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Distinct expression of interleukin (IL)-36 α , β and γ , their antagonist IL-36Ra and IL-38 in psoriasis, rheumatoid arthritis and Crohn's disease

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SUMMARY

Interleukin (IL)-36 α , IL-36 β and IL-36 γ are expressed highly in skin and are involved in the pathogenesis of psoriasis, while the antagonists IL-36Ra or IL-38, another potential IL-36 inhibitor, limit uncontrolled inflammation. The expression and role of IL-36 cytokines in rheumatoid arthritis (RA) and Crohn's disease (CD) is currently debated. Here, we observed that during imiquimod-induced mouse skin inflammation and in human psoriasis, expression of IL-36 α , γ and IL-36Ra, but not IL-36 β and IL-38 mRNA, was induced and correlated with IL-1 β and T helper type 17 (Th17) cytokines (IL-17A, IL-22, IL-23, CCL20). In mice with collagen-induced arthritis and in the synovium of patients with RA, IL-36 α , β , γ , IL-36Ra and IL-38 were all elevated and correlated with IL-1 β , CCL3, CCL4 and macrophage colony-stimulating factor (M-CSF), but not with Th17 cytokines. In the colon of mice with dextran sulphate sodium-induced colitis and in patients with CD, only IL-36 α , γ and IL-38 were induced at relatively low levels and correlated with IL-1 β and IL-17A. We suggest that only a minor subgroup of patients with RA (17–29%) or CD (25%) had an elevated IL-36 agonists/antagonists ratio, *versus* 93% of patients with psoriasis. By immunohistochemistry, IL-36 cytokines were produced by various cell types in skin, synovium and colonic mucosa such as keratinocytes, CD68⁺ macrophages, dendritic/Langerhans cells and CD79 α ⁺ plasma cells. In primary cultures of monocytes or inflammatory macrophages (M1), IL-36 β and IL-36Ra were produced constitutively, but IL-36 α , γ and IL-38 were produced after lipopolysaccharide stimulation. These distinct expression profiles may help to explain why only subgroups of RA and CD patients have a potentially elevated IL-36 agonists/antagonists ratio.

Keywords: Crohn's disease, IL-36, psoriasis, rheumatoid arthritis

INTRODUCTION

The interleukin (IL)-1 family of cytokines includes seven agonists (IL-1 α , IL-1 β , IL-18, IL-33, IL-36 α , IL-36 β and IL-36 γ) and four members with established or hypothetical antagonist or anti-inflammatory activity (IL-1Ra, IL-36Ra, IL-37 and IL-38) [1](#), [2](#). Agonistic cytokines are involved in shaping innate and adaptive immunity, while antagonists limit uncontrolled inflammation and immune responses. IL-36 α / β / γ recruit and activate the same receptor composed of the specific chain IL-36R (IL-1Rrp2) and the common chain IL-1R accessory protein (IL-1RAcP). All three IL-36 agonists similarly induce proinflammatory cytokines, chemokines and co-stimulatory molecules, thus promoting neutrophil influx, dendritic cell (DC) activation, polarization of T helper type 1 (Th1) and IL-17-producing T cells ($\alpha\beta$ T cells and $\gamma\delta$ T cells) and keratinocyte proliferation [3](#), [4](#). IL-36Ra also binds to IL-36R, but does not recruit IL-1RAcP. It is therefore a true antagonist of IL-36 cytokines [5](#). More recently, it has been shown that IL-38 binds to IL-36R and exerts antagonistic effects similar to those of IL-36Ra on blood mononuclear cells [6](#). However, the exact molecular mechanism by which IL-38 modulates inflammation is still controversial, and *in-vivo* data are needed to ascertain its antagonistic role.

Based on several mouse models of skin inflammation and genetic studies in patients with generalized pustular psoriasis, it is recognized that IL-36 cytokines are involved in the pathogenesis of psoriasis, and that IL-36Ra deficiency strongly exacerbates skin inflammation [3](#), [7](#). Indeed, IL-36 α , β , γ and IL-36Ra were found over-expressed in human psoriatic lesional skin, as well as in various mouse models of psoriasis [8](#). They are produced mainly by keratinocytes, but mononuclear cells, inflammatory macrophages and DCs were also found positive for expression of IL-36 cytokines [7](#), [8](#), [9](#). Inducers of IL-36 encompass inflammatory cytokines [IL-1 β , tumour necrosis factor (TNF)- α , IL-17, IL-22] and Toll-like receptor (TLR) ligands such as lipopolysaccharide (LPS) or double-stranded RNA [10](#), [11](#), [12](#). Post-translational processing (N-terminal truncation) markedly increased the biological activity of IL-36 agonists. However, the proteases involved in IL-36 processing are unknown [13](#).

Considering their expression by epithelial cells, macrophages and DC, it is not surprising that some of these cytokines were detected in other inflamed organs. In the lung, IL-36 γ is induced in various models of asthma [14](#) and can be produced by bronchial epithelial cells in response to viral infection, smoke or inflammatory cytokines [3](#), [14](#). IL-36 γ , but not IL-36 α or IL-36 β , is over-expressed by macrophages in lung granulomatous lesions following mycobacterial infection [15](#). However, IL-36R deficiency does not compromise mouse survival or lung bacterial clearance [15](#). In several mouse models of kidney disease, IL-36 α is produced by tubular epithelial cells [16](#). In a model of liver injury, only IL-36 γ is induced, presumably in hepatocytes, and administration of the antagonist IL-36Ra disturbs tissue recovery [17](#). In patients with primary Sjögren's syndrome, IL-36 α and IL-38 are over-expressed in the salivary gland, IL-36 α being produced mainly by T cells and macrophages [18](#). In the synovial membrane of rheumatoid arthritis (RA) patients, IL-36 α and IL-36Ra are expressed highly by plasma cells [19](#). Moreover, synovial fibroblasts express the IL-36R and respond to IL-36 β by induced expression of IL-6 and IL-8, suggesting that IL-36 β could contribute to joint inflammation in RA [20](#). However, experiments using blocking anti-IL-36R antibodies or mice deficient for IL-36R demonstrated that IL-36 cytokines have no significant role in different mouse models of arthritis [21](#), [22](#).

Overall, the biology of IL-36 cytokines, and in particular potential differential functions or expression patterns of the three IL-36 agonists, are still poorly understood. It is suspected that

their expression and activation are regulated differentially, but their relative expression in different organs or tissues such as psoriatic skin or arthritic joints has not been explored thoroughly. Moreover, in numerous other diseases such as inflammatory bowel disease, no data were available.

Here we compared the relative expression and cell sources of IL-36 α , β and γ , of their antagonist IL-36Ra and of IL-38 in patients with psoriasis, RA and Crohn's disease (CD), as well as in the corresponding mouse models of inflammation. Our results indicated that IL-36 cytokines and IL-38 have different expression profiles in these chronic inflammatory diseases, and suggested that only a minor subgroup of RA and CD patients have an elevated IL-36 agonists/antagonists ratio. *In situ* and in cell cultures, these cytokines were indeed produced at different levels by various cell types such as keratinocytes, monocytes/macrophages and plasma cells, their expression being also regulated differently by TLR ligands or inflammatory cytokines.

MATERIALS AND METHODS

Mouse models of inflammation

All research involving animals was conducted following institutional guidelines and was approved by the French ethical committee CEEA.2012.187, by local veterinary services (licence no. D-44015) or by the Geneva cantonal authority for animal experimentation (licence no. 1005/3935/2). Experiments were carried out under the supervision of authorized investigators. Mice were purchased from Janvier Laboratories (Le Genest, France) and were housed in pathogen-free or conventional (Geneva) facilities, and under light (12- h light/dark cycle)-, temperature (22–25°C)- and humidity (50–60%)-controlled conditions.

For imiquimod-induced skin inflammation, the back skin of male C57BL/6J mice at 8–10 weeks of age was shaved and remaining hairs were removed using a depilatory cream (Veet[®]; Reckitt Benckiser, Massy, France). Psoriasis-like skin disease was induced as described previously by van der Fits *et al.* [23](#). Shaved back skin was treated daily with 62.5 mg Aldara[®] cream [5% imiquimod (IMQ); 3M pharmaceuticals, St Paul, MN, USA] for 6 days. Control mice were treated similarly with vaseline (VAS) (Vaseline Lanette cream; Fagron, Waregem, Belgium). Two days after IMQ application, skin inflammation with erythema, scaling and thickness was observed, which increased further by day 4 (early phases of inflammation), and the disease severity reached a plateau by day 6 (peak of inflammation) [24](#).

For collagen-induced arthritis (CIA), 8-week-old male DBA/1 mice were immunized 21 days before the beginning of the experiment by intradermal injection in the tail with an emulsion of type II collagen at 2 mg/ml (Sigma-Aldrich, St Louis, MO, USA) and complete Freund's adjuvant (CFA; Sigma-Aldrich) at 1 mg/ml [25](#). At day 0, an intradermal injection of type II collagen and incomplete Freund's adjuvant (IFA; Sigma-Aldrich) emulsion in the same concentrations was performed. Mice were killed at days 3 (early phase), 7 (peak phase), 10 (stabilization phase) and 14 (resolution phase) after the first inflammatory symptoms assessed by a daily hind paw clinical scoring. Control mice were non-immunized, phosphate-buffered saline (PBS)-injected mice.

