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Th17 Trafficking Cells in Behcet's Disease Skin Lesions

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1. Introduction

Behçet's disease (BD) is a vasculitis characterized by oral, genital ulcers and uveitis with varying other manifestations associated with vascular inflammation. Additional target organ, including vascular, neurological, and gastrointestinal manifestations, were added to the disease spectrum [Yazici et al., 2003]. The etiology of BD is considered to be a complex systemic vasculitis, caused by T-helper-1 (Th1) cytokine skewed neutrophilic and lymphohistiocytic inflammation [Suzuki et al., 2006; Kulaber et al., 2007; Koarada et al., 2004; Keller et al., 2005].

The pathogenesis of BD is still unclear, but immune dysfunction, viral and bacterial agents, such as Staphylococcus spp. and herpes simplex virus, have been postulated [Onder et al., 2001]. Cytokines play crucial roles in the inflammatory responses in BD [Hamzaoui et al., 2002; Direskeneli et al., 2003]. BD as many autoimmune diseases are considered to be T cell-regulated diseases, further classified as Th1-mediated diseases, with Th1-like diseases featuring a high production of IFN-γ. However, this classification fails to explain the involvement of inflammatory cells as seen in many autoimmune/inflammatory diseases. A unifying feature of the inflammation observed in BD is the nonspecific hyperreactivity of tissue to minor trauma, termed the skin pathergy reaction (SPR), which remains the most diagnostically relevant lesion in BD patients, where an exaggerated inflammatory response develops in the skin of BD patients that is characterized by dermal infiltration of activated dentritic cells (DCs) and the presence of a Th1-type immunological cascade [Melikoglu et al., 2006]. The immunohistochemistry of patients with sterile, pustular skin eruptions in the context of a systemic autoinflammatory disease revealed a substantially denser, lymphocyte rich cell infiltrate (mainly $CD4^+$ and some $CD8^+$ T cells than in normal skin). The majority of T cells detected were immigrating, inflammatory T cells, as they expressed CCR6, the receptor for CCL20 (MIP-3α) [Keller et al., 2005].

Studies show that $CD4^+$ $IL\text{-}17^+$ and $CD8^+$ $IL\text{-}17^+$ T cells (Th17) play an active role in inflammation and autoimmune diseases in murine systems [Komiyama et al., 2006; Bettelli et al., 2007; Kryczek et al. 2007], and have never been studied in skin lesions from BD patients. The question addressed in this study is why Th1 and Th17 cells often colocalized in

pathological environments and what is the mechanism and pathological relevance of this colocalization. We studied skin lesions from BD patients. Previous studies implicated Th1 cells promoting cytokine in skin lesions from BD patients [Melikoglu et al., 2006].
In the current investigation, we explored the phenotype and function of IL-17-secreting T cells in BD and healthy skin, and the factors supporting their trafficking to and induction in lesional skin. Specifically, we show that IFN-γ is demonstrated as a potent promoter of IL-17$^+$ T cell trafficking, induction, and function. Our observations support a model wherein Th1 and IL-17$^+$ T cells mechanistically interact and collaboratively contribute to BD skin pathogenesis.

2. Materials and methods

2.1 Patients skin testing, and tissue samples

The study was approved by the Ethical Committee of our University. A total of 12 patients with active BD (3 females, 9 males) fulfilling the International Study Group Criteria for BD [ISG. 1990] were enrolled into this study. BD patients were aged: 39 years (range 26-47 years) and the mean disease duration were 76 months (range 10-132 months). Disease activity was evaluated according to published criteria [Lawton & Bhakta, 2004]. Of 12 patients, all had oral ulcerations, 8 had genital ulcers, 6 had erythema nodosum, 10 had papulopustular lesions, 8 had arthritis, 7 had uveitis, 6 had deep venous thrombosis, and 11 had a positive skin pathergy reaction (SPR). Consistent with previous published reports, there were no demographic or clinical differences discernible between BD patients with a positive or negative SPR in our study [Krause et al., 2000].
The skin lesions were scored [Diri et al., 2001]: 0 = no lesions; 1 = 1-5 lesions; 2 = 6-10 lesions; 3 = 11-15 lesions; 4 = 16-20 lesions; and 5 = more than 20 lesions. Table I describes BD patients with skin lesions. Patients were treated with steroids and colchicines. Seven donors of healthy human skin were included in this study. Punch-biopsy specimens (4 mm) were obtained from affected skin (pustular eruption) and were divided in two equal parts, one for T cell elution and one for RT-PCR analysis. All skin biopsy samples were obtained with a circular dermal punch after injection of 1% lidocaine solution into the hypodermis. Biopsy samples were snap frozen directly in liquid nitrogen for mRNA extraction and RT-PCR analysis.

2.2 Immune cell isolation

Single cell suspensions were prepared from PBMC and skin tissue samples. Skin biopsy samples were incubated in 50 U/ml dispase (BD Biosciences) at 37°C for 90 min. The skin portions were then cut into 1-mm pieces and digested in collagenase for 2 h at room temperature. Single cell suspensions of epidermal portions were generated by incubation in Cell Dissociation buffer (Invitrogen) at 37°C for 2 h. Skin explant cultures of T cells from skin biopsies were prepared as described by Clark et al. [Clark et al., 2006].
Immune cells including T cells and CD14$^+$ or CD11c$^+$ myeloid APCs were enriched using paramagnetic beads (StemCell Technologies) and sorted from stained single cell suspensions using a high-speed cell sorter (FACSAria; BD Immunocytometry Systems) as described by Curiel et al [Curiel et al., 2003]. Cell purity was >98% as confirmed by flow cytometry (LSR II; BD Immunocytometry Systems). CD14$^+$ or CD11c$^+$ myeloidAPCs were used to stimulate T cells as indicated.

