luo et al., 2011). Because the experiment was designed to explore how IPI-145 affects T-cell differentiation in vivo during an adaptive response, the compound was not added to the cultures. After 72 hours, 1 μCi of [³H]thymidine was added to each well and the cells were incubated for an additional 24 hours. The cells were harvested, and [³H]thymidine incorporation was determined using a scintillation counter and a stimulation index was then calculated. IL-17 concentration in the culture supernatant after day 42 was measured using an immunoassay according to the manufacturer’s protocols (Bio-Rad).

Statistical Analysis

Results are expressed as the mean ± S.E.M. Statistical differences were assessed using one-way or two-way repeated-measures analysis of variance with Tukey’s multiple comparison test. In some cases, the analysis was followed by Dunnett’s multiple comparison test or a t test for comparison of each group. A P value < 0.05 was considered significant.

Results

Adjuvant Arthritis in Rats

Effect of PI3Kδ,γ Inhibition on the Onset and Progression of CIA. After evaluating the role of PI3Kδ,γ in AA, we examined their contribution to a model dependent on autoantibodies against CII. Mice were divided into four groups: 10 mg/kg IPI-145 starting on day 20, 50 mg/kg IPI-145 starting on day 20, 50 mg/kg IPI-145 starting on day 30, and vehicle. CIA mice that were treated with IPI-145 beginning on day 20 developed minimal signs of arthritis, with significant benefit observed at both doses (see Fig. 5A). Delayed therapy with IPI-145 modestly decreased joint swelling but had no effect on arthritis scores (Fig. 5, A and B). Histology scores on day 42 demonstrated a significant decrease in the early treatment regimens but not with delayed therapy compared with the vehicle treatment group (P < 0.05) (Fig. 5, C and D).

Regulation of MMP Expression in AA by PI3Kδ,γ Inhibition. To evaluate the mechanism of action for IPI-145, the effect of the compound on synovial MMP expression was determined by quantitative real-time polymerase chain reaction. As with AA, expression of MMP-13 mRNA in ankle
joints was reduced by IPI-145 in the early treatment group compared with the vehicle treatment group (Fig. 6). Note that MMP-3 expression was also decreased by IPI-145 in this model.

Effect of PI3Kδ,γ Inhibition on the T-Cell Response in CIA. PI3Kδ,γ can potentially regulate T-cell development, proliferation, and cytokine production (Hirsch et al., 2000; Sasaki et al., 2000), whereas PI3Kδ is involved in T-cell differentiation and expansion (Oak et al., 2006). To determine the effect of PI3Kδ,γ inhibition on CIA-specific T-cell responses in vivo, splenocytes from the 50 mg/kg IPI-145 day 20 treatment group and the vehicle treatment group were isolated and stimulated with the CII CB11 fragment. There was no significant effect of IPI-145 on CII-stimulated T-cell proliferation as determined by [3H]thymidine incorporation, indicating that in vivo T-cell differentiation was not affected by exposure to the compound during the adaptive immune response (Fig. 7A). However, IL-17 production in response to CII peptide was significantly reduced in splenocytes from the IPI-145–treated group (n = 3 for each group; P < 0.05) (Fig. 7B). Only very small amounts of interferon-γ and IL-4 were
produced in treated and control splenocytes and the PI3K inhibitors had no significant effect (data not shown).

**Effect of PI3Kδ,γ Inhibition on Anti-CII Antibodies in CIA.** PI3Kδ regulates antibody production from B cells, whereas PI3Kγ appears to play a minimal role in B cell function (Henley et al., 2008). To determine the effect of IPI-145 on B cells in vivo, the levels of anti-CII autoantibodies in CIA were measured in the vehicle and treatment groups. Serum concentrations of anti-CII total IgG were significantly lower in the 10 and 50 mg/kg IPI-145 early treatment groups than in the vehicle treatment \( (P < 0.005) \) or 50 mg/kg late treatment \( (P < 0.05) \) groups (Fig. 8, A and B). Because anti-CII IgG2a is associated with binding to cartilage and complement fixation, we separately analyzed relative amounts of IgG2a. The anti-CII IgG2a/total IgG ratio was modestly decreased in the 10 mg/kg IPI-145 treatment and 50 mg/kg early treatment groups \( (P < 0.005 \) and \( P < 0.05 \), respectively) compared with the vehicle treatment group, whereas there was no difference of the ratio of IgG1 to total IgG in all groups (Fig. 8C). This result suggests that IPI-145 modestly suppresses the generation of pathogenic autoantibodies to CII, especially anti-CII IgG2a.

