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Impaired Peripheral Th1 CD4+ T Cell Response to *Escherichia coli* Proteins in Patients with Crohn's Disease and Ankylosing Spondylitis

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Abstract
Background To clarify the impact of T cell responses towards enteric antigens for chronic intestinal inflammation, we determined T helper 1 reactivity towards conserved *Escherichia coli* proteins in patients with Crohn's disease (CD) and healthy individuals and patients with ankylosing spondylitis (AS), who also often show microscopic inflammatory lesions within the gut or even develop overt inflammatory bowel disease.
Methods We determined the frequency of IFNγ+CD40L+ cells/CD4+ T cells after stimulation of whole blood with pools of *E. coli* proteins.
Results The *E. coli*-specific Th1 response was significantly reduced in CD patients and to a lower extent also in AS patients.
Conclusions *E. coli* is a target for polyclonal Th1 responses in healthy individuals. The impairment of these responses in CD and AS patients might be due to recruitment of enterobacteria-specific Th1 cells to the gut or might reflect inadequate priming of adaptive immune response.

Keywords Intestinal inflammation · Th1 response · *E. coli*

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Introduction

Inflammatory bowel disease (IBD) is a chronic disorder of the gastrointestinal tract. Crohn's disease (CD) and ulcerative colitis (UC) are the two prototypes of IBD. CD is characterized by granulomatous inflammation which can occur throughout the entire gastrointestinal tract and most often includes the terminal ileum [1]. In contrast, UC is characterized by chronic continuous inflammation in the colorectum without granuloma formation [2]. Apart from the intestinal inflammation, IBD patients often develop extraintestinal manifestations. For instance, up to 10% of IBD patients develop ankylosing spondylitis (AS) which is characterized by inflammatory lesions of the spine. On the other hand, endoscopic and microscopic studies showed that up to 60% of AS patients have subclinical, microscopic

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inflammation within the intestine and up to 10% progress to overt IBD suggesting an etiological linkage between IBD and AS [3–5]. This is also supported by genetic association studies: AS is strongly linked to the presence of HLA-B27, but besides that, strong association of AS was found with single nucleotide polymorphisms in *STAT3*, *IL-23R*, and *IL-12B* genes, which are also disease susceptibility genes of CD [6].

In addition to the genetic risk factors, environmental factors as well as a dysregulated mucosal immune response towards commensal bacteria are thought to contribute to the pathogenesis of IBD [7, 8]. In particular, T helper cell responses are instrumental for the development of disease as suggested by the severe combined immunodeficiency (SCID) transfer model [9, 10]. Animal models and data from humans indicate that the inflammatory process in CD is driven by an excessive Th1 response while UC is dominated by a Th2 response [11]. In addition, Th17 cells were shown to be involved in both diseases [12].

In animal models of IBD, such as the SCID transfer model, the interleukin (IL)-2 and IL-10 knockout models, the TCR-α-chain-deficient mouse, and the dextran sodium sulfate colitis model, inflammation is absent in germ-free rodents demonstrating that the normal enteric bacterial flora plays a crucial disease-driving role [7, 11, 13]. Also in CD patients, an association of enteric bacterial species, in particular *Escherichia coli*, and chronic intestinal inflammation was reported in several studies [14–19].

The isolation of peripheral and intestinal T cell clones from IBD patients with cross-reactivity towards antigens shared by different enteric bacterial species including *E. coli* suggests a pathogenic role of conserved antigens of intestinal bacteria in IBD [20]. Also in reactive arthritis triggered by the intestinal pathogen *Yersinia enterocolitica*, highly conserved antigens, such as the 50S ribosomal protein L23 and the heat-shock protein 60 were identified as immunodominant antigens [21, 22].

To elucidate the importance of *E. coli*-specific T cell responses in the pathogenesis of CD and AS which are both characterized by chronic intestinal inflammation, we performed in vitro stimulation of peripheral CD4+ T cells with recombinant *E. coli* proteins. These proteins included proteins which were selected according to their high phylogenetic conservation as described by Ergin et al. as well as *E. coli*-specific pathogenicity factors [23]. Responding *E. coli*-specific Th1 cells were identified by expression of the surface molecule CD40 ligand (CD40L) which is suitable for detection of antigen-specific CD4+ T cells and the Th1 cytokine IFNγ in patients with CD and AS in comparison with healthy individuals [24].

Materials and Methods

Patients and Healthy Controls

Heparinized whole blood was obtained from 39 CD patients, 27 AS patients, and 21 healthy individuals. The disease state of CD patients was determined by the Crohn's Disease Activity Index (CDAI) [25]. A CDAI of >150 was considered as active disease, and a CDAI ≤150 was considered as clinical remission. The disease state of AS patients was determined by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [26]. A BASDAI of ≥4 was considered as active disease, and a BASDAI <4 as inactive disease. The study was approved by the local ethics committee. The characteristics of CD and AS patients included in the study are given in Table I.

Bacterial Antigens Used for T Cell Stimulation

One hundred ninety-six highly conserved proteins of *E. coli* were expressed and purified as described previously in detail [23]. Briefly, after homologous expression, recombinant proteins were purified via affinity chromatography using Ni-NTA agarose (Qiagen, Hilden, Germany). Bacterial lipopolysaccharide was removed using magnetic beads coated with polymyxin B (Chemicell, Berlin, Germany). The proteins were adjusted to 500 μg/ml final concentration in PBS and pooled according to their molecular weight. Each protein pool contained 10 conserved proteins, except for pool 20 that contained only 6 proteins. Thus, 196 conserved proteins were included in 20 pools.

Furthermore, a set of 13 pathogenicity factors was purified as described [23]. Each pathogenicity factor was adjusted to 500 μg/ml final concentration in 0.1% acetic acid and tested on single protein level.