For antigen-induced arthritis (AIA), 7-week-old female C57BL/6J mice were immunized 7 days before the beginning of the experiment by subcutaneous injection at the base of the tail

of an emulsion of CFA (1 mg/ml) and methylated bovine serum albumin (mBSA, 8 mg/ml; Sigma-Aldrich). At day 0, mBSA at 10 µg/ml was injected into the right knee and mice were boosted with 20 µg lipopolysaccharide (Sigma) in 100 µl PBS by intraperitoneal injection. Mice were killed at days 3 (early phase), 7 (peak phase), 10 (stabilization phase) and 14 (resolution phase).

For collagen antibody-induced arthritis (CAIA), 8-week-old male DBA/1 mice were injected intraperitoneally with 1.5 mg of four-clone cocktail (Arthrogen-CIA[®] arthritogenic monoclonal antibodies; Chondrex, Redmond, WA, Australia). Three days later, intraperitoneal injections of 50 µg of LPS (Chondrex) were administered. Mice were killed at day 7 (peak of hind paw inflammation).

Colitis was induced in 10-week-old C57BL/6J mice by adding 3% of dextran sulphate sodium (DSS; MP Biomedicals, Heidelberg, Germany) to the drinking water. Weight loss and reduced colon length peaked at day 7 (peak of inflammation).

Back skin, ankle, knee and distal colon samples were stored at –80°C for reverse transcription–quantitative polymerase chain reaction (RT–qPCR) analysis.

Patients

All enrolled patients gave their formal consent. The study was approved by the local ethics committee of the Nantes or Poitiers Hospitals and by the French Research Ministry (no. 2008-402). The research was carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki). The following tissue biopsies were used for RT–qPCR and/or histology.

Skin biopsies were obtained from the back skin lesions of 15 patients with moderate to severe plaque psoriasis [psoriasis area and severity index (PASI) >10; all patients were Caucasians] who did not receive any therapy for >4 weeks (Table 1, see Supporting information, File S1). Normal control skin biopsies were obtained from surgical samples of healthy breast skin.

Synovial biopsies were obtained surgically at the time of arthroplasty from 21 patients with RA [disease activity score in 28 joints (DAS28) >3.2] and six patients with osteoarthritis (OA) (Table 3; see Supporting information, File S1). Patients fulfilled the American College of Rheumatology classification for RA [26](#) and OA [27](#), respectively. The histopathological severity of synovitis was graded as described by Krenn *et al.* [28](#).

Colonic biopsy specimens were obtained at the time of colectomy from 16 patients with active CD [Crohn's Disease Activity Index (CDAI) >150] (Table 2, see Supporting information, File S1), as described previously [29](#). Control colonic biopsies were obtained from the same patients from macroscopically and microscopically unaffected colonic areas, as well as from healthy donors.

In addition to these tissue biopsies, synovial fluids were obtained from 30 RA patients and 29 OA patients during an arthrocentesis due to a flare of their disease (Table 4, see Supporting information, File S1). Cells were removed by centrifugation (4900 g, 15 min) and fluids were stored immediately at –80°C.

Cell cultures

Normal human epidermal keratinocytes (NHEK) were obtained from two healthy donors, as described previously [30](#). NHEK were then cultured in keratinocyte serum-free medium supplemented with bovine pituitary extract (25 µg/ml) and recombinant epidermal growth factor (EGF) (0.25 ng/ml; all from Invitrogen Life Technologies, Cergy-Pontoise, France).

Rheumatoid arthritis synovial fibroblasts (RASf) were obtained from three RA patients after incubation of synovial membranes in collagenase A (Sigma-Aldrich) at 1 mg/ml in RPMI-1640 medium (Lonza, Basel, Switzerland) for 2 h under stirring. After 70 µm filtration, cells were cultured in RPMI-1640 medium supplemented with 10% of fetal bovine serum (ThermoScientific, Courtaboeuf, France) and 1% of penicillin/streptomycin (Lonza). After 24 h, non-adherent cells were removed and adherent RASf were used between passages 3 and 8 [31](#).

The Caco-2 cell line (ATCC, Manassas, VA, USA) was cultured in Dulbecco's modified Eagle's medium (DMEM) medium (Lonza), supplemented with 10% of fetal bovine serum and 1% of penicillin/streptomycin.

To obtain primary myeloid cells, peripheral blood mononuclear cells (PBMC) from three healthy donors were isolated by centrifugation over Ficoll gradient (Sigma-Aldrich). CD14⁺ monocytes were labelled magnetically with CD14 microbeads and selected positively by magnetic-activated cell sorting (MACS) technology (Miltenyi Biotec, Bergisch Gladbach, Germany). For M1 and M2 macrophages, CD14⁺ monocytes were cultured in alpha minimum essential medium (α-MEM; Lonza) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin and treated, respectively, for 5 days with granulocyte-macrophage colony-stimulating factor (GM-CSF; R&D Systems, Minneapolis, MN, USA) (25 ng/ml), together with interferon (IFN)-γ (R&D Systems) (50 ng/ml) or macrophage colony stimulating factor (M-CSF; R&D Systems) (25 ng/ml) together with IL-10 (R&D Systems) (50 ng/ml) [32](#). To obtain monocyte-derived DCs (moDC), CD14⁺ monocytes were cultured for 7 days with GM-CSF (25 ng/ml) and IL-4 (50 ng/ml). For osteoclast generation, CD14⁺ monocytes were cultured for 11 days with M-CSF (25 ng/ml) and receptor activator of nuclear factor kappa-B ligand (RANKL) (25 ng/ml) [33](#).

NHEK, RASf and Caco-2 cells were then treated for 24 h with or without polyI:C (25 µg/ml; Sigma-Aldrich) or a proinflammatory cytokine cocktail (M5) composed of recombinant human IL-17A, oncostatin M (OSM), TNF-α, IL-22 and IL-1β (10 ng/ml each, all purchased from R&D Systems) [34](#). Monocytes, M1, M2 macrophages, moDC and osteoclasts were treated for 8 h with *Escherichia coli* lipopolysaccharide (LPS, 100 ng/ml; Sigma-Aldrich).

RNA extraction and RT-qPCR analysis

Mouse and human tissue samples (50–100 mg) were homogenized with a DI25 Ultra-Turrax homogenizer (IKA, Staufen, Germany) in TriReagent (Molecular Research Center, Cincinnati, OH, USA). Cell cultures were scraped in TriReagent. First-strand cDNA was synthesized from 1 µg total RNA using the Maxima H Minus First Strand cDNA Synthesis Kit (ThermoScientific, Saint Aubin, France). The qPCR mix contained 200 ng reverse-transcribed total RNA, SyBr green buffer (Applied Biosystems, Saint Aubin, France) and 300 nM primers [32](#), [35](#). The qPCR was carried out on a CFX96 Real-Time PCR Detection System (BioRad, Marnes-la-Coquette, France). Analysis was performed using hypoxanthine-guanine

phosphoribosyltransferase (HPRT) as invariant control and results were expressed as $2^{-\Delta Ct}$. Calculation of the agonists/antagonists ratio was performed with the following formula: (IL-36 α -fold increase + IL-36 β -fold increase + IL-36 γ -fold increase)/(IL-36Ra-fold increase + IL-38-fold increase). A ratio of 1.5 indicates no change compared to control samples.

Enzyme-linked immunosorbent assays (ELISA) and multi-parametric luminex assays

ELISAs for the detection of IL-36 α , β and γ , IL-36Ra and IL-38 were carried out in 96-well precoated plates from MyBioSource (San Diego, CA, USA). Synovial fluids were diluted 1 : 2 prior to performing the assay according to the supplier's instructions. Absorbance at 450 nm was determined on a Victor2 plate reader (PerkinElmer, Courtaboeuf, France).

As described previously [32](#), we also measured levels of IL-1 β , IL-1Ra, TNF- α , IL-12p70, IL-17A, IL-6, IFN- γ , IL-8, CCL3, CCL4 and M-CSF in synovial fluids using Luminex technology (Bio-Plex Pro Assays from Bio-Rad and Milliplex MAP kits from Millipore, Molsheim, France). OSM levels were determined by ELISA (R&D Systems).