**Effect of PI3Kδ,γ on IgE Levels in CIA.** PI3Kδ is a negative regulator of IgE production in murine B cells that suppresses class switch recombination from IgG1 to IgE (Zhang et al., 2008). Therefore, PI3Kδ inhibition could potentially increase serum IgE levels. To evaluate this possibility, serum IgE concentrations were measured in the CIA mice. As shown in Fig. 8D, serum IgE was higher in the 50 mg/kg day 20 treatment group than in the vehicle treatment or the 10 mg/kg treatment groups. The IPI-145 50 mg/kg day 30 treatment group also has higher serum levels of IgE than the IPI-145 early treatment group.
10 mg/kg treatment group (Fig. 8B). These results suggest that a PI3Kδ,γ inhibitor attenuates suppression of IgE class switching.

Discussion

Orally bioavailable small molecules could have significant advantages over protein therapeutics, and several classes of signal transduction inhibitors are being developed for RA. With the approval of a Janus kinase inhibitor in the United States, this alternative approach to biologics could become increasingly important. PI3K is another signaling pathway that is potentially relevant as a therapeutic target in inflammatory diseases because it plays a key role in both innate and adaptive immunity. Previous animal studies with PI3Kδ and PI3Kδ inhibition show that they independently regulate the development and progression of arthritis (Camps et al., 2005; Hayer et al., 2009; Gruen et al., 2010). Combined deficiency or...
inhibition of PI3K and PI3Kδ has striking therapeutic impact with near complete suppression of arthritis (Randis et al., 2008).

IPI-145 is a novel small molecule inhibitor of PI3Kδ,γ that reversibly inhibits both kinases. The \( K_D \) values for IPI-145 are 0.023 nM for PI3Kδ, 0.24 nM for PI3Kγ, 1.56 nM for PI3Kδ, and 25.9 nM for PI3Kα, and the details of this compound are published elsewhere (Winkler et al., 2013). IPI-145 is much less active against PI3Kα and has a modest effect on PI3Kβ. Although the role of PI3Kβ is uncertain due to embryonic lethality in knockout mice, some data suggest that it can regulate neutrophil function (Kulkarni et al., 2011). Nevertheless, it is more likely that the pharmacologic activity observed in AA and CIA models is primarily due to an action on δ and γ isoforms.

In the present study, we show that a potent PI3Kδ,γ inhibitor, IPI-145, decreases disease severity in two models of chronic inflammatory arthritis (i.e., rat AA and mouse CIA). The induction of CIA requires both innate and adaptive immune responses through generation of pathogenic CII-specific T cells and production of CII-specific antibodies (Brand et al., 2003). On the other hand, AA requires T cell and neutrophils but appears to be independent of complement or B cells (Joe et al., 1999). Robust clinical and histopathologic evidence of improvement was observed in pretreatment regimens in both models. Delayed therapy for treatment of established disease was prominent in AA, whereas it was limited in CIA. The pathogeneses of the two models are quite distinct, with one being antibody and complement mediated and the other mediated by T cells and cannot be transferred by serum. Therefore, the differences likely are due to the role of PI3Kγ or δ in the two different mechanisms of disease, with a greater role in immune complex-mediated inflammation in CIA models. Because of the greater effect of innate immunity, the greater benefit in delayed disease might make sense in AA (Yanaba et al., 2007).

Cartilage and bone damage in RA is, in part, a consequence of an imbalance between excessive MMPs and inadequate

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**Fig. 6.** Effect of IPI-145 on the expression of MMP genes in CIA arthritic joints. Expression of MMP-3 and MMP-13 mRNA in ankle joint tissues was determined by quantitative real-time polymerase chain reaction. Data were normalized to hypoxanthine guanine phosphoribosyltransferase and presented in relative cell expression units. (A) MMP-3 mRNA expression was reduced in mice in the IPI-145 early treatment (d20) group compared with the vehicle treatment group. (B) MMP-13 mRNA expression was reduced in mice in the IPI-145 50 mg/kg (d20) treatment group compared with the vehicle treatment group. Values are presented as the mean ± S.E.M. (n = 3 per group). *P < 0.05. d20, day 20; d30, day 30; HPRT, hypoxanthine guanine phosphoribosyltransferase.

