differential, and transformation of skin KCs.

Data availability statement
Data sets related to this article were previously published (Ryan et al., 2019) and are available at ncbi.nlm.nih.gov/geo under accession # GSE106281.

ETHICS STATEMENT
The use of human cells was approved by University of Colorado Institutional Biosafety Committee, and no patient consent was necessary as human tissue or cells were obtained from third party commercial sources. The use of tissues from animal experiments was approved by University of Colorado Institutional Animal Care and Use Committee.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.03.940.

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AUTHOR CONTRIBUTIONS
Formal Analysis: ME, ECT; Investigation: SKC, POI, SM, ECT; Resources: ME, ECT; Visualization: SKC, POI, SM, ECT; Writing - Original Draft Preparation: SKC, ECT

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Differential Effects of Biologics on Psoriasis-Related Vascular Inflammation and Risk of Thrombosis


TO THE EDITOR
The high prevalence of atherosclerosis in patients with psoriasis is consistent with a chronic low-grade vascular inflammation (Puig, 2017), a symptom associated with a variety of cardiovascular (CV) events. The possibility that biological anti-inflammatory drugs used to treat the cutaneous manifestations of psoriasis also target this accompanying vascular inflammation raises their therapeutic potential.

This study addresses this issue by means of a comprehensive experimental protocol incorporating an in vitro functional approach using blood samples from patients with psoriasis and a well-established translational animal model of the disease. Our aim was three-fold: (i) to evaluate if psoriasis has any impact on leukocyte and endothelium interactions, a hallmark of vascular inflammation that represents an early
stage of the atherosclerotic plaque formation and precedes the generation of thrombi; (ii) to explore the influence of several of the currently used biologics on such interactions; and (iii) to determine how psoriasis-associated vascular dysfunction impacts on thrombi formation—an end-stage event—and its modulation by biologics.

Our initial experiments assessed the interaction of leukocytes from patients with moderate-to-severe psoriasis (PASI and/or body surface area ≥ 10 and Dermatology Life Quality Index ≥ 10) with healthy human vascular endothelial cells (Supplementary Materials and Methods). This study was approved by the Hospital Universitario Dr. Peset’s ethical review board and all patients signed informed consent forms before participating in the study. The results obtained were consistent with the presence of an inflammatory milieu in the blood of patients with psoriasis, as indicated by a significant decrease in the rolling velocity of polymorphonuclear cells (PMNs) and an increase in the number of rolling PMNs and number of PMNs adhering to the endothelial monolayer (Figure 1a–c; Supplementary Videos S1 and S2). The adhesion of PMNs is mediated by the interaction of β2-integrins with ICAM-1. These three β2-integrins share a common β subunit (CD18) and have a specific α subunit (CD11a for LFA-1, CD11b for Mac-1, and CD11c for CR4). The effect of biologics on the expression of these subunits was evaluated: (d) CD11a, (e) CD11b, (f) CD11c, and (g) CD18. Results are expressed as mean ± SEM, n = 10. *P < 0.05, **P < 0.01, ***P < 0.001, or ****P < 0.0001 versus healthy volunteers; #P < 0.05, ##P < 0.01 versus naïve patients (if distribution was normal: two-tailed, unpaired Student’s t-test; if no normal distribution was given: two-tailed, Mann-Whitney test). ADA, adalimumab; HUVEC, human umbilical vein endothelial cell; PMN, polymorphonuclear cell; SEC, secukinumab; UST, ustekinumab.

