

Treatment of severe persistent asthma with IL-6 receptor blockade

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Clinical Implications

- The use of tocilizumab in 2 children with severe persistent, steroid-resistant asthma resulted in immunological improvement and suggestive of clinical improvement.

TO THE EDITOR:

Asthma is a chronic lung inflammatory disease with multiple phenotypic manifestations, underlined by several disease endotypes that reflect distinct pathophysiological mechanisms.¹ A T-helper

cell type 2 high (T_H2^{High}) and mixed T_H2/T_H17 endotypes have been associated with severe asthma.² T_H2^{High} is characterized by an eosinophilic whereas a T_H2/T_H17 by a mixed eosinophilic/neutrophilic airway inflammation. T_H2/T_H17 endotype manifests as a difficult-to-control, steroid-resistant disease.^{3,4} Past research has demonstrated high sputum levels of IL-6 in patients with mixed eosinophilic/neutrophilic airway inflammation.⁵ IL-6 blockade has been proposed as a treatment for asthma.⁶ The IL-4 receptor alpha chain variant R576 (IL-4R α -R576) drives mixed T_H2/T_H17 airway inflammation.^{7,8} The treatment of *IL4ra*^{R576} mice with an anti-IL-6 monoclonal antibody (mAb) protected against severe airway inflammation.⁵ There are no reported cases of IL-6 pathway blockade for pediatric asthma. Here, we analyzed the response of 2 patients with severe persistent, nonatopic asthma with evidence of T_H2/T_H17 inflammation treated with tocilizumab, a humanized anti-IL-6 receptor (anti-IL-6R) mAb.

PATIENT 1

This is a 6-year-old boy with severe persistent, nonatopic asthma, homozygous for the *IL4R*^{R576} allele (mutant allele). He had severe life-threatening asthma (with documented reversibility of airway obstruction on bronchodilation; [Tables I and II](#)) with 18 intensive care unit (ICU) admissions (4 requiring intubation

TABLE I. Clinical response: childhood asthma control test, pulmonary function testing, and hospital admissions: patient 1

ACT and pulmonary function testing				
Date	C-ACT	FVC: L (% predicted)	FEV ₁ : L (% predicted)	FEV ₁ /FVC (% predicted)
July 22, 2016*				
Pre-albuterol	24	1.10 (87%)	0.82 (71%)	74 (81%)
Post-albuterol		1.46 (115%)	1.10 (95%)	75 (82%)
October 27, 2016†				
Pre-albuterol	21	1.48 (114%)	0.9 (76%)	61 (67%)
December 28, 2016				
Pre-albuterol	27	0.98 (75%)	0.58 (49%)	59 (69%)
May 17, 2017				
Pre-albuterol	27	1.68 (89%)	1.28 (81%)	76 (86%)
May 26, 2017				
Pre-albuterol	27	1.77 (125%)	1.16 (91%)	66 (73%)
December 13, 2017				
Pre-albuterol	27	1.90 (125%)	1.30 (99%)	68 (77%)
Admissions				
Year	General inpatient	ICU	Total	Inpatient days
2014	0	5	5	12
2015	0	5	5	68
2016	0	2	2	14
2016	0	0	0	0
2017	1 (10/17)	1 (2/17)	2	17
2018	0	0	0	0

Bold values indicate time period before initiation of tocilizumab.

C-ACT, Childhood Asthma Control Test (for children 4-11, max score 27); FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ICU, intensive care unit.

*Pre/post-spirometry. Baseline obtained and then patient received albuterol 2 puffs, 90 mcg/inh. Post-albuterol spirometry demonstrated FEV₁ reversibility $\geq 12\%$ and an absolute increase in FEV₁ of ≥ 200 mL over baseline value.

†Initiation of tocilizumab.

TABLE II. Clinical response: childhood asthma control test, pulmonary function testing, and hospital admissions: patient 2

Date	C-ACT	FVC: L (% predicted)	FEV ₁ : L (% predicted)	FEV ₁ /FVC (% predicted)
December 7, 2016	11			
February 23, 2017*				
Pre-albuterol	Not done	1.27 (118%)	1.22 (122%)	96 (103%)
July 25, 2017				
Pre-albuterol	20	1.51 (126%)	1.16 (106%)	77 (84%)
August 10, 2017				
Pre-albuterol	20	1.53 (128%)	1.22 (111%)	79 (86%)
August 28, 2017†				
Pre-albuterol	22	1.62 (136%)	1.33 (121%)	82 (89%)
April 30, 2018‡				
Pre-albuterol	21	1.56 (116%)	1.10 (91%)	71 (78%)
Post-albuterol		1.79 (133%)	1.54 (127%)	86 (95%)
Admissions				
Year	General inpatient	ICU	Total	Inpatient days
2015	1	0	1	2
2016	4	3	7	32
2017	1	0	1	6
2018	0	0	0	0

Bold values indicate time period before initiation of tocilizumab.