2.3 APC activation and cytokine production

Fresh peripheral blood $CD11c^+$ APCs (0.5×10^6/ml) were incubated for 72 h with or without recombinant human IFN-γ (200 ng/ml; R&D Systems). These cells were washed and used for T cell stimulation or activated for 12 h with LPS (1 μg/ml; Sigma-Aldrich) or incubated 3 days (1×10^6 cells/ml) in the presence of LPS to detect cytokine levels in supernatants. All Abs were from R&D Systems.

2.4 T cell culture system

Myeloid APCs were cocultured with peripheral blood T cells in ratios from 1:3 to 1:10 (0.5×10^6 T cells/ml) for 4 days in the presence of anti-CD3 (2.5 - 5 μg/ml) and anti-CD28 (1.2 - 2.5 μg/ml) mAbs (BD Biosciences). Different cytokines and neutralizing antibodies (Abs) or their combinations including IL-1α (2.5 ng/ml), IL-1β (2.5 ng/ml), IL-23 (10 ng/ml), anti-IL-4 (1 μg/ml), anti-IFN-γ (2 μg/ml), anti-IL-1 (1 μg/ml anti-IL-1R plus 1 μg/ml anti-IL-1 α) were used as indicated (all from R&D Systems). Cells were subjected to flow cytometric phenotyping, intracellular cytokine staining, and transcript detection by real-time PCR. Culture supernatants were collected for detection of IL-17 by ELISA (R&D Systems). For flow cytometry analysis, cells were first stained extracellularly with specific monoclonal antibodies (Abs), then fixed and permeabilized with Perm/Fix solution (eBioscience), and finally stained intracellularly with specific Abs against the indicated cytokines (BD Biosciences). Samples were acquired on a LSR II (BD Biosciences) and data were analyzed with DIVA software (BD Biosciences).

2.5 Migration assays

Migration assays were performed in a Transwell system with a polycarbonate membrane of 6.5 mm diameter with a 3-μm pore size as described by Curiel et al. [Curiel et al., 2004]. Purified T cell subsets were added to the upper chamber, and CCL20 (5 ng/ml; R&D Systems) was added to the lower chamber. After 4 h of incubation at 37°C, the phenotype and number of T cells in the upper and lower chambers was determined by FACS.

2.6 Quantification of gene expression

Quantitative real-time PCR was performed on control and lesional skin samples from 12 BD patients and 7 normal healthy controls as we have recently reported [Hamzaoui et al., 2008]. Quantification of gene expression in the cultured myeloid APCs was performed as described by Kryczek et al [Kryczek et al., 2007]. The gene transcripts were quantified in a MasterCycler RealPlex system (Eppendorf Scientific) and expressed as mRNA quantities normalized to GAPDH levels.

2.7 Immunohistochemistry

Skin biopsy samples were stained with anti-CD4 or anti-CD8 Ab as described [Hamzaoui et al., 2008]. The staining was detected using the one-step avidin-biotin complex technique (BD Pharmingen).

2.8 Statistical calculations

The Wilcoxon rank-sum test was used to determine pairwise differences and the χ^2 test used to determine differences between groups. A value for $p < 0.05$ was considered as significant. Differences in phenotype of T cell subsets were tested with the paired Student's *t* test.

Correlation was tested by Spearman's test. All statistical analysis was done on Statistical software (StatSoft).

3. Results

3.1 IL-17$^+$ T cells are increased in BD skin lesions

We investigated the distribution of CD4$^+$ and CD8$^+$ IL-17$^+$ T cells in BD-skin lesions and in healthy donors [Figure 1A and Figure 1B]. High levels of CD3$^+$ IL-17$^+$ T cells were observed in active BD compared to healthy control skin. The highest percentage of CD4$^+$ and CD8$^+$ IL-17$^+$ T cells were observed in BD-skin lesions. Notably, we observed an increased number of CD8$^+$ IL-17$^+$ T cells in BD-skin [Figure 1B]. We further analyzed the expression of IFN-γ and IL-17 per single cell level. Interestingly, 40–60% IL-17$^+$ T cells coexpressed IFN-γ in the BD-skin [Figure 1C].

The data demonstrate the prevalence, phenotype, and distribution of IL-17$^+$ T cells in patients with BD, and indicate that BD-skin lesions is an environment containing abundant IL-17$^+$ T cells including Th17 cells and CD8$^+$ IL-17$^+$ T cells.