**Fig. 7.** T-cell proliferation and cytokine production response to CII. (A) Splenocytes from mice treated with 50 mg/kg IPI-145 starting on day 20 (n = 3) or vehicle (n = 3) were isolated and stimulated with CII CB11 fragment. T-cell proliferation was determined by \[^{3}H\]thymidine incorporation. Data are presented as the stimulation index (cpm of CII-stimulated/cpm of unstimulated). There was no significant effect of IPI-145 on CII-stimulated T-cell proliferation. (B) IL-17 production in response to CII CB11 fragment was measured by immunoassay. IL-17 production was significantly reduced in IPI-145–treated animals compared with vehicle-treated animals. Values are presented as the mean ± S.E.M. (n = 3 per group). *P < 0.05. d20, day 20; d30, day 30. NS, not significant.
compensation of their inhibitors (Firestein, et al., 1991; Mohammed et al., 2003). MMP-3 and MMP-13 are highly expressed in pannus tissues of RA joints and they contribute to the irreversible joint destruction. IPI-145 reduced MMP gene expression in the two models and likely contributes to the observed protective effect. Decreased MMP mRNA could be due to a direct effect of PI3Kδ inhibition on the signaling mechanisms required for MMP production. Alternatively, IPI-145 could secondarily decrease protease production by suppressing production of other inflammatory cytokines involved in induction of MMP, such as IL-1β or TNF. For example, TNF-mediated FLS activation is blocked by a PI3Kδ inhibitor, suggesting that PI3Kδs intersect with proinflammatory cytokine signaling (Bartok et al., 2012). This has also been observed in microglia in which MMP expression is PI3Kδ dependent (Ito et al., 2007). Thus, the protective effect of IPI-145 could result from directly suppressing MMP expression or by decreasing cytokines that induce gene expression in FLSs.

We also evaluated whether IPI-145 blocks activation of its major downstream effector molecule, Akt. PI3K generates PI3,4,5-triphosphate that binds to Akt at the plasma membrane, leading to activation of mammalian target of rapamycin complex 1 (Wu and Hu, 2010). The PI3K/Akt/mammalian target of rapamycin pathway plays a major role regulating cellular growth, survival, proliferation, and migration (Manning and Cantley, 2007). Our data confirm the biologic relevance of PI3Kδ, y blockade in vivo because Akt phosphorylation in joint tissues was decreased by IPI-145. Therefore, IPI-145 benefit is most likely due to direct modulation of the PI3K pathway.

The pathogenesis of CIA requires the generation of CII-specific T cells, which in turn, leads to the production of pathogenic antibodies. PI3Kδ can clearly affect generation of autoreactive clones by regulating thymocyte maturation, T-cell proliferation and cytokine production by stimulation of the T-cell receptor, and immunologic synapse organization (Sasaki et al., 2000; Fruman and Cantley, 2002; Alcázar et al., 2007). PI3Kδ also participates in T-cell maturation, differentiation, and trafficking by regulating lymph node homing receptor CD62L (Oak et al., 2006; Sinclair et al., 2008). Thus, altered T-cell responses could play a role in the beneficial effects of IPI-145. Despite the well defined role of PI3Kδs in T cells, there was no effect of IPI-145 on T-cell proliferative responses to CII peptide.

The proliferation data suggested that the IPI-145 might also control inflammatory arthritis through other mechanisms. One possibility is that the compound alters T-cell cytokine production, most notably IL-17, which is a critical cytokine in autoimmunity (Kunz and Ibrahim, 2009). Because PI3Kδ regulates differentiation in T-cell subsets, we evaluated the T-cell cytokine response to CII peptide. Splenocytes from IPI-
145–treated mice produced significantly less IL-17 in response to CII compared with vehicle-treated mice. The IL-17 data raise the possibility that IPI-145 acts in part by modulating generation of T-helper 17 (Th17) cells. This hypothesis is supported by a recent study showing that p110δ inhibition impairs Th17 but not Th1 differentiation (Kurebayashi et al., 2012).

