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CXCL4 is a novel inducer of human Th17 cells and correlates with IL-17 and IL-22 in psoriatic arthritis

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CXCL4 regulates multiple facets of the immune response and is highly upregulated in various Th17-associated rheumatic diseases. However, whether CXCL4 plays a direct role in the induction of IL-17 production by human CD4$^+$ T cells is currently unclear. Here, we demonstrated that CXCL4 induced human CD4$^+$ T cells to secrete IL-17 that co-expressed IFN-γ and IL-22, and differentiated naïve CD4$^+$ T cells to become Th17-cytokine producing cells. In a co-culture system of human CD4$^+$ T cells with monocytes or myeloid dendritic cells, CXCL4 induced IL-17 production upon triggering by superantigen. Moreover, when monocyte-derived dendritic cells were differentiated in the presence of CXCL4, they orchestrated increased levels of IL-17, IFN-γ, and proliferation by CD4$^+$ T cells. Furthermore, the CXCL4 levels in synovial fluid from psoriatic arthritis patients strongly correlated with IL-17 and IL-22 levels. A similar response to CXCL4 of enhanced IL-17 production by CD4$^+$ T cells was also observed in patients with psoriatic arthritis. Altogether, we demonstrate that CXCL4 boosts pro-inflammatory cytokine production especially IL-17 by human CD4$^+$ T cells, either by acting directly or indirectly via myeloid antigen presenting cells, implicating a role for CXCL4 in PsA pathology.

Keywords: CXCL4 · IL-17 · IL-22 · Psoriatic arthritis

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Introduction

CD4$^+$ T cells orchestrate immune responses in physiological and pathological conditions by secreting a wide array of cytokines. To gain effector functions, CD4$^+$ T cells require the engagement of T cell receptor and costimulatory molecules, in the presence of specific cytokines and chemokines, resulting in distinct subsets of T helper cells (Th). The interferon gamma (IFN-γ)-secreting type 1 cells (Th1) and IL-4/IL-5/IL-13-producing type 2 cells (Th2) form the classical subsets of CD4$^+$ T cells, that have been expanded by discoveries of other T helper cells, such as the IL-17-secreting type 17 cells (Th17 cells) [1]. Characterized by expression of IL-17, IL-21, IL-22 and other pro-inflammatory mediators, Th17 cells are potent inducers of immune responses needed for

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pathogen clearance, but are also pivotal in the development of autoimmunity. Psoriasis (Pso), psoriatic arthritis (PsA), systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis, are amongst the diseases where IL-17 involvement in the pathogenesis is evident [2–6]. In PsA, IL-17 producing cells accumulate in skin lesions and synovial fluid and play a destructive role by inducing tissue inflammation and bone erosion [7–9]. Therefore, it is important to understand the key upstream drivers of IL-17 activation in PsA.

CXCL4, previously known as platelet factor 4 (PF4), is an immunomodulatory chemokine produced by multiple immune cells that can target virtually all cells in the vasculature [10]. Besides the crucial role of CXCL4 in maintaining homeostasis, it has been implicated in many inflammatory conditions [11]. We and others reported elevated levels of CXCL4 in various Th17-associated rheumatic diseases [12–16], yet the role of CXCL4 in driving the Th17 pathway is largely unexplored. While it has been indicated previously that blocking CXCL4 in human platelet-CD4$^+$ T cells co-cultures led to a reduction of IL-17 production [17, 18], direct effects of CXCL4 on Th17 responses have never been studied. Also, these findings of CXCL4 promoting Th17 activity were not supported by mouse studies [19, 20].

Here we sought to investigate whether CXCL4 contributed to Th17 activation in human CD4$^+$ T cells and by which mechanisms it acted, directly on CD4$^+$ T cells or indirectly via antigen presenting cells (APCs). To support these findings, we went on to assess whether CXCL4 was also related to Th17 cytokines in a disease setting-in the inflamed joints of patients with PsA, a prototypical Th17 mediated disease.

Results

CXCL4 increases IL-17 producing cells in CD3/CD28-activated CD4$^+$ T cells

To investigate CXCL4 effect on T helper cell responses, CD4$^+$ T cells isolated from peripheral blood of healthy individuals were stimulated with α-CD3/CD28 in the absence or presence of CXCL4. CXCL4 significantly increased IL-17 production by CD4$^+$ T cells as compared to CD3/CD28 stimulation alone (Fig. 1A and B). This was supported by de novo synthesis of *IL17A* mRNA in CD4$^+$ T cells upon CXCL4 treatment (Supporting Information Fig. 1). CXCL4 did not significantly alter the levels of other T helper cytokines (Fig. 1C, Supporting Information Fig. 2A) nor did it affect proliferation (Supporting Information Fig. 3A). In contrast, CXCL4 treatment induced co-expression of IFN-γ and IL-22 in IL-17 positive cells (Fig. 1D and E). Therefore, our data indicates that CXCL4 directly induces human CD4$^+$ T cells to produce IL-17 in co-expression with other pro-inflammatory cytokines such as IFN-γ and IL-22.

To further dissect CXCL4 effect on Th17 differentiation, we purified naïve CD4$^+$ T cells from peripheral blood of healthy individuals, and stimulated them with α-CD3/CD28 in the absence or presence of CXCL4. Similar to bulk CD4$^+$ T cells, CXCL4-treated naïve CD4$^+$ T cells showed an increased IL-17 production as compared to CD3/CD28 stimulation alone (Fig. 2A and B). CXCL4 did not change the levels of other T helper cytokines (Fig. 2C), however CXCL4 elevated the ratio of IL-17$^+$IL-22$^+$ expressing cells (Fig. 2D). Thus, naïve CD4$^+$ T cells preferentially differentiate into Th17 cells when exposed to CXCL4.