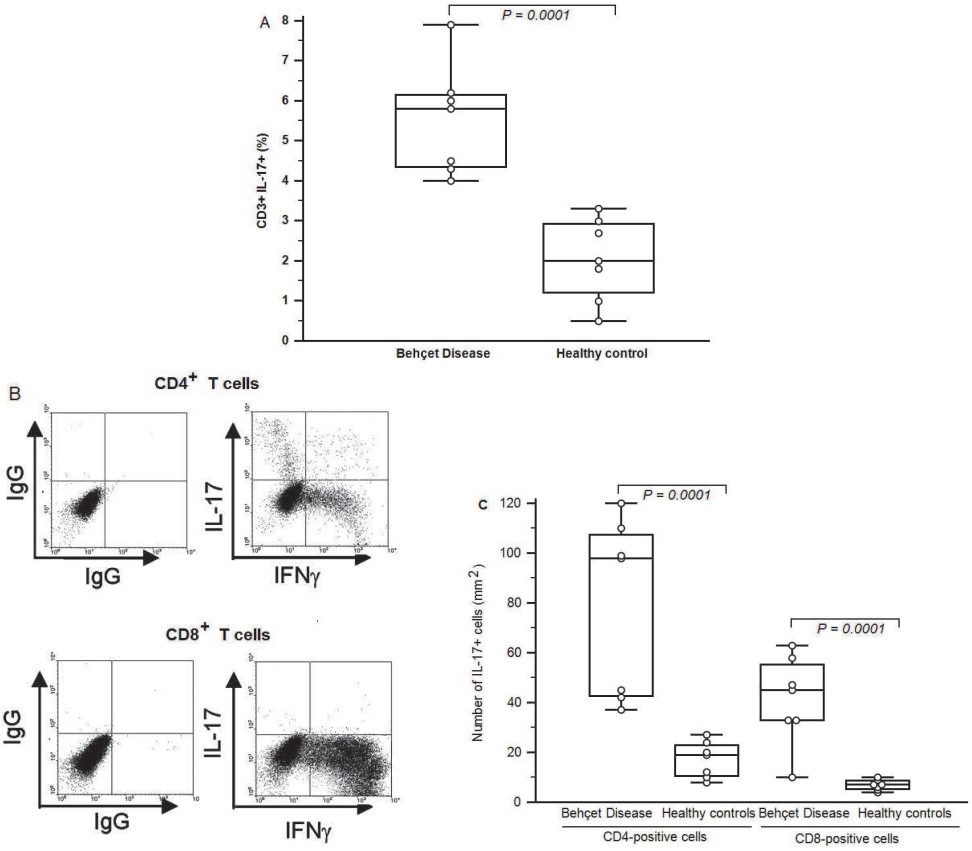


Fig. 1. IL-17$^+$ T cells in BD skin lesions.

Skin biopsy from healthy donors and patients with Behcet's disease (BD) were stained with specific antibodies as described in Materials and Methods. IL-17+ T cells were analyzed with FACS. (A): Results show mean percentage of IL-17+ T cells in T cells. Error bar indicates SD. Representative dot plots are shown. Total number of IL-17+ T cells in the skin. Total number of IL-17+ T cells was calculated by multiplying the percentage of IL-17+ T cells by the absolute number of T cells/mm2 of skin, as determined previously [51]. Results shown are mean number ± SD, for *n* = 12 BD patients and 7 healthy donors. (B): Coexpression of IL-17 and IFN-γ on IL-17+ T cells. Single cell suspensions were made from skin tissues in healthy donors and patients with BD. The expression of IFN-γ and IL-17 were analyzed by intracellular cytokine staining gated on CD3+ T cells. (C): Total numbers of CD4+ and CD8+ IL-17+ T cell subsets are shown as mean number ± SD.

3.2 IFN-γ+ T cells in patients with BD

As BD was related as Th1 disease, we examined the distribution of CD4+, CD8+, and IFN-γ+ T cells in BD-skin lesion. Consistent with previous reports [9], we observed a large number of CD4+ and CD8+ T cells in BD skin lesion [9; 20]. The distribution of CD4+ and CD8+ IFN-γ+ T cells is similar for T cells expressing IFN-γ and IL-17 in BD-skin. High levels of IFN-γ+ T cells [BD: 180.08 ± 62.46 cells/ mm2; HC: 25.71 ± 14.05 cells/ mm2; P = 0.0001][Figure 2A] and IFN-γ transcripts [BD: 27.75 ± 6.98 relative IFNγ mRNA expression (10-3); HC: 4.5 ± 1.87 relative IFNγ mRNA expression (10-3); P = 0.0001] [Figure 2B] were also detected in BD skin lesions compared to healthy controls.

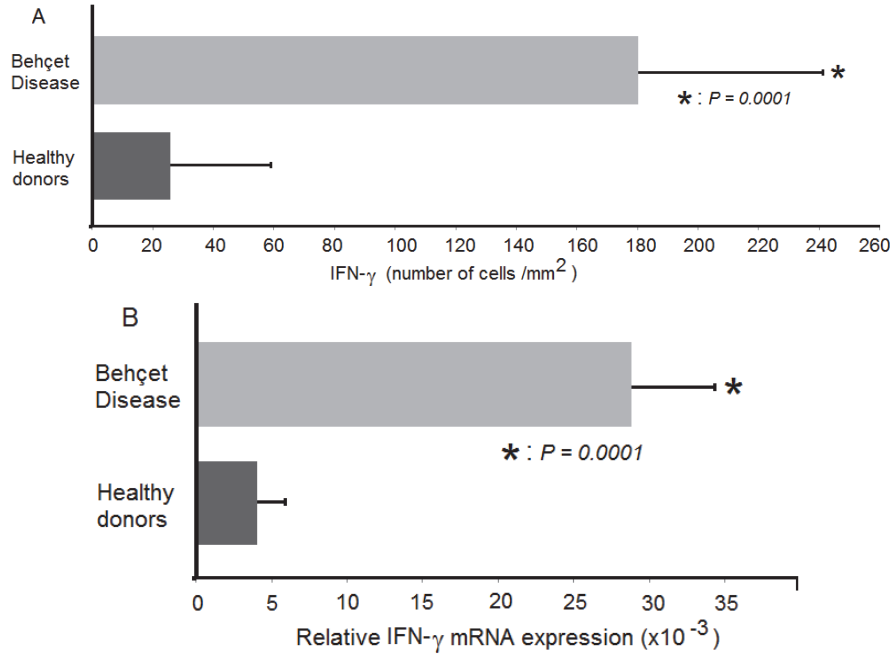


Fig. 2. IFN-γ+ T cells in BD skin lesions. [A] and [B]: IFN-γ+ T cells in BD lesions.