PI3Kδ is also critical for B cell development, proliferation, and antibody production, and PI3Kδ-deficient B cells display reduced antibody production (Clayton et al., 2002; Okkenhaug et al., 2002). To determine the effect of IPI-145 on autoantibody production, we measured the serum level of anti-CII antibody subclasses in CIA. In the early treatment groups, anti-CII antibody production was reduced compared with control or delayed IPI-145 treatment. The onset and pathogenesis of CIA is associated with a predominance of pathogenic IgG2a because it binds to the cartilage and can fix complement. To determine the effect of IPI-145 on the predominance of anti-CII IgG2a, we confirmed that the anti-CII IgG2a/total IgG ratio was lower in the IPI-145 early treatment group than in the vehicle treatment group. Reduced anti-CII IgG2a production by IPI-145 is another factor that could contribute to decreased arthritis development and severity. It is not surprising, however, that delayed therapy has no effect. Previous studies using anti-CD20 antibodies to deplete B cells show that delayed therapy is ineffective in CIA and that benefit is only observed if B cells are depleted very early in the model (Yanaba et al., 2007).

IgE production, in contrast, was enhanced by PI3Kδ inhibition in CIA. Genetic or pharmacological blockade of PI3Kδ p110δ augments class switch recombination from IgG1 to IgE despite the decrease in autoantibody IgG2a antibodies (Zhang et al., 2008, 2012). Oral treatment with another PI3Kδ inhibitor, IC87114 (Sadhu et al., 2003), demonstrated a similar effect, implicating the δ isoform activity rather than γ in our studies (Zhang et al., 2008). Although the role of IgE in RA is not well defined, the presence of IgE anticitrullinated protein antibodies in RA patients supports a possible relationship of IgE and RA (Schuerwegh et al., 2010). Interestingly, phase I studies in humans have not shown an increase in IgE levels and suggest that this might be a rodent-specific phenomenon (Infinity Pharmaceuticals, unpublished observations).

The contribution of PI3Kδ inhibition to the efficacy of the inhibitor observed in the two animal models could be through multiple mechanisms, but most likely relates to its critical role in chemokine signaling (Li et al., 2000; Barbi et al., 2008; Barberis et al., 2009). Many chemokines and chemotactants act through G protein-coupled receptors, in which signaling converges on PI3Kδ. Selective PI3Kδ depletion or deficiency decreases disease severity in murine lupus and arthritis models (Barber et al., 2005; Camps et al., 2005). Although it is difficult to assess cell migration in chronic models, histological assessment shows decreased synovial infiltration in PI145–treated animals that is consistent with an effect on migration. As with PI3Kδ, PI3Kγ could also act by decreasing activation of innate immune cells such as mast cells or neutrophils in addition to cell recruitment (Hirsch et al., 2000; Lafargue et al., 2002).

In conclusion, IPI-145 is a novel and potent PI3Kδ,γ inhibitor that attenuates the inflammation and joint damage in two animal models of RA. PI3Kδ,γ inhibition reduces synovial inflammation and joint destruction through suppression of MMP expression in joint tissues, decreased autoantibody levels, and diminished IL-17 production. By blocking the partially overlapping activities of two isoforms, it is likely that the benefit would be greater than blocking a single enzyme. In addition, targeting PI3Ks with a restricted distribution while preserving functions of ubiquitous isoforms, especially PI3Kδ, could decrease the risk of systemic toxicity. These data suggest that a targeted molecular PI3Kδ,γ inhibitor may be an important new therapeutic option for RA.

**Authorship Contributions**

**Participated in research design:** Boyle, Kim, Bartok, Firestein.

**Conducted experiments:** Kim, Topolewski.

**Performed data analysis:** Boyle, Kim, Topolewski, Firestein.

**Wrote or contributed to the writing of the manuscript:** Boyle, Kim, Firestein.

**References**

Alcazar I, Marqués M, Kumar A, Hirsch E, Wymann M, Carrera AC, and Barber DF (2002) PI3Kδ isoform activity rather than δ inhibition to the efficacy of the phosphoinositide 3-kinase (PI3Kδ) inhibitor, IC87114 (Sadhu et al., 2003), demonstrated a similar benefit is only observed if B cells are depleted very early in the model. **FASEB J** 16:2932–2940.


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