Figure 1. Effect of biologics on human leukocyte-endothelium interactions and on the expression of adhesion molecules. The interactions between PMNs isolated from patients with psoriasis and healthy human umbilical vein endothelial cells (HUVECs) were assessed using a parallel plate flow chamber in vitro model in which the flow conditions mimic the in vivo fluid dynamic environment. Among the patients with psoriasis, two cohorts were differentiated: biologic-naïve (naïve) and biologic-treated with ADA, SEC, or UST. Images were recorded in a single field of view over a 5-minute period during which (a) rolling velocity, (b) rolling flux, and (c) adhesion to a confluent HUVEC monolayer were determined. The adhesion of PMNs to endothelial cells is mediated by the interaction of leukocyte β2-integrins LFA-1, Mac-1, and/or CR4 with ICAM-1, their endothelial ligand. These three β2-integrins share a common β subunit (CD18) and have a specific α subunit (CD11a for LFA-1, CD11b for Mac-1, and CD11c for CR4). The effect of biologics on the expression of these subunits was evaluated: (d) CD11a, (e) CD11b, (f) CD11c, and (g) CD18. Results are expressed as mean ± SEM, n = 10. *P < 0.05, **P < 0.01, ***P < 0.001, or ****P < 0.0001 versus healthy volunteers; #P < 0.05, ##P < 0.01 versus naïve patients (if distribution was normal: two-tailed, unpaired Student’s t-test; if no normal distribution was given: two-tailed, Mann-Whitney test). ADA, adalimumab; HUVEC, human umbilical vein endothelial cell; PMN, polymorphonuclear cell; SEC, secukinumab; UST, ustekinumab.
Biologics and Psoriasis-Related Vascular Inflammation

We employed the ferric chloride model of vascular injury to explore the consequences of this increase of leukocyte-endothelium interactions with respect to the formation of thrombi (Koupenova et al., 2017). A low concentration of ferric chloride (50 mM) does not directly elicit thrombosis in the cremasteric arterioles of healthy animals (Supplementary Figure S2). However, if this dose was superfused onto the arterioles of mice with IMQ-induced psoriasis, a rapid occlusion followed, consistent with a prothrombotic milieu and in line with the aforementioned induction of endothelial-leukocyte interactions (Figure 2b, Supplementary Video S5). This enhanced sensitivity to occlusion was evident as early as two days after application of IMQ and was paralleled by the development of dermal damage and the enhanced leukocyte recruitment, peaking when the psoriatic lesion was fully established. Our results are consistent with clinical studies pointing to an association between severe psoriasis and increased CV risk (Mehta et al., 2011).

Treatment with anti-TNFα and anti-IL-17 drugs, but not anti-IL-12/23, reversed the psoriasis-related increase in the well-established translational model of imiquimod (IMQ)-induced psoriasis. All animal procedures complied with Spanish law and were approved by the Ethics Committee of the Generalitat Valenciana (A1487302585981). In this setting, in which both leukocytes and endothelial cells are exposed to the same milieu, we observed a marked increase in leukocyte adhesion to the vascular wall (Figure 2a; Supplementary Videos S3 and S4). This effect was concurrent with the appearance of cutaneous injury and peaked when the psoriatic lesion was fully established (6 days) (Supplementary Figure S1).
in leukocyte-endothelial interactions in both our in vitro (with human samples) and animal models. Leukocytes from patients in clinical remission (PASI < 3) and treated with either anti-TNFα (adalimumab) or anti–IL-17 (secukinumab) drugs exhibited a level of leukocyte-endothelium interactions similar to those in healthy volunteers. However, leukocytes from subjects with similar values of PASI but treated with an anti–IL-12/23 (ustekinumab) displayed a level of interaction comparable with that obtained in biologically naive patients, thus suggesting a vascular inflammatory milieu (Figure 1a–c). Likewise, in blood from adalimumab- or secukinumab-treated patients with psoriasis, the profile of CD11c and CD18 was similar to that in healthy volunteers but remained high in patients treated with ustekinumab. The CD11b subunit was unaffected and remained upregulated in all cases (Figure 2d–g).

Continuous administration of the evaluated biologic treatments during the entire period of IMQ application could have hampered the vascular inflammatory cascade; thus, once the psoriatic condition was fully established (6 days), we assessed the actions of single doses of anti–TNFα, anti–IL-17 and anti–IL-12/23 drugs. As expected, acanthosis was not influenced by any of the biological drugs assessed (Supplementary Figure S3). However, single doses of the three anti–TNFα drugs (adalimumab, infliximab, and etanercept) or the anti–IL-17 drug reversed the leukocyte-endothelium interactions that accompanied IMQ-induced psoriasis (Supplementary Video S6), whereas the anti–IL-12/23 drug did not reverse leukocyte recruitment (Figure 2c). Furthermore, the enhanced sensitivity to thrombi formation observed in non-treated psoriatic mice was dose-dependently prevented by anti–TNFα (Supplementary Video S7) and anti–IL-17 drugs but not by anti–IL-12/23 (Figure 2d) (Supplementary Results).