Stimulation of Peripheral CD4+ T Cells and Flow Cytometric Analysis

Heparinized whole blood (1 ml) was stimulated for 6 h at 37°C and 5% CO_2 using 12 ml cell culture tubes (Greiner Bio-one, Frickenhausen, Germany) in the presence of 1 μg/ml anti-CD28 antibody (BD Pharmingen, Heidelberg, Germany) with or without the following antigens: 5 μg/ml of *E. coli* proteins either in pools or as single proteins, 10 μg/ml of *Staphylococcus aureus* enterotoxin B (SEB; Sigma, Deisenhofen, Germany), or 4 μg/ml cytomegalovirus pp65 recombinant protein (CMV-pp65; Miltenyi, Bergisch Gladbach, Germany) or 20 Limes flocculation (Lf)/ml tetanus toxoid (Chiron-Behring, Marburg, Germany). To prevent cytokine secretion, 10 μg/ml brefeldin A (Sigma-Aldrich, Deisenhofen,

Table I Patient characteristics

	CD (n=39)	AS (n=27)
Age, mean (min–max)	46.1 (19–70)	41.7 (25–63)
Gender	18 males/21 females	17 males/10 females
Disease duration, mean (min–max)	16.9 years (<1–43)	10.1 years (<1–34)
Disease location: ileal/ileocecal	17	n.a.
Disease location: ileal + colonic	7	n.a.
Disease location: colonic	15	n.a.
Surgery: ileal/ileocecal	12	n.a.
Surgery: colon	8	n.a.
Disease activity	8 active disease	13 active disease (BASDAI>4)
	9 drug-free remission	6 remission (BASDAI<4)
	22 drug-dependent remission	8 n.d.
Medication	15× azathioprine (50–200 mg/day), 2× Purinethol (50 mg/day), 2 infliximab 1× MTX (15 mg/day), 8× systemic prednisolone (5–40 mg/day), mesalazine (0.5–4 g/day)	2 sulfasalazine (3 g/day), 1 MTX (10 mg), 9 NSAIDs, 1× morphine, 1 novalminsulfone

n.a. not applicable, *n.d.* not done

Germany) was added for the last 4 h of stimulation. Erythrocytes were lysed and leukocytes were fixed and permeabilized using the FACS Permeabilizing Solution 2 (BD Pharmingen).

The following antibodies were used for further cytometric analysis (all from BD Pharmingen): anti-CD4-peridinin chlorophyll A protein (anti-CD4-PerCP, clone SK3), anti-CD40L-phycoerythrin (anti-CD40L-PE, clone TRAP), anti-IFNγ-Allophycocyanin (anti-IFNγ-APC, clone B27), and anti-CD40L-APC (clone TRAP1). Anti-Vβ17-PE (clone ET185) was purchased from Immunotech (Hamburg, Germany). All antibody stainings were performed in the presence of 1 mg/ml Beriglobin (Chiron-Behring) in order to avoid non-specific binding of antibodies to Fc receptors.

CD4+ T cells (2×10^5) per sample were analyzed using a FACSCalibur flow cytometer and the Cell Quest Pro software (both from BD Pharmingen). Antigen-specific CD4+ Th1 cells were quantified as the frequency of IFNγ+ CD40L+ cells among CD4+ T cells. In a subgroup of patients, the percentage of Vβ17+CD4+ T cells and CD40L+Vβ17+ CD4+ cells was assessed by flow cytometry.

Statistical Analysis

Statistical analysis was performed with the GraphPad Prism version 4 (GraphPad Software, San Diego, USA). We used the parametric-free Mann–Whitney test. Differences were considered as significant at $p<0.05$.

Results

Stimulation of Peripheral CD4+ T Cells with Protein Pools of *E. coli*

To analyze the Th1 responsiveness towards *E. coli*, whole blood of CD and AS patients as well as of healthy individuals was stimulated with recombinant *E. coli* proteins. The frequency of CD40L+IFNγ+ cells among CD4+ T cells was determined as shown in Fig. 1a. Stimulation for 6 h with the addition of brefeldin A for the last 4 h was tested to be the best time frame to detect the *E. coli*-specific Th1 response (Fig. 1b). In Fig. 2, results of the stimulation of whole blood of 39 CD patients, 20 AS patients, and 21 healthy individuals with pools 1–20 are shown. None of the protein pools elicited a Th1 response exclusively in patients with CD or AS. Rather, CD patients showed a significantly reduced Th1 response to most (18 out of 20) of the *E. coli* protein pools (Fig. 2). In 10 out of 20 pools, we also observed a reduced Th1 response towards *E. coli* protein pools in AS patients (Fig. 2).

To look whether disease activity contributes to the reduced Th1 responsiveness, we subanalyzed the data for CD patients with active disease. Pools 4, 9, 10, 13, 14, and 18 were selected for this subanalysis since stimulation with these pools elicited a strong response in healthy individuals (mean % CD40L+IFNγ+ cells/CD4+ T cells>0.03) and a strongly reduced response in CD patients ($p<0.01$) according to Fig. 2. As shown in Fig. 3, patients with active disease also

