Dual Blockade of Interleukin-1 β and Interleukin-17A Reduces Murine Arthritis Pathogenesis but Also Leads to Spontaneous Skin Infections in Nonhuman Primates^[S]

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ABSTRACT

Despite the efficacy of biologics for treatment of rheumatoid arthritis (RA), many patients show inadequate responses and likely require neutralization of multiple mediators. Neutralization of both interleukin (IL)-1 β and IL-17A with monoclonal antibodies showed greater efficacy than either agent alone in a mouse arthritis model with cooperative inhibition of key inflammatory factors, IL-6, granulocyte colony-stimulating factor (G-CSF), and CXC chemokine ligand (CXCL1). Given the potential clinical benefit in RA, we generated a human dual variable domain antibody Ig, ABBV-615, capable of simultaneous binding and neutralization of IL-1 β and IL-17A. ABBV-615 was characterized and evaluated in cynomolgus monkeys for pharmacokinetics and toxicity to enable clinical development. ABBV-615 exhibited affinities (K_D) of 12 and 3 pM on human IL-1 β and IL-17A, respectively, and potencies (IC_{50}) of 3 and 58 pM, respectively,

as well as excellent drug-like properties. ABBV-615 pharmacokinetics in cynomolgus monkeys was dose proportional from 20 to 100 mg/kg with a mean half-life of 16 days. However, a 13-week repeat-dose toxicity study in cynomolgus monkeys revealed time-dependent spontaneous infections exclusively in skin at all doses tested and not historically seen with single-agent anti-IL-1 α/β or anti-IL-17A. Consistent with reduced resistance to skin infections, IL-1 β - and IL-17A-stimulated human keratinocytes demonstrate cooperative or compensatory production of key antibacterial and inflammatory mediators such as lipocalin-2, G-CSF, CXCL1, IL-8, tumor necrosis factor, and IL-6, which aid in defense against skin bacterial infections. These results illustrate the skin-specific antimicrobial mechanisms of IL-1 β and IL-17A and highlight the importance of understanding unique combinatorial effects of biologic agents.

Introduction

Although there have been significant advancements made in the treatment of autoimmune diseases such as rheumatoid arthritis (RA), a number of patients remain partially responsive or unresponsive to therapy. In an attempt to address this unmet need, we generated an anti-interleukin (IL)-1 β /IL-17A dual-variable domain (DVD)-Ig molecule to concomitantly neutralize IL-1 β and IL-17A, both of which are involved in RA disease pathophysiology (Mateen et al., 2016).

IL-1 α and β (IL-1) are proinflammatory cytokines that drive innate immune responses by inducing chemokines and cytokines to activate and recruit immune cells, thereby promoting inflammation. In addition, IL-1 stimulates adaptive immune

responses by activating and promoting T helper (Th)17 cells, which produce IL-17A, among other cytokines. IL-17A stimulates chemokine production to recruit neutrophils and other immune cells to sites of inflammation. In support of their role in inflammation, blockade of IL-1 or IL-17A shows significant efficacy in autoinflammatory syndromes and psoriasis, respectively (Jesus and Goldbach-Mansky, 2014; Canavan et al., 2016). Both IL-1 and IL-17A have been suggested to contribute to RA pathogenesis through induction of other proinflammatory cytokines from fibroblasts and monocytes, recruitment of other immune cells, as well as osteoclast activation, osteoclastogenesis, and cartilage damage (Choy, 2012). Single-agent IL-17A and IL-1 antagonists were only modestly effective (Cohen et al., 2004; Cardiel et al., 2010; Alten et al., 2011; Genovese et al., 2013) in RA, but leave open the possibility for superior combinatorial effects. The potential combinatorial effects of dual IL-17A and IL-1 β targeting have been suggested in mouse models where treatment with anti-IL-17A and anti-IL-1 β neutralizing antibodies or a bispecific anti-IL-1/IL-17A antibody resulted in a greater

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ABBREVIATIONS: ANOVA, analysis of variance; AUC_{0–168h}, area under the concentration-time curve from time 0–168 hours; CIA, collagen-induced arthritis; C_{max}, maximal concentration; CXCL, CXC chemokine ligand; DVD, dual variable domain; G-CSF, granulocyte colony-stimulating factor; GLP, good laboratory practice; HC, heavy chain; IL, interleukin; LC, light chain; LCN2, lipocalin 2; mAb, monoclonal antibody; RA, rheumatoid arthritis; Th, T helper; TNF, tumor necrosis factor.

reduction in severity of arthritis, bone damage, and cartilage destruction (Zhang et al., 2013; Wu et al., 2016). A separate study evaluating anti-IL-17A treatment of IL-1-deficient human transgenic tumor necrosis factor (TNF) mice also impaired the spontaneous development of arthritis to a greater degree than anti-IL-17A treatment or IL-1 deficiency alone (Zwerina et al., 2012).

These present studies confirm and extend upon the combinatorial efficacy of anti-IL-1 β and anti-IL-17A in a mouse arthritis model, and describe the generation and characterization of an anti-IL-1 β /IL-17A DVD-Ig molecule, ABBV-615, and testing of this agent in a repeat-dose cynomolgus monkey toxicology study to determine feasibility for clinical development.

Materials and Methods

Collagen-Induced Arthritis Mouse Study. Male DBA/1J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and used at 6–8 weeks of age. All procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health as well as the Institutional Animal Care and Use Committee and monitored by an attending veterinarian.

Type II bovine collagen was obtained from MD Biosciences (St. Paul, MN). Zymosan A was obtained from Sigma-Aldrich (St. Louis, MO). Surrogate mouse monoclonal antibodies (mAbs) for anti-IL-1 β and anti-IL-17A were used in the mouse model. Mouse anti-human IL-17A IgG2a/k mAb (1D10) was produced at AbbVie in a HEK-293-6E cell line utilizing Entrez human gene sequence 3605 as this mAb showed significant cross-reactivity to and neutralization of mouse IL-17A. Mouse anti-mouse IL-1 β IgG2a/k mAb (10G11.B11) was prepared at Harlan Bioproducts (Indianapolis, IN) utilizing Entrez mouse gene sequence 16176.

Arthritis was induced with an intradermal injection at the base of the tail with 100 μ l emulsion containing 100 μ g type II bovine collagen in 0.1 N acetic acid and 100 μ l complete Freund's adjuvant containing 100 μ g *Mycobacterium tuberculosis* H37Ra (BD Difco, Franklin Lakes, NJ). A boost of 1.0 mg zymosan A in 200 μ l phosphate-buffered saline (Life Technologies, Grand Island, NY) was given 21 days later i.p. Disease onset occurred within 3–7 days following the boost, and mice were monitored daily for a change in paw swelling with a caliper thickness gauge (310-115; Dyer, Lancaster, PA). Mice were enrolled for the study between days 24 and 28 at the first clinical signs of disease and treated with 5 mg/kg anti-IL-1 β and 12 mg/kg anti-IL-17A twice per week by i.p. injection, which were previously established maximally efficacious doses in this model (data not shown). At the termination of the experiment, serum was collected for mAb exposure and paws were collected for histopathology or homogenization for mRNA or cytokine/chemokine protein analysis.