C-ACT, Childhood Asthma Control Test (for children 4–11, max score 27); FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ICU, intensive care unit.

*Initiation of tocilizumab.

†Tocilizumab discontinued.

‡Pre/post-spirometry. Baseline obtained and then patient received albuterol 2 puffs, 90 mcg/inh. Post-albuterol spirometry demonstrated FEV₁ reversibility ≥12% and an absolute increase in FEV₁ of ≥200 mL over baseline value.

and multiple requiring noninvasive positive pressure ventilation [NIPPV]). Past workup demonstrated negative testing to aeroallergens (skin prick testing [SPT] and serum allergen-specific IgE [sIgE]), normal total serum IgE, normal immune evaluation, sweat test not suggestive of cystic fibrosis, and mild peripheral eosinophilia without evidence for Churg-Strauss syndrome. His antineutrophil cytoplasmic antibody testing was negative and vocal cord dysfunction was thought to be unlikely. Modified barium swallow (MBS) showed deep laryngeal penetration of thin liquids, and rigid bronchoscopy revealed type 1 laryngeal cleft. Flexible bronchoscopy and bronchoalveolar lavage (BAL) revealed columnar epithelium admixed with numerous eosinophils and scattered neutrophils, macrophages, and lymphocytes (consistent with mixed T_H2/T_H17 inflammation).

Despite fluticasone-salmeterol, montelukast, azithromycin, prednisolone (QOD), omeprazole, nectar-thickened liquids, and careful assessment of adherence to medications in November 2015, he developed status asthmaticus requiring mechanical ventilation, isoflurane, continuous albuterol, heliox, and terbutaline with a prolonged ICU course. Theophylline and intravenous immunoglobulins (IVIG, at 1 g/kg q4wk) were added to his treatment regimen. His insurance denied coverage of omalizumab. Compassionate use for mepolizumab was not approved. Dupilumab was not yet Food and Drug Administration (FDA) approved. When stabilized, he underwent successful laryngeal cleft repair. After repair, his MBS was normal and thickened feeds were discontinued.

Although he initially improved after cleft repair and theophylline/IVIG, he had another severe asthma exacerbation in September 2016 requiring NIPPV. Given ongoing life-threatening exacerbations and

known T_H2/T_H17 airway inflammation, he started tocilizumab on October 27, 2016, at 10 mg/kg IV q4wk. Tocilizumab was approved through his insurance. Six months later, he was admitted to ICU in status asthmaticus, so tocilizumab was increased to 8 mg/kg/dose q2wk and IVIG to 1 g/kg q2wk. On this regimen, he developed neutropenia (absolute neutrophil count: 680 cells/μL). Accordingly, the tocilizumab dose was readjusted to 10 mg/kg q4wk, on which he is currently maintained, in addition to budesonide/formoterol (160–4.5 mcg, 2 puff BID), montelukast (10 mg QD), azithromycin (200 mg 3 times/wk), theophylline (450 mg QD), prednisolone (9 mg QOD), and IVIG 1 g/kg q2wk.

Overall, he exhibited sustained clinical and immunologic responses to tocilizumab over the past 26 months. Clinically, he had decreased hospital admissions, inpatient hospital days, and improved pulmonary function testing (forced expiratory volume in 1 second from 76% to 99%) (Tables I and II; Figure E1, A, available in this article's Online Repository at www.jaci-inpractice.org). He has also had a marked immunological response to tocilizumab with decreased circulating IL-4⁺Foxp3⁺ (T_H2-like) and IL-17⁺Foxp3⁺ (T_H17-like) Treg cells, implicated in disease pathogenesis (Figure 1, A and C),⁹ as well as decreased T_H2 and T_H17 effector cells (Figure E2, A, available in this article's Online Repository at www.jaci-inpractice.org).