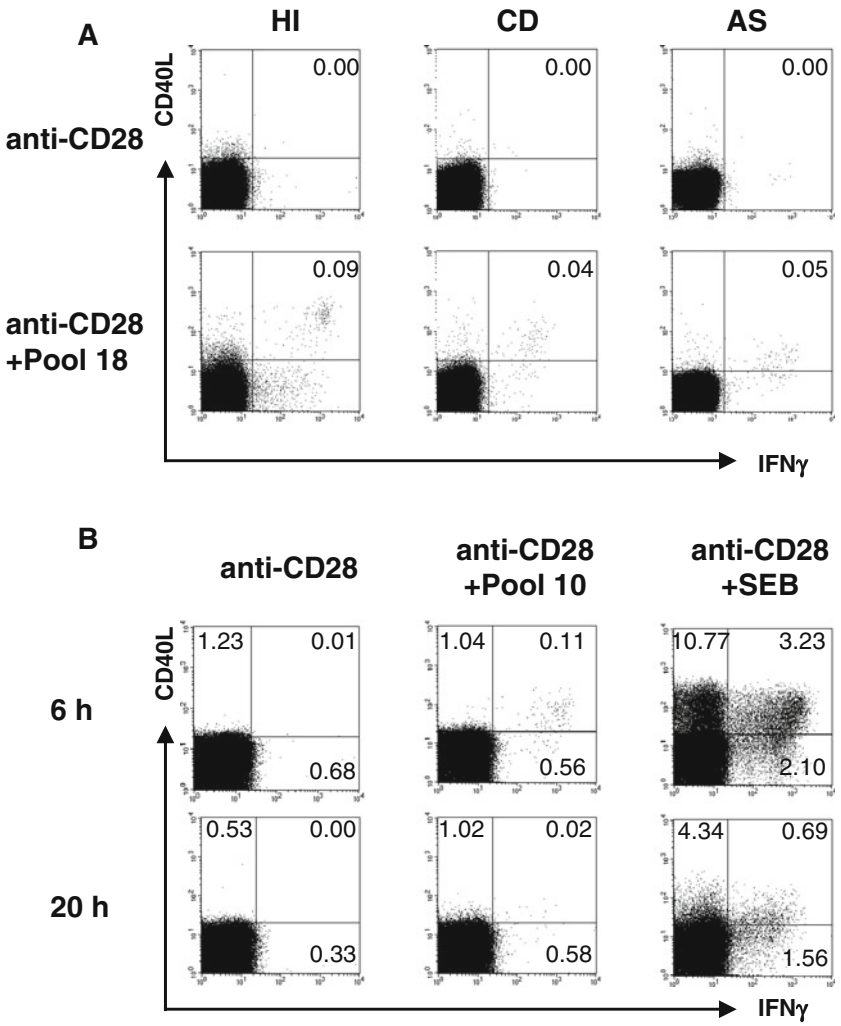


Fig. 1 Th1 response towards protein pools of *E. coli*. **a** Representative examples of IFNγ and CD40L staining of CD4+ T cells after stimulation of whole blood of healthy individuals (*HI*), Crohn's disease (*CD*), and ankylosing spondylitis (*AS*) for 6 h with anti-CD28 and anti-CD28 + *E. coli* protein pool 18 are shown. **b** One representative example out of four experiments of whole blood stimulation of healthy individuals for 6 and 20 h with anti-CD28, anti-CD28 + *E. coli* pool 10, and anti-CD28 + SEB. The percentage of CD40L+IFNγ+ was determined by intracellular staining. Brefeldin A was added for the last 4 h of stimulation. *Numbers* indicate the percentage of positive cells per quadrant

showed a reduced Th1 response to all of these pools tested. Due to the low number of patients with active disease (n=8 tested for pools 4, 9, and 10; n=4 tested for pools 13, 14, and 18), the difference was statistically significant only for pool 9. To test whether immunosuppression could contribute to this impairment, we analyzed the Th1 responsiveness of patients that were in drug-free remission. Th1 reactivity towards the abovementioned pools was also reduced in these patients, and again the numbers of patients in this subgroup were rather low (n=9 tested for pools 4, 9, and 10; n=5 tested for pools 13, 14, and 18; Fig. 3).

Stimulation of Peripheral CD4+ T Cells with Pathogenicity Factors of *E. coli*

In addition to the conserved *E. coli* protein pools, we tested T cell reactivity towards 13 pathogenicity factors. We observed again a reduced Th1 responsiveness in CD patients (Fig. 4). The Th1 response of AS patients to the pathogenicity factors was not significantly reduced.

Identification of Individual *E. coli* Proteins Eliciting Th1 Cell Responses

As indicated in Fig. 2, the strength of the Th1 response to the protein pools differed. A strong response (mean>0.03% CD40L+IFNγ+ T cells/CD4+ T cells) was found in healthy donors after stimulation with pools 4, 9, 10, 13, 14, 16, 17, 18, and 20. Of these nine pools, five pools (9, 13, 17, 18, and 20) were selected. The 50 single proteins contained within these pools were used to stimulate the whole blood of 2 CD patients, 2 AS patients, and 1 healthy individual (HI) who had strongly (>0.03% CD40L+IFNγ+ T cells/CD4+ T cells) responded to these protein pools before. Nine of the 50 tested proteins elicited a strong Th1 response (>0.03% CD40L+IFNγ+ cells/CD4+ T cells) in each of the tested individuals. The names of these single proteins and their distribution in the pools are listed in Table II. To test the Th1 response of CD and AS patients to these single proteins, we stimulated the whole blood of other patients with CD and AS (10 each) and 12 HI with these selected