For histopathological evaluation, left and right rear paws were fixed in neutral buffered formalin, decalcified in Cal-Rite, routinely processed in paraffin, sectioned, and stained with H&E. All joints in each paw section were evaluated, and the paw was assigned a total score for synovial cell proliferation/cellular infiltrates (pannus), cartilage damage, and bone erosion by a board-certified veterinary pathologist. Slides were scored blinded as follows: 0 = no significant lesion, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe. A total of 26 animals per group was evaluated for paw swelling, and 10 animals per group were evaluated by histologic scoring. A two-way analysis of variance (ANOVA) with Bonferroni post-test was used to determine statistical significance.

Paws for homogenization for either protein or RNA analysis were frozen immediately in liquid nitrogen and stored at -80°C until processing. For protein analysis, tissues were pulverized frozen with a Bio-Pulverizer (BioSpec Products, Bartlesville, OK) and transferred to

Bullet Blender Bead Lysis tubes (Next Advance, Averill Park, NY) on water ice. Ice-cold 750 μ l radioimmunoprecipitation assay lysis buffer containing a protease inhibitor cocktail for use with mammalian cell and tissue extracts (Sigma-Aldrich) was added to each tube and homogenized twice using a bullet blender at maximum speed, for 30 seconds each. Once homogenized, tubes were spun for 10 minutes at 10,000 G, and the supernatant was removed to a 96-well plate for analysis. Cytokines and chemokines were analyzed by MesoScale Discovery (Rockville, MD) electrochemiluminescence (Mouse ProInflammatory 7-Plex Kit). For gene expression analysis, paws were first pulverized on dry ice, and bone fragments were placed into Qiazol (Qiagen, Germantown, MD) and refrozen. A standard chloroform extraction and isopropanol precipitation was performed to isolate the RNA. Total RNA was converted to complementary RNA using the Affymetrix 3' in vitro transcription (IVT) labeling kit, and 10 μ g IVT product was hybridized onto the gene strip array according to the manufacturer's protocol (Affymetrix, Santa Clara, CA). Raw data were converted to expression in Expression Console (Affymetrix) and imported into Excel for additional analysis. Three to four animals per group were assessed for gene expression analyses and five animals per group for paw homogenate protein. Statistical significance was determined with a one-way ANOVA with Bonferroni post-test.

Generation of ABBV-615. ABBV-615, also known as DVD3418, is a potent human anti-IL-1 β /IL-17A DVD-Ig that was engineered from mAbs to human IL-1 β and IL-17A. The design and generation of DVD-Igs were carried out, as previously described (Wu et al., 2007; Lacy et al., 2015). Briefly, the two variable domains of the anti-IL-1 β mAb and the anti-IL-17A mAb were fused in frame with glycine-serine peptide linkers in each DVD-Ig heavy chain (HC) and light chain (LC) using overlapping polymerase chain reaction. The polymerase chain reaction products encoding DVD-Ig HC and LC were subcloned into mammalian expression vectors. ABBV-615 protein was generated by transient transfection of HEK 293-6E cells with HC- and LC-expressing plasmids or in a Chinese hamster ovary cell line stably expressing ABBV-615, and purified by protein A Sepharose affinity chromatography. In ABBV-615, the anti-IL-1 β variable domain is at the 5'-end as the outer domain, and the anti-IL-17A variable domain is at the 3'-end as the inner domain. ABBV-615 is a human IgG1 with mutations in the Fc region (L234A, L235A) (based on European Union numbering system) that impair its effector function by reducing interaction with complement and human Fc γ receptors.

Binding Affinity Measurement of ABBV-615 by Surface Plasmon Resonance. The binding affinities of ABBV-615 to IL-1 β and IL-17A from human, cynomolgus monkey (*Macaca fascicularis*), rabbit, rat, and mouse were determined by surface plasmon resonance analysis using Biacore T200 instrument (Serial Number 1464285; GE Healthcare Life Sciences, Piscataway, NJ). ABBV-615 was captured by a goat anti-human IgG Fc polyclonal antibody (Thermo Scientific, Waltham, MA) that was covalently immobilized across a CM5 biosensor chip via amino groups. Serially diluted cytokine solutions (human IL-1 β and IL-1 β species at 0.39–50 nM, human IL-17A at 0.78–100 nM, and IL-17A species at 0.195–200 nM) were injected at a flow rate of 50 μ l/min. After a dissociation step, surface was regenerated with 10 mM glycine, pH 1.5, at 100 μ l/min. Association and dissociation rate constants, as well as overall affinity (equilibrium dissociation constant K_D), were calculated by the instrument evaluation software based on the values extracted from the data using global fit analysis.

Sequential Binding of IL-17A and IL-1 β to ABBV-615 by Surface Plasmon Resonance. ABBV-615 was captured by the goat anti-human IgG Fc polyclonal antibody immobilized on CM5 chip. IL-17A and IL-1 β , both at 200 nM, were injected sequentially to attain saturation. Then the experiment was repeated in the reverse order of sequential injection of the cytokines. Surfaces were regenerated with 10 mM glycine, pH 1.5, at a flow rate of 100 μ l/min. Binding stoichiometry was calculated according to GE Healthcare guidelines utilizing binding response signals and respective mol. wt. of the molecules.

Bioassays for Determination of ABBV-615 Neutralization Potency. ABBV-615 was tested in vitro for its IL-1 β and IL-17A neutralization activity by IL-1 β bioassay and IL-17A bioassay, respectively. Neutralization of human, cynomolgus monkey, and rabbit IL-1 β by ABBV-615 was measured in an IL-8 release assay in a human fetal lung fibroblast cell line MRC-5 (American Type Culture Collection, Manassas, VA), which produces IL-8 in response to human or cynomolgus monkey IL-1 β , as described previously (Wu et al., 2007; Lacy et al., 2015). Briefly, MRC-5 cells were plated in a 96-well plate at a density of 10^4 cells/well and incubated overnight in basal medium (Life Technologies) containing 10% fetal bovine serum (Hyclone), 1% L-glutamine (Life Technologies), 1% sodium bicarbonate (Life Technologies), 1% sodium pyruvate (Life Technologies), and 100 U/ml penicillin and 100 μ g/ml streptomycin (Life Technologies), in a 37°C, 5% CO₂ incubator. Next day, serial dilutions of ABBV-615 were preincubated with IL-1 β (human or cynomolgus monkey IL-1 β at 200 pg/ml; rabbit IL-1 β lysate generated from crude *Escherichia coli* at 1:62,500 dilution) for 1 hour and then added to the cells with a final IL-1 β concentration at approximately 3 pM. After an overnight incubation, supernatants were transferred to a 96-well round-bottom plate and tested for human IL-8 using a Meso Scale Discovery human IL-8 assay kit, according to manufacturer's instructions. The neutralization potency of ABBV-615 was determined by calculating average percent inhibition relative to the IL-1 β -alone control values. IC₅₀ values were calculated by the GraphPad Prism 5 software (La Jolla, CA) using four-parameter logistic nonlinear regression curve-fitting model.

