Expression of IL-17RA in Innate Cells of Patients with Common Variable Immunodeficiency (CVID) and its Clinical Implications

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ABSTRACT

Background: Common Variable Immunodeficiency (CVID) is a primary immunodeficiency disorder characterized by B-cell dysfunction and immunoglobulin production deficiency. Dysregulation of interleukin-17 (IL-17) and its receptor IL-17RA have been reported in various immune disorders. This study aimed to investigate the expression of IL-17RA in innate immune cells of CVID patients and its correlation with clinical manifestations.

Methods: A cross-sectional study included 22 CVID patients and 14 age- and sex-matched healthy controls. IL-17RA expression was assessed in various immune cell subsets using flow cytometry. Demographic and clinical data were collected, and statistical analysis was performed.

Results: CVID patients had elevated IL-17RA expression in neutrophils, non-classical monocytes, and dendritic cells compared to healthy controls. Patients with a history of intestinal microbial colonization, particularly with *Campylobacter jejuni* and *Giardia intestinalis*, showed significantly higher IL-17RA expression in innate cells. Elevated IL-17RA expression in monocytes and dendritic cells also correlated with higher fecal calprotectin levels in CVID patients, regardless of microbial colonization.

Conclusions: The study suggests that despite previous reports of reduced circulating Th17 cells and IL-17 levels in CVID patients, IL-17RA expression in innate cells may be elevated, potentially indicating altered IL-17 signaling. This heightened IL-17RA expression could contribute to a persistent pro-inflammatory state, possibly due to microbial translocation or other inflammatory factors. The association of IL-17RA expression with gastrointestinal microbial colonization and its correlation with fecal calprotectin underscores the complexity of IL-17RA's role in CVID pathophysiology. Further research in larger cohorts could elucidate the implications of IL-17RA expression in both infectious and non-infectious inflammatory aspects of CVID.

Keywords: CVID, primary immunodeficiency, interleukin-17, inflammation, innate immunity

Impact Statement

Despite low circulating IL-17 levels, CVID patients appear to have heightened IL-17RA expression in innate immune cells. This altered IL-17 signaling may sustain a pro-inflammatory state, influenced by microbial colonization.

Introduction:

Common Variable Immunodeficiency (CVID) is the most common symptomatic inborn error of immunity (1). It is mainly characterized by failure of B-cell differentiation and decreased production of immunoglobulins (Igs). This leads to a predominantly humoral immunodeficiency, despite also being associated with cell-mediated deficiencies (2). Aside from B-cell dysfunction, other immunological abnormalities, such as T-cell dysfunction, monocyte/macrophage hyperactivity and abnormal cytokine production, with subsequent inflammatory dysregulation, are observed in many patients (3). This wide presentation of immunological defects reflects CVID's heterogeneous genetic abnormalities and it leads to diverse clinical manifestations – recurrent sinopulmonary infections, autoimmune disorders, granulomatous diseases, enhanced risk of malignancy, and impaired antibody response (4).

The interleukin-17 (IL-17) family comprises a group of six pro-inflammatory cytokines (from the main cytokine IL-17A, also known as IL-17, through IL-17F) produced mainly by Th17 lymphocytes, but also by $CD8^+$ T-cells, γδ T-cells, and various innate immune cell populations (5–7). IL-17 signals through the IL-17 receptor A (IL-17RA, CD217) and IL-17RC subunits. IL-17F, the most closely related family member, also binds this receptor complex [7]. Whereas IL-17RA is ubiquitously expressed (with particularly high expression by innate immune cells such as macrophages and dendritic cells), lower IL-17RC expression limits IL-17 signaling in non-hematopoietic epithelial and mesenchymal cells (9).

The physiologic expression of IL-17 has a significant impact in innate immunity, playing a role in responses against extracellular bacteria, fungi and parasites by directly recruiting monocytes and indirectly influencing neutrophils, mainly through interaction with epithelial cells (6,10–12). It is also important for the barrier function of the skin and of the gut, by maintaining the tight junctions of the intestinal epithelium, stimulating tissue regeneration and upregulating antimicrobial proteins, such as β-defensins and calprotectin to control infections (13). By contrast, IL-17 chronic overactivity may elicit pathological responses, with Th17 cells having a major role in both cancer and autoimmune diseases (e.g. psoriasis, rheumatoid arthritis or autoimmune encephalitis) (14).

Defects in the IL-17 pathway have been described for inborn errors of immunity (15). Studies in patients with CVID have demonstrated a severe reduction of circulating Th17 and innate lymphoid cells, with a similar trend for serum levels of IL-17 (3,16). No relation was found between this decrease and autoimmune disorders, which occur frequently in CVID patients (3,16,17). However, the expression of IL-17RA, a receptor that is crucial for IL-17 signaling and innate immunity regulation, is largely unknown in the innate immune cells of CVID patients.

Therefore, we examined the expression of IL-17RA in the circulating innate immune cells of CVID patients and looked to determine how it correlated with the clinical manifestations of the disease.

Materials and methods:

Study design and subject recruitment

A cross-sectional study was conducted at the Allergy and Clinical Immunology Unit of Coimbra University Hospital, Portugal. A total of 22 patients with a clinical diagnosis of CVID, according to the diagnostic criteria of the European Society for Immunodeficiencies (ESID) Registry Working Party, were consecutively enrolled from 2018 to 2022 and results were compared with 14 age and sex-matched healthy controls (absence of infectious disease in the previous three months, as well as neoplastic, autoimmune, or lymphoproliferative disease) (18).