Our results are in line with observational data from large cohorts of anti-TNF–treated patients, suggesting a reduced risk of major adverse cardiac events (Sajja et al., 2018) (Supplementary Discussion), and with the recently published results of the CARIMA clinical trial (von Stebut et al., 2019), which indicate that secukinumab has a beneficial effect on CV risk by improving the endothelial function of patients. Although IL-12 is assumed to be proatherogenic (Lee et al., 1999), meaning that its inhibition should confer some vascular protection, most clinical data concerning ustekinumab suggest a neutral profile with respect to adverse CV events (Armstrong et al., 2014; Hugh et al., 2014; Reich et al., 2011) or even an increase in the rate of observed major adverse cardiac events (Tzellos et al., 2013). Furthermore, it is important to point out that clinical trials with another anti–IL-12/IL-23, briakinumab, have been discontinued pending further investigation about mechanistic links to major adverse cardiac events (Ryan et al., 2011), and vigilance for CV risk factors is recommended when initiating dermatological treatment with any drug belonging to this family of biologics.

Overall, our results demonstrate that psoriasis coexists with a state of vascular inflammation conductive to an increased risk of cardiovascular events such as thrombosis. Anti–TNFα and anti–IL-17 drugs, but not anti–IL-12/23, prevent this state, thus fueling the ongoing debate about the appropriateness of earlier initiation of treatment with selected biologics in patients with psoriasis at a high risk of developing CV events.

Data availability statement

Datasets related to this article can be found at https://doi.org/10.17632/mkgmnp3nd.1, hosted at Mendeley Data (Andújar, 2019).

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CONFLICT OF INTEREST

JVE has consulted or participated in teaching activities sponsored by Abbvie, Gilead, MSD, and Pfizer. None of the other authors state any conflicts of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: JVE, IA; Data Curation: PGM, AMP; Formal Analysis: PGM, ABG, IA; Funding Acquisition: JVE, AA, IA; Investigation: PGM, VCD, CVV; Methodology: VCD, ABG, IA; Project Administration: JVE, AA; Resources: AMP, AA; Supervision: JVE, IA; Validation: SRL; Visualization: PGM, IA; Writing - Original Draft Preparation: JVE, IA; Writing - Review and Editing: VCD, AMP, SRL, ABG, AA, IA

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.02.039.

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Loss of Wild-Type CDKN2A Is an Early Event in the Development of Melanoma in FAMMM Syndrome

TO THE EDITOR

The development of melanoma involves a sequence of genetic and epigenetic alterations. Somatic mutations typically sequentially induce MAPK pathway activation (BRAF and NRAS), upregulation of telomerase (TERT), and disruption of the G1/S cell cycle checkpoint (CDKN2A), in addition to other pathogenic alterations (Shain et al., 2018). Biallelic CDKN2A loss is the most common genetic alteration distinguishing melanocytic nevi from invasive melanomas (Shain et al., 2015). Homozygous deletion of CDKN2A has been reported in a small proportion of dysplastic nevi but never in common nevi. Studies in primary human melanocytes have revealed that CDKN2A deletions confer migratory and invasive behavior (Tran et al., 2002; Zeng et al., 2018).