Immunohistochemistry (IHC)

After 48 h of formalin fixation, synovial, skin and colon samples were dehydrated and paraffin-embedded. Three- μ m-thick sections were taken and immunohistochemistry (IHC) was performed as described previously [35](#) with the following primary antibodies at the concentrations indicated: rabbit anti-human IL-36 α (Sigma-Aldrich; dilution 1 : 200), rabbit anti-human IL-36 β (Sigma-Aldrich; dilution 1 : 50), mouse anti-human IL-36 γ (Proteintech, Manchester, UK; dilution 1 : 50), mouse anti-human CD79 α (Novus Biologicals, Littleton, CO, USA; dilution 1 : 400), rabbit anti-human CD55 (Sigma-Aldrich; 1 : 200) and mouse anti-human CD68 (Dako, Les Ulis, France; 1 : 200). The counterstain was performed with Mayer's haematoxylin. Specificity of the IHC staining was confirmed using isotype control antibodies and by preincubation of the primary antibody with a 10-fold excess of recombinant IL-36 α , β or γ (R&D Systems) (see Supporting information, File S4). IHC sections of RA synovium were analysed by two observers using a five-point scale (0–4), as described elsewhere [19](#). All analyses were performed in multiple randomly selected high-power microscopic fields (original magnification \times 400) after scanning the slides with the NanoZoomer 2.0-RS system (Hamamatsu, Massy, France). Minor differences between the two observers were resolved by mutual agreement or the mean score was used.

For multiple fluorescent labelling, sections were incubated sequentially with anti-CD79 α antibody; Alexa Fluor (AF) 647-conjugated goat anti-mouse antibody (Invitrogen, Saint Aubin, France; 1 : 200); anti-CD68 antibody; biotinylated goat anti-mouse antibody (Dako; 1 : 300); AF488-conjugated streptavidin (Invitrogen; 1 : 400); anti-IL-36 α , β or γ antibody; biotinylated goat anti rabbit antibody (Dako; 1 : 600) or biotinylated goat anti-mouse antibody (Dako; 1 : 200); AF594-conjugated streptavidin (Invitrogen; 1 : 200). Prolong[®] Gold Mountant with 4',6-diamidino-2-phenylindole (DAPI) (Life Technologies) was used. The stained sections were visualized with a Nikon A1 Rsi confocal laser microscope (Champigny sur Marne, France). Images were exported into Fiji software (NIH, Bethesda, MD, USA) to generate the composite figures.

Statistical analysis

Differences between groups were evaluated by the Mann–Whitney or unpaired Student's *t*-test as indicated in the figure legends using GraphPad Prism version 6 software (La Jolla, CA, USA). Correlations were evaluated by Pearson's bivariate correlation analysis. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Distinct expression of IL-36 agonists and antagonists in mouse models of skin, joint and colon inflammation

To directly compare the expression of IL-36 α , β and γ , IL-36Ra and IL-38 in different pathophysiological conditions, we first analysed their mRNA levels in relevant mouse models of skin, joint and colon inflammation by RT–qPCR. As shown in Fig. [Fig. 1, 1](#), all five cytokines were expressed constitutively in normal mouse skin. Only IL-36 α and γ and IL-36Ra were further induced at the peak of IMQ-induced skin inflammation, whereas IL-38 mRNA levels were reduced significantly.

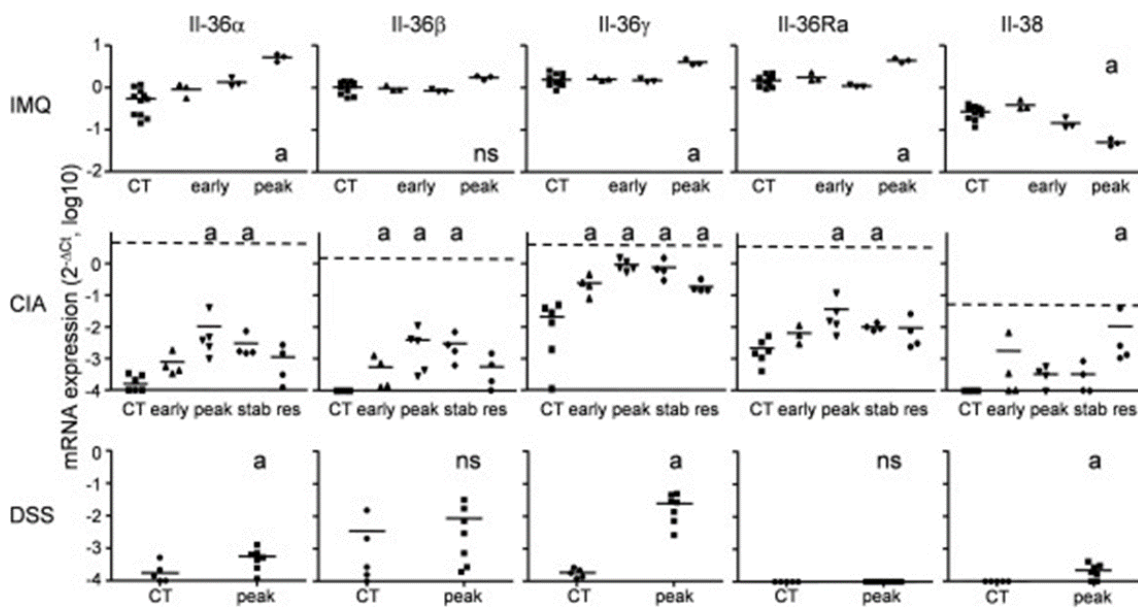


Figure 1: Expression of interleukin (IL)-36 cytokines in mouse models of inflammation. Expression of IL-36 α , β and γ , IL-36Ra and IL-38 mRNA in mouse skin during imiquimod (IMQ)-induced inflammation ($n = 3$ mice per time-point, upper panels), in hind paws of mice with collagen-induced arthritis (CIA) ($n = 4$ – 5 mice per time-point, middle panels) or in the distal colon of mice with dextran sulphate sodium (DSS)-induced colitis ($n = 7$ mice, lower panels) at early, peak, stabilized (stab) and/or resolution (res) phases of inflammation (see Materials and methods), as assessed by reverse transcription–quantitative polymerase chain reaction (RT–qPCR). Control groups (CT) comprised non-induced mice at the different time-points. The results represent cytokine mRNA expression relative to hypoxanthine–guanine phosphoribosyltransferase (HPRT) and are shown as individual values and mean. The dotted line indicates the cytokine mRNA expression level observed in mouse skin at the peak of inflammation. (a) $P < 0.05$ compared to CT group, as assessed by Mann–Whitney *U*-test.

In comparison to skin, constitutive expression of these cytokines was 100–10 000-fold lower in mouse joint (either hind ankles or knees) or colon (Fig. (Fig.1).1). During the course of collagen-induced arthritis (CIA), IL-36 α , β and γ and IL-36Ra mRNA levels were induced significantly in the joints, especially at the peak of inflammation, whereas IL-38 mRNA levels were increased further during the resolution of inflammation. Similar results were obtained in the collagen antibody-induced arthritis model (CAIA, Supporting information, File S2). In a third arthritis model, however, (antigen-induced arthritis or AIA), induction of all five cytokines was lower, and was significant only for IL-36 α and γ and IL-36Ra (Supporting information, File S2). In the DSS-induced colitis model, only IL-36 α and γ and IL-38 were induced significantly in the colon (Fig. (Fig.11).

In the DSS or IMQ models, later time-points were not included and thus expression of IL-36 cytokines or IL-38 was not analysed in the resolution of skin or colon inflammation. Overall, however, these results indicate that IL-36 agonists, IL-36Ra and IL-38 were expressed differently in several mouse models of inflammation.

IL-36 cytokines are also expressed differently in patients with psoriasis, RA and Crohn's disease

To extend these observations to patients with the corresponding chronic inflammatory diseases, we next analysed several cohorts of patients with psoriasis, RA or CD. As observed in IMQ-induced skin inflammation, patients with psoriasis had significantly higher skin mRNA levels of IL-36 α and γ and IL-36Ra, whereas IL-38 was reduced (Fig. (Fig.2a).2a). With regard to the animal models, expression of these cytokines was several-fold lower in the joints (synovial tissue) or in the colon of patients with RA or CD, respectively. As observed in the mouse CIA or CAIA arthritis models, levels of IL-36 α , β and γ and IL-38 mRNA were increased significantly in the synovial tissue of patients with RA compared to patients with OA (Fig. (Fig.2a).2a). IL-36Ra mRNA levels were not increased significantly in RA, but a few patients (two of 17) over-expressed this cytokine markedly (>80-fold) compared to OA patients. Expression of IL-38 was regulated inversely in RA (induced by 7.4-fold) *versus* psoriasis (reduced by 2.4-fold), whereas expression of IL-36 β was induced in RA (390-fold) but not in psoriasis. As observed in the mouse DSS-induced colitis model, IL-36 α and γ and IL-38 were up-regulated in inflamed colonic biopsies of patients with CD compared to unaffected biopsies from the same patients, but IL-36 β and IL-36Ra were not. This was significant for IL-36 α and IL-38, but only a tendency was observed for IL-36 γ ($P = 0.08$). Moreover, the mean fold increase of IL-36 α (2.7-fold) or IL-36 γ (2.2-fold) in CD was modest in comparison to that of patients with psoriasis (72- and 11-fold, respectively) or RA (4.6- and 48-fold, respectively) (Fig. (Fig.2a).2a). Similar results were obtained when comparing to colonic biopsies from healthy donors ($n = 15$): IL-36 α was induced 1.6-fold ($P = 0.04$), IL-36 γ was induced 2.5-fold ($P = 0.054$) and IL-38 was induced 1.6-fold ($P = 0.03$), whereas IL-36 β and IL-36Ra were not expressed differently (data not shown).