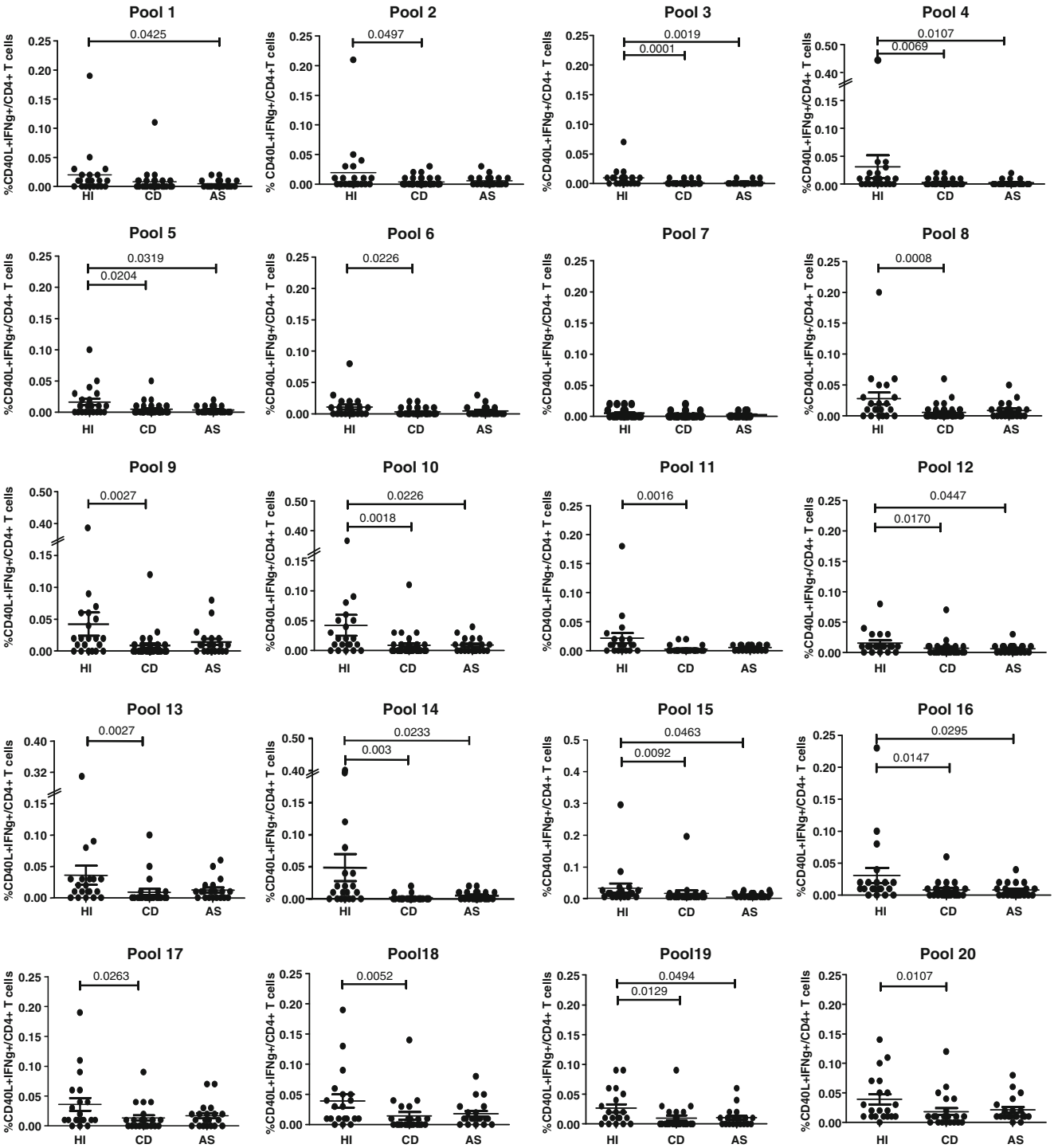


Fig. 2 Th1 response towards 20 protein pools of *E. coli*. The frequency of CD40L+IFNγ+ cells/CD4+ T cells (minus background levels of anti-CD28 stimulation) after stimulation of whole blood of 39 CD patients, 20 AS patients, and 21 healthy individuals with anti-CD28 + 20 different pools of *E. coli* proteins is shown. The *p* value of significant differences (Mann–Whitney *U* test) is given

conserved proteins. The Th1 response of CD patients to seven of these nine tested single proteins was reduced in comparison to HI (Fig. 5). Also AS patients showed reduced responses to six of the nine tested proteins (Fig. 5).

Altogether, CD and AS patients showed an impaired Th1 cell reactivity towards conserved *E. coli* proteins. Th1 responsiveness towards pathogenicity factors of *E. coli* was selectively reduced in CD patients.

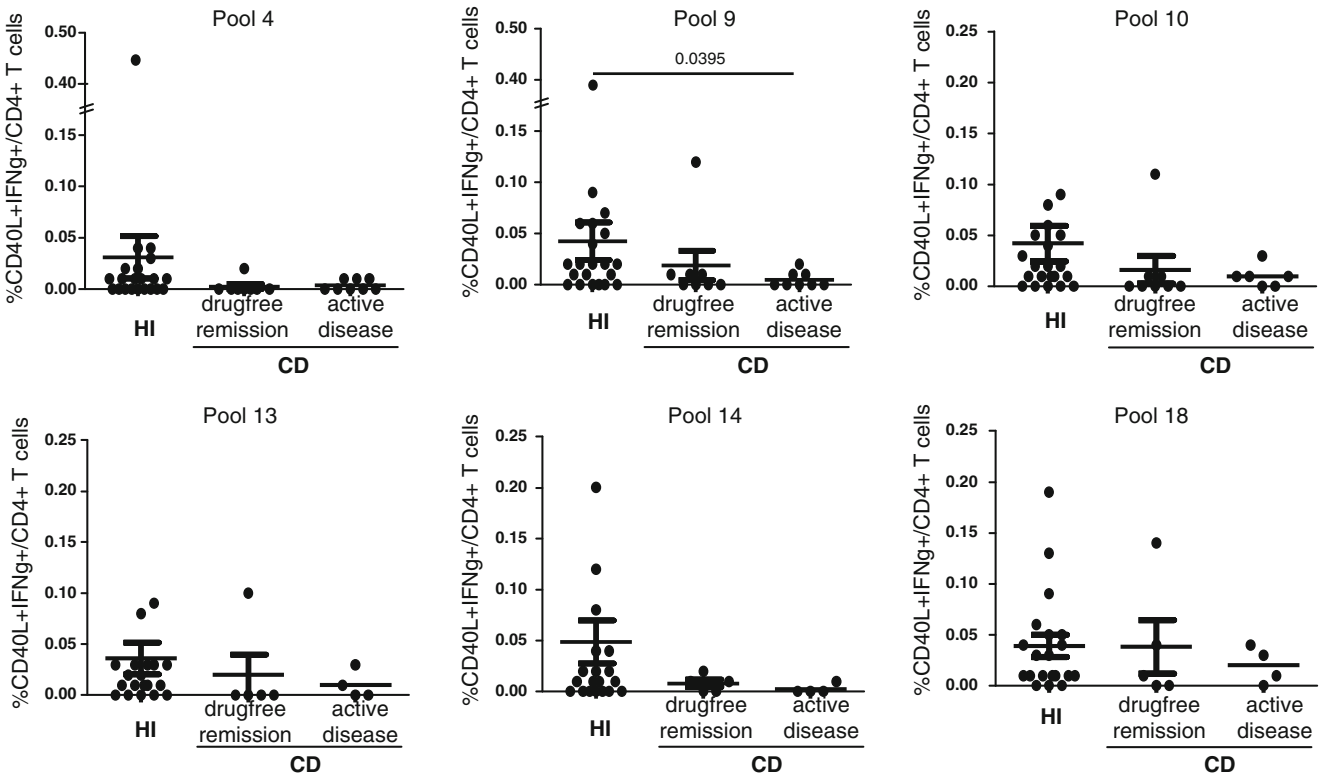


Fig. 3 Impact of immunosuppression and disease activity on the *E. coli*-specific Th1 response. The frequency of CD40L+IFNγ+ cells/CD4+ T cells (minus background levels of anti-CD28 stimulation) after stimulation of whole blood of CD patients with active disease or drug-free remission and healthy individuals with anti-CD28 + the indicated pools of *E. coli* proteins is shown. The *p* value of significant differences (Mann–Whitney *U* test) is given