At least one third of hereditary melanoma cases are caused by heterozygous germline mutations of CDKN2A, designated as familial atypical multiple mole melanoma (FAMMM) syndrome (Bergman et al., 1990). This dominant high penetrance melanoma susceptibility gene encodes two tumor suppressor proteins that are translated in alternate reading frames. The α transcript encodes p16\(^{\text{Nkx2}}\), a protein that mediates G1 arrest by inhibiting the phosphorylation of the cyclin D1–CDK4/6 complex. The β transcript encodes p14\(^{\text{ARF}}\), which inhibits MDM2, thereby promoting p53 activity (Sharpless and DePinho, 1999). In carriers of germline CDKN2A mutations, the wild-type allele is functionally inactivated in melanoma by a second somatic event, commonly through deletion (Curtin et al., 2005). In the Netherlands, a specific founder mutation, a 19–base pair deletion in exon 2 of CDKN2A (c.225_243del, p.(A76Cfs\(^*\)64)) known as the p16\(^{\text{Leiden}}\) mutation, is the most frequent cause of hereditary melanoma (Gruis et al., 1995b). CDKN2A loss-of-heterozygosity (LOH) has been demonstrated previously in primary and metastatic melanomas of patients with FAMMM syndrome (Gruis et al., 1995a; Hashemi et al., 1999). The timing of wild-type CDKN2A inactivation in hereditary melanoma development because of germline CDKN2A mutation is unknown.

The objective of this study was to investigate CDKN2A LOH in melanocytic neoplasms of patients with FAMMM syndrome. To analyze allelic imbalances at the CDKN2A locus, we developed a custom single nucleotide polymorphism (SNP)-based digital PCR method enabling absolute quantification of both alleles (Nell et al., 2019). The rs2811708 SNP, located in intron 1 of CDKN2A 2 kilobases upstream of the p16\(^{\text{Leiden}}\) mutation, has a minor allele frequency of 26% in the Dutch population. A second SNP, rs3731237, located 4 kilobases downstream of the p16\(^{\text{Leiden}}\) mutation with a minor allele frequency of 26%, was included to validate the presence of allelic imbalances at 9p21 (Boomsma et al., 2014). Capillary sequencing analysis was performed on blood DNA of homozygous and heterozygous p16\(^{\text{Leiden}}\) mutation carriers to show linkage of rs2811708-[T] and rs3731237-[C] with the p16\(^{\text{Leiden}}\) mutation (Figure 1b and c). Combined, this SNP-based digital PCR approach allows for quantification of CDKN2A allelic imbalances and losses in melanocytic neoplasms of patients with FAMMM syndrome.

The pathological diagnosis of all lesions was made by two melanoma pathologists independently. Patient consent was not necessary as the genetic analysis of nevus and melanoma biopsy material was in compliance with the Dutch code of conduct for responsible use of human tissue in the

Abbreviations: FAMMM, familial atypical multiple mole melanoma syndrome; LOH, loss-of-heterozygosity; SNP, single nucleotide polymorphism

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SUPPLEMENTARY RESULTS
These results expand on those published by Li et al. (2018), using a different model of thrombosis (photochemical injury) and treating mice for 6 weeks with specific antibodies that target mouse IL-12/23 and IL-17A. Although the same protective effects are observed with anti–IL-17, there is a discrepancy between our results with ustekinumab and their results with anti–IL-12/23 mouse antibodies that would require further investigation and that could be due to the different thrombotic trigger used and/or the time frame in regard to drug administration and exposure.

SUPPLEMENTARY DISCUSSION
By differentiating between the protective effects of anti-TNFα drugs and a lack of action of the anti–IL-12/23 ustekinumab, our data are in keeping with in vitro results concerning leukocyte-endothelial cell interactions (Ríos-Navarro et al., 2015). The former agents inhibited adhesion molecules in both leukocytes and endothelial cells, whereas ustekinumab displayed a more discreet profile, having no effect on leukocyte recruitment by the endothelium (Ríos-Navarro et al., 2015). Previous studies (Edwards et al., 1993; Simon et al., 1992) carried out on skin biopsies reported that the leukocytes from patients with psoriasis expressed significantly higher levels of CD11b, CD11c, and CD18 subunits in dendritic cells and keratinocytes; however, the vascular relevance, if any, was not evaluated.