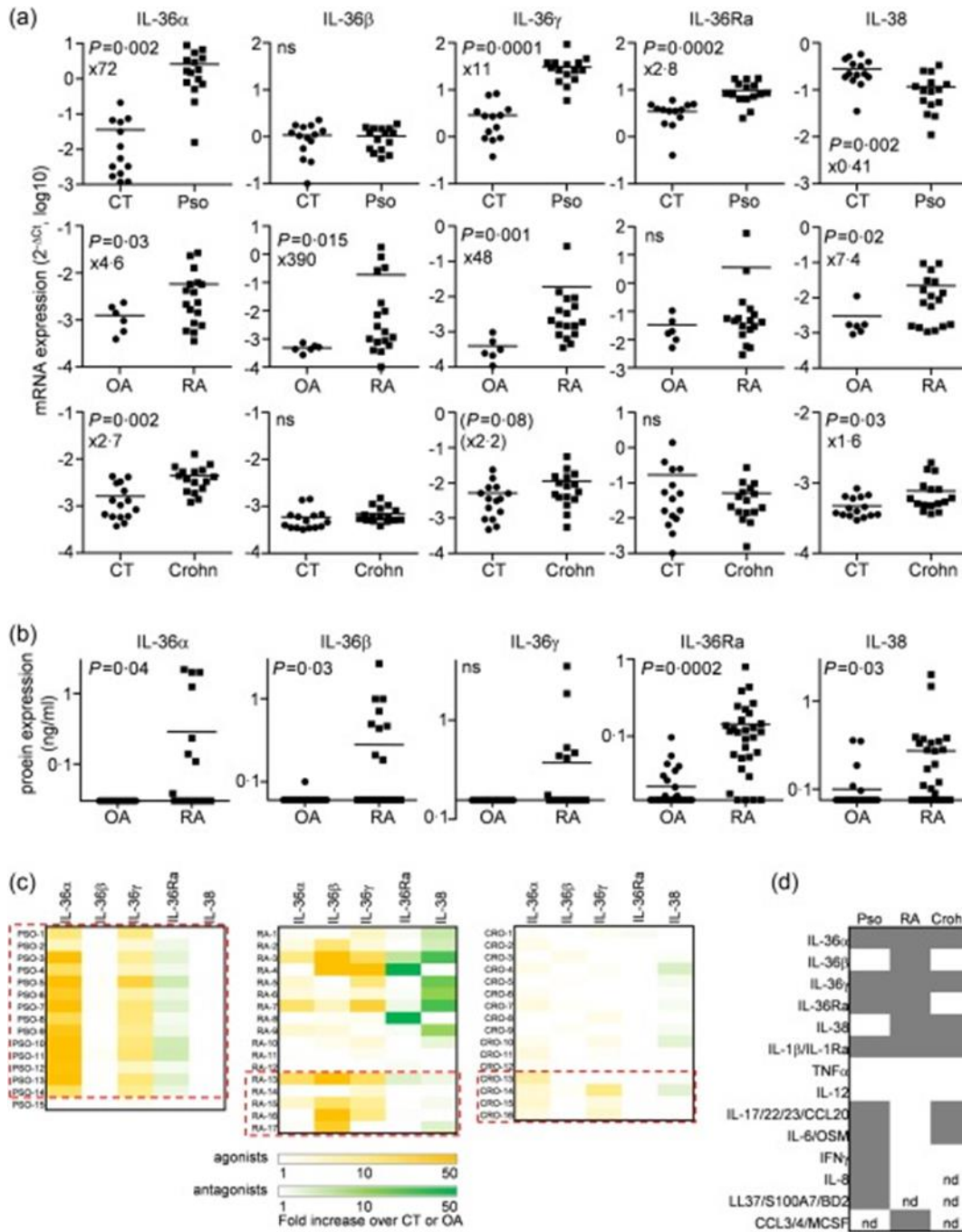


Figure 2

Expression of interleukin (IL)-36 cytokines in patients with psoriasis, rheumatoid arthritis (RA) and Crohn's disease (CD). (a) Expression of IL-36α, β and γ, IL-36Ra and IL-38 mRNA in human healthy skin (CT, $n = 14$) and psoriasis samples (PSO, $n = 15$) (upper panels), in synovial tissue of patients with OA ($n = 6$) or RA ($n = 17$) (middle panels) and in colonic biopsies of patients with CD from normal, non-inflamed (CT, $n = 15$) or inflamed (CD, $n = 16$) areas (lower panels). The results represent cytokine mRNA expression relative to hypoxanthine–guanine phosphoribosyltransferase (HPRT) and are shown as individual values and mean. The different P -values are indicated, as assessed by unpaired Student's t -test, as well as the mean fold increase in expression over the CT group. (b) Cytokine protein levels, as assessed by enzyme-linked immunosorbent assay (ELISA) in synovial fluid of RA patients ($n = 30$) versus osteoarthritis (OA) patients ($n = 29$). The results are shown as individual

values and mean. The different P -values are indicated, as assessed by unpaired Student's t -test. (c) Induction of cytokine mRNA expression in PSO, RA or CD (CRO) is presented in individual patients as fold increase over the mean value calculated in the CT or OA groups. Agonists are presented using the indicated yellow colour-code, antagonists with the green colour-code. The dotted areas identify patients with an elevated agonists/antagonists ratio. (d) Cluster of genes with positive statistically significant correlations ($P < 0.05$, as assessed by Pearson's bivariate correlation analysis) at the mRNA and/or protein expression level in patients with PSO, RA or CD are presented in grey. In white are presented genes whose expression does not correlate with the grey ones; n.d. = not done. See Tables 1–4 (Supporting information, File S1) for expression of these genes in PSO, RA and CD patients.

To obtain further insight into the protein production and release from cells, we next measured IL-36 agonists, IL-36Ra and IL-38 protein levels in the synovial fluids of patients with RA (Table 4, Supporting information, File S1) by ELISA. As shown in Fig. [Fig. 2b, 2b](#), IL-36 α and β , IL-36Ra and IL-38 protein levels were significantly higher in RA than in OA patients. For IL-36 γ , six of 30 patients had elevated protein levels, but this did not reach statistical significance (Fig. [\(Fig. 2b, 2b\)](#)). Overall, similar results were obtained at the mRNA and protein level, although the percentage of RA patients expressing higher IL-36 levels than OA controls varied between the two cohorts. For example, >50% of RA patients over-expressed IL-36Ra at the protein levels against 12% of RA patients at the mRNA level. Inversely, 20% over-expressed IL-36 γ protein, against >50% at the mRNA level (compare Fig. [Fig. 2a, 2a](#) and [b](#)). Although several post-transcriptional regulatory mechanisms could be implicated, it is important to note that the two cohorts comprised different patients and that a strict comparison of mRNA and protein expression was not possible.

Taken together, it became apparent that only a subpopulation of patients with RA or CD over-expressed IL-36 cytokines, whereas a majority of patients with psoriasis over-expressed IL-36 α and γ and IL-36Ra. If we consider IL-36Ra and IL-38 as potential antagonists of IL-36 cytokines, the subpopulation of RA or CD patients with an elevated agonists/antagonists ratio was reduced further [29% (five of 17) of patients with RA, mean ratio = 133; 25% (four of 16) of patients with CD, mean ratio = 6.2; 93% (14 of 15) of patients with psoriasis, mean ratio = 24] (Fig. [\(Fig. 2c, 2c\)](#), mRNA level). At the protein level, 17% (five of 30, mean ratio = 19.5) of patients with RA had an elevated agonists/antagonists ratio (Supporting information, File S3).