PATIENT 2

This is a 5-year-old boy with mild atopic dermatitis, eosinophilic esophagitis, and severe persistent, nonatopic asthma who exclusively carried the dominant *IL4R*^{Q576} allele. Although showing improvement of obstruction on bronchodilation (Tables I and II), he had

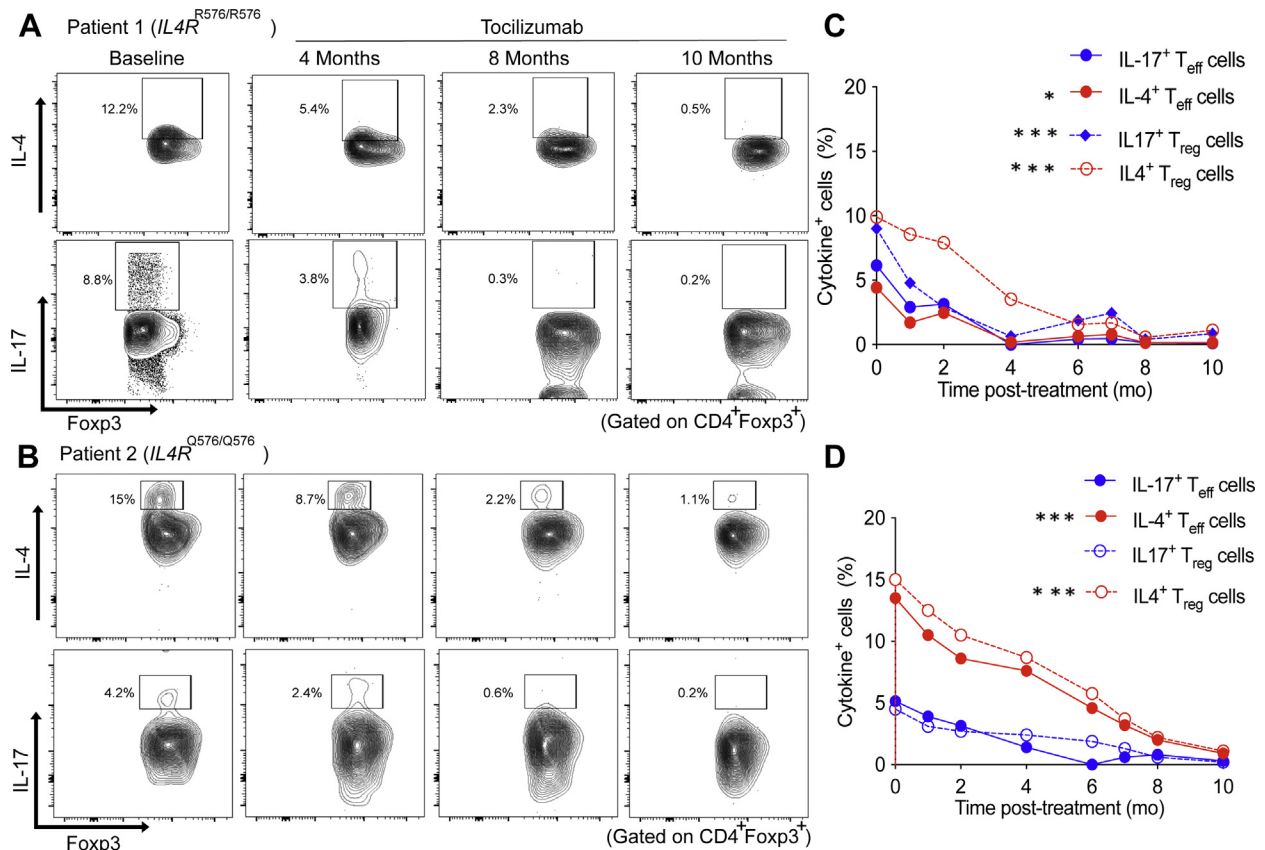


FIGURE 1. Analysis of cytokine production by Tregulatory (Treg) cells in patients 1 and 2. **A, B,** Flow cytometric analysis of IL-4 and IL-17 expression in Treg cells just before therapy and at 4, 8, and 10 months after tocilizumab treatment in **(A)** patient 1 and **(B)** patient 2. **C, D,** Graphical presentation of IL-4 and IL-17 expression in Treg and T-effector (Teff) cells in **(C)** patient 1 and **(D)** patient 2. **P* < .05, ****P* < .001, by 2-way analysis of variance with Tukey post-test analysis.