CD45+CD3+ T cells were analyzed by FACS in cell suspensions obtained from healthy skin and BD skin lesions. [A]: IFN-γ+ T cells were analyzed by FACS and quantified in the skin. Results shown are the mean number of cells/mm² of skin ± SD for n = 5 healthy donors. [B]: Expression of IFN-γ in skin from healthy donors and patients with BD. IFN-γ was quantified by real-time PCR. Results shown are mean value of relative expression ± SD for 12 BD patients and 7 healthy donors.

3.3 Myeloid APCs induce IL-17+ T cells

We sorted both CD14+ and CD11c+ myeloid APCs from peripheral blood and skin lesions from BD patients and healthy donors [Figure 3A]. CD14+ and CD11c+ myeloid APCs induced similar levels of Th17 cells [Figure 3B]. Then we investigated the potential role for myeloid APCs in inducing IL-17+ T cells [Figure 3C]. Interestingly, APCs from BD peripheral blood and skin lesions were significantly more efficient than those from healthy donors in inducing IL-17 production [Figure 3C]. Myeloid APCs induced both CD4+ and CD8+ IL-17+ T cells [Figure 3D]. We performed similar experiments with responder T cells from normal donors and BD patients. Our data indicate that BD myeloid APCs potently induce IL-17+ T cells, and may thereby stimulate and maintain the IL-17+ T cell pool in BD patients.

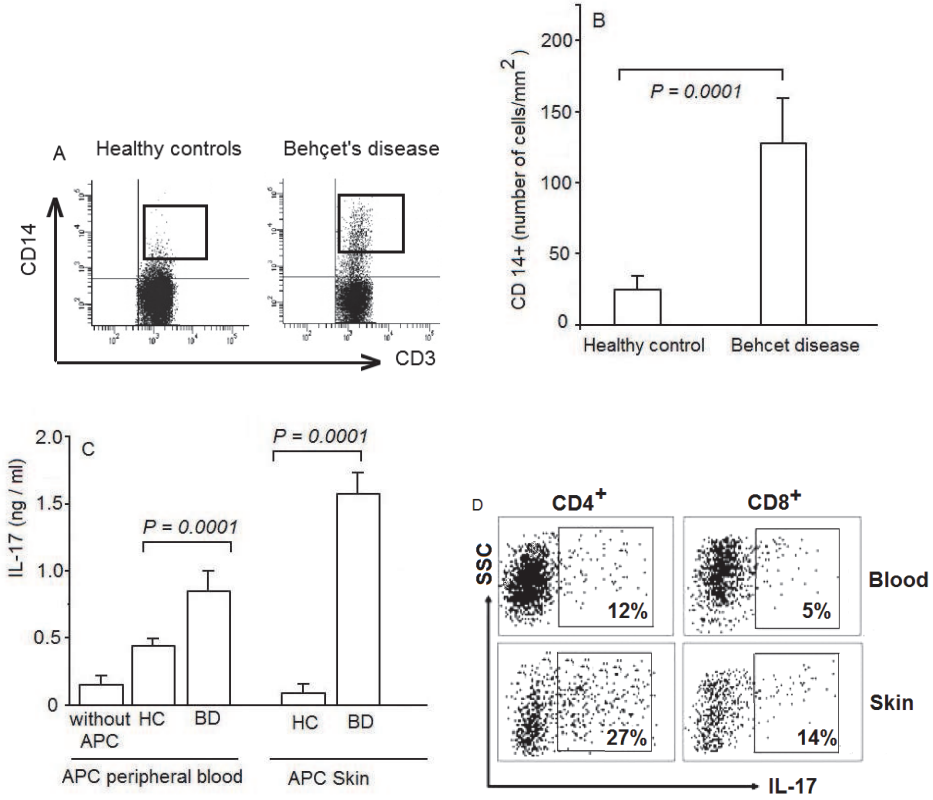


Fig. 3. Behçet's disease (BD) myeloid APCs stimulate IL-17+ T cell expansion.

Myeloid APCs ($CD45^+CD14^+$) were analyzed by FACS in cell suspensions obtained from healthy skin and BD skin lesions. [A]: One representative dot plot of $CD14^+$ leukocytes from the skin of a healthy donor and a patient with BD. Total number of $CD3^+$ or $CD14^+$ cells is indicated. [B]: Myeloid APCs were analyzed by FACS and quantified in the skin. Results shown are mean number of cells/mm^2 of skin ± SD for 5 BD patients and 4 healthy controls. [C and D]: BD myeloid APCs induce $IL\text{-}17^+$ T cells. Normal peripheral blood T cells were stimulated for 5 days with myeloid APCs derived from skin and blood of healthy donors or patients with BD in the presence of anti-CD3 and anti-CD28. [C]: IL-17 was detected in the culture supernatants. Results shown are mean ± SD, for the same number of BD patients and controls.