CXCL4 induces IL-17 production by CD4$^+$ T cells when co-cultured with myeloid antigen-presenting cells

Next we addressed whether CXCL4-induced IL-17 induction was APCs-dependent. For this purpose, human CD4$^+$ T cells were co-cultured with APCs loaded with superantigen SEB. In an autologous co-culture of monocytes and CD4$^+$ T cells, we found that CXCL4 increased the secretion of IL-17 as compared to superantigen SEB alone (Fig. 3A). CXCL4 treatment also seemed to slightly induce IL-5 and IL-22 production. We further assessed the effect of CXCL4 on CD4$^+$ T cell cytokine production using three other APCs: myeloid dendritic cells (mDCs), plasmacytoid dendritic cells (pDCs) and B cells. We observed that CXCL4 significantly enhanced IL-17 production of CD4$^+$ T cells when co-cultured with mDCs (Fig. 3B), but not with pDCs or B cells. Previous data from others and our group indicate clear immunomodulatory effects of CXCL4 at higher doses [21, 22]. In co-culture with monocytes, increasing amount of CXCL4 up to 5 μg/mL did not additionally enhance the IL-17 induction (Supporting Information Fig. 4). The effect of CXCL4 on CD4$^+$ T cell proliferation upon activation with superantigen SEB-loaded APCs was minimal (Supporting Information Fig. 3B and C). Thus, also in the presence of myeloid APCs, CXCL4 promotes the production of the pro-inflammatory IL-17 production by CD4$^+$ T cells.

CXCL4-primed monocyte-derived dendritic cells enhance CD4$^+$ T cell activation

Previous works have shown that during monocyte differentiation into dendritic cell or macrophage, the addition of CXCL4 resulted in an altered expression of cell surface markers and a distinct transcriptomic profile [22–24]. They also differed in their capacity to activate T cells, yet the effect on IL-17 production was not assessed. We added CXCL4 to monocytes differentiating into dendritic cells (moDCs) during culture with GM-CSF and IL-4. After differentiation, moDCs were co-cultured with autologous CD4$^+$ T cells in the presence of SEB. CXCL4-treated moDCs induced a higher IL-17 production by CD4$^+$ T cells as compared to conventional moDCs (Fig. 4A and B). CXCL4 treatment also increased the percentage of IFN-γ-producing cells (Fig. 4C), but not IL-4 or IL-10 producing cells (Fig. 4D). Interestingly, CXCL4-treated moDCs increased the percentage of co-expressing IL17$^+$IL-22$^+$ cells (Fig. 4E). CXCL4-treated moDCs also significantly potentiated CD4$^+$ T cell proliferation (Fig. 4F, Supporting Information Fig. 3D). Therefore, our data suggest that CXCL4 modulates moDCs to induce proliferation of CD4$^+$ T cells and production of pro-inflammatory cytokines, especially IL-17.