persistent severe asthma symptoms despite nectar thick liquids, mometasone/formoterol, fluticasone, montelukast, theophylline, prednisolone (QOD), and omeprazole. Azithromycin was discontinued because of lack of clinical benefit. Theophylline was discontinued because of side effects. Evaluation revealed negative SPT and sIgE to aeroallergens, normal total IgE, reassuring immune evaluation, sweat test not suggestive of cystic fibrosis, normal ciliary biopsy, and negative allergic bronchopulmonary aspergillosis workup. He had mild peripheral eosinophilia without evidence for Churg-Strauss syndrome. MBS showed deep laryngeal penetration of thin liquids. His rigid bronchoscopy revealed type 1 laryngeal cleft. He underwent flexible bronchoscopy and BAL that demonstrated airway eosinophilia. BAL culture grew moderate *Streptococcus pneumoniae*, for which he was treated with Augmentin. Nevertheless, he continued to have persistent exacerbations requiring oral steroids. Therapies with other biologics were considered but were not available. Omalizumab and anti-IL-5 therapy were not approved for his age (also has nonatopic history). Dupilumab was not at that time FDA approved. His insurance denied IVIG therapy but approved tocilizumab for the treatment of asthma. He started tocilizumab on February 23, 2017, at 10 mg/kg IV q4wk. Because of ongoing asthma symptoms, tocilizumab was increased to 8 mg/kg q2wk on May 2017. He had 1 episode of neutropenia (absolute neutrophil count: 840 cells/ μ L) that spontaneously resolved after tocilizumab was held for 2 weeks. He

discontinued tocilizumab on August 28, 2017, given commitment/challenges of traveling for IV infusions.

While on tocilizumab, the patient demonstrated favorable clinical response with decreased hospital admissions (Tables 1 and II; Figure E1, B, available in this article's Online Repository at www.jaci-inpractice.org), and was weaned off oral prednisolone. Flow cytometric analysis at baseline demonstrated T_H2^{High} skewing affecting his Treg and Teff cells with a lesser T_H17 cell response as compared with patient 1 (Figure 1, B). Tocilizumab therapy suppressed his circulating IL-4⁺Foxp3⁺ (T_H2-like) and IL-17⁺Foxp3⁺ (T_H17-like) Treg cells (Figure 1, B and D) and T_H2 and T_H17 cells (Figure E2, B, available in this article's Online Repository at www.jaci-inpractice.org). His immunological improvement remained sustained at 4 months after tocilizumab therapy, the last time his studies were repeated. Since discontinuation of tocilizumab, he underwent laryngeal cleft repair with subsequent normal MBS so nectar thick liquids were discontinued.

In summary, both patients demonstrated clinical and immunological responses to tocilizumab therapy. There were no adverse infections on tocilizumab despite developing mild neutropenia that spontaneously resolved. Neither patients' peripheral eosinophilia was impacted by tocilizumab therapy (Figure E3, available in this article's Online Repository at www.jaci-inpractice.org). Patient 1, homozygous for the *IL4R*^{R576}

mutant allele, continues on tocilizumab with clinical and immunologic response. Patient 2, homozygous for the dominant *IL4R*^{Q576} allele, discontinued tocilizumab but also demonstrated clinical and immunological improvement. Clinical response for both patients must also be evaluated in the setting of other confounding factors including polypharmacy, cleft repair, and the natural history of asthma, which are limitations for this case series.

Tocilizumab therapy may be effective in patients with severe persistent, steroid-resistant asthma by virtue of suppressing both T_H2 and T_H17 cell responses. Further studies are needed to determine the role of anti-IL-6R therapy in severe asthma and the utility of *IL4R* genotyping in directing better patient outcomes.

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ONLINE REPOSITORY

METHODS

Study approval

Studies on patient peripheral blood samples were all performed at the Boston Children's Hospital and were approved by the Institutional Review Board.