SUPPLEMENTARY MATERIALS AND METHODS
Human study population
Peripheral blood was obtained from a sample of 50 participants, 40 patients with psoriasis and 10 age- and sex-matched healthy volunteers (age range, 30–61 years old). None of the patients had been on systemic therapy during at least 4 weeks before their enrollment in the study. Among the patients with psoriasis, two cohorts were differentiated: biologic-naïve (10 patients) and biologic-treated (10 patients per treatment group). Participants were considered eligible to be included in the study if they were aged ≥ 18 years and diagnosed with moderate-to-severe psoriasis (PASI and/or body surface area ≥ 10 and Dermatology Life Quality Index ≥ 10). The demographics and clinical characteristics of the cohort were consistent with moderate-to-severe disease (predominantly male, with an elevated BMI, Supplementary Table S1). Patients with a history of cardiopathy and patients taking nonsteroidal anti-inflammatory drugs were excluded. The diagnosis of psoriasis was confirmed and quantified by a dermatologist. Patients in the biologic-naïve group had shown failure or contraindication/intolerance in at least one systemic therapy regimen (including cyclosporine, methotrexate, and phototherapy). The treatment group included patients under treatment with biologic drugs used in monotherapy (adalimumab, secukinumab, or ustekinumab). All treated patients were considered to be in clinical remission (PASI < 3). Corresponding healthy volunteers were recruited to undergo the same testing as the patients with psoriasis. The study was approved by the Hospital Universitario Dr. Peset’s ethical review board, and all patients signed informed consent forms before participating in the study.

Whole blood from patients was collected in sodium citrate tubes (BD Vacutainer, Beckton Dickinson S.A., Madrid, Spain), and samples were incubated with dextran (3%) for 45 minutes. Human polymorphonuclear cells in the supernatant were separated by gradient density centrifugation (250g, 25 minutes) with Ficoll-Paque Plus (GE Healthcare, Chicago, IL). Leukocytes were washed (Hank’s Balanced Salt Solution without Ca2+ or Mg2+) (Lonza, Basel, Switzerland) after red blood cell lysis and resuspended in complete RPMI media (Gibco, Thermo Fisher Scientific, Waltham, MA).

Adhesion assay under flow conditions
Human umbilical vein endothelial cell culture. Cells were harvested from freshly obtained umbilical cords, and transferred to fibronectin-coated (5 μg/ml) 25-mm plastic coverslips (Nunc, Thermofisher Scientific) until confluence (about 48 hours) for the adhesion experiments (De Pablo et al., 2010b).

Flow cytometry studies of human samples
Leukocyte adhesion molecules (CD11a, CD11b, CD11c, and CD18) were analyzed in a FACS Calibur cytomter (BD Biosciences, San Jose, CA) within 2 hours of blood collection. Samples (40 μl of blood) were incubated on ice with saturating amounts of FITC-conjugated antibody (CD11a and CD18) or phycoerythrin-conjugated antibody (CD11b and CD11c) for 20 minutes in the dark. The antibodies were assayed at the previously described doses (De Pablo et al., 2010b).
Blood samples were lysed using BD FACS Lysing Solution (BD Biosciences, Allschwil, Switzerland). Neutrophils were identified in the flow cytometer by their specific size (forward-angle light scatter) and granularity (side-angle light scatter).

In each sample, the mean of the specific median fluorescence intensity was employed as a marker of the expression of the respective epitope, and all samples were compensated for using the appropriate isotype-matched negative control. In each case, 10,000 cells were analyzed. Analysis was performed in an EPICS XL-MCL cytometer (Coulter Electronics).

Mouse model of psoriasis
Wild-type C57BL/6 male mice (9 weeks old, 22–30 g) (Charles River Laboratories, Barcelona, Spain) were used for the induction of psoriasis. An area on the mice’s backs of approximately 3 × 2 cm was shaved 24 hours before initiating the experiment. Following the previously described protocol for the induction of psoriasis (van der Fits et al., 2009), a daily dose of 62.5 mg of a commercially available imiquimod cream (5%) (Aldara; 3M Pharmaceuticals, Maplewood, MN) was applied to the shaved backs of the mice for six consecutive days. Vehicle mice were treated similarly with Vaseline. To supplement fluid loss associated with imiquimod treatment, 100 ml of saline was injected intraperitoneal (i.p.) on days 2 and 4 into all the animals (imiquimod- and Vaseline-treated mice) (Swindell et al., 2017). On day 7, mice underwent the thrombosis protocol described hereafter.