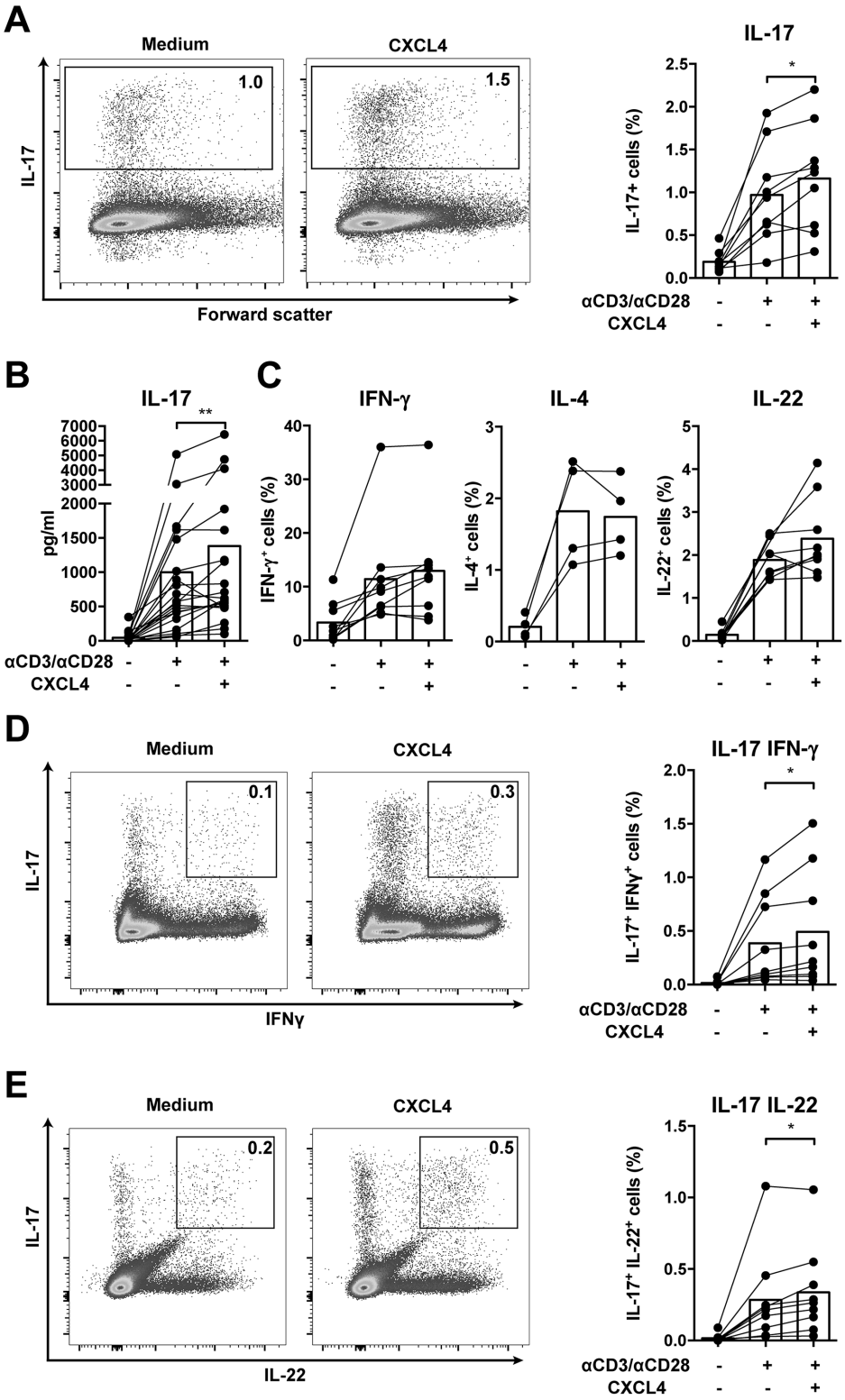

Figure 1. CXCL4 increases the percentage of IL-17 producing cells in CD3/CD28-stimulated human $CD4^+$ T cells. $CD4^+$ T cells were isolated from healthy donors and cultured with CD3/CD28 coated Dynabeads and CXCL4 for five days. (A, B) The effect of CXCL4 on IL-17 production by $CD4^+$ T cells was assessed by (A) flow cytometric intracellular cytokine staining and (B) enzyme-linked immunosorbent assay. (C) The percentage of of IFN-γ-, IL-4- and IL-22-producing $CD4^+$ T cells were measured by flow cytometry. (D, E) The amount of IL-17 producing cells co-expressing IFN-γ (D) or IL-22 (E) were measured by flow cytometry. Cells were gated on live, single cells. Means (bars) and values from each donor are shown. Data are pooled from two to four independent experiments, except for panel B from 14 independent experiments, with one to four donor samples per experiment. Each dot on the bar graphs represent a single donor and paired *t*-test was used for statistical analysis. $^*p<0.05$, $^{**}p<0.01$.

CXCL4 is increased in Th17 diseases and correlates with Th17 cytokines at the site of inflammation

To assess potential clinical relevance of our findings above, we measured the level of circulating CXCL4 in patients with Pso and PsA, both known to be type 17-driven autoimmune diseases. The level of CXCL4 in the circulation was previously shown to be increased in Pso patients [14]. Here we found that the plasma level of CXCL4 was increased in both Pso and PsA patients as compared to healthy individuals (Fig. 5A). We then examined