Correlation between IL-36 cytokines and other inflammatory cytokines

We next searched for correlations between expression of IL-36 cytokines, clinical data and expression of other inflammatory cytokines. In patients with psoriasis, mRNA expression of IL-36 α and γ and IL-36Ra were correlated positively ($r^2 > 0.46$, $P < 0.001$), but not with IL-36 β or IL-38 (Fig. [\(Fig. 2d, 2d\)](#)). Moreover, expression of IL-36 α and γ and IL-36Ra correlated with disease severity (PASI, $r^2 > 0.22$, $P < 0.02$), as well as mRNA expression of IL-1 β , Th17 cytokines (IL-17A, IL-22, IL-23 and CCL20), OSM (an IL-6 family member), IFN- γ , IL-8 and anti-microbial proteins (LL37, S100A7, BD2) (Fig. [\(Fig. 2d, 2d\)](#) and Table 1, see Supporting information, File S1). Interestingly, mRNA expression of IL-38 correlated negatively with the PASI ($r^2 = 0.14$, $P < 0.05$) and IL-17A expression ($r^2 = 0.19$, $P < 0.02$) but correlated positively with CK10 expression ($r^2 = 0.14$, $P < 0.05$), a marker of keratinocyte differentiation, which was also decreased in patients with psoriasis (Table 1,

Supporting information, File S1). In patients with RA, IL-36 agonists, IL-36Ra and IL-38, correlated with each other at the mRNA and protein level (Fig. (Fig.2d).2d). There was no statistically significant correlation with disease severity (DAS28), synovitis score 28, treatment or any other clinical characteristics, but expression of IL-36 γ , IL-36Ra and IL-38 proteins correlated with the total leucocyte count in the synovial fluid, especially the number of monocytes ($r^2 > 0.43$, $P < 0.001$). In RA, expression of IL-36 agonists, IL-36Ra and IL-38 also correlated with expression of IL-1 β , IL-1Ra, CCL3, CCL4 and M-CSF, but not with TNF- α , Th17 cytokines or IL-6 (Fig. (Fig.2d)2d and Tables 3 and 4, see Supporting information, File S1). In patients with CD, IL-36 α and γ and IL-38 correlated together, but not with IL-36 β or IL-36Ra. They also correlated with IL-1 β , IL-17A and IL-6, as observed in psoriasis (Fig. (Fig.2d).2d). Unfortunately, the number of RA or CD patients with a potentially elevated agonists/antagonists ratio was too low to identify statistically significant correlations with clinical data or with expression of other cytokines (not shown).

Thus far, these results indicated that IL-36 cytokines and IL-1 β formed a cluster of correlative cytokines, but that distinct expression patterns were observed in psoriasis (enhancement of IL-36 α and γ , IL-36Ra), RA (enhancement of IL-36 α , β and γ , IL-36Ra, IL-38) and CD (enhancement of IL-36 α and γ , IL-38). Moreover, additional correlations were found with other cytokines: Th17 cytokines in psoriasis and CD; CCL3, CCL4 and M-CSF in RA.

IL-36 cytokines are expressed by different cell types *in situ*

To analyse further the protein production of these cytokines and their tissue distribution in psoriasis, RA and CD, we next used immunohistochemistry on skin, synovial and colon tissues. The specificity of the staining was confirmed with antibodies against IL-36 α , β and γ (see Supporting information, File S4) but our attempts with several commercially available antibodies against IL-36Ra or IL-38 were unsuccessful (data not shown). As shown in Fig. Fig.3a,3a, IL-36 α , β and γ were found mainly in keratinocytes, but other cells such as endothelial cells or infiltrating mononuclear cells were also positive in psoriatic or healthy skin. With the help of additional staining on serial sections (Fig. (Fig.3b),3b), IL-36 α was found in keratinocytes, endothelial cells, CD68⁺ macrophages/DCs/Langerhans cells and, to a lower extent, in CD55⁺ dermal fibroblasts and in rare CD79 α ⁺ B cells (Fig. (Fig.3a).3a). In comparison, IL-36 β was found in keratinocytes (cytoplasmic and nuclear/nucleolus staining) and endothelial cells, but at a much lower level in macrophages, DCs and Langerhans cells. IL-36 γ was found in keratinocytes, macrophages, DCs and Langerhans cells, but not in endothelial cells.

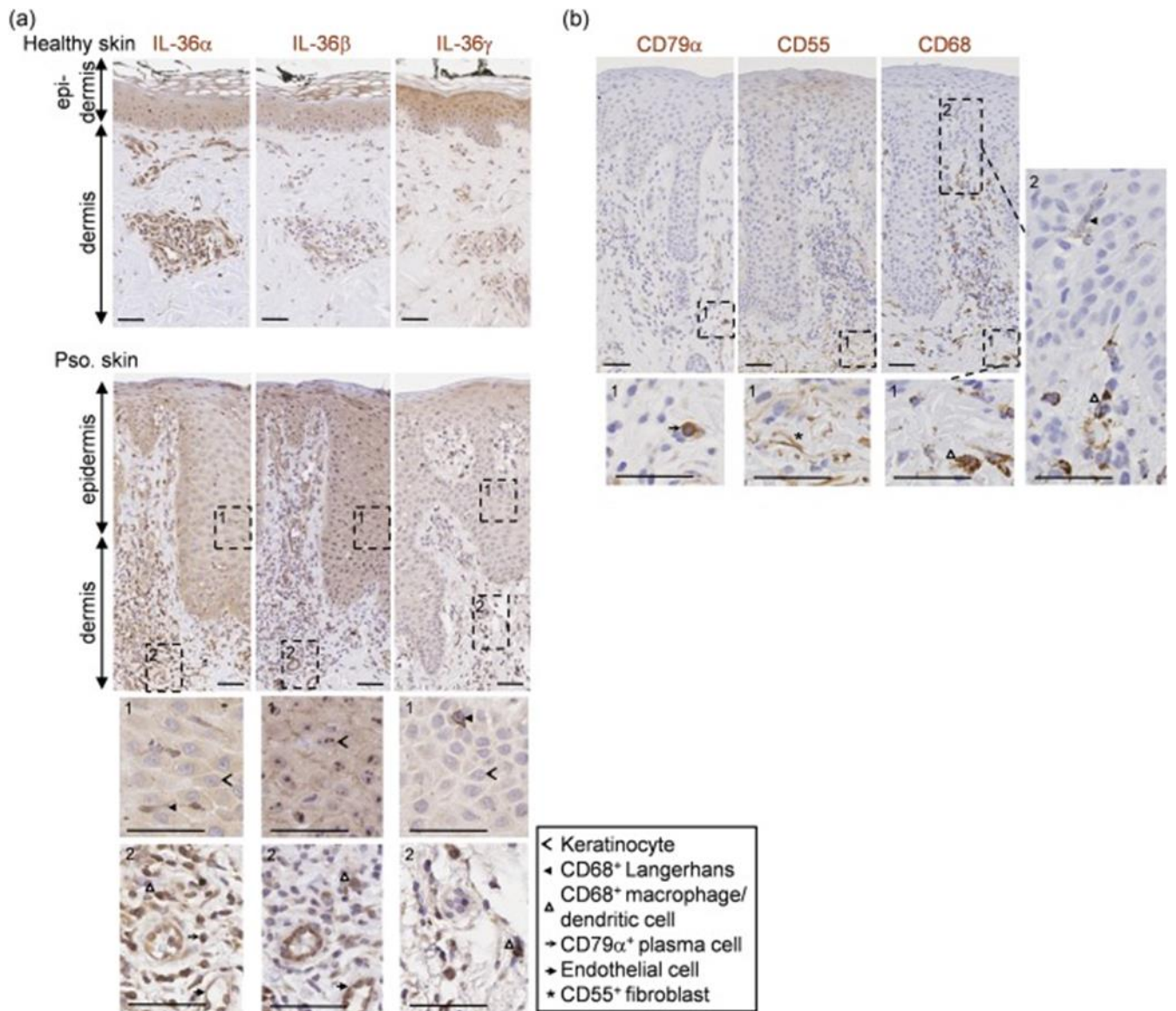
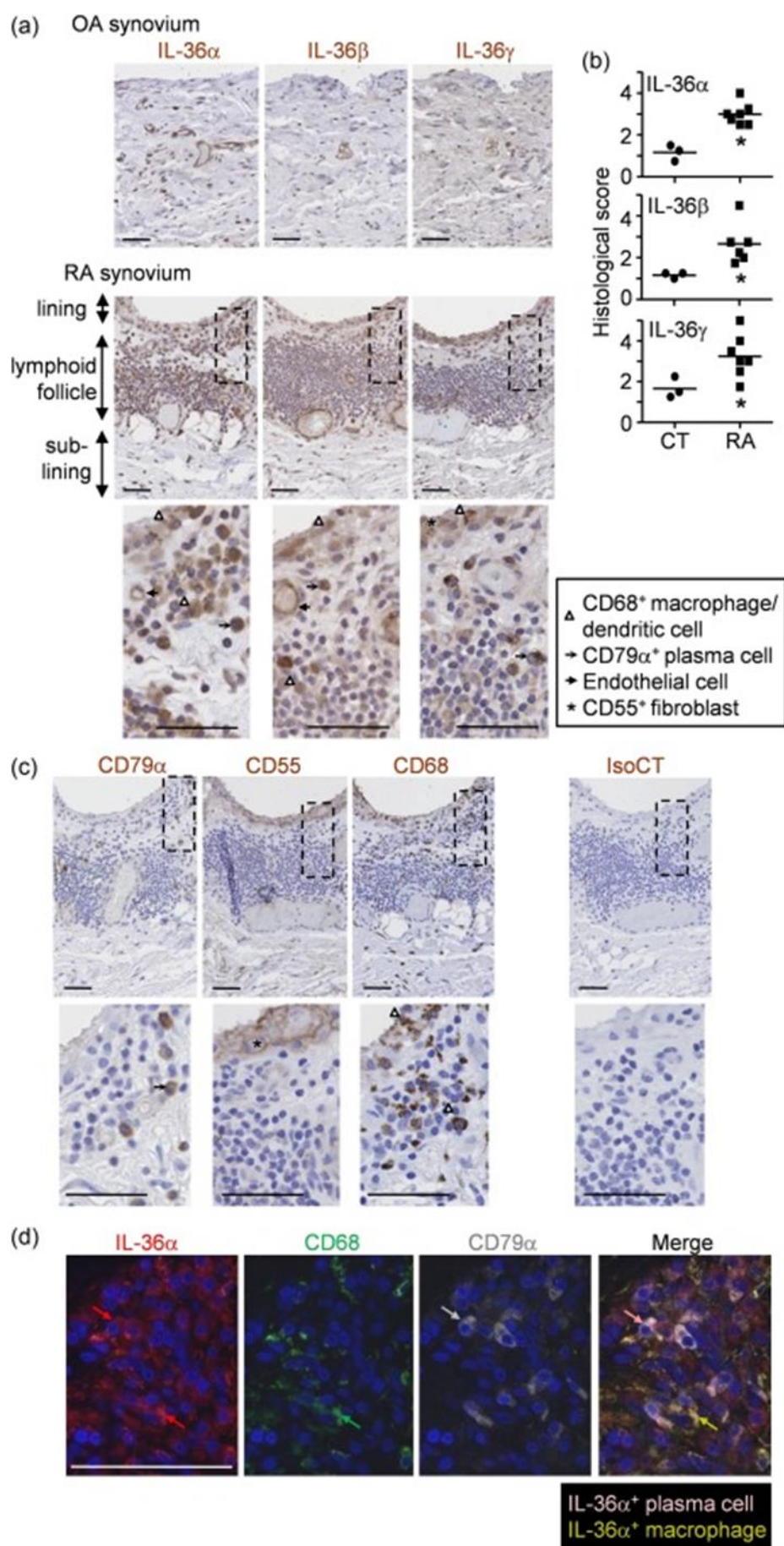