Neutralization of rat and mouse IL-1 β by ABBV-615 was measured in a CXCL chemokine ligand (CXCL1) release assay in a rat lung fibroblast cell line RLF-6 (American Type Culture Collection). RLF-6 cells were determined to secrete rat CXCL1 in response to rat or mouse IL-1 β stimulation. The RFL-6 bioassay was essentially the same as MRC-5 bioassay, except that the rat CXCL1 levels were measured by using a Meso Scale Discovery rat CXCL1 assay kit.

Neutralization of human and cynomolgus monkey IL-17A by ABBV-615 was measured in an IL-6 release assay in a human foreskin fibroblast cell line HS27, which secretes IL-6 in response to IL-17A. HS27 cells were cultured in Dulbecco's modified Eagle's medium high glucose medium (Life Technologies) containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μ g/ml streptomycin, and 1 mM sodium pyruvate. Serial dilutions of ABBV-615 were preincubated with human or cynomolgus monkey IL-17A and then added to HS27 cells plated in a 96-well plate at a density of 2×10^4 cells/well. The final concentration of each IL-17A was approximately 60 pM. After an overnight incubation, supernatants were collected and tested for human IL-6 using a human IL-6 assay kit (Meso Scale Discovery, Gaithersburg, MD). The neutralization potency of ABBV-615 was determined by calculating average percent inhibition relative to the IL-17A-alone control values. IC₅₀ values were calculated by the GraphPad Prism 5 software.

Neutralization of mouse, rat, and rabbit IL-17A by ABBV-615 was measured in a mouse embryo fibroblast cell line NIH3T3 (American Type Culture Collection), which secretes IL-6 in response to IL-17A and TNF. The NIH3T3 bioassay was similar to the HS27 bioassay except that approximately 20-fold more NIH3T3 cells were incubated

with ABBV-615/IL-17A–preincubated mixture plus mouse TNF at a final concentration of 10 pM and IL-6 production was measured by using a Meso Scale Discovery mouse IL-6 assay kit.

Pharmacokinetics Study in Cynomolgus Monkey. The in vivo studies with cynomolgus monkeys were carried out at AbbVie (North Chicago, IL) or MPI Research (Mattawan, MI), in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health, and were approved by the Institutional Animal Care and Use Committee. Female cynomolgus monkeys ($n = 2$ /group) were administered ABBV-615 i.v. at 1 or 5 mg/kg (10 mg/ml in 30 mM histidine, 8% w/v sucrose, 0.02% polysorbate 80, pH 6.0). Blood samples were collected from each monkey at 5 minutes; 4, 12, and 24 hours; and 3, 7, 10, 14, 21, and 28 days postdose. Serum samples were analyzed using Meso Scale Discovery assay employing biotinylated human IL-1 β for capture and Sulfo-Tag–labeled human IL-17A for detection. Pharmacokinetic parameters for each animal were calculated with PLASMA rev 2.6.12 by noncompartmental analysis and linear trapezoidal method.

Dose Range Finding and Tolerability Study in Cynomolgus Monkey. Cynomolgus monkeys (1/sex/group) were administered dosages of 0, 20, or 100 mg/kg per dose of ABBV-615 at 0, 10, and 50 mg/ml concentration, respectively, in 15 mM histidine, 7.5% sucrose, and 0.016% Tween 80, pH 6.0, via i.v. injection once weekly for 6 weeks or 20 mg/kg per dose (at 20 mg/ml concentration) via s.c. injection once weekly for 6 weeks. Serial blood serum samples for an exposure-time profile of ABBV-615 were collected and analyzed from each animal on day 1 (dose 1) and day 36 (dose 6). Predose 4- and 6-week blood samples were also collected and analyzed. Other parameters that were evaluated in the study included the following: mortality, clinical signs, body weight, hematology, coagulation, and clinical chemistry. Dose proportionality was defined as proportional if mean maximal concentration (C_{max})/dose and area under the concentration-time curve from time 0–168 hours (AUC_{0-168h})/dose were within 0.5 to $2 \times$ of each other and the S.D. overlap between dose groups in non-good laboratory practice (GLP) and GLP cynomolgus monkey studies.

Thirteen-Week Cynomolgus Monkey GLP Toxicology Study. ABBV-615 was administered i.v. or s.c. once weekly for 13 weeks (13 doses total) to cynomolgus monkeys (six/sex/group in control and 200 mg/kg per week i.v. and s.c., respectively; four/sex/group in low- and mid-dose groups), and reversibility or progression of any observed changes was assessed following a 12-week recovery period. The study details are summarized in Table 1. Dose proportionality was defined as proportional if mean C_{max} /dose and AUC_{0-168h} /dose were within 0.5 to $2 \times$ of each other and the S.D. overlap between dose groups in non-GLP and GLP cynomolgus monkey studies.

Assessment of toxicity was based on mortality, clinical observations, injection site observations, and body weight; ophthalmoscopic and electrocardiographic examinations; and clinical and anatomic pathology. Determination of ABBV-615 concentration and antidrug antibody analysis was conducted on serum samples. Predose and 15-minute postdose plasma samples from study days 1 and 36 were analyzed for complement activation (i.e., CH50 increase with concomitant increase of the activation product C3a). Details are included in

TABLE 1
Study design for 13-week cynomolgus monkey toxicology study

Dose Level (mg/kg per Week)	Dose Volume (ml/kg per Week)	Dose Concentration (mg/ml)	Number of Males	Number of Females
0/0 (i.v./s.c.)	2/2 ^a	0/0	6 ^b	6 ^b
20 s.c.	2	10	4	4
65 s.c.	2	32.5	4	4
200 s.c.	2	100	6 ^b	6 ^b
200 i.v.	2	100	6 ^b	6 ^b

^aAnimals received vehicle (15 mM histidine, 75 mg/ml sucrose, 0.0163% Tween 80, pH 6.0) both i.v. and s.c., about 5 minutes apart.