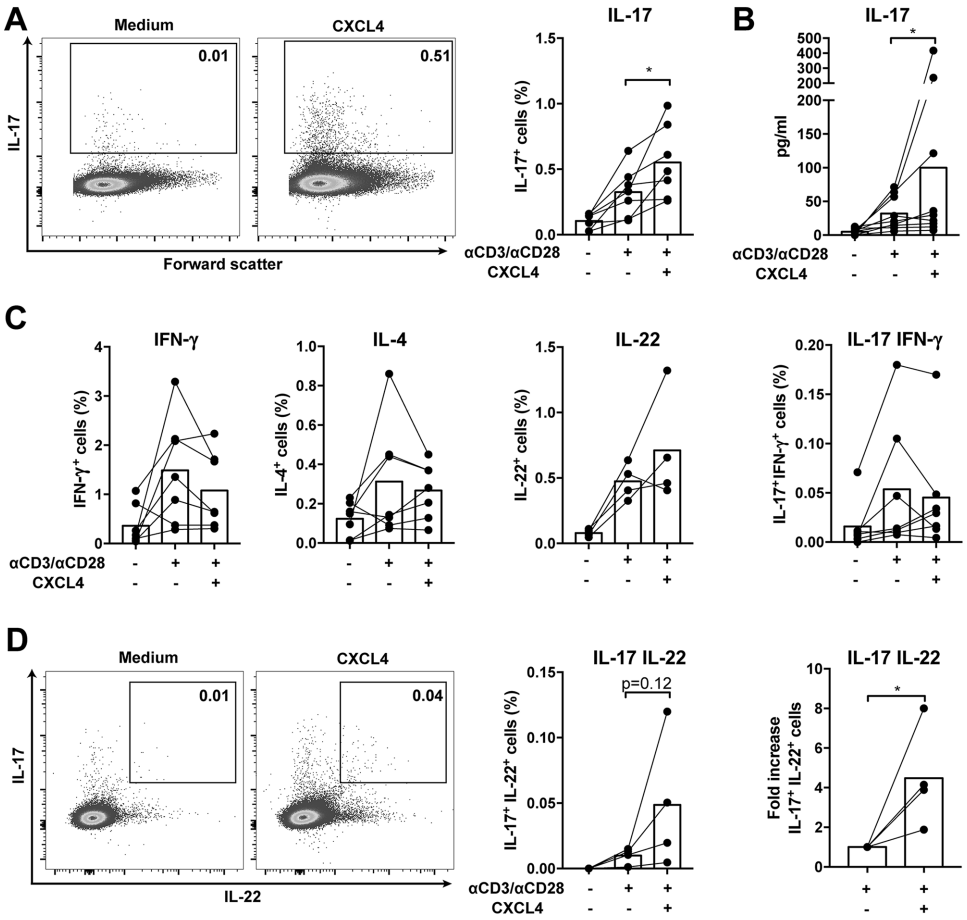

Figure 2. CXCL4 induces IL-17 producing cells differentiated from naïve human CD4$^+$ T cells. Naïve CD4$^+$ T cells were purified by fluorescence-activated cell sorting and cultured with CD3/CD28 coated Dynabeads and CXCL4 for seven days. (A, B) The effect of CXCL4 on IL-17 production by CD4$^+$ T cells was assessed by (A) flow cytometric intracellular cytokine staining and (B) enzyme-linked immunosorbent assay. (C) The levels of IFN-γ-, IL-4-, IL-22-, and IL-17/IFN-γ-producing CD4$^+$ T cells were measured by flow cytometry. (D) The amount of IL-17 producing cells co-expressing or IL-22 was measured by flow cytometry. Cells were gated on live, single cells. Means (bars) and values from each donor are shown. Data are pooled from four to 10 independent experiments, with one to two donor samples per experiment. Each dot on the bar graphs represents a single donor and paired *t*-test was used for statistical analysis. $^*p<0.05$, $^{**}p<0.01$.

intra-articular level of CXCL4 from patients with PsA and patients with osteoarthritis as a non-autoimmune disease control group. In SF, we found a trend toward increased CXCL4 levels in patients with PsA as compared to those with osteoarthritis (Fig. 5B, $p = 0.066$). To determine whether CXCL4 mediates Th17 activation in vivo at the site of inflammation, we measured CXCL4 and T cell-derived cytokines in the SF of patients with PsA. Remarkably, CXCL4 strongly correlated with both IL-17 ($r = 0.713$, $p < 0.01$) and IL-22 ($r = 0.620$, $p < 0.01$) (Fig. 5C), whereas CXCL4 did not correlate with IFN-γ, IL-5, IL-10, nor GM-CSF in the SF of PsA patients, clearly mimicking our in vitro results. The enhanced IL-17 production by CD4$^+$ T cells upon CXCL4 treatment was also observed in PsA patients (Fig. 5D and E). Additionally, we had five donors from which multiple synovial fluid samples were collected multiple times at different time points. CXCL4 level completely mirrored the changes of IL-17 amount in PsA SF over time in four out of five PsA patients (Supporting Information Fig. 5). These data suggest that in PsA, higher CXCL4 levels are associated with increased Th17 cytokines locally at the site of inflammation.

Discussion

IL-17 producing cells have been implicated to play a major role in multiple autoimmune and chronic inflammatory disorders. Here we show that CXCL4 – a chemokine shown to be highly present in many of these disorders – directly and indirectly promotes the production of IL-17 by human CD4$^+$ T cells (Fig. 6). In addition to that, CXCL4 enhances the levels IL-17 secreting cells that also produce IFN-γ and IL-22. Moreover, in the Th17-mediated disease context of PsA, CXCL4 level in PsA SF is significantly associated with IL-17 and IL-22 levels.

Previous reports on CXCL4 effects on the regulation of T cells have been inconsistent. CXCL4 was described to inhibit the expression of IFN-γ (Th1) while favoring IL-13 (Th2) on cultured