Figure 3

Immunohistochemical analysis of interleukin (IL)-36 cytokines in psoriasis. (a) Sections from human healthy skin ($n = 3$ donors, upper panels) or from patients with psoriasis ($n = 3$, lower panels) were stained for IL-36 α , β or γ . Representative images are shown. Enlarged images 1 and 2 correspond to the respective boxed areas. Scale bar = 50 μm . (b) Serial sections from psoriatic skin were stained for CD79 α (pan-B lymphocytes), CD55 (pan-fibroblasts) or CD68 (macrophages, dendritic and Langerhans cells). Enlarged images 1 and 2 correspond to the respective boxed areas. Scale bar = 50 μm . The box presents the symbols used to show representative cell types.

The same approach was undertaken on synovial sections from RA and OA patients. IL-36 α , β and γ staining was enhanced in the synovial tissue of RA patients compared to OA patients (Fig. (Fig.4a,b).4a,b). In the order of staining intensity, IL-36 α staining was detected mainly in CD79 α ⁺ B cells, whose morphology was compatible with plasma cells, CD68⁺ macrophages/DCs and endothelial cells. IL-36 β was detected in endothelial cells, plasma cells

and macrophages/DCs. IL-36 γ was detected mainly in macrophages/DCs, plasma cells and synovial fibroblasts (Fig. (Fig.4a,c).4a,c). The two important cell sources, macrophages/DCs and plasma cells, were confirmed by confocal microscopy after triple staining for IL-36/CD68/CD79 α (Fig. (Fig.4d4d and Supporting information, File S5).



[Figure 4](#)

Immunohistochemical analysis of interleukin (IL)-36 cytokines in rheumatoid arthritis (RA). (a) Sections of human synovial tissue from patients with OA ($n = 3$, upper panels) or from patients with RA ($n = 7$, lower panels) were stained for IL-36 α , β or γ . Representative images are shown. Enlarged images correspond to the respective boxed areas. Scale bar = 50 μm . (b) Semiquantitative analysis of the immunohistochemistry (IHC) stainings. The results represent individual values and mean. $*P < 0.05$ compared to CT osteoarthritis (OA) group, as assessed by Mann–Whitney U -test. (c) Serial sections from RA synovium were stained for CD79 α (pan-B lymphocytes), CD55 (pan-fibroblasts), CD68 (pan-macrophages and dendritic cells) or with an isotype control antibody (IsoCT). Enlarged images correspond to the respective boxed areas. Scale bar = 50 μm . The box presents the symbols used to show representative cell types. (d) Co-localization of IL-36 α with CD68 (pan-macrophages and dendritic cells) or with CD79 α (pan-B lymphocytes) in the synovial tissue of RA patients as assessed by confocal microscopy. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (blue). Representative images are shown. Examples of IL-36 α ⁺CD68⁺ (yellow arrow) and IL-36 α ⁺CD79 α ⁺ (pink arrow) cells are shown in the merged image. Scale bar = 50 μm .

In the colon mucosa of patients with CD, IL-36 α was found mainly in CD79 α ⁺ plasma cells, CD68⁺ macrophages/DCs and endothelial cells in the lamina propria (Fig. (Fig.5a).5a). Enterocytes in the crypts were only slightly positive. IL-36 β staining was found mainly in plasma cells, enterocytes and endothelial cells, and again seemed lower in macrophages/DCs. IL-36 γ was found mainly in macrophages/DCs and plasma cells, staining in enterocytes being very low (Fig. (Fig.55a).

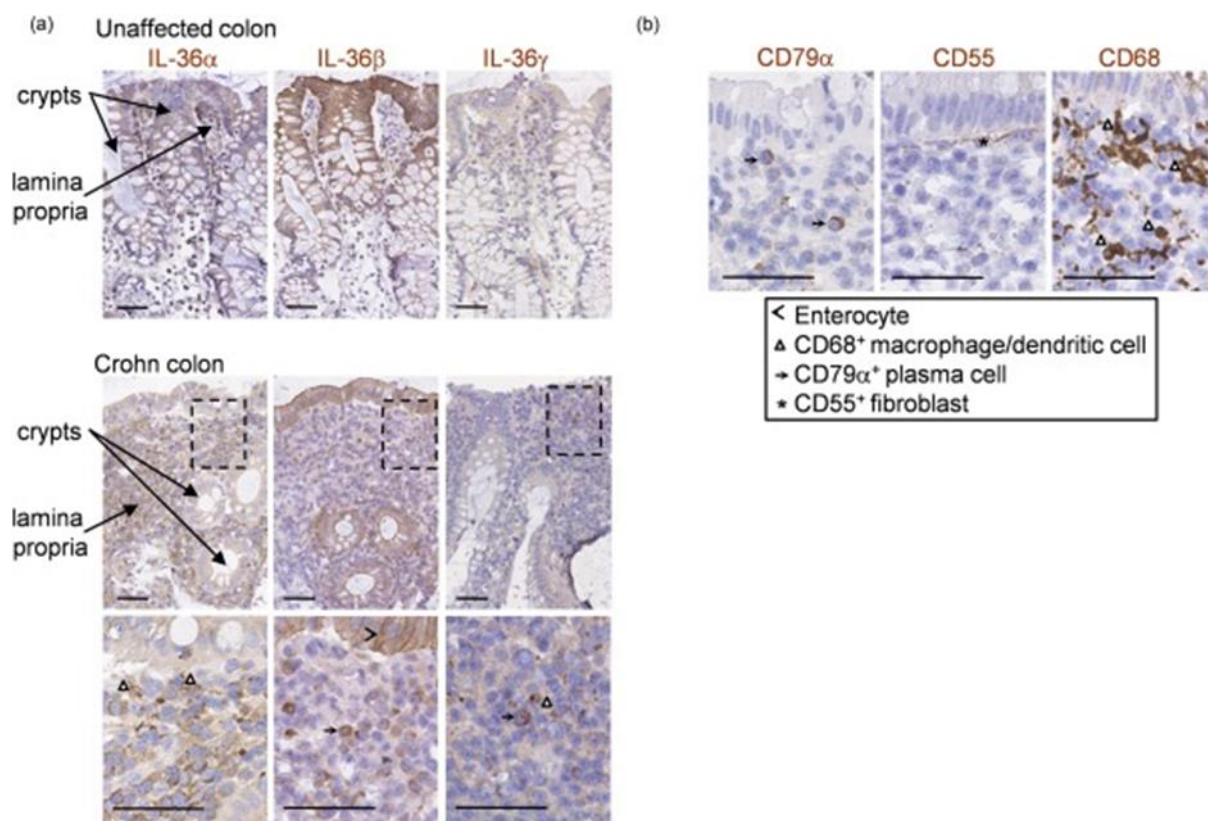


Figure 5

Immunohistochemical analysis of interleukin (IL)-36 cytokines in Crohn's disease (CD). (a) Sections from human colonic biopsies of patients with CD from normal, non-inflamed (unaffected, $n = 4$ donors, upper panels) or inflamed (CD, $n = 4$, lower panels) areas were stained for IL-36 α , β or γ . Representative images (mucosa) are shown. Enlarged images correspond to the respective boxed areas. Scale bar = 50 μm . (b) Serial sections from inflamed CD colon were stained for CD79 α (pan-B lymphocytes), CD55 (pan-fibroblasts) or CD68 (macrophages and dendritic cells). Scale bar = 50 μm . The box presents the symbols used to show representative cell types.