^bTwo animals/sex/group were maintained for a 12-week recovery period.

Fig. 1. Combination treatment with anti-IL-1 β and anti-IL-17A is superior to monotherapy in a therapeutic model of mouse CIA. (A) Paw (footpad) thickness of CIA mice was measured daily by caliper. Baseline was subtracted for each individual mouse before the onset of disease. Data are expressed as change in paw thickness (millimeter). (B) Histopathology was performed on rear paws and visually scored from 0 to 4 based on the severity of inflammation, cartilage damage, and bone destruction. * $P < 0.05$ versus vehicle; ^ $P < 0.05$ versus monotherapy ($n = 12$). Data are expressed as the mean $\pm$ S.E. and is a representative of three experiments.

Supplemental Material and Supplemental Table 1. Blood neutrophil counts were specifically evaluated for changes using a two-way ANOVA with Bonferroni post-test.

In Vitro Human Keratinocyte Assays. Adult primary human keratinocytes from three healthy females were obtained from Thermo Fisher and maintained in Epilife medium (Thermo Fisher, Waltham, MA) with growth supplements (Thermo Fisher). IL-17A was prepared by transiently overexpressing IL-17A in HEK293 cells using polyethylenimine (Sigma-Aldrich). Three days post-transfection, supernatants were harvested and IL-17A was purified using standard immobilized metal affinity column (GE Healthcare, Little Chalfont, UK) chromatography. IL-17A was further purified using a Superdex 75 column (GE Healthcare). IL-1 β was obtained from Sigma-Aldrich. To determine the keratinocyte response to IL-17A and IL-1 β , cells were plated at a density of 10,000 cells/well in 96-well plates using Epilife medium with growth supplements except for the hydrocortisone. The next day, the media was exchanged and increasing concentrations of IL-17A and IL-1 β were added. Culture supernatants were collected after 24 and 48 hours. Concentrations of CXCL1 (R&D Systems, Minneapolis, MN), granulocyte colony-stimulating factor (G-CSF; Meso Scale Discovery), lipocalin 2 (LCN2; R&D Systems), TNF (Meso Scale Discovery), IL-6 (Meso Scale Discovery), S100A8/9 (R&D Systems), and β -defensin 2 (myBiosource, San Diego, CA) were determined using commercially available kits. The maximum fold inductions for each secreted factor were extrapolated from the data by dividing the maximum induction for each treatment group (i.e., IL-17A alone, IL-1 β alone, or IL-17A/IL-1 β combo) by background levels of the secreted factor.

Results

Combinatorial Efficacy and Inhibition of Inflammatory Mediators in Mouse Collagen-Induced Arthritis.

In support of dual neutralization of IL-1 β and IL-17A as a

potential therapy for RA, we confirmed previously published studies in the collagen-induced arthritis (CIA) model in mice (Zwerina et al., 2012; Zhang et al., 2013). Although treatment with anti-IL-1 β or anti-IL-17A mAb alone partially reduced paw swelling in the mouse CIA model, treatment with both antibodies significantly decreased paw swelling relative to the single antibody treatments (Fig. 1A). In addition, histologic evaluation confirmed significantly greater inhibition of inflammation of the joint as well as prevention of cartilage damage and bone erosion with combination treatment compared with monotherapy (Fig. 1B). Similar levels of serum anti-cytokine antibody levels were detected 96 hours after the last dose in animals receiving either mono or combination therapy, indicating that consistently high levels of treatment antibodies were present throughout the course of the study. Anti-IL-1 β levels were 43 ± 3 and 38 ± 3 μ g/ml in mono versus combination therapy, and anti-IL-17A levels were 71 ± 4 and 71 ± 5 μ g/ml, respectively. There were no treatment-related adverse events observed with single-agent or combination antibody treatment, including weight loss, dehydration, hair loss, lethargy, or other health concerns (data not shown).

To define the molecular basis for the improved efficacy, microarray and protein analysis of paw homogenate was performed on mice given single versus dual cytokine inhibition. This analysis identified cytokines and chemokines, including IL-6, CXCL-1, and G-CSF, which showed greater inhibition with the combination of both antibodies compared with single-agent treatment (Table 2).

TABLE 2

Dual neutralization of IL-1 β and IL-17A cooperatively inhibits CXCL1, IL-6, and G-CSF gene and protein expression in paw homogenate from mouse CIA model

Cytokine/Chemokine	Paw Homogenate Gene Expression Inhibition Relative to Untreated ^a			Paw Homogenate Protein Inhibition Relative to Untreated ^a		
	Anti-IL-1 β	Anti-IL-17A	Combination	Anti-IL-1 β	Anti-IL-17A	Combination
G-CSF	None	None	19 $\pm$ 4	30 $\pm$ 32	23 $\pm$ 5	92 $\pm$ 4 ^b
IL-6	34 $\pm$ 27	33 $\pm$ 16	87 $\pm$ 4 ^b	2 $\pm$ 28	13 $\pm$ 17	72 $\pm$ 12 ^b
KC (CXCL1)	47 $\pm$ 10	33 $\pm$ 6	73 $\pm$ 7 ^b	11 $\pm$ 18	8 $\pm$ 39	66 $\pm$ 14

^aData shown are mean percentage change $\pm$ S.E. from four or five animals per group for gene and protein inhibition, respectively.

^b $P < 0.05$.

Fig. 2. Construction and characterization of anti-IL-1 β /IL-17A DVD-Ig ABBV-615. (A) Schematic diagram of ABBV-615 DVD-Ig with the anti-IL-1 β variable domain in the outer position linked to the anti-IL-17A variable domain in the inner position. (B) Affinity measurement of ABBV-615 by surface plasmon resonance. Dissociation was monitored for 1500 seconds for human IL-1 β binding. Due to the very slow off-rates for human IL-17A binding, dissociation was monitored for 3900 seconds for high human IL-17A concentration (50 and 100 nM) and for 850 seconds for lower concentrations (1.56–25 nM). The affinity (K_D) (mean $\pm$ S.D.) based on three independent experiments is 11.6 ± 1.1 pM for human IL-1 β binding and 3.1 ± 0.9 pM for IL-17A binding. Representative sensorgrams are shown. (C) Sequential binding of human IL-17A and IL-1 β to ABBV-615 by surface plasmon resonance. Left: human IL-17A was injected first, followed immediately by injection of human IL-1 β . Right: human IL-1 β was injected first, followed immediately by injection of human IL-17A. Representative sensorgrams with sequential injections of antigens are shown. (D) ABBV-615 potency by cell-based bioassay. Neutralization potency of ABBV-615 on human IL-1 β and human IL-17A was determined by IL-8 release assay in MRC-5 cells and IL-6 release assay in HS27 cells, respectively. ABBV-615 neutralization potency (IC_{50}) (mean $\pm$ S.E.) on human IL-1 β and human IL-17A calculated from three independent experiments is 3.3 ± 0.3 and 58.1 ± 4.7 pM, respectively. Representative inhibition data are shown. Plotted in y-axis is percentage of inhibition relative to the human IL-1 β or human IL-17A alone control.