3.4 IFN-γ induces myeloid APCs to stimulate $IL\text{-}17^+$ T cells

We next studied why BD myeloid APCs are potent inducers of $IL\text{-}17^+$ T cells. The contribution of IFN-γ was investigated, as IFN-γ is increased in serum of BD patients [Hamzaoui et al., 2007]. IFN–γ+ T cells are enriched in BD skin lesions as reported in Figure 2 and all studies implicated IFN-γ (Th1 cells) in BD pathogenesis. To test the hypothesis that IFN-γ may program myeloid APCs to stimulate $IL\text{-}17^+$ T cells, $CD11^+$ cells from the blood of donors were conditioned with IFN-γ and tested for their capacity to induce $IL\text{-}17^+$ T cells. IFN–γ profoundly increased the capacity of CD11c+ cells to elicit $IL\text{-}17^+$ T cells [Figure 4A, B, and C]. We also conditioned peripheral blood myeloid APCs from BD patients with exogenous IFN-γ, and observed that IFN-γ was able to further enhance the ability of healthy controls and BD myeloid APCs to induce IL-17-secreting T cells [Figure 4]. The data suggest that IFN-γ released by BD T cells may condition myeloid APCs to induce $IL\text{-}17^+$ T cells.

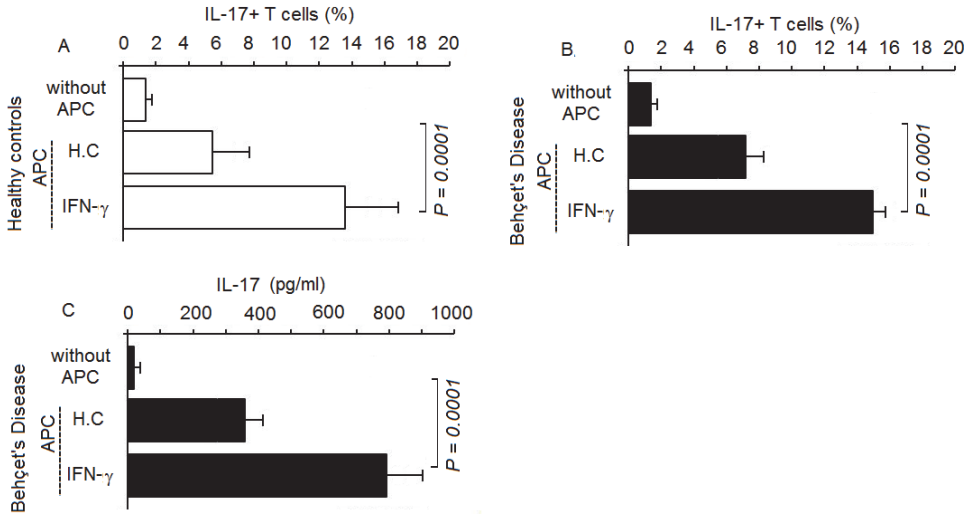
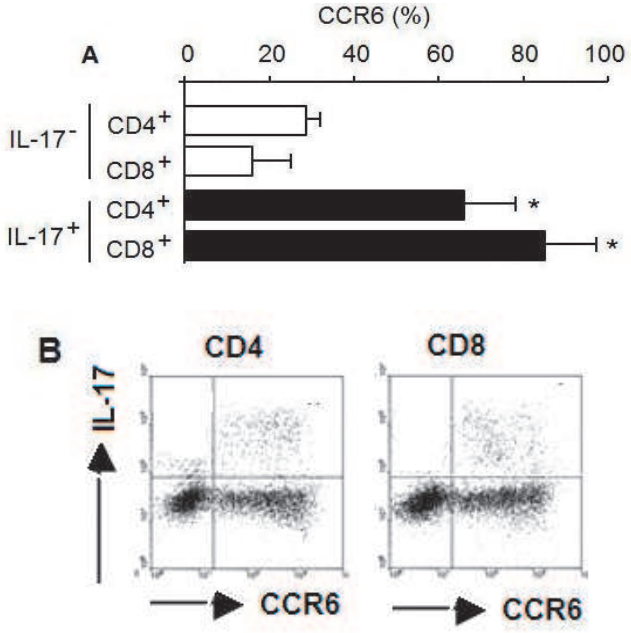


Fig. 4. IFN-γ induces BD- APCs to stimulate $IL\text{-}17^+$ T cells.

Healthy control (A) or BD patients (B) blood-derived ex vivo CD11c+ cells were conditioned for 72 h with or without IFN- γ, and then cultured with normal T cells for 5 days in the presence of anti-CD3 and anti-CD28. [A]: IL-17+ T cells were detected by FACS. Results shown are mean percentage ± SD of IL-17+ T cells in T cells. (C): IL-17 was measured in the supernatants by ELISA. Results shown are mean percentage ± SD of IL-17. Five patients with BD and 4 healthy controls were investigated.

3.5 CCR6+ IL-17+ T cells and CCL20 in Behçet's disease

We examined how IL-17+ T cells traffic to the BD skin environment. We found that CD4+ and CD8+ IL-17+ T cells derived from BD skin lesions highly expressed CCR6 [Figure 5A and B]. We therefore asked whether IL-17+ T cells could migrate toward CCL20, the ligand for CCR6. We observed that T cells efficiently migrated in response to CCL20, and that the migrating cells were enriched for IL-17+ T cells (from 0.2% IL-17– cells in the upper chamber to 18% IL-17+ T cells in the lower chamber) [Figure 5C]. We further tested the role of IFN-γ in CCL20 production. We observed that IFN–γ stimulated CCL20 production from CD11c+ APCs [Figure 5D]. High levels of CCL20 mRNA were detected in lesional BD skin [Figure 5E]. The data suggest that IFN-γ derived from BD T cells induces CCL20 and promotes homing of IL-17+ T cells to the BD environment. In addition to CCR6, we observed that BD- IL-17+ T cells highly expressed CD103 as compared with IL-17– T cells [Figure 5F]. CD103 may play a specific role in IL-17+ T cell trafficking.