IL-36 cytokines are expressed differently and regulated in cultured cells

To examine further the differential expression of IL-36 cytokines by tissue resident and infiltrating cells, we next analysed their mRNA expression in a large panel of cultured cells stimulated with inflammatory cytokines or TLR ligands (Fig. (Fig.6a).6a). Confirming our immunohistochemical results, keratinocytes in primary culture appeared to be the main producers of IL-36 α , β and γ , IL-36Ra and IL-38, especially after stimulation with the TLR-3 ligand polyI:C or with a combination of five inflammatory cytokines (IL-17A, IL-22, OSM, IL-1 β , TNF- α ; M5). In comparison, synovial fibroblasts from RA patients (RASf) or Caco2-transformed enterocytes expressed 10–1000-fold less IL-36 α , β and γ and IL-36Ra, even after stimulation with inflammatory cytokines or polyI:C (Fig. (Fig.6a).6a).

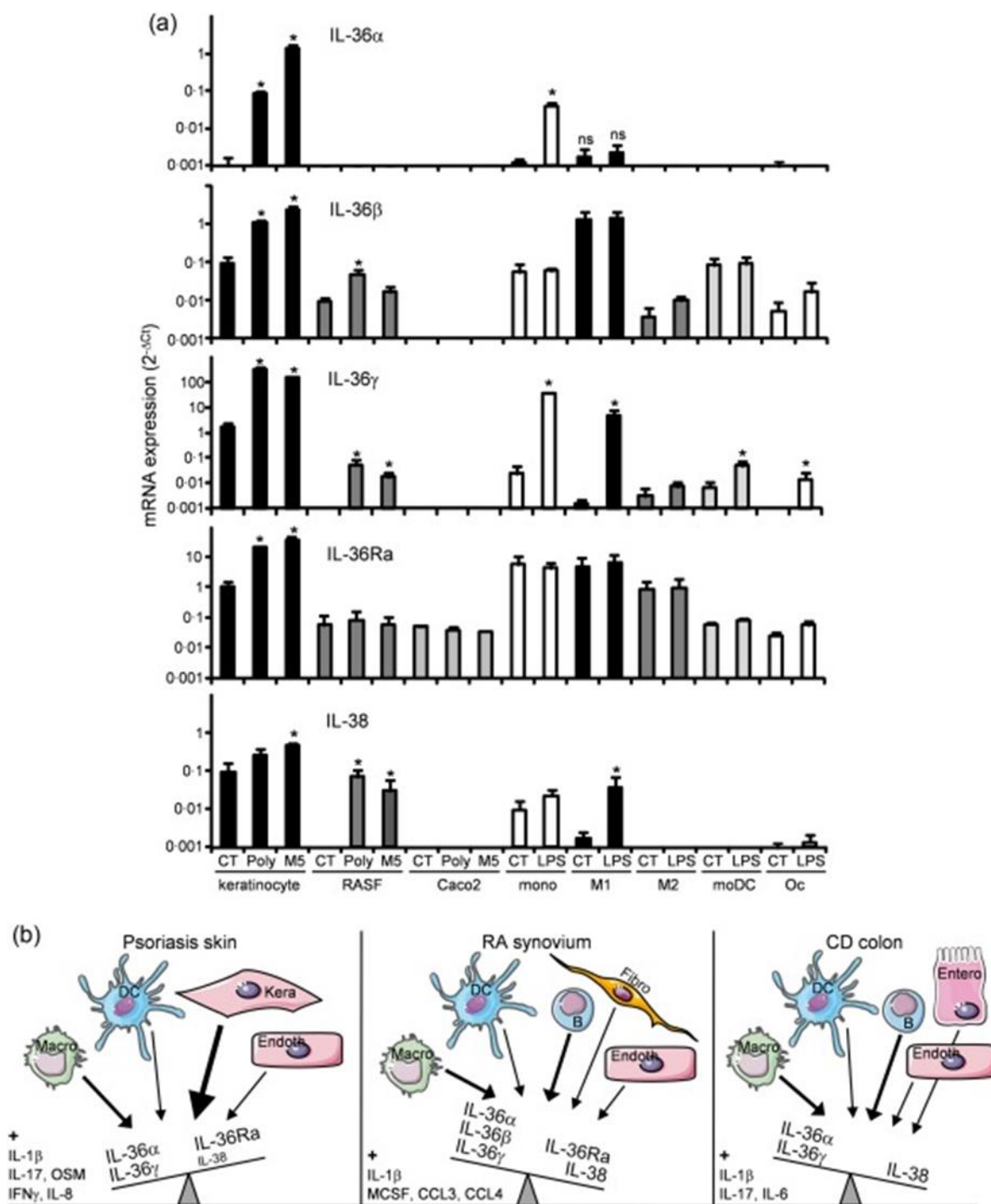


Figure 6

Expression of interleukin (IL)-36 cytokines in cultured cells. (a) Primary cultures of normal human epidermal keratinocytes from two different healthy donors, synovial fibroblasts from three different rheumatoid arthritis (RA) patients [rheumatoid arthritis synovial fibroblasts (RASF)] or Caco2 enterocytes were stimulated for 24 h with polyI:C (25 μg/ml) or a combination of five cytokines [M5: IL-17A, IL-22, oncostatin M, IL-1β, tumour necrosis factor (TNF)-α, all at 10 ng/ml]. CD14⁺ monocytes (mono), M1 macrophages, M2 macrophages, monocyte-derived dendritic cells (moDC) and osteoclasts (Oc) were prepared from three different healthy blood donors and stimulated for 8 h with lipopolysaccharide

(LPS) (100 ng/ml). Expression of IL-36 α , β , γ , IL-36Ra and IL-38 mRNA was assessed by reverse transcription–quantitative polymerase chain reaction (RT–qPCR). The results represent cytokine mRNA expression relative to hypoxanthine–guanine phosphoribosyltransferase (HPRT), shown as mean \pm standard error of the mean (s.e.m.).

* $P < 0.05$ compared to unstimulated cultures (CT), as assessed by Mann–Whitney U -test. (b) Summary of the cell sources and different expression profiles of IL-36 cytokines in psoriasis, RA and CD. Macro = macrophage; DC = dendritic cells; Kera = keratinocyte; Endoth = endothelial cell; B = plasma cell; Fibro = fibroblast; Entero = enterocyte. Other cytokines, which correlate with IL-36 cytokines are also indicated (+).

Other important producers of IL-36 cytokines were monocytes and/or inflammatory macrophages (M1) (Fig. (Fig.6a).6a). In comparison, anti-inflammatory macrophages (M2), monocyte-derived DCs and osteoclasts were poor producers in culture. Expression of IL-36 α and γ and IL-38 was induced by LPS (TLR-4 ligand) in monocytes and/or inflammatory macrophages, but IL-36 β and IL-36Ra were produced in the absence of LPS stimulation (Fig. (Fig.6a).6a). IL-38 was also expressed in RASF stimulated with polyI:C or the combination of five inflammatory cytokines (IL-17A, IL-22, OSM, IL-1 β , TNF- α) (Fig. (Fig.66a).

DISCUSSION

Although the role of IL-36 cytokines in the pathogenesis of psoriasis is well documented [7](#), their implication in other inflammatory diseases is currently debated. Previous studies indicated that some of these cytokines (IL-36 α and IL-36Ra) are over-expressed in RA and psoriatic arthritis synovium [19](#), but neutralization of the IL-36 receptor demonstrated that these cytokines have no significant role in mouse joint inflammation [21](#), [22](#). In mouse models of arthritis and in patients with RA, we show here that expression of IL-36 α , β and γ is enhanced in inflamed joints, but that levels are globally lower than in the skin. IL-36Ra and IL-38 are induced in arthritic joints, whereas only IL-36Ra is induced in psoriatic skin, IL-38 even being reduced. In mice with colitis and in patients with CD, levels of IL-36 α and γ are increased, but at a relatively low level. If we consider both IL-36Ra and IL-38 as potential IL-36 antagonists, only a minor subpopulation of patients with RA (17–29%) or CD (25%) have an elevated agonists/antagonists ratio, compared to virtually all patients with psoriasis (>90%). These results are in accordance with a key role of IL-36 cytokines in psoriasis [7](#), but not in RA [21](#), [22](#) or CD. None the less, they also suggest that these cytokines could have a role in a subgroup of patients with RA or CD.