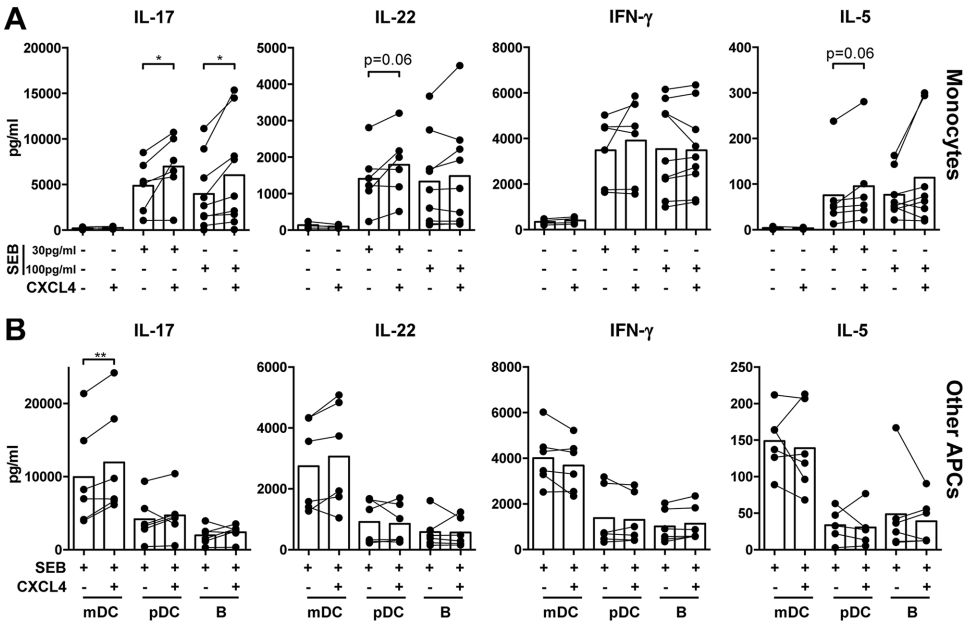

Figure 3. CXCL4 induces IL-17 production in autologous antigen-presenting cells (APCs)-CD4$^+$ T cells co-culture. Monocytes, B cells, myeloid dendritic cells (mDCs), plasmacytoid dendritic cells (pDCs), and CD4$^+$ T cells were isolated from healthy individuals, co-cultured in the absence or presence of superantigen from Staphylococcal enterotoxin B (SEB) and CXCL4 for three days and restimulated with PMA and ionomycin. (A) Supernatant from co-culture of monocytes and CD4$^+$ T cells stimulated with superantigen SEB and CXCL4 were measured for IL-17, IL-22, IFN-γ, and IL-5. (B) The effect of CXCL4 treatment on 100 pg/mL superantigen SEB-activated CD4$^+$ T cells co-cultured with myeloid dendritic cells (mDCs), plasmacytoid dendritic cells (pDCs), or B cells, on IL-17, IL-22, IFN-γ, and IL-5 production was assessed. Cytokines produced were determined using a Luminex-based assay. Means (bars) and values from each donor are shown. Data are pooled from two to five independent experiments, with one to four donor samples in duplicate per experiment. Each dot on the bar graphs represent a single donor paired *t*-test was used for statistical analysis. $^*p<0.05$, $^{**}p<0.01$.

naïve CD4$^+$ T cells [25]. However, another study suggested that CXCL4 only induced the proliferation and cytokine production of CD4$^+$CD25$^+$ T cells, but not CD4$^+$CD25$^-$ T cells [26]. Our data are the first evidence revealing a direct CXCL4 effect on driving IL-17 production by CD4$^+$ T cells. Moreover, multiple studies showed that platelets, that can secrete a large amount of CXCL4, promoted CD4$^+$ T cells IL-17 production in a co-culture, thus supporting our findings [17, 18, 27]. CXCL4 also increased the amount of IL-17$^+$ double producers with IFN-γ and IL-22. These cells have been found in many human inflammatory conditions [28–32], therefore the induction of IL-17$^+$ cells and their co-expression with IFN-γ and IL-22 by CXCL4 may exacerbate pathological processes.

CXCL4 is known to elicit inflammatory response on myeloid cells. On monocytes, CXCL4 has been shown to promote their survival and pro-inflammatory cytokines production, such as IL-6, TNFα, as well as reactive oxygen species [33, 34]. While there is little known about the effect of CXCL4 on mDC, we previously showed that CXCL4 could enhance IFN-α production by pDCs upon toll-like receptor stimulation [12]. We recently demonstrated that CXCL4 potentiated moDCs cytokine production upon toll-like receptor stimulation [22]. Through regulating APC function, CXCL4 can promote an inflammatory environment that results in an increased IL-17 production by CD4$^+$ T cells.

SF from PsA patients contains many soluble mediators that recruit immune cells and promote tissue inflammation. The source of CXCL4 in PsA SF has yet to be identified. Macrophages have been suggested to contribute to the overexpression of CXCL4 in rheumatoid arthritis synovium [13]. mDCs and pDCs, both capable of producing CXCL4, are increased in PsA and rheumatoid arthritis SF [35–37]. Furthermore, in addition to CD4$^+$ T cells, there are other type 17 cells enriched in the PsA SF, including the type 3 innate lymphoid cells [38], CD8 T cells [39], and γδ T cells [40]. CXCL4 contribution to the IL-17 regulation in these cells still needs to be evaluated.

Compelling evidence suggests a pro-inflammatory role for CXCL4 in multiple mouse inflammation models [12, 41–43], however it is intriguing that some studies showed CXCL4 to suppress IL-17 production [19, 20]. The underlying mechanism is unclear, the apparent species-specific prerequisite for Th17 development in human and mice may contribute to this discrepancy [44–46]. Furthermore, human Th17 cells also did not seem to co-produce GM-CSF as seen in mice studies [47], and our data showed that CXCL4 did not influence GM-CSF production by human CD4$^+$ T cells in vitro. In support of this, no correlations were found between CXCL4 and GM-CSF in inflamed PsA joint.

In conclusion, we have identified CXCL4 as a new Th17 driver, that is able to directly and indirectly promote IL-17 production in human CD4$^+$ T cells, and that it correlates with Th17 cytokines levels intra-articularly, at inflammatory site of PsA patients. These data strongly suggest CXCL4 to play a significant role in Th17 regulation in PsA. Further research to dissect the molecular mechanisms involved and to assess the CXCL4 contribution in other Th17-mediated diseases is necessary.