The combinatorial efficacy and inhibition of inflammatory mediators in mouse CIA prompted us to generate a dual specific antibody that simultaneously targets both human IL-1 β and IL-17A and explore its feasibility for clinical evaluation in RA by testing in pharmacokinetics and toxicology studies in cynomolgus monkey.

Generation and Characterization of Anti-Human IL-1 β /IL-17A DVD-Ig ABBV-615. To generate a dual specific antibody that neutralizes both human IL-1 β and IL-17A, a DVD-Ig molecule ABBV-615 was engineered from the variable domains of mAbs to human IL-1 β and IL-17A (Fig. 2A). The anti-IL-1 β domain E26.35 in ABBV-615 is similar to the

TABLE 3
Species cross-reactivity of ABBV-615

Species	IL-1 β Cross-Reactivity ^a		IL-17A Cross-Reactivity ^a	
	Potency (IC ₅₀ , pM)	Affinity (K _D , pM)	Potency (IC ₅₀ , pM)	Affinity (K _D , pM)
Human	11 $\pm$ 6	6.2 $\pm$ 2.1	60 $\pm$ 9	$\leq$ 2
Cynomolgus	7 $\pm$ 3	2.6 $\pm$ 1.4	163 $\pm$ 24	$\leq$ 8.3
Mouse	11,233 $\pm$ 7,105	10,800 $\pm$ 809	>50,000	Low binding ^b
Rat	>50,000	Low binding ^b	12,000 $\pm$ 3,237	Low binding ^b
Rabbit	1,422 $\pm$ 685	ND ^c	2,900 $\pm$ 1,000	400 $\pm$ 98

^aData shown are mean $\pm$ S.E. from three independent experiments.

^bPoor binding response with very low signal measured in the surface plasmon resonance (BIAcore) assay.

^cNot determined due to the lack of purified rabbit IL-1 β protein.

anti-IL-1 β domain E26.13 in the anti-IL-1 α /IL-1 β DVD-Ig ABT-981 (Lacy et al., 2015); both share the same LC and differ in only four amino acids in HC (one in CDR3 and three in frameworks 3). The anti-IL-17A domain in ABBV-615 was generated by PROfusion mRNA display technology. As determined by surface plasmon resonance analysis, ABBV-615 (generated from a Chinese hamster ovary stable cell line) exhibited high-affinity binding to human IL-1 β (K_D = 11.6 $\pm$ 1.1 pM) and human IL-17A (K_D = 3.1 $\pm$ 0.9 pM), with fast on-rates at 10⁵ (M⁻¹s⁻¹) range and slow off-rates at 10⁻⁶ (s⁻¹) range (Fig. 2B). To determine whether binding of one antigen to ABBV-615 interferes with binding of the other antigen under saturating conditions, the antigens were injected over captured ABBV-615 immediately one after another without any dissociation phase. Human IL-1 β was able to bind captured ABBV-615 when human IL-17A was already bound (Fig. 2C). Likewise, human IL-17A was able to bind captured ABBV-615 when human IL-1 β was already bound. Based on the amount of the bound antigens and ABBV-615 capture levels, the apparent stoichiometry for each antigen was determined to be 1.9–2.2. The stoichiometry data demonstrated that human IL-1 β bound to both ABBV-615 binding domains even after both human IL-17A binding domains were saturated, and vice versa, implying that ABBV-615 was physically capable of simultaneously binding two human IL-1 β molecules and two human IL-17A molecules.

The neutralization potency of ABBV-615 on human IL-1 β and IL-17A was measured by analyzing cytokine production from MRC-5 and HS27 cells, respectively. Inhibition curves were generated by titrating increasing concentrations of ABBV-615 against human IL-1 β (3 pM) or human IL-17A (60 pM), and the IC₅₀ values were derived as described in *Materials and Methods*. Consistent with the high affinity, ABBV-615 exhibited high neutralization potency on human IL-1 β and human IL-17A, with IC₅₀ at 3.3 $\pm$ 0.3 and 58.1 $\pm$ 4.7 pM, respectively (Fig. 2D).

The species cross-reactivity of ABBV-615 was determined by potency assays and affinity measurement (Table 3). ABBV-615 (generated from HEK 293 cells) bound and neutralized cynomolgus monkey IL-1 β and IL-17A with high affinity and potency, comparable to the human proteins. ABBV-615 neutralized rabbit IL-1 β and IL-17A with moderate potencies with IC₅₀ values at 1.4 $\pm$ 1.2 and 2.9 $\pm$ 1.8 nM, respectively, which are 50- to 130-fold reduced from the potencies on the human proteins. ABBV-615 exhibited poor binding and significantly reduced neutralizing activity on mouse and rat IL-1 β and IL-17A (Table 3); thus, this agent could not be used in the murine CIA model of arthritis.

Pharmacokinetics with ABBV-615 in Cynomolgus Monkey.

Because ABBV-615 is able to effectively neutralize both monkey IL-17A and IL-1 β , with comparable potency to the human cytokines, cynomolgus is a pharmacologically relevant animal species to conduct GLP toxicology studies. In initial pharmacokinetic studies in cynomolgus monkeys, ABBV-615 exhibited a biphasic concentration versus time profile with low clearance (mean clearance = 0.065 ml/h/kg) and low volume of distribution (mean volume of distribution = 39.8 ml/kg) following a single i.v. dose of 1 and 5 mg/kg in cynomolgus monkey (Fig. 3A; Supplemental Table 2). Following six weekly doses in monkey, the C_{max} and AUC_{0–168h} were approximately dose proportionally increased from 20 to 100 mg/kg i.v. doses (Fig. 3, B and C). ABBV-615 mean half-life in monkey was approximately 16 days, and mean s.c. bioavailability was approximately 68% (Supplemental Table 2). Lower exposure occurred following the sixth dose in one animal/group, probably due to the development of anti-drug antibodies. Administration of six weekly dosages of ABBV-615 up to 100 mg/kg per dose by i.v. bolus, or 20 mg/kg per dose by s.c. injection was well tolerated in cynomolgus monkeys (one animal/sex/group). No adverse findings or any signs of toxicity were observed in this limited assessment (data not shown).