Antibodies: flow cytometry and intracellular staining

Single-cell suspensions were stained with the indicated antibodies and analyzed on an LSRIIFortessa cytometer (Becton Dickinson, Franklin Lakes, NJ). Cytokine expression in CD4⁺ T cells was determined by stimulating cells with phorbol myristate acetate (20 ng/mL) plus ionomycin (1 µg/mL) for 4 hours in the presence of Golgi-plug (BD Biosciences, Billerica, MA) followed by intracellular staining for the respective cytokine using the eBioscience Fixation/Permeabilization buffer following the manufacturer's instructions. Fluorescence-conjugated monoclonal antibodies (mAbs) used were obtained from BD Biosciences, Biolegend, and eBioscience: anti-CD3-APC-Cy7 (H1T3a), anti-CD4-PerCP-Cy5.5 and PE (PRA-T4), anti-CD25-PE (CD25-4E3), anti-CD127-PE-Cy7 (A019D5), anti-CRTH2-FITC (BM16), anti-CXCR3-APC (G025H7), anti-CCR4-BV605 (L29H14), anti-CCR6-Amcyan (G034E3). For intracellular staining, the following mAbs were used from BD Biosciences, Biolegend, and eBioscience: anti-IFNG-PE-Cy7 (45.B3), anti-IL-13-PerCP-Cy5.5 (JE510-SA2), anti-IL-4-BV605 (MP4-25D2), anti-IL-17-APC (BL168), and anti-FOXP3-Pacific Blue (PCH101).

Cell preparation

Blood was obtained from the patients after a written consent. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll (GE-Healthsciences, Woburn, Mass). Shortly, 4 mL of Ficoll was layered in a 15 mL tube. Afterward, the blood was layered on top of Ficoll very slowly to build 2 separate phases. The ficoll/blood mixture was centrifuged at 300 *g* for 20 minutes without breaks to have the PBMCs caught in the

middle layer. The cells were aspirated into a new 15 mL tube and washed twice with 10 mL phosphate buffer solution (PBS). The cell pellet was then used for the PMA/ionomycin/Golgi-plug stimulation and fluorescence-activated cell sorting (FACS) staining.

Data analysis

The flowcytometric analysis of the data was done using Flowjo software (FlowJo, LLC). The graphs and statistical analysis were done using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, Calif: www.graphpad.com). The time course of cytokine expression after therapy was analyzed by 2-way analysis of variance with Tukey post-test analysis. Within each cell type and for each cytokine, the values obtained after therapy were compared with the pretherapy baseline value. A *P* value <.05 was considered statistically significant.

Statistical process control (SPC) charts were created to demonstrate days between admissions for patients 1 and 2. SPC charts demonstrate data over time illustrating common cause and special cause variation. A t-chart is a measure of time between disease incidences for a specified period of time.^{E1} Common cause variation demonstrates randomly distributed variation around the center line appearing between upper and lower control limits.^{E2} Common cause variation represents variation inherent in the process. Control limits are positioned 3 standard deviations from the center line (3-sigma limits).^{E1} Special cause variation demonstrates nonrandom variation. Identification of nonrandom variation is defined by rules of SPC. The Western Electric rules for special cause variation include: (1) 1 or more points beyond a 3-sigma limit; (2) 2 of 3 successive points beyond a 2-sigma limit; (3) 4 of 5 successive points beyond a 1-sigma limit; and (4) 8 consecutive points above or below the center line.^{E3} SPC t-charts were created for the days between admissions in QI Macros, SPC Software for Excel (<https://www.qimacros.com>) to analyze measures.