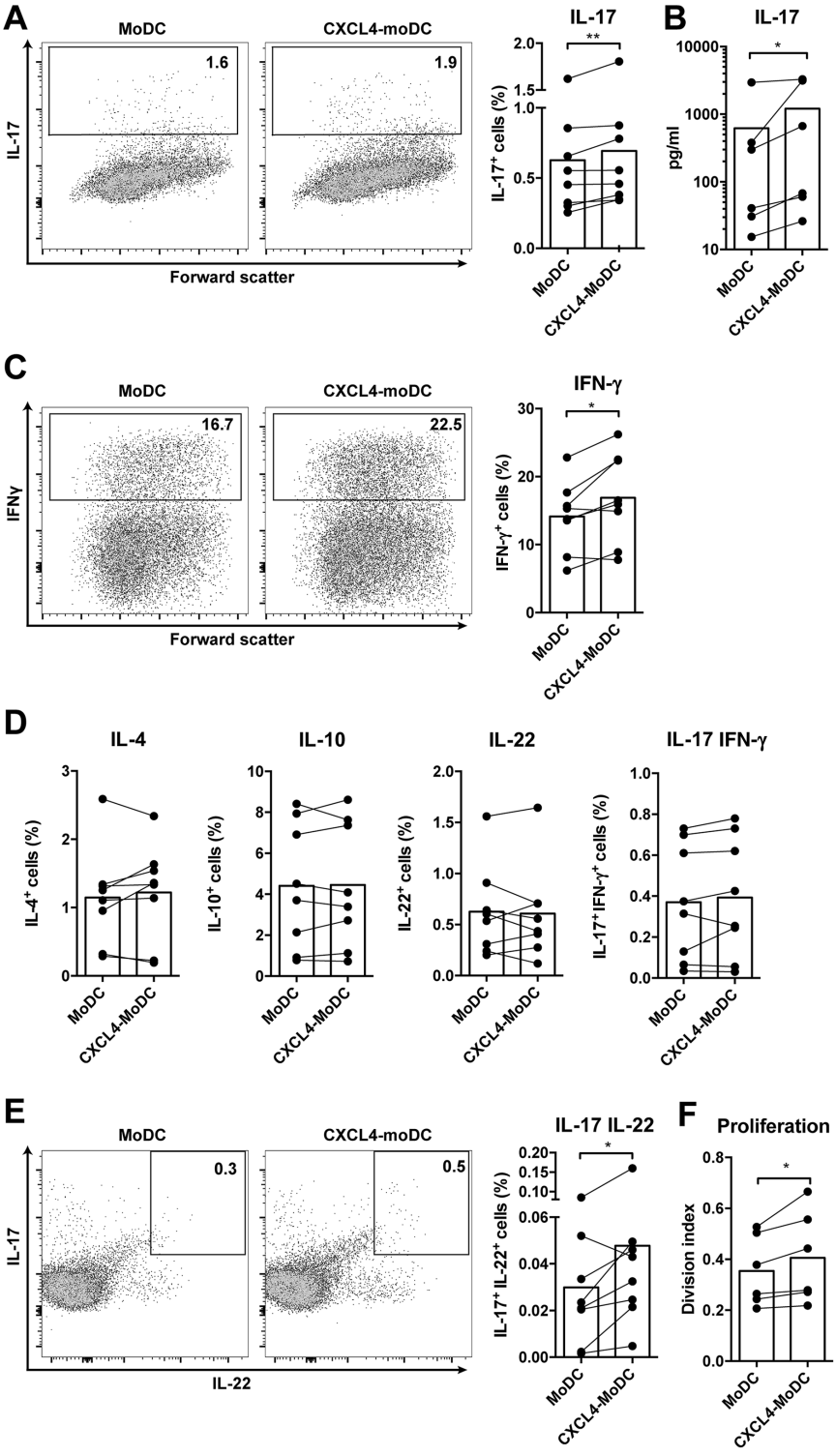

Figure 4. CXCL4-differentiated monocyte-derived dendritic cells enhance pro-inflammatory cytokine production and proliferation by $CD4^+$ T cells. Monocytes from healthy donors were isolated and differentiated into dendritic cells in the absence or presence of CXCL4 (moDCs or CXCL4-moDCs). moDCs were then co-cultured with autologous $CD4^+$ T cells in the presence of superantigen from Staphylococcal Enterotoxin B (SEB) for three days and restimulated with PMA and ionomycin. (A-C) Comparison of co-culture with moDC or CXCL4-moDC on IL-17 or IFN-γ production by $CD4^+$ T cells was assessed by (A, C) intracellular cytokine staining and (B) enzyme-linked immunosorbent assay are shown. (D) Intracellular cytokine staining was performed for the measurement of $IL\text{-}4^+$, $IL\text{-}10^+$, $IL\text{-}22^+$, and $IL\text{-}17^+IFN\text{-}\gamma^+$ cells gated on live $CD4^+$ T cells. (E) The amount of IL-17 producing cells co-expressing IL-22 as measured by flow cytometry. (F) $CD4^+$ T cells were labeled with CellTrace Violet prior co-culture and proliferation was analyzed as division index. Cells were gated on live, single, $CD4^+$ T cells. Means (bars) and values from each donor are shown. Data are pooled from two to three independent experiments, with two to three donor samples per experiment. Each dot on the bar graphs represent a single donor and paired *t*-test was used for statistical analysis. $^*p<0.05$, $^{**}p<0.01$.

Materials and methods

Patient's population

Peripheral blood and synovial fluid (SF) were obtained in accordance with the local Institutional Review Board's approval and patients gave their written informed consent. For plasma measurement, blood was collected from 10 healthy controls, 10 patients with Pso, and 10 patients with PsA. Venous blood was collected in a 10 mL EDTA vacutainer (#367864, BD Biosciences), centrifuged at 1700 × *g* for 10 min, and plasma was collected. SF samples were isolated from 17 patients with PsA and nine patients with osteoarthritis. All SF samples were collected from effusion of