Stimulation of Peripheral CD4+ T Cells with SEB

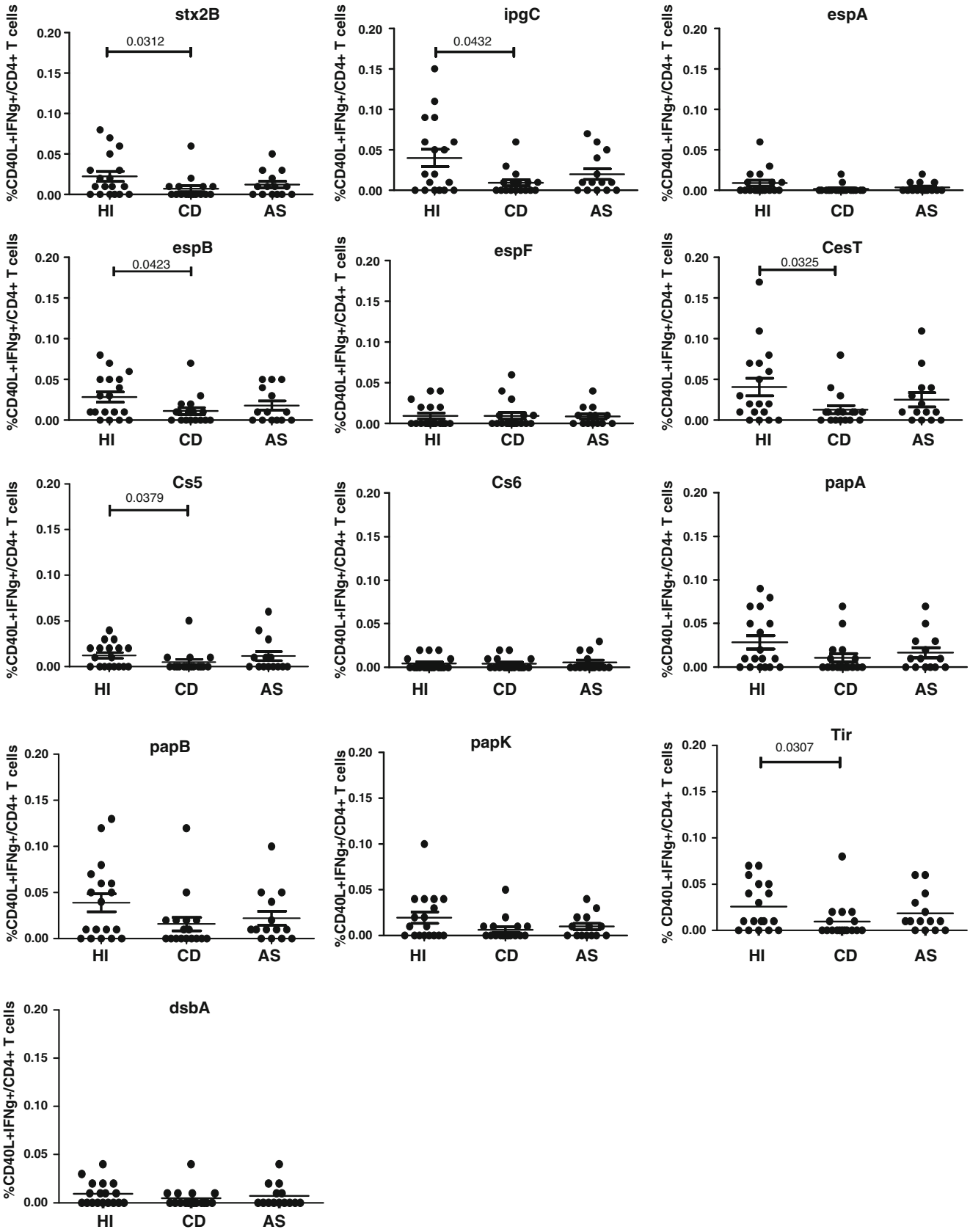
To analyze whether the impaired *E. coli*-specific T cell response in CD patients reflects a general impairment of Th1 cell responsiveness, we stimulated peripheral CD4+ T cells of HI, CD, and AS patients with SEB. SEB is a superantigen that bypasses antigen presentation and activates T cells by cross-linking Vβ chains of the T cell receptor with MHC II [27, 28].

Interestingly, also superantigen-dependent Th1 cell responses were significantly decreased in patients with CD and AS compared with HI (Fig. 6a). Again, immunosuppression of CD patients did not seem to affect reactivity. The median frequencies (and range) of IFNγ+CD40L+ cells/CD4+ T cells in CD patients with immunosuppression, patients with mesalazin treatment, and patients in drug-free remission were 2.12% (0.37–5.69%), 2.25 (0.46–6.42%), and 1.59% (0.26–7.07%), respectively.

SEB preferentially stimulates Vβ17-positive cells among CD4+ T cells. To investigate if the decreased SEB-induced Th1 response in patients with CD and AS is due to numeric deficiencies of this CD4+ T cell subset, we assessed the frequencies of Vβ17+ cells among CD4+ T cells but found no differences between HI and CD and AS patients. The frequencies (median, range) of Vβ17+CD4+ T cells were 5.46% (4.06–7.07%) in HI, 5.66% (4.37–6.46%) in CD patients, and 5.33% (4.79–6.27%) in AS patients, respectively.

In addition, we analyzed the frequencies of activated CD40L+ cells among Vβ17+CD4+ T cells upon stimulation with SEB. Patients with CD showed also significantly decreased frequencies of activated CD40L+ cells among Vβ17+CD4+ T cells when compared with HI (Fig. 6b), confirming the clearly impaired SEB-dependent T cell response in CD patients. In AS patients, the percentage of CD40L+ cells/Vβ17+CD4+ T cells was not significantly reduced in comparison to HI.