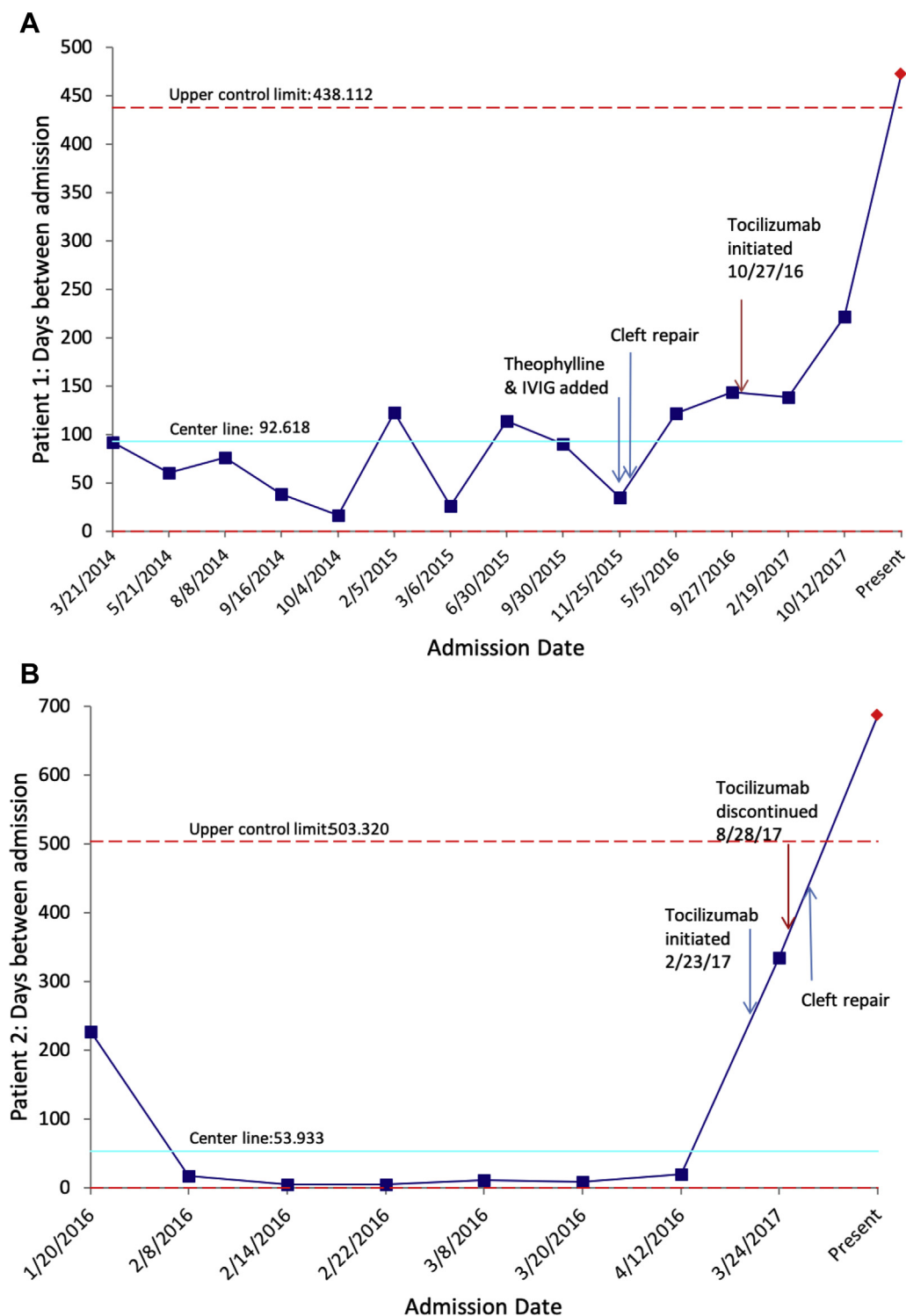


FIGURE E1. Statistical process control t-chart illustrating days between inpatient hospital admissions in patient 1 (A) and patient 2 (B). Note: red points indicate special cause variation. IVIG, Intravenous immunoglobulins.