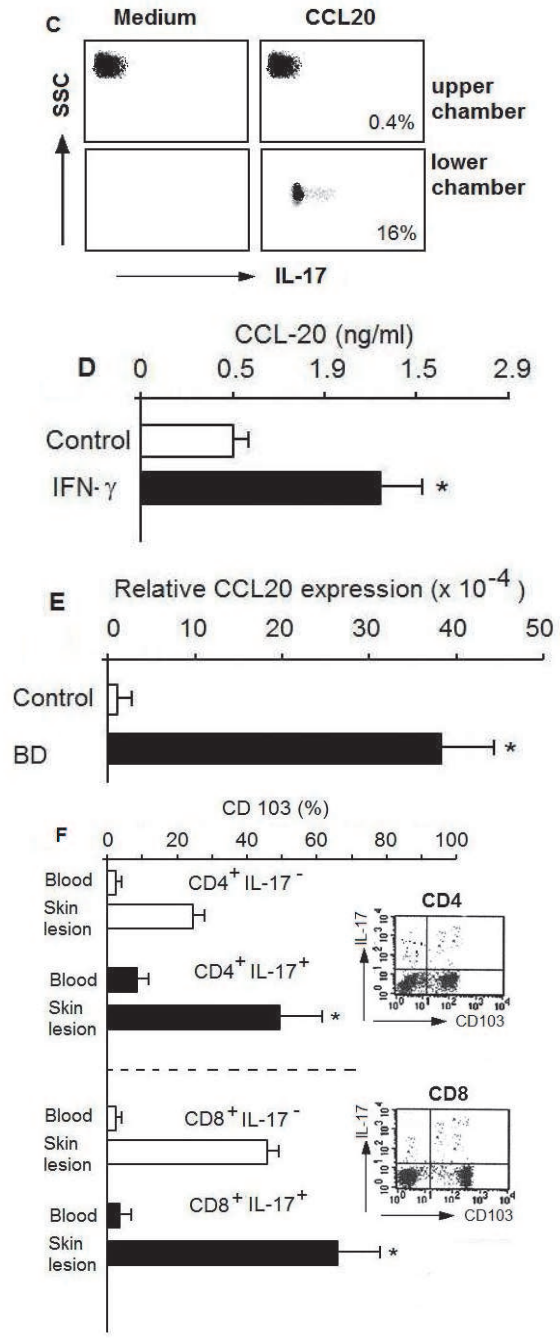


Fig. 5. CCR6+ IL-17+ T cells and CCL20 in the BD environment.

[A,B]: BD-IL-17+ T cells highly express CCR6. Expression of CCR6 was determined by FACS on IL-17+ and IL-17–T cells in BD skin. Results shown are mean of CCR6+ T cells in IL-17+ T cells or IL-17– T cells ± SD for 5 healthy donors and 6 BD patients. (*): P < 0.05 compared with IL-17– T cells. [C]: IL-17+ T cells migrate in response to CCL20. Migration assay was performed as described in Materials and Methods. The migrated T cells were subjected to intracellular staining for IL-17. The percentage of IL-17+ T cells in the upper and lower chambers is shown. [D]: IFN-γ induces CCL20 production. Blood CD11c+ cells were stimulated for 3 days with or without IFN- γ. CCL20 was detected by ELISA in the supernatants. Results shown are mean value ± SD for 8 healthy controls. (*): P < 0.05 compared with control. [E]: High expression of CCL20 in BD skin. CCL20 transcript was quantified by real-time PCR. Results shown are mean value of relative 1 expression ± SD for 5 healthy donors. (*): P = 0.004 compared with healthy skin control. [F]: High expression of CD103 on BD IL-17+ T cells. CD103 expression was determined by FACS on IL-17+ and IL-17– T cell subsets. Results shown are mean ± SD of CD103+ T cells in each T cell subset, for 5 healthy donors. (*): P < 0.05 compared with IL-17– T cells. One representative dot plot of BD CD103+ IL-17+ T cells is shown gated each on CD4+ and CD8+ T cells.

3.6 Functional characterization of CD8+IL-17+ and CD4+IL-17+ cells from BD-skin lesions

The ability of CD8+IL-17+ and CD4+IL-17+ cells-producing T cells derived from BD-skin lesions to proliferate in response to TCR-mediated stimulation was also assessed. For this purpose, CCR6-sorted and expanded cells were challenged with anti-CD3/CD28 mAb. A well-characterized Th17 line was used as a control. The response of the Th17 cells from the BD-skin lesions was significantly increased to those of the CD8+IL-17+ and CD4+IL-17+ T cells derived from healthy controls [Figure 6].