Toxicology of ABBV-615 in Cynomolgus Monkey. To examine longer-term, repeat-dose toxicology in a larger number of cynomolgus monkeys, ABBV-615 was administered by s.c. injection at 20, 65, and 200 mg/kg per week or by i.v. bolus injection at 200 mg/kg per week for 13 weeks (Table 4). From 20 to 200 mg/kg per week s.c. dose levels, C_{max} and AUC_{0–168h} were increased approximately dose proportionally (Supplemental Table 2). Five of 40 animals dosed with ABBV-615 (one each at 20 and 65 mg/kg per week, two at 200 s.c., and one at 200 i.v.) showed a confirmed anti-ABBV-615 antibody titer (anti-drug antibody) (Supplemental Material). In this study, administration of ABBV-615 resulted in three unscheduled deaths, one male at 65 mg/kg per week with s.c. dosing, one female at 200 mg/kg per week with i.v. dosing, and another female at 200 mg/kg per week with i.v. dosing because of a broken humerus (Table 4). The death/morbidity of the first two animals was due to generalized infection/septicemia and was considered to be indirectly related to ABBV-615, but adverse (Supplemental Material). Both animals exhibited clinical signs of declining health status, such as decreased activity/lethargy, hunched posture, dehydration, and/or impaired hind limb function. In addition, they showed changes in clinical pathology parameters that were secondary to inflammation, including

Fig. 3. Single and repeat dosing of ABBV-615 in cynomolgus monkeys demonstrates acceptable pharmacokinetic properties. (A) Individual ABBV-615 serum concentration versus time profile following single i.v. dose of 1 and 5 mg/kg (two female/group). (B) Individual ABBV-615 serum concentration versus time profile following six weekly i.v. doses of 20 and 100 mg/kg and s.c. dose of 20 mg/kg after dose 1. (C) Individual ABBV-615 serum concentration versus time profile following 6-week weekly i.v. doses of 20 and 100 mg/kg and s.c. dose of 20 mg/kg after dose 6 (one female + one male/group).

a decrease in red cell mass, increase in fibrinogen and globulin, as well as reduced food intake and/or general ill health such as decreases in sodium, chloride, phosphorus, and total protein (Supplemental Table 4). At the terminal necropsy, ABBV-615-related generalized infection/septicemia was also present in one 65 mg/kg per week male with s.c. dosing, and as with the unscheduled death animals was

deemed adverse (Supplemental Material; Table 4). ABBV-615-related clinical signs for surviving animals included nodules at 200 mg/kg per week with s.c. dosing and 200 mg/kg per week with i.v. dosing and abscesses at 20 and 65 mg/kg per week with s.c. dosing and 200 mg/kg per week with i.v. dosing (Supplemental Material; Table 4), which correlated to secondary decreases in red cell mass (Supplemental Table 3, A and B). There were increases in globulin and/or fibrinogen and/or decreases in albumin, albumin/globulin ratio that were evidence of an inflammatory response at 200 mg/kg per week with s.c. or i.v. dosing. Although increased skin infections can be associated with neutropenia (Gonzalez-Barca et al., 2001), there were no consistent neutrophil changes over dose or time (Fig. 4), and other hematologic parameters remained unchanged (Supplemental Table 3, A and B). Complement activation analysis did not show any evidence for complement activation by measuring total classic pathway activity (CH50 assay) and level of the activation product C3a in predose and 15-minute postdose samples from study days 1 and 36 (Supplemental Table 1). Furthermore, there were also no notable histopathological changes to the skin outside of the abscesses or injection sites. Changes in clinical pathology parameters were generally reversible following the 12-week recovery period (data not shown). At the terminal necropsy, ABBV-615-related nonadverse mononuclear cell infiltration consisting of focal infiltrates of macrophages and lymphocytes was present in s.c. injection sites from males at ≥ 65 mg/kg per week with s.c. dosing and females at ≥ 20 mg/kg per week with s.c. dosing (Supplemental Material).

In contrast to the observations with ABBV-615, data generated in separate experiments with agents that neutralize only IL-17A (anti-IL-17A mAb) or IL-1 [anti-IL-1 α /anti-IL-1 β DVD-Ig molecule (Lacy et al., 2015)] showed no evidence for skin abscesses or increased sensitivity toward septicemia in 13-week repeat-dose toxicology studies in cynomolgus monkeys using identical high-dose levels of 200 mg/kg per week with i.v. and s.c. dosing (for more details, see Table 5). As the affinities, potencies, and in vivo exposure levels of the anti-IL-17A and IL-1 α / β DVD-Ig were similar to treatment with ABBV-615 (Table 5), these results clearly demonstrate that neutralization of both IL-17A and IL-1 β is needed to increase the sensitivity for skin infections in cynomolgus monkey.

Production of Antimicrobial Factors by Human Epidermal Keratinocytes. In an effort to understand the mechanisms contributing to increased susceptibility to skin infections with dual neutralization of IL-1 β and IL-17A, we explored the effects of IL-1 β and IL-17A costimulation on cytokine, chemokine, and antimicrobial peptide secretion from normal human epidermal keratinocytes in vitro. IL-1 β , IL-17A, or combination treatment significantly increased the production of IL-6, TNF, LCN2, CXCL1, G-CSF, and IL-8 (Fig. 5). We also evaluated β -defensin 2 and S100A8/9 levels in these cultures; however, β -defensin 2 was not upregulated by either IL-17A or IL-1 β (data not shown), and increases in S100A8/9 did not consistently reach statistical significance (data not shown).

IL-1 β treatment had the strongest effect on IL-6 and TNF, producing eightfold increases over untreated control. In contrast, IL-17A strongly increased LCN2 and G-CSF (9- to 11-fold). Lastly, IL-8, CXCL1, and G-CSF demonstrated greater induction with costimulation relative to single IL-1 β

TABLE 4

Overview on incidence of infections and time of onset, skin abscesses, and moribundity

Dose Group (mg/kg per Week, Route)	Study Week	Abscesses: Incidence per Dose Group ^a	Findings and Location
20, s.c.	13	1/8	Abscess, corner of mouth
65, s.c.	6	1/8	Abscess at lower lip and under chin ^b
65, s.c.	8	2/8	Euthanized because of septicemia/inflammation (day 38) ^{b, c}
65, s.c.	12	2/8	Nodule/Abscess on dorsal surface ^b
65, s.c.	13	3/8	Abscess, left and right foot ^b
200, i.v.	4	1/12	Abscess, dorsal surface
200, i.v.	7	2/12	Abscess at base of tail
200, i.v.	7	3/12	Euthanized in extremis with broken humerus (day 51)
200, i.v.	8	4/12	Left inguinal nodule/abscess
200, i.v.	11	N/A	Swelling on back of knee/abscess right hind limb
200, i.v.	12	5/12	Abscess on left and right forelimb
200, i.v.			Euthanized because of septicemia/inflammation (day 74); animal did not show clinically an abscess ^b
200, i.v.			Abscess, left hind limb

^aStudy design: six animals/group/sex in control, 200 i.v. and 200 s.c.; four animals/group/sex at 20 and 65 s.c.^bMicroscopy showed inflammation and septicemia in various organs (heart, lung, muscle, etc.) in these animals.^cMoribund monkey; Gram-positive cocci bacterial colonies were observed in the heart and subcutis using Brown-Brenn Gram staining.

or IL-17A alone. G-CSF in particular was highly sensitive to costimulation with a 162-fold increase over untreated control, while showing only 6- and 11-fold increases with IL-1 β or IL-17A alone, respectively. Thus, the single-agent and combinatorial treatments with IL-17A and IL-1 β showed different patterns of induction for key cytokines, chemokines, and antimicrobial factors that are important for defense against skin infections.