IL-17RA expression analysis

The expression of IL-17RA (CD217 antigen) was assessed by flow cytometry in myeloid dendritic cells (mDC), plasmacytoid dendritic cells (pDC), three subsets of monocytes (classical, intermediate and non-classical), and neutrophils. The following antibodies (and respective clones) were used: CD16 FITC (CLB/FcGRAN1), CD278 PE (C398.4A), CD3 PerCPCy5.5 (SK7), HLA-DR PE-Cy7 (L243), CD8 APC-H7 (SK1), CD45 V500c (2D1), CD217 APC (W15177 A), and CD14 APC-H7 (M Ø P9). Gating strategy for monocytes and dendritic cells is represented in Figure 1.

Data collection

Demographic and clinical data were collected from CVID patients through review of clinical records. Demographic variables included: sex, age at CVID diagnosis and age at cytometry flow analysis. Clinical variables included the following: phenotypes (updated 2012 criteria proposed by Chapel *et al.* – autoimmune cytopenia, polyclonal lymphocytic infiltration and unexplained enteropathy), history of malignancy, asthma/COPD, chronic rhinosinusitis, pneumonia, chronic diarrhea; hepatomegaly, splenomegaly and/or bronchiectasis; infectious colonization and isolated microorganisms; IgG through levels' measurements; and blood cell count, IgG and maximum fecal calprotectin measurements up to the time of cytometry analysis (19). Infectious colonization was defined as the analysis of microorganisms (parasites, bacteria and fungi) in the respiratory and gastrointestinal tracts – particularly, sputum and stool cultures, as well as antigen measurement in stool samples. Patients with identification of microorganisms in these samples were defined as colonized. Collected information was then analyzed with the flow cytometry data.

Statistical analysis

All statistical analyses were done using STATA software (version 16.1, StataCorp LLC, Texas, USA). Categorical data was described as a proportion and continuous data either as mean or median, depending on normality. P-values of <0.05 for correlations were considered statistically significant. Chi-squared or Exact Fisher tests were used for associations between categorical variables. T-test or Mann-Whitney U statistical analysis was also conducted, with the test choice depending on observation of normality. After univariate analysis, a multivariate linear regression model was built in order to assess the impact of intestinal tract colonization in the association between mean fecal calprotectin levels and CD217 cell expression.

Results:

Demographic characteristics

CVID patients (n=22) had a median age at the time of flow cytometry analysis of 48 years (*IQR 44-56*). Ten patients were male (45.5%). The median age of CVID diagnosis was 35 years (*IQR*

28-45). Healthy controls (n=14) were age- and sex-matched and, therefore, not significantly different from patients (Table 1).

Clinical characteristics of CVID patients

Regarding Chapel phenotypes, 11 (50%) patients presented with autoimmune cytopenia, 10 (45.5%) with polyclonal lymphocytic infiltration and 6 (27.3%) with chronic enteropathy (Table 2). Four patients (18.2%) had no disease-related complications, and 7 (31.8%) presented with more than one phenotype. Imaging studies showed hepatomegaly in 5 (22.7%) patients, splenomegaly in 9 (40.9%) and bronchiectasis in 14 (63.6%). Asthma/COPD was present in 7 patients and chronic rhinosinusitis in 18 (81.8%). A past history of pneumonia was registered in 13 patients (59.1%), chronic diarrhea in 12 (54.5%) and malignancy in 3 (13.6% - gastric, colorectal and thyroid cancer).

Fourteen patients had a history of microbial colonization: 11 in the respiratory airways and the same number in the gastrointestinal tract. The microorganisms isolated in sputum were *H. influenzae* (n=10), *P. aeruginosa* (n=2), *M. catarrhalis* (n=2), *S. pneumoniae* (n=2), *S. aureus* (n=1), and *Aspergillus niger* (n=1). In the gastrointestinal tract, the isolated bacteria were *H.pylori* (n=10), *C. jejuni* (n=7) and *S. enterica* (n=1), as well as the parasite *Giardia intestinalis* in 4 patients.

Relative blood cell count

In peripheral blood, CVID patients had no significant differences in leucocyte cell absolute count in comparison to controls (median 6850 vs 6200 x 10^3/L, *p=0.390*), but lymphocyte count was lower (median 1521 vs 2064 x 10^3/L, *p=0.048*). Among the lymphocyte subgroup, the proportion of B-cells was significantly lower in CVID patients (5.1 vs 21.2%, *p<0.001*), with no difference between T-cells; CVID patients also had a lower proportion of CD4+ T-cells when compared to controls (60.8 vs 64.8%, *p=0.014*), which contrasted with higher CD8+ (35.4 vs 26.8%, *p=0.007*) and triple-negatives (CD4-CD8-γδ- T-cells – 1.7 vs 1.3%, *p=0.039*), with no significant differences in γδ T-cells. Regarding innate cells, the overall proportion of mDCs (0.15 vs 0.26%, *p<0.001*), pDCs (0.04 vs 0.14%, *p<0.001*), and circulating non-classical monocytes (5.0 vs 11.2%, *p<0.001*) was significantly lower in CVID patients. In contrast, there were significantly higher numbers of neutrophils (63.2 vs 52.4%, *p=0.003*) and classical monocytes (83.7 vs 79.0%, *p=0.014*).

CD217 expression in innate cells, based on the Mean Fluorescent Intensity (MFI)

Overall, CD217 expression was significantly higher in most innate cell types of CVID patients compared to controls (Table 1): neutrophils (1908 vs 1474, *p=0.003*), non-classical monocytes (1132 vs 864, *p=0.012*), mDCs (1438 vs 1135, *p<0.001*) and pDCs (509 vs 344, *p<0.001*). No significantly different expression of CD217 was found in classical and intermediate monocytes between the two groups.

We then compared how CD217 expression was related to CVID clinical manifestations. Patients with a history of intestinal colonization had a significantly higher expression of this receptor in comparison with non-colonized CVID patients. No