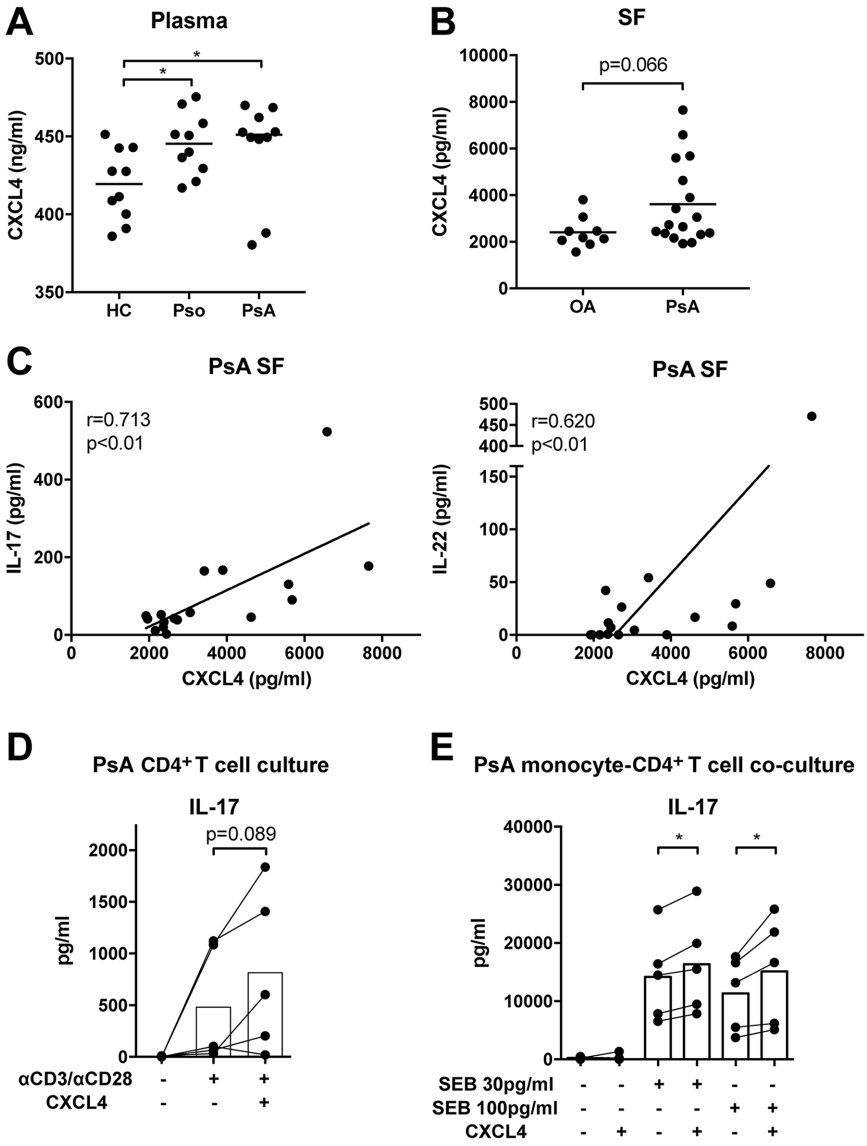

Figure 5. CXCL4 expression is upregulated in Th17-mediated diseases, correlates with Th17 cytokine levels in the synovial fluid of psoriatic arthritis joints, and induces IL-17 production in psoriatic arthritis patients. Plasma was obtained from healthy controls (HC), psoriasis (Pso), or psoriatic arthritis (PsA) patients, and synovial fluid (SF) was collected from PsA and osteoarthritis (OA) patients. Monocytes and $CD4^+$ T cells were isolated from PsA patients and CXCL4 effect was assayed in (co-) cultures. (A) CXCL4 was measured in the plasma of HC, Pso, or PsA patients by enzyme-linked immunosorbent assay. Kruskal-Wallis test was used for statistical analysis. (B) The levels of CXCL4 was measured in SF from OA and PsA patients using a Luminex-based assay. Mann-Whitney test was used for statistical analysis. (C) The intraarticular levels of CXCL4, IL-17, and IL-22 in PsA SF were measured using Luminex-based assay. Correlation between cytokine levels was assessed by Spearman's correlation. (D, E) The effects of 2 μg/mL CXCL4 on secreted IL-17 by $CD4^+$ T cells from PsA patients upon (D) CD3/CD28 stimulation or (E) co-culture with autologous monocytes in the absence or presence of superantigen from Staphylococcal Enterotoxin B (SEB) as assessed by enzyme-linked immunosorbent assay are shown. Data are pooled from three independent experiments, with one to two patient samples in duplicate per experiment. Means (bars) and values from each patient are shown and paired t-test was used for statistical analysis. $^*p<0.05$.

the knee as part of routine clinical care. For SF collection, fluids were centrifuged at 2300 × *g* for 10 min at 4°C to remove cells and debris. All samples were aliquoted and immediately frozen at −80°C until further use. Patients with PsA fulfilled Classification of Psoriatic Arthritis Study Group criteria and their characteristics are summarized in Supporting Information Table 1.

Cell isolation

Peripheral blood mononuclear cells from healthy donors and PsA patients were isolated by Ficoll gradient (#17-1440-02, GE Healthcare). Cells were processed for further isolation using magnetic beads for plasmacytoid dendritic cells (pDCs, #130-090-532), myeloid dendritic cells (mDCs) and B cells (#130-094-487), monocytes (#130-050-201), and $CD4^+$ T cells (#130-096-533) on autoMACS Pro Separator according to manufacturer's instructions, all from Miltenyi Biotec. Naïve $CD4^+$ T cells ($CD127^+$ $CD25^-$ $CD27^+$ $CD4^+5RO^-$) were further purified using fluorescence-activated cell sorting on BD FACSAria (BD Biosciences). Purity was routinely above 99% for naïve $CD4^+$ T cells, 95% for bulk $CD4^+$ T cells, and above 90% for other cells as assessed by flow cytometry. Cells were washed and cultured with complete medium of RPMI-GlutaMAX (#61870-010, Thermo Fisher Scientific) supplemented with 10% FBS (Biowest) and Penicillin-Streptomycin (#15070063, Thermo Fisher Scientific).