Discussion

As a strategy to improve efficacy in RA and potentially other inflammatory diseases, we used DVD-Ig technology to simultaneously target both IL-1 β and IL-17A. Similar to other previously reported results (Zwerina et al., 2012; Zhang et al., 2013; Wu et al., 2016), depletion or blockade of both IL-1 β and IL-17A improved efficacy in a mouse model of arthritis over individual agents, supporting the potential combinatorial effects for treatment of disease. A DVD antibody, ABBV-615, was generated that neutralizes human and cynomolgus monkey IL-1 β and IL-17A with high potency, binding both cytokines simultaneously and shows antibody-like pharmacokinetics in cynomolgus monkeys. In a 13-week repeat-dose cynomolgus monkey toxicology study, ABBV-615 demonstrated an increased incidence of skin infections in a time-dependent manner at all doses tested, whereas historic data with either anti-IL-17A or anti-IL-1 α/β DVD-Ig neutralizing antibodies did not demonstrate any evidence of increased infections in monkeys at similar exposure levels and similar treatment duration. The increase in infections was not attributable to decreases in circulating neutrophils or changes in other hematologic parameters. These results provide evidence in nonhuman primates that the presence of either IL-1 β or IL-17A is required specifically for defense against skin infections.

Although preclinical and clinical studies with single-agent IL-1 or IL-17A neutralization show minimal safety risk, there is evidence that these two cytokines are each important in defense against skin infections (Miller and Cho, 2011). IL-17A is important in defense against *Candida albicans*, as patients with mutations in IL-17A or IL-17 receptor show susceptibility to chronic candidiasis as well as *Staphylococcus aureus*

(Puel et al., 2011; McDonald, 2012). IL-17 receptor knockout, IL-1 β , or IL-1 receptor knockout mice show reduced dermal *S. aureus* bacterial clearance and increased abscess size and reduced neutrophil recruitment (Miller et al., 2007; Cho et al., 2010, 2011; Maher et al., 2013). In addition, IL-1 β and IL-17A are both able to induce a variety of antimicrobial and neutrophil chemotactic factors in human keratinocytes or cell lines, including β -defensins, cathelicidin, S100 calcium-binding proteins (S100) A8 and A9, CXCL1, CXCL5, and IL-8, which have been shown to contribute to antibacterial defense (Olaru and Jensen, 2010; Krishna and Miller, 2012).

The emergence of skin infections in cynomolgus monkeys following dual blockade with IL-1 β and IL-17A supports that these cytokines play a redundant role in the protection from skin infection. Anakinra, a recombinant form of IL-1 receptor antagonist, had a similar safety profile to placebo, with the exception of injection-site reactions, in a clinical trial in methotrexate-inadequate responder RA patients (Cohen et al., 2004). AMG 108, an antibody targeting IL-1 receptor, showed a similar lack of adverse events in a RA trial (Cardiel

Fig. 4. Neutrophil counts (mean $\pm$ S.E.) from monkeys (males and females combined) over the course of the 13-week toxicology study with ABBV-615 (group 1 = control s.c./i.v.; groups 2–4 = 20, 65, and 200 mg/kg per week s.c.; and group 5 = 200 mg/kg per week i.v.; six animals/sex in groups 1, 4, and 5; four animals/sex in groups 2 and 3).

TABLE 5
ABBV-615, IL-17A-mAb, and IL-1α/IL-1β DVD-Ig

A comparison of 1) exposure data after dosing for 13 weeks at 200 mg/kg per week i.v. or s.c. in cynomolgus monkey; 2) potency and affinity regarding IL-17A and IL-1β; 3) total incidence of skin abscesses at top doses of 200 mg/kg per week; and 4) total incidence of skin abscesses at all dose levels tested. Unit: C_{max} is milligrams per milliliter; AUC is AUC_{0–168h} at milligrams × hour per milliliter; affinity and potency IC₅₀ are picomolar.

Compound	200 mg/kg per Week i.v.		200 mg/kg per Week s.c.		Affinity		Potency (IC ₅₀)		Overall Incidence Skin Abscesses at 200 mg/kg i.v. and s.c.	Overall Incidence Skin Abscesses at All Dose Levels Tested
	C _{max}	AUC	C _{max}	AUC	IL-17A	IL-1β	IL-17A	IL-1β		
Anti-IL-17A mAb ^a	9.44	792	4.39	569	0.8	—	9	—	0/16	0/32
Anti-IL-1α/IL-1β DVD-Ig ^b	10.9	723	2.90	369	—	28	—	26	0/20	0/40
ABBV-615	11.9	1160	4.95	720	3	12	58	3	5/24	9/40

^aStudy design of 13-week study: control (i.v./s.c.), 20, 60, and 200 (all i.v.) and 200 s.c.; four animals/group/sex (= a total of 32 animals dosed with anti-IL-17A mAb).
^bStudy design of 13-week study: control (i.v./s.c.), 50, 100, and 200 (all i.v.) and 200 s.c.; six animals/group/sex in control, 50 and 200 i.v., four animals at 100 mg i.v. and 200 s.c. (= a total of 40 animals dosed with the DVD-Ig).

et al., 2010). Furthermore, canakinumab, a human anti-IL-1β mAb in RA patients, showed comparable adverse events compared with placebo with no increase in infections (Alten et al., 2011). Finally, the anti-IL-17A monoclonal antibody, secukinumab, in RA also demonstrated a comparable safety profile to other biologics, with no dose-dependent effects on the incidence of adverse events. There was a slightly higher incidence of upper respiratory infections, but no noted skin infections (Genovese et al., 2013). Thus, in contrast to dual neutralization, single IL-1 or IL-17A neutralizing agents showed little evidence of increasing cutaneous infectious risk in either monkeys or humans.