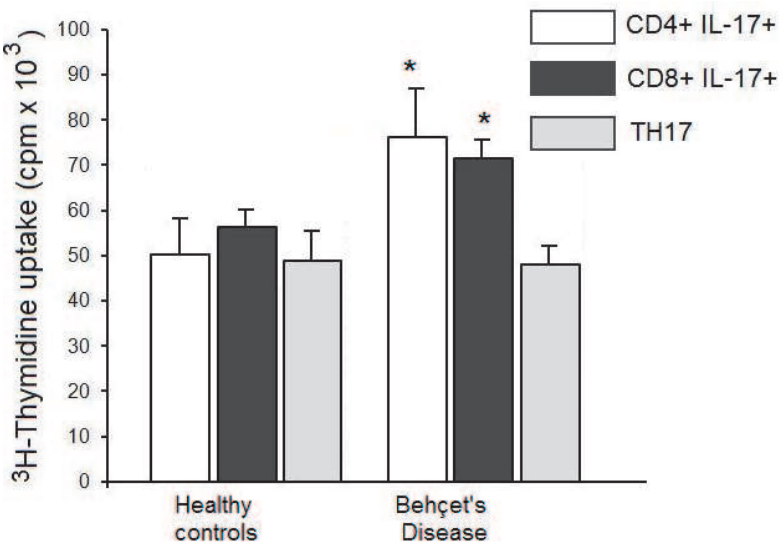


Fig. 6. Proliferative response of BD-skin-derived CD8+IL-17+ and CD4+IL-17+ T cells and a Th17 cell line.

Proliferative responses of $CD8^+IL\text{-}17^+$ and $CD4^+IL\text{-}17^+$ T cells from BD patients were more important than in healthy controls. Columns represent mean ± SD values (n=8) of proliferative response and asterisks, statistical significance [*]: $P < 0.05$.

4. Discussion

In this report we have investigated the phenotype and function of $IL\text{-}17^+$ T cells in skin healthy controls and BD patients with skin lesions. We show that $CD3^+$ T cells expressing IL-17 were increased in BD-skin lesions compared to skin biopsies from healthy controls. $CD3^+$ $IL\text{-}17^+$ T cells (Th17) are postulated to play a role in inflammatory/autoimmune pathogenesis [Wilson et al., 2007; Zaba et al., 2007; Murphy et al., 2003; Aggarwal et al., 2003]. Recent data from Melikoglu et al [Melikoglu et al., 2006] reported increased cytokines (IFN-γ, IL-12 p40, IL-15), chemokines (MIP3-α, IP-10, Mig, and iTac), and adhesion molecules (ICAM-1, VCAM-1) in the skin of BD patients with SPR^+ but not in the skin of normal controls. These results suggested that BD patients experience marked cellular influx into the injury site, leading to an exaggerated lymphoid Th1-type response. Our results agree the inflammatory state in skin BD patient; our present results added a Th17-type response. We show that both $CD4^+$ and $CD8^+$ T cells express IL-17 in BD-skin lesions. We observed high levels of $CD8^+$ $IL\text{-}17^+$ T cells in the BD skin lesions. $CD8^+$ $IL\text{-}17^+$ T cells are ideally positioned to respond to potential keratinocyte autoantigens on HLA class I molecules. $CD8^+$ $IL\text{-}17^+$ T cells have been genetically implicated in skin lesions from autoimmune/inflammatory diseases [Nair et al., 206]. Our observations support the hypothesis that $CD8^+$ $IL\text{-}17^+$ T cells are critical mediators of the persistently altered epidermal growth and differentiation and the local inflammation that is characteristic of BD skin lesions. $CD8^+$ $IL\text{-}17^+$ T cells were observed in cancers [12] and in psoriasis [Kryczek et al., 2008]. Our data provide the first evidence that $CD8^+$ $IL\text{-}17^+$ T cells are important in BD as observed in autoimmune/inflammatory diseases [Wilson et al., 2007]. The presence of $CD8^+$ T cells is necessary for the epidermal hyperproliferative response [Conrad et al., 2007].
We characterized the phenotype of skin BD lesions $IL\text{-}17^+$ T cells: $CD103^+CCR6^+$ $IL\text{-}17^+$ T cells are effector T cells often found in environments with chronic inflammation [Gudjonsson et al. 2004; Krolls et al., 2006]. $IL\text{-}17^+$ T cells highly express CD103, which may facilitate trafficking of $IL\text{-}17^+$ cells into inflammatory tissues [Conrad et al., 2007; Pauls et al., 2001]. We confirm that BD skin is an environment enriched with CCL20, and show that CCL20 is triggered by IFN-γ in myeloid APCs. We reported that BD Th1 cells are one of the major sources of IFN-γ, as reported recently [Hamzaoui et al., 2007], and that $IL\text{-}17^+$ T cells efficiently migrate toward a CCL20-enriched BD environment via CCR6. Our data lead us to propose a first mode of potential interaction between Th1 and $IL\text{-}17^+$ T cells in BD: IFN-γ derived from Th1 cells promotes trafficking of $IL\text{-}17^+$ T cells to the environment BD lesions through the induction and maintenance of local CCL20 production.
Regarding the contribution of epidermal $CD8^+IL\text{-}17^+$ cells to the pathogenesis of BD skin lesions, we have proved that $CD4^+IL\text{-}17^+$ and $CD8^+IL\text{-}17^+$ proliferate and secrete cytokines efficiently in response to CD3/TCR-mediated stimulation and to certain mediators. In Behcet's disease, high mRNA levels of IL-8, IFN-γ, IL-12, IL-10, and MCP-1were found in lesional skin and pathergy sites [Melikoglu et al., 2006].
$CD4^+CD25^+$ regulatory T (Treg) cells were found increased in the peripheral circulation of BD patients [Hamzaoui, 2007]. Treg cells and interleukin 17 (IL-17)-producing T helper