Fig. 4 Th1 cell responses towards pathogenicity factors of *E. coli*. ▶ Whole blood of 18 healthy individuals (*HI*), 17 Crohn's disease (*CD*) patients, and 14 ankylosing spondylitis (*AS*) patients was stimulated with *E. coli* pathogenicity factors. The percentage of CD40L+IFNγ+ cells/CD4+ T cells (minus background levels elicited by anti-CD28) for each group is given for individual pathogenicity factors. The *p* value of significant differences (Mann–Whitney *U* test) is given


stx2B
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espB
0.0423
espF
CesT
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Cs5
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Cs6
papA
papB
papK
Tir
0.0307
dsbA
%CD40L+IFNg+/CD4+ T cells
% CD40L+IFNg+/CD4+ T cells
0.20
0.15
0.10
0.05
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HI
CD
AS

Table II Names of single immunogenic *E. coli* proteins and their distribution within pools

#	Protein	Pool
1	NAD-linked malate dehydrogenase	13
2	Phosphoribosylaminoimidazole synthetase; AIR synthetase	9
3	Predicted carbohydrate kinase	18
4	Putative control proteins (lipoprotein NlpI)	20
5	RecA	9
6	30S ribosomal subunit protein S13	18
7	50S ribosomal subunit protein L14	18
8	50S ribosomal subunit protein L2	17
9	Ffh (signal recognition particle (SRP) component with 4.5S RNA)	17

Whole blood of one healthy individual and two patients each either with Crohn's disease or ankylosing spondylitis classified as strong responder towards protein pools 9, 13, 17, 18, and 20 was re-stimulated with single conserved proteins contained in the respective pools. Proteins #1–9 elicited Th1 cell responses in each of these individuals. The table shows the protein name and number of the pool that contained the protein

Stimulation of Peripheral CD4+ T Cells with Recall Antigens CMV-pp65 and Tetanus Toxiod

To analyze whether CD and AS patients have also impaired Th1 responses to recall antigens, we measured Th1 responses upon stimulation with CMV-pp65 and tetanus toxoid (TT).

CMV-pp65 is an immunodominant target of CD4+ T cell responses towards CMV [29]. The Th1 response to CMV was unimpaired in CD patients while AS patients showed a reduced response (Fig. 7a). The T cell response towards TT was determined by measuring the entire population of activated T cells, i.e., the percentage of CD40L+ cells/CD4+ T cells since TT stimulation of CD4+ T cells elicits production of several cytokines including IL-2, tumor necrosis factor-β (TNFβ), and IFNγ [30]. No significant difference in the percentage of CD40L+ cells/CD4+ T cells elicited by TT stimulation was found in CD and AS patients compared to HI (Fig. 7b).

Discussion

Commensal bacteria are crucial for disease development in most animal models of chronic colitis [7, 11, 13]. Also in IBD patients, an association between enteric bacterial species, in particular *E. coli*, and chronic intestinal inflammation has been suggested by several studies [14–20]. However, it is unclear whether T cell responses towards intestinal bacteria contribute to the excessive and tissue-damaging inflammation characteristic for IBD.

Since Th1 cytokine production is thought to drive inflammation in Crohn's disease [11], we analyzed Th1 reactivity towards conserved proteins and pathogenicity factors of *E. coli* in the blood of HI and patients with CD or AS. By analyzing the frequency of IFNγ+CD40L+ cells among CD4+ T cells after stimulation with highly conserved *E. coli* proteins, we detected a Th1 response in healthy individuals to most of the protein pools. This indicates that *E. coli* is a target of the CD4+ and particularly Th1 immune response even in healthy individuals. We identified nine conserved proteins and several pathogenicity factors that were most immunogenic, but not one single immunogenic protein, suggesting that T cell responses towards conserved *E. coli* antigens are polyclonal.

None of the *E. coli* proteins or pathogenicity factors elicited Th1 responses selectively in patients with CD or AS. Instead and surprisingly, we observed a significantly reduced Th1 responsiveness towards conserved *E. coli* proteins in the peripheral blood of patients with CD or AS compared to healthy individuals. The subanalysis of CD patients with active disease and patients in drug-free remission showed reduced Th1 responses in both groups. This suggests that an impaired *E. coli*-specific Th1 response is a characteristics of CD patients which is not dependent on disease activity and not caused by the immunosuppression. This is also supported by the fact that AS patients included in this study did not receive immunosuppression but showed impaired *E. coli*-specific Th1 responses.

E. coli is one of over 400 species of microbes colonizing the human ileum and colon. If the *E. coli*-specific response reflects Th cell responses towards other commensal enterobacteria, the global Th1 cytokine production should be affected in CD and AS patients. In fact, decreased frequencies of IFNγ- and TNFα-producing CD4+ T cells upon polyclonal stimulation have been reported before both in the blood of AS and CD patients [31, 32]. Furthermore, reduced ex vivo IFNγ messenger RNA expression in peripheral blood cells was found in CD and UC patients [33]. In line with these studies, we also found a reduction of the frequency of IFNγ+CD40L+ cells among CD4+ T cells after SEB stimulation by about 50% in CD and AS patients compared to HI. As we found unimpaired recall responses towards TT in CD and AS patients and in CD patients also to CMV, the impairment of the polyclonal Th1 response seems to reflect a selective impairment of Th1 responses towards distinct bacterial antigens or to antigens encountered within distinct sites like the intestinal mucosa in CD and AS patients. Further own preliminary data regarding Th responses towards fungal antigens such as *Candida albicans* antigens showing no impairment of this Th1 response in CD patients support the view of germ-specific alterations of the Th response in CD patients (Syrbe and Scheffold, unpublished data).