In psoriasis, expression of IL-36 α and γ and IL-36Ra correlates with disease severity, IL-1 β and Th17 cytokines, in accordance with their implication in the pathogenesis of the disease. Similar correlations were found previously, highlighting the T cell dependency of psoriasis and that IL-36 cytokines are indeed part of the IL-17 cytokine framework, one of the main cytokine targets in this disease [10](#), [36](#). Expression of IL-36 cytokines is regulated not only by IL-17 cytokines; they also potentiate the function of IL-17 cytokines [10](#). IL-1 and IL-36 α also induce each others' expression and co-operate to regulate psoriasis-like skin inflammation [37](#). Similarly in CD, expression of IL-36 α and γ and IL-38 correlates with IL-17A, sustaining a role for these cytokines in the pathogenesis of CD, at least in a subgroup of patients [38](#), [39](#). In sharp contrast, expression of IL-36 cytokines does not correlate with IL-17 in RA, in line with a separate molecular pathophysiology [36](#).

IL-36 γ was found previously to be increased in psoriasiform skin lesions of patients with CD, but until recently there was no reports regarding the association between IL-36 cytokines and

chronic intestinal inflammation [39](#), [40](#). In mouse and human inflamed colon, we observe an increased expression of IL-36 α and γ and IL-38. Very recently, Medina-Contreras *et al.* also showed induced colonic expression of IL-36 γ in mice with colitis and patients with CD [41](#). Moreover, IL-36R-deficient mice have decreased colitis at early time-points as well as defective recovery from mucosal injury [41](#). These results suggest a major role for IL-36 γ in colonic inflammation and its resolution, and additional studies are necessary to understand more clearly the role of other IL-36 cytokines and IL-38 in CD.

Whereas IL-36 β is induced in RA, it is not increased in CD or in psoriasis, indicating a distinct cell source or regulation of its expression. Similarly, IL-36Ra is induced in RA and psoriasis but not in CD, whereas IL-38 is induced in RA and CD but not in psoriasis. It is important to note that several other studies described an elevated expression of IL-36 β in psoriasis skin lesions, both in humans and in several mouse models of psoriasis [8](#), [10](#). However, all studies noticed a low expression of IL-36 β in psoriasis in comparison to IL-36 α and γ and IL-36Ra. In a recent study using the IMQ-induced skin inflammation, IL-36 α was identified as the most induced IL-36 cytokine. Moreover, IL-36 α -deficient mice were protected from skin pathology, whereas IL-36 β or IL-36 γ -deficient mice developed normal skin disease [37](#). These results indicate that IL-36 cytokines are produced or regulated differently in inflammatory diseases, and thus do not have totally redundant roles.

By IHC and in cell cultures, our results suggest that keratinocytes, monocytes/macrophages and plasma cells are main producers of IL-36 cytokines and that each individual cytokine has a particular expression pattern (see below). Overall, the IHC and cell culture approaches gave concordant results, but several limitations can be found. First, IL-36 cytokines are expressed in many different cell types, the main cell sources being therefore difficult to ascertain. Secondly, the stimulation of cells in primary cultures with TLR agonists or inflammatory cytokines does not necessarily reflect the situation in diseases. Thirdly, IL-36 β was found in enterocytes by IHC but not in the Caco2-transformed cell line. Analysis of IL-36 expression in primary cultures of enterocytes would be necessary to confirm these results.

Taking these limitations into consideration, we suggest that IL-36 α and γ and IL-36Ra are induced in psoriasis, produced mainly by keratinocytes after stimulation with inflammatory cytokines or TLR-3 ligands, whereas the expression of IL-38 appears to be regulated differently. Indeed, IL-38 expression is reduced in psoriasis in correlation with the loss of CK10 expression, suggesting that it is associated uniquely with keratinocyte dedifferentiation [34](#). Reduced expression of IL-38 is also observed in dedifferentiated keratinocytes compared to differentiated keratinocytes cultured in high-calcium media [42](#). In our *in-vitro* experiments, keratinocytes cultured with inflammatory cytokines undergo both inflammatory stimuli and dedifferentiation [34](#), resulting in mild elevation of IL-38 expression. The loss of IL-38 could have an important role in the pathogenesis of psoriasis, to unleash inflammation and immune responses driven, for example, by IL-36 agonists. Conversely, induced production of IL-38, possibly by synovial fibroblasts, monocytes and macrophages, could limit the impact of IL-36 agonists in the pathogenesis of RA or CD. Future studies will certainly unravel important new clues on the role of IL-38 in the pathophysiology of chronic inflammatory diseases, and there is an urgent need to decipher more accurately its antagonistic potency against IL-36 cytokines or other inflammatory triggers *in vitro* and *in vivo*.

In addition to keratinocytes, mononuclear infiltrates are often found positive for IL-36 cytokine expression [8](#). As described previously, plasma cells express IL-36 α and IL-36Ra especially in RA, which is characterized by a high number of infiltrating B cells in the joints

19. In psoriasis (this study) or Sjögren's syndrome [18](#), IL-36 α ⁺ plasma cells can be detected, but are rare. We found that plasma cells are also positive for expression of other IL-36 agonists, and thus could be an important cell source for all these cytokines in RA and CD. Endothelial cells express IL-36 α and β , and could therefore represent an additional source, especially considering that angiogenesis is an important component in many chronic inflammatory diseases such as psoriasis, RA or CD [43](#).

Other cells which are present in many, if not all, inflammatory diseases are monocytes and macrophages. Previous studies demonstrated that macrophages and DCs produce IL-36 cytokines, especially IL-36 α and γ [7](#), [9](#), [44](#). Vigne *et al.* described that LPS stimulates expression of IL-36 α and γ in bone marrow-derived DC, whereas IL-36 β and IL-36Ra were not detected [44](#). moDC and M1 macrophages over-express IL-36 γ after stimulation with LPS or inflammatory cytokines by a mechanism dependent on the transcription factor T-bet [9](#), whereas CCAAT/enhancer-binding protein beta (C/EBP β) and nuclear factor kappa B (NF- κ B) are key transcriptional regulators of IL-36 α in LPS-treated macrophages [45](#). Macrophages and/or DCs were also found positive for IL-36 α by *in-situ* hybridization or immunohistochemistry [15](#), [18](#) and IL-36 γ was found highly expressed in inflammatory colonic macrophages isolated from DSS-treated mice [41](#). Using IHC and/or in cell culture, we observed in this study that monocytes and M1 macrophages can produce IL-36 cytokines but with a distinct expression profile. For example, in culture, IL-36 α is produced mainly by monocytes, whereas IL-36 β is produced mainly by M1 macrophages. IL-36 α and γ and IL-38 are produced after myeloid cell stimulation with LPS, whereas IL-36 β and IL-36Ra are produced constitutively by these cells. This basal, non-inducible expression of IL-36 β could be related to the low macrophage IL-36 β staining observed by IHC, especially in psoriasis and CD (see Figs [Figs2,2](#), [,3,3](#), [,4](#)).4). Using IHC, DCs and Langerhans cells are found positive, especially for IL-36 α and γ , but moDC are relatively poor producers in culture.

Taken together, and although uncertainties persist at the level of the main cell sources and the exact antagonistic role of IL-38, these results can be summarized as presented in Fig. [Fig.6b.6b](#). In psoriasis skin, the agonists IL-36 α and γ and the antagonist IL-36Ra are induced, the balance being in favour of the agonists. They are produced mainly by keratinocytes and presumably also myeloid cells (inflammatory M1 macrophages and DCs), and their expression correlates with Th17 cytokines. In RA synovium, all three IL-36 agonists, IL-36Ra and IL-38 are induced, the balance being in favour of these potential antagonists in a majority of patients. They are produced by many different cell types, such as myeloid cells and plasma cells, and their expression correlates with MCSF, CCL3 and CCL4. In CD colon, IL-36 α and γ and IL-38 are induced at a low level, the balance being in favour of the potential antagonist IL-38 in a majority of patients. They are produced by different cell types, such as myeloid cells and plasma cells, and their expression correlates with Th17 cytokines. Additional cells, such as endothelial cells, fibroblasts and enterocytes, could also participate in psoriasis, RA and/or CD. Because expression of the different IL-36 cytokines is regulated differently and their cell sources are not identical, this helps to explain the different expression profiles observed in three chronic inflammatory diseases and why only a subgroup of RA and CD patients have, possibly, an elevated IL-36 agonists/antagonists ratio. Additional studies are necessary to better identify these patients and to investigate whether they would benefit from IL-36 neutralization.

DISCLOSURE

All authors declare that they have no disclosures.

AUTHOR CONTRIBUTIONS

M.A.B., G.B., M.G., J.A. and C.C. performed the experiments and analysed the data. B.B. performed the confocal analysis. F.M. and J.C.L. provided human and mouse skin biopsies, performed keratinocyte cultures and gene expression studies. M.R.D. and A.B. provided colonic biopsies and performed gene expression studies. S.V., C.G. and G.P. performed the mouse colitis model. B.L.G. provided synovial samples, performed mouse arthritis models, planned studies, analysed the data and wrote the manuscript. F.B. planned studies, analysed the data and wrote the manuscript. All authors read and approved the final manuscript.

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