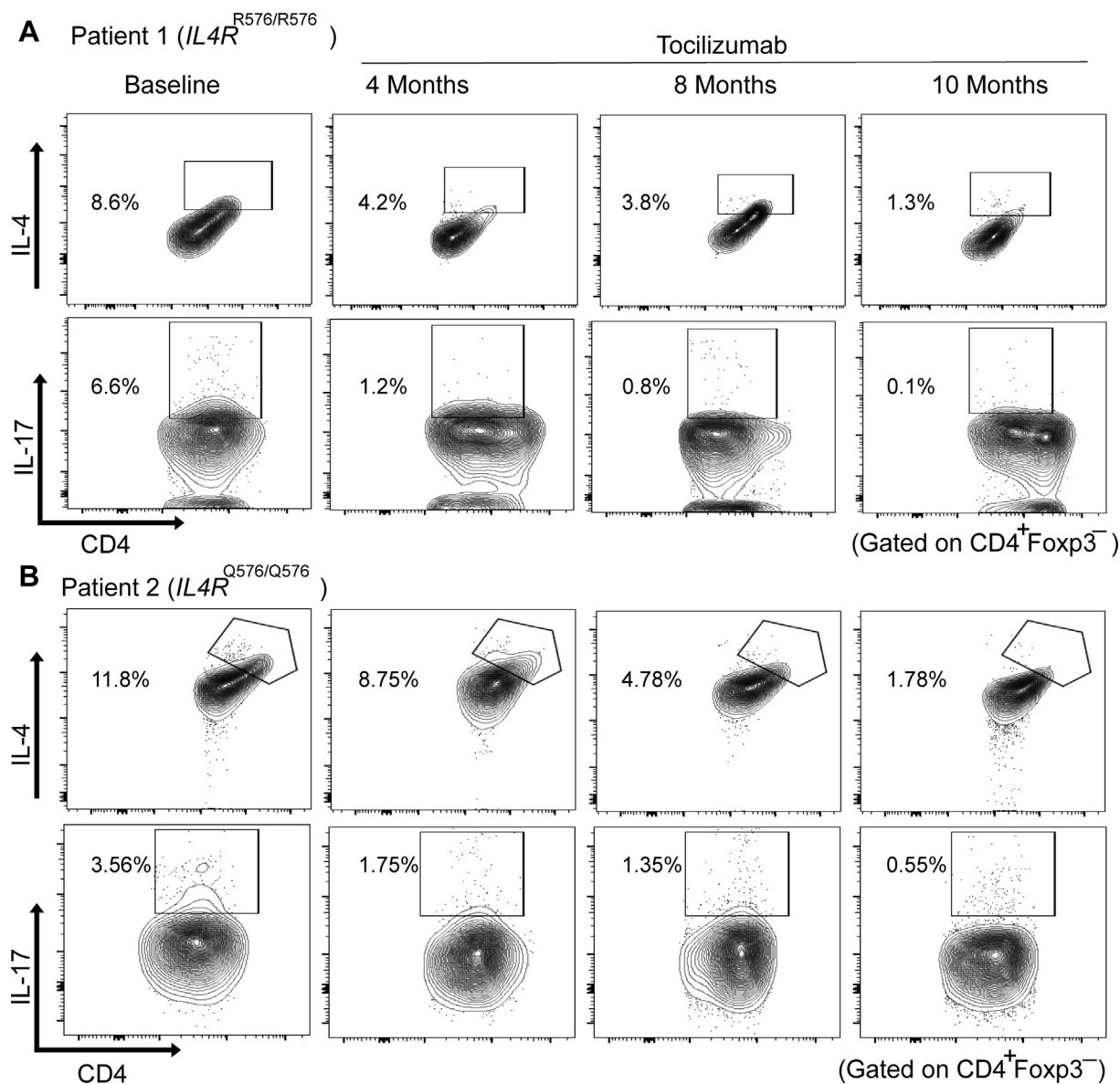


FIGURE E2. Analysis of cytokine production by T effector (Teff) cells in patients 1 and 2. **A, B,** Flow cytometric analysis of IL-4 and IL-17 expression in Teff cells at baseline and at 4, 8, and 10 months after tocilizumab treatment in **(A)** patient 1 and **(B)** patient 2.

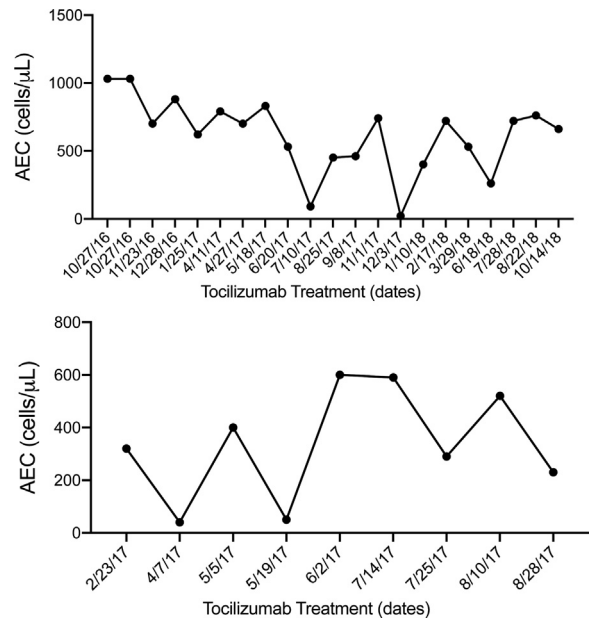


FIGURE E3. Peripheral blood absolute eosinophilic count (AEC) at baseline and with each tocilizumab infusion in (A) patient 1 and (B) patient 2.

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