Intravital assessment of vascular inflammation and thrombosis
Mice were anesthetized (ketamine hydrochloride 100 mg/kg and xylazine hydrochloride 10 mg/kg i.p.) and the cremaster muscle was exteriorized. An orthostatic microscope (Nikon Optiphot-2, SMZ1, Nikon) with an incorporated video camera (Nikon DS-Fi3, Nikon) was used to visualize the transilluminated tissue. During the whole procedure, the tissue was superfused continuously with bicarbonate-buffered saline (pH 7.4, 37°C, 2 ml/min). The NIS Elements AR program was used to capture images of single unbranched cremasteric vessels (x1,300). Diameter (20–35 μm in arterioles; 25–40 μm in venules) was measured with a video caliper (Microcirculation Research Institute, Texas A&M University, College Station, TX). An optical Doppler velocimeter (Microcirculation Research Institute) was used to measure center-line red blood cell velocity. Blood flow and wall shear rate were calculated as described elsewhere (Alvarez et al., 2004).

Pharmacological treatment of mice with psoriasis
The different drugs under evaluation were administered in a single dose 24 hours before the thrombosis-induction protocol was initiated. Particular emphasis was placed on employing clinically relevant concentrations of the drugs (Nast et al., 2015). However, to avoid the problems arising from using human antibodies in a mouse model, dosing was equivalent in mg/kg to that administered in a clinical context, which we based ourselves on studies evaluating the degree of binding of adalimumab to mouse TNFα (Zhu et al., 2016). In this sense, the range of doses tested was as follows: adalimumab (2.2–4.4 mg/kg, i.p.), infliximab (5–10 mg/kg, intravenous), etanercept (0.7–1.4 mg/kg, i.p.), secukinumab (2.1–4.3 mg/kg, i.p.), and ustekinumab (0.7–1.3 mg/kg, i.p.). Body weight was measured before psoriasis induction and every day until sacrifice.

Tissue harvesting and histology
Following thrombosis, animals were euthanized, and skin was collected and fixed in formalin. Paraffin-embedded skin was sectioned (5 μm) and stained with H&E, and epidermal thickness was measured by a researcher who was blind to the treatment.

Materials
Xylazine, ferric chloride, dextran, fibronectin, HEPES, HSA and methotrexate were acquired from Sigma, St. Louis, MO; DPBS + and DPBS − were acquired from Lonza; ketamine (Merial, Lyon, France), imiquimod cream (5%) (Aldara; 3M Pharmaceuticals), adalimumab (Humira), infliximab (Remicade), etanercept (Enbrel), secukinumab (Consentyx), and ustekinumab (Stelara) were used in the form of their clinically available preparations. FITC mouse IgG1 κ isotype, FITC mouse anti-human CD18 and FITC mouse anti-human CD11a, as well as PE mouse IgG1 κ isotype, PE mouse anti-human CD11b/Mac-1 and PE mouse anti-human CD11c were all acquired from BD Pharmingen, BD Biosciences, Allschwil, Switzerland. Unless stated otherwise, chemical reagents were supplied by Sigma.
Ethics and statistics
Human studies were approved by the Hospital Universitario Dr. Peset’s ethical review board, and all patients signed informed consent forms before participating in the study. All animal procedures complied with Spanish law and were approved by the Ethics Committee of the Generalitat Valenciana (A1487302585981).

Statistical analysis was performed with GraphPad Prism software (GraphPad, La Jolla, CA). The data was first analyzed for normal distribution (Kolmogorow–Smirnow test). For human samples, when normal distribution was given, we applied the unpaired two-tailed Student’s t-test. If no normal distribution was given, Mann-Whitney test was used to compare ranks. A P-value < 0.05 was considered significant. In animal experiments, when normal distribution was given, we applied the one-way ANOVA test with Tukey post-hoc test; if no normal distribution was given, Kruskal-Wallis test with Dunn’s multiple comparison was used.