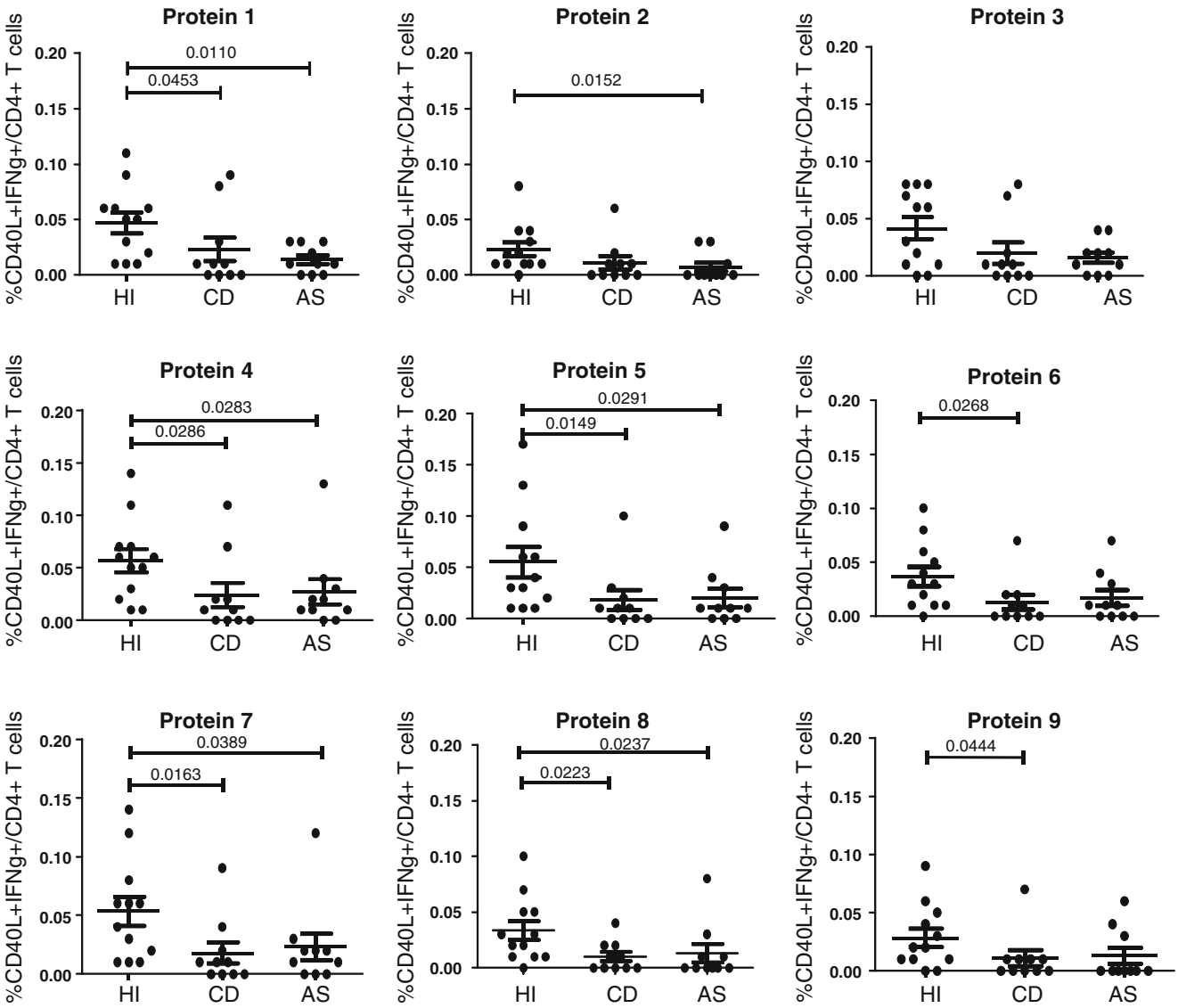

Fig. 5 Th1 cell responses towards nine selected conserved proteins of *E. coli*. Whole blood of 12 healthy individuals (*HI*) and of 10 patients each with either Crohn's disease (*CD*) or ankylosing spondylitis (*AS*) was stimulated with individual conserved proteins #1–9 according to Table II. The percentage of CD40L+IFNγ+ cells/CD4+ T cells (minus background levels elicited by anti-CD28) per group is given for individual proteins. The *p* value of significant differences (Mann–Whitney *U* test) is given

The impairment of peripheral Th1 responses in CD and AS patients could result from a disturbed T cell priming resulting in compensatory enhanced induction of other Th phenotypes. We started to address this question in CD patients by using an enrichment method of activated T cells and subsequent analysis of rare cytokine-producing T cells. Preliminary data confirmed the impairment of IFNγ production after stimulation with *E. coli* antigens and preclude a compensatory enhancement of IL-17 or IL-22 production in CD patients (Syrbe and Scheffold, unpublished data).

Furthermore, deprivation of effector T cells from the blood into acute inflamed sites could also result in reduced numbers of circulating effector T cells. In fact, several studies reported an accumulation of IFNγ-producing T cells in the *lamina propria* of the inflamed intestinal mucosa of CD patients [34, 35]. However, if this is indeed a major factor, one would expect an impact of the disease activity.

On the other hand, there are studies showing an impaired innate immune response to *E. coli* in patients with CD [36, 37]. Subcutaneous injection of heat-inactivated *E. coli* elicited a much lower acute local inflammation as measured by blood flow and leukocyte immigration in CD patients than in healthy individuals. Moreover, bacterial clearance was delayed in CD patients. Further studies suggested that a