cells (TH17) carry out opposite functions, the former maintaining self-tolerance and the latter being involved in inflammation and autoimmunity [Deknuydt et al., 2009]. Several recent studies have indicated the existence of a close interplay between Treg and Th17 cells in regulating some autoimmune diseases. Whereas murine Treg cells suppress both Th1 and Th2 cells, in BD patients they enhance IL-17 secretion, likely through production of TGF-β [Mangan et al., 2006; Veldhoen et al., 2006]. Interplay between Treg cells and $CD4^+$ IL-17^+ cells have to be investigated in BD patients [Hamzaoui et al., 2011a; 2011b]. Memory Treg actually showed a more pronounced proficiency to give rise to Th17 cells than conventional memory $CD4^+$ T cells, suggesting that they may be at least partially committed towards the T_H17 differentiation pathway. This is consistent with the fact that memory Treg populations contain high proportions of cells expressing CCR6, the receptor of macrophage inflammatory protein3α (MIP-3α/CCL20) that has been shown to characterize the T_H17 lineage [Acosta-Rodriguez et al., 2007; Tosello et al., 2008].

The findings reported in the present study have several implications. First, one can imagine a scenario in which, during skin BD inflammation, Treg cells stimulated by APC activated by microbial products participate to innate immunity and transiently down-regulate suppressor functions, to allow the development of adaptive immunity. In a second scenario, sustained induction of Treg into Th17 cells *in vivo* will likely occur under defined permissive conditions, such as those that have been reported to lead to autoimmune/inflammatory reactions including chronic inflammation in the presence of interleukin (IL)-1 and IL-2. IL-1 was highly expressed in BD [Bilginer et al., 2010; Kôtter et al., 2005].

There are <1% $CD4^+$ and <0.5% $CD8^+$ IL-17^+ T cells in peripheral blood of healthy humans [Kryczek et al., 2007]. Peripheral blood of active BD patients exhibited high levels of Th17 cells in their peripheral circulation [Hamzaoui et al., 2002; Direskeneli et al.; 2003; Hamzaoui et al., 2011] and in cerebrospinal fluid [Hamzaoui et al., 2011]. High levels of Th17, $CD4^+$ IL-17^+ and $CD8^+$ IL-17^+ T cells are observed in BD inflammatory skin. How does this induction occur? In addition to migration from peripheral blood, IL-17^+ T cells may be induced within the BD lesions environment. It has been reported that myeloid cell-derived genes contribute to pathogenic manifestations in inflammatory diseases [Haider et al., 2008; Szegedi et al., 2003]. We demonstrate that myeloid APCs including macrophages and myeloid dendritic cells potently induce human IL-17^+ T cells. Our observation may also explain why IL-17^+ T cells are often found in inflammatory tissues and organs. In support of this concept, we show that myeloid APCs isolated from BD patients potently stimulate IL-17^+ T cells. Increased circulating IFN-γ [Szegedi et al.,2003] may activate circulating APCs, allowing them to enter tissue and promote expansion of IL-17^+ T cells. Th1 cells can suppress Th17 cell differentiation through IFN-γ [Kolls et al., 2004; Weaver et al., 2006; Teunissen et al., 1998].

IFN-γ triggers myeloid APCs to produce IL-1 and IL-23, and in turn induce IL-17^+ T cells [Kryczek et al 2008]. Our data therefore demonstrate the potential mode of interaction between Th1 and IL-17^+ T cells in BD skin lesions: IFN-γ derived from Th1 cells promotes IL-17^+ T cell development through IL-1 and IL-23 in the skin lesion environment in Behcet disease. Therefore, IFN-γ may possibly play dual roles in regulating the IL-17^+ T cell pool: IFN-γ targets APCs to initiate and promote Th17 polarization [Teunissen et al., 1998; Kolls et al., 2004; Szabo et al., 1998] to suppress Th17 polarization.

In summary, we show that IFN-γ may promote trafficking, induction, and function of IL-17^+ T cells in patients with BD. This study clarify the interaction between Th1 and Th17 cells,

challenges the view that Th1 cells suppress Th17 cell development, and suggests a collaborative contribution of Th1 and Th17 to autoimmune/inflammatory diseases. Ongoing research is addressing this aspect as well as attempting to define better whether $CD8^+$ IL-17^+ lymphocytes act as cytotoxic cells (Tc17 cells) which contribute to initiate, stabilize, or inhibit the BD process in the skin.

5. References

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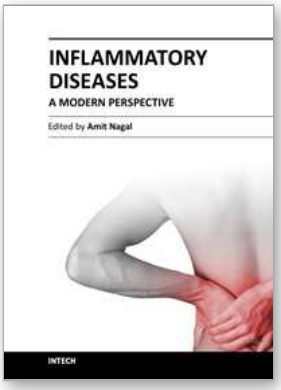

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"Inflammatory Diseases - A Modern Perspective" represents an extended and thoroughly revised collection of papers on inflammation. This book explores a wide range of topics relevant to inflammation and inflammatory diseases while its main objective is to help in understanding the molecular mechanism and a concrete review of inflammation. One of the interesting things about this book is its diversity in topics which include pharmacology, medicine, rational drug design, microbiology and biochemistry. Each topic focuses on inflammation and its related disease thus giving a unique platform which integrates all the useful information regarding inflammation.

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