T cell stimulation

The 50 000 $CD4^+$ T cells were cultured in a complete medium on a 96-well round bottom plate at 37°C for 3–7 days. In $CD4^+$ T cells monoculture, cells were activated with Dynabeads Human T-Activator CD3/CD28 (#111.31D, Thermo Fisher Scientific) at bead-to-cell ratio of 1:5. In autologous co-culture with antigen-presenting cells (APCs), $CD4^+$ T cells were cultured with pDCs,

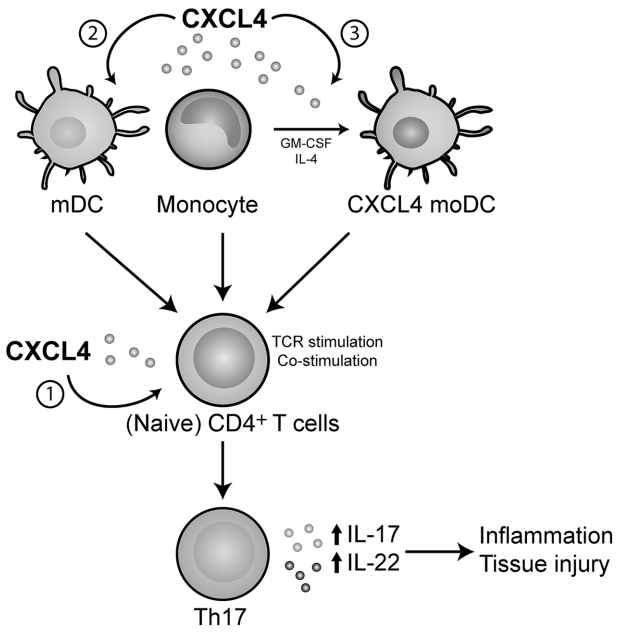

Figure 6. Proposed mechanisms of CXCL4 as a novel Th17 driver. CXCL4 promotes IL-17 production in human $CD4^+$ T cells by acting (1) directly on CD3/CD28-activated human (naïve) $CD4^+$ T cells, and (2) indirectly in a co-culture of $CD4^+$ T cells with monocytes and myeloid dendritic cells (mDCs). (3) CXCL4 also primed monocytes-derived dendritic cells (CXCL4-moDCs) to induce IL-17 production and proliferation in activated $CD4^+$ T cells. These IL-17-producing cells also co-produce IL-22, which results in an increased immune response at the site of inflammation, such as a psoriatic arthritis joint.

mDCs, B cells, monocytes, or monocytes-derived DCs (moDCs) at APC-to-$CD4^+$ T cell ratio of 1:5, in the presence of superantigen from Staphylococcal Enterotoxin B (SEB) (#S4881, Sigma Aldrich). Recombinant human CXCL4 (#300-16, Peprotech) was added as indicated. For restimulation, cells were stimulated with phorbol 12-myristate 13-acetate (PMA, #P8139) and ionomycin (#I0634, all Sigma Aldrich) overnight. For intracellular cytokine staining, PMA, ionomycin and GolgiStop (#554724, BD Biosciences) was added for the final four hours of culture. For proliferation analysis, $CD4^+$ T cells were labeled with CellTrace Violet (1.5 μM; #C34557 Thermo Fisher Scientific) prior culture.

Monocyte-derived dendritic cells

Monocytes were cultured at a density of 1 million cells/mL in complete medium with 800 IU/mL recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF, #204-IL, R&D Systems), 500 IU/mL recombinant human IL-4 (#215-GMP, R&D Systems), in the presence or absence of 10 μg/mL recombinant human CXCL4 for six days, with medium and cytokines refreshment on day three. Cells were harvested and remaining cytokines were washed in complete medium and rested for 1–2 h at 37°C prior to co-culture with $CD4^+$ T cells from the same donor.

Flow cytometry

For intracellular cytokine staining, cells were stained with Fixable Viability Dye (#65-0866, eBioscience) for dead cell exclusion, then cells were fixed and permeabilized using Foxp3/Transcription Factor Staining Buffer Set (#00-5523, eBioscience), and stained for IL-17A (#11-7179), IL-22 (#17-7222), IFN-γ (#45-7319, #12-7319, eBioscience), IL-4 (#564112, BD Biosciences), IL-10 (#554706, BD Biosciences), GM-CSF (#502317, Biolegend), CD3 (#48-0038), and $CD4^+$ (#25-0049 eBioscience). Cells were acquired on BD LSRFortessa (BD Biosciences). Cells were gated to exclude debris, doublets, and dead cells and analyzed by FlowJo software (Tree Star). Division index was calculated as a measure of proliferation, following FlowJo guidelines. Alternatively, percentage of proliferated cells (CellTrace $Violet^-$) was shown.

Cytokine measurement

Cytokines in cell-free supernatant, plasma, or synovial fluid, were measured using enzyme-linked immunosorbent assay (IL-17A, #88-7176, eBioscience; CXCL4, #DY795, R&D Systems; GM-CSF, #88-8337, eBioscience) or using a multiplex immunoassay based on xMAP technology (Luminex) at the MultiPlex Core Facility of the Laboratory of Translational Immunology, University Medical Center Utrecht [48]. For the Luminex-based assay, acquisition was performed with a Biorad FlexMap3D system using Xponent 4.2 software and analyzed using Bio-Plex Manager 6.1.1.

Statistical analysis

Paired *t*-test, Mann–Whitney test, Kruskal–Wallis test, or Spearman's correlation analysis, were calculated using GraphPad Prism 6.0 Software. Differences of $p < 0.05$ were considered significantly different.

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Conflict of interest: The authors declare no financial or commercial conflict of interest.

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Abbreviations: **APC**: antigen presenting cell · **mDC**: myeloid dendritic cell · **moDC**: monocyte-derived dendritic cell · **pDC**: plasmacytoid dendritic cell · **PsA**: psoriatic arthritis · **Pso**: psoriasis · **SEB**: Staphylococcal Enterotoxin B · **SF**: synovial fluid · **Th**: T helper

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