Although we did not definitively identify the nature of the bacterial infection seen in cynomolgus monkeys treated with

ABBV-615, neutrophilic abscesses are a hallmark of *S. aureus* infections in addition to the reported roles of IL-1 and IL-17A in defense against this infection. Additionally, we did observe Gram-positive cocci in one of the animals exhibiting an abscess. Immune activation in the skin by *S. aureus* appears to be through Toll-like receptor 2 activation, which induces IL-1β, IL-6, and IL-23 (Miller and Cho, 2011; Krishna and Miller, 2012). IL-1β and IL-23 induce IL-17A production from natural killer T cells, Th17 cells, α/β T cells, and natural killer cells (Cua and Tato, 2010), and can do so in the absence of T cell receptor engagement (Dungan and Mills, 2011). The IL-17A and IL-1β also induce keratinocytes to produce chemokines (CXCL1, CXCL2, IL-8) to recruit neutrophils and antimicrobial peptides/proteins (β-defensins, cathelicidin,

Fig. 5. Primary human epidermal keratinocyte production of antibacterial factors upon treatment with IL-1β and IL-17A. Secreted levels of IL-6, TNF, LCN2, G-CSF, CXCL1, and IL-8 in response to titrations of IL-17A (200–0.2 ng/ml) and IL-1β (50–0.2 ng/ml) at 48 hours poststimulation are shown. Three different induction patterns are illustrated: (A and B) IL-1β driven, (C) IL-17A driven, and (D–F) costimulation driven. The maximum fold induction versus untreated control for each secreted factor after treatment with IL-17A, IL-1β, or IL-17A/IL-1β costimulation (G) is also shown. A total of three individual human keratinocyte donors was tested (one experiment per donor). Data are expressed as mean ± S.E. from one of three representative donors.

Fig. 6. Illustration of combinatorial IL-1 β and IL-17A activities contributing to bacterial clearance in the skin (A) and proposed how ABBV-615 increases skin infection susceptibility by blockade of both IL-1 β and IL-17A (B) [modified from Ryu et al. (2014)]. Colonization and infection of the skin by *S. aureus*: immune system evasion and the response to cationic antimicrobial peptides. <http://www.mdpi.com/1422-0067/15/5/8753>. Copyright © 2014 by the authors; licensee MDPI, Basel, Switzerland. This image is open access distributed under the terms and conditions of the Creative Commons Attribution license, <http://creativecommons.org/licenses/by/3.0/>.

lipocalin 2), which have bacteriocidal or bacteriostatic activities (Olaru and Jensen, 2010; Dungan and Mills, 2011; Ryu et al., 2014). IL-1 β also appears to limit the production of the immune-suppressive cytokine, IL-10, by Th17 cells that are specific for *S. aureus* (Zielinski et al., 2012). Amplification of the recruitment can occur through IL-1 β - and IL-17A-induced antimicrobial proteins and chemokines, such as CCL20, which recruit additional immune cells, including Th17 cells (Miller and Cho, 2011; Ryu et al., 2014). All of these activities highlight the cooperative role of IL-1 β and IL-17A in defense against skin infections that was confirmed in the present studies.

The skin-restricted susceptibility to infection with dual IL-1 β and IL-17A neutralization suggests a central role for skin-resident stromal and/or parenchymal cells such as keratinocytes, propagating the induction of neutrophil-attracting chemokines and antimicrobial proteins in response to IL-17A and IL-1 β (Miller and Cho, 2011). Our *in vitro* results suggest that both IL-17A and IL-1 β have a unique keratinocyte signature with certain secreted factors showing more IL-17A sensitivity (LCN2), others showing more IL-1 β sensitivity (TNF and IL-6), and still others (IL-8, CXCL1, and G-CSF) being most sensitive to costimulation with IL-17A and IL-1 β . These differences in the keratinocyte secretome highlight the increased impact of neutralizing two different cytokines.

This apparent overlap between the immune response and the keratinocyte response, as well as a potential model for the infection susceptibility with ABBV-615, is summarized in Fig. 6. In this model of skin infection, invading bacteria initiate an IL-1 β -producing myeloid response and an IL-17A-producing lymphocyte response in parallel to resolution of the infection. Both of these parallel pathways converge at skin-resident stromal and parenchymal cells, which directly help fight off the infection with bactericidal peptides/proteins (e.g., LCN2) and also amplify the immune response by producing cytokines and chemokines (e.g., IL-8, IL-6, TNF, CXCL1). In this model, each stimulus can access some unique outputs from keratinocytes as well as some shared outputs.

Inhibiting only IL-17A or IL-1 β may leave enough of the keratinocyte response intact to prevent infection, but neutralizing both IL-1 β and IL-17A simply reduces too many key chemokines and antimicrobial peptides.

The induction of G-CSF, CXCL1, and IL-6 production by IL-17A and/or IL-1 β proteins in keratinocytes was consistent with the inhibition of these factors at the gene and protein levels in paw homogenates from the mouse arthritis model following treatment with anti-IL-17A and anti-IL-1 β . Thus, although playing a key role in a disease setting (arthritis), IL-1 β and IL-17A also appear to play a protective role specifically in skin infections and reinforce the hypothesis that dual inhibition of IL-1 β and IL-17A has a much broader impact than single cytokine inhibition.

Increased infection risk has been observed clinically in short-term studies with other combinations of approved therapies tested in RA, including anakinra (IL-1 receptor antagonist) plus etanercept (soluble TNF receptor that blocks TNF) as well as abatacept (blocking costimulation of T cells) plus anti-TNF (Genovese et al., 2004; Weinblatt et al., 2006, 2007). A few patients developed cellulitis, and there were also cases of respiratory, gastrointestinal, ear or urinary infections, as well as herpes, candidiasis, and pneumonia infections (Genovese et al., 2004; Weinblatt et al., 2006, 2007). Neither of these combinations noted an increase in abscessing cutaneous infections, as we saw in the cynomolgus monkey study with ABBV-615. Thus, the specificity to abscessing skin infections may be unique to the dual neutralization of IL-1 β and IL-17A, although this would have to be verified in a human clinical study. Importantly, these results of dual inhibition are likely not related to the nature of the bispecific molecule used in this study but would likely have been found in any other dual specific format or even with separate dosing of two antibodies. Therefore, caution should be exercised in combining anti-IL-1 and anti-IL-17A biologic agents in patients.

In summary, the increase in skin infections in monkeys upon dual neutralization of IL-1 and IL-17A with ABBV-615 demonstrated the importance of leaving either IL-1 β or IL-17A pathways intact in a system relevant to humans.

Although cytokines often show redundant activities, this particular combination blockade for IL-1 β and IL-17A revealed a specific role of these two cytokines in cutaneous infections and highlights the need to better understand immune interactions.

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Authorship Contributions

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