Datasets related to this article can be found at https://doi.org/10.17632/mkygmbp3nd.1, hosted at Mendeley Data (Andújar, 2019).

SUPPLEMENTARY REFERENCES
Supplementary Figure S1. Acanthosis, measured as epidermal thickness, in IMQ-treated mice. Results are expressed as mean ± SEM, n = 6. *P < 0.05, ***P < 0.001, or ****P < 0.0001 versus corresponding value of 0 days. (One-way ANOVA test with Tukey post-hoc test). Bottom panels are representative images of dorsal skin stained with H&E. Bar = 200 µm. IMQ, imiquimod.

Supplementary Figure S2. Thrombus formation induced by ferric chloride in cremasteric arterioles of WT animals. After surgery, ferric chloride (25–100 mM solution) was applied to the top of the cremasteric arteriole by superfusion. Arterioles were then visualized until blood flow ceased, or for 8 minutes if no vessel occlusion occurred. Results are expressed as mean ± SEM, n ≥ 6. *P < 0.05 or **P < 0.01 versus corresponding value in ferric chloride 25 mM-treated WT group (Kruskal-Wallis with Dunn’s multiple comparisons test). WT, wild type.
Supplementary Figure S3. Acanthosis, measured as epidermal thickness, is not modified 24 hours after treatment with biological drugs. IMQ-treated mice were treated with different biological drugs used in therapy for psoriasis (ADA, 2.2 and 4.4 mg/kg, i.p.; INF, 5 and 10 mg/kg, i.v.; ETA, 0.7 and 1.4 mg/kg, i.p.; SEC, 2.1 and 4.3 mg/kg, i.p.; and UST, 1.3 and 8.6 (supratherapeutical) mg/kg, i.p.). Results are expressed as mean ± SEM, n = 6. ****P < 0.0001 versus corresponding value in Veh group (normal distribution, one-way ANOVA test with Tukey post-hoc test). Bottom panels are representative images of dorsal skin stained with H&E. Bar = 200 μm. ADA, adalimumab; ETA, etanercept; IMQ, imiquimod; INF, infliximab; i.p., intraperitoneal; i.v., intravenous; SEC, secukinumab; UST, ustekinumab; Veh, vehicle.
**Supplementary Table S1. Characteristics of Patients Enrolled in the Study**

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Naïve Mean (SEM)</th>
<th>All Mean (SEM)</th>
<th>ADA Mean (SEM)</th>
<th>SEC Mean (SEM)</th>
<th>UST Mean (SEM)</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>45.6 (2.6)</td>
<td>49.8 (1.9)</td>
<td>48.4 (3.3)</td>
<td>47.6 (3.7)</td>
<td>51.1 (2.3)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.9 (1.8)</td>
<td>168.1 (1.7)</td>
<td>167.1 (3.9)</td>
<td>168.3 (2.0)</td>
<td>168.8 (2.9)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.8 (4.2)</td>
<td>79.3 (2.7)</td>
<td>84.3 (6.5)</td>
<td>71.7 (3.0)</td>
<td>82.1 (3.0)</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>102.2 (2.8)</td>
<td>99.1 (2.9)</td>
<td>101.4 (3.8)</td>
<td>90.5 (2.6)</td>
<td>105.3 (6.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.7 (1.3)</td>
<td>28.1 (0.9)</td>
<td>30.0 (1.7)</td>
<td>25.4 (1.2)</td>
<td>29.0 (1.3)</td>
</tr>
<tr>
<td>D-dimer (%)</td>
<td>132.9 (29.1)</td>
<td>88.2 (10.6)</td>
<td>78.8 (12.0)</td>
<td>62.7 (11.6)</td>
<td>74.6 (24.6)</td>
</tr>
<tr>
<td>Gender: male</td>
<td>80</td>
<td>60</td>
<td>60</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>Inflammatory arthritis</td>
<td>20</td>
<td>30</td>
<td>50</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Never smoked</td>
<td>20</td>
<td>30</td>
<td>10</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

Abbreviations: ADA, adalimumab; BMI, body mass index; SEC, secukinumab; UST, ustekinumab.