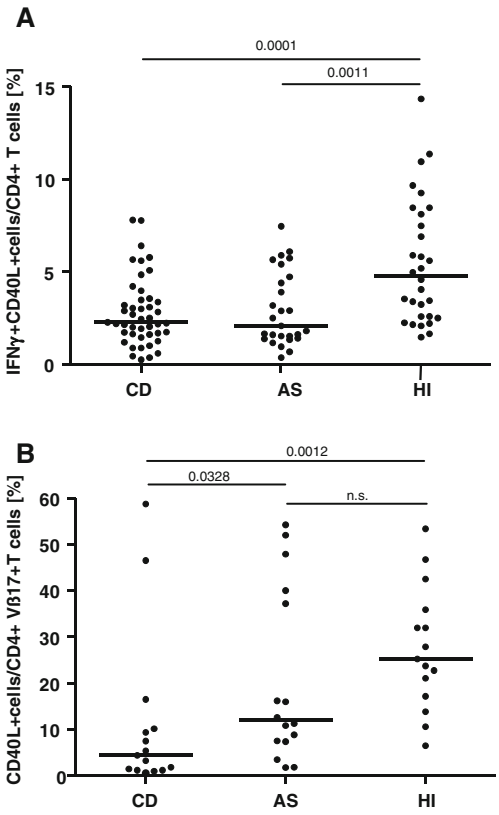

Fig. 6 CD4+ T cell responses towards SEB. **a** Whole blood of 30 healthy individuals (*HI*), 45 patients with Crohn's disease (*CD*), and 27 patients with ankylosing spondylitis (*AS*) was stimulated with SEB, and the frequency of IFNγ+CD40L+ cells/CD4+ T cells was quantified. Individual measurements and the median are shown. For calculations of the *p* values, the Mann–Whitney test was used. **b** Whole blood of 15 HI, 15 CD patients, and 16 AS patients was stimulated with SEB and the frequency of CD40L+ cells among CD4+Vβ17+ T cells was quantified. Individual measurements and the median are shown. For calculations of the *p* values, the Mann–Whitney *U* test was used

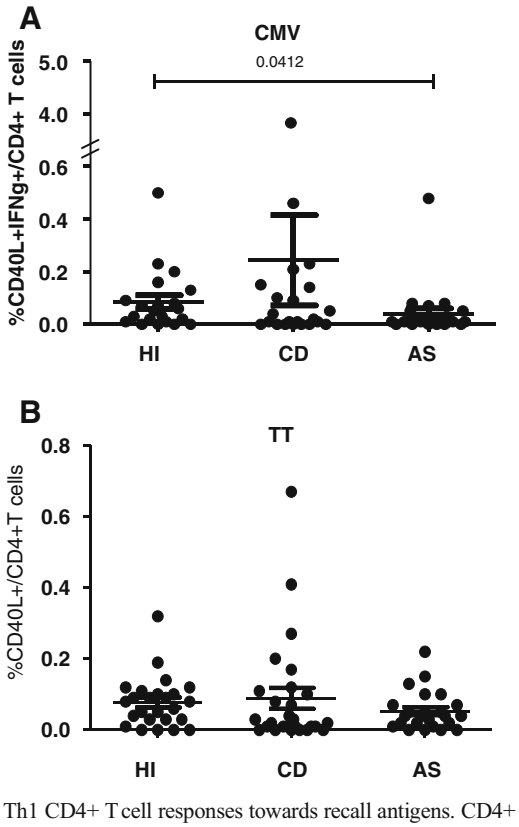

Fig. 7 Th1 CD4+ T cell responses towards recall antigens. CD4+ T cell response towards CMV-pp65 protein (**a**) and tetanus toxoid (*TT*; **b**) was analyzed in 25 healthy individuals (*HI*), 27 Crohn's disease (*CD*) patients, and 24 ankylosing spondylitis (*AS*) patients. **a** The frequency of IFNγ+CD40L+/CD4+ T cells (minus background levels elicited by anti-CD28) to CMV-pp65A stimulation was determined for HI, AS, and CD patients. The *p* value of significant differences (Mann–Whitney *U* test) is given. **b** The frequency of IFNγ+CD40L+/CD4+ T cells (minus background levels elicited by anti-CD28) to TT stimulation was determined for HI, AS, and CD patients. The *p* value of significant differences (Mann–Whitney *U* test) is given

disordered macrophage stimulation causes the impaired acute inflammation and bacterial clearance in Crohn's disease [37].

In addition to these experimental data, also whole genome association studies showed an association between CD and genes of innate immune functions. Thus, CD is strongly associated with risk variants in the genes nucleotide-binding oligomerization domain-containing-2 (*NOD2*), autophagy-related protein 16L1 (*ATG16L1*), and immunity-related GTPase family, M (*IRGM*) [38]. *NOD2* is an intracellular sensor of bacterial muramyldipeptide and ligation of *NOD2* triggers autophagy and MHC II antigen presentation in dendritic cells. CD patients expressing the CD risk variants of *NOD2* were deficient in autophagy induction and T cell stimulation [39]. Similar effects were observed in patients expressing the Crohn-specific risk variants of the gene *ATG16L1* [39]. Impaired autophagy induction was associated with defective handling and elimination of intracellular bacteria. Such impaired innate immune function could also result in reduced or inefficient Th cell priming. The broad impairment of the *E. coli*-specific Th1 response observed in this study suggests, however, that it is independent from the disease-linked NOD2 gene variants which are only found in about one fourth of the CD patients [40]. Thus, impaired systemic *E. coli*-specific Th1 responses might be a common result of different pathogenic conditions or secondary to the chronic intestinal inflammation. Similar to that, impaired CARD15 signaling was found in CD patients without disease-linked variants [41]. Furthermore, NOD2-independent defects in innate functions have been found, as GM-CSF production by PBMNCs of CD patients was not only reduced after stimulation by NOD-ligand but also after stimulation by TLR4 agonist lipopolysaccharide [42].

AS is most strongly associated with the presence of *HLA-B27*, but the role of this molecule for the development of the disease is controversial [43]. Interestingly, HLA-B27 has been shown to affect antibacterial responses. For

instance, survival of *Salmonella* was promoted in HLA-B27-transfected macrophages [44], suggesting similar deficiencies in intracellular bacterial handling as observed in cells expressing CD-associated risk variants of the *NOD2* or *ATG16L1* gene. Furthermore, impaired antigen presentation has been described in a HLA-B27 transgenic rat model [45]. Interestingly, AS patients showed impaired responses towards the conserved proteins but not (or much less) towards the pathogenicity factors. Whether this differential responsiveness might reflect disease-specific exposure to particular *E. coli* strains, such as pathogenic vs. non-pathogenic, i.e., commensal *E. coli* or preferential exposure to adherent-invasive *E. coli* strains in Crohn's disease, which express pathogenicity factors different from those used here for T cell stimulation, is currently unclear. Further studies are required that link genetic background, local gut microbiota, and functional data to delineate the causes of impaired *E. coli*-specific T cell responses in CD and in AS patients, which will help understand the pathogenesis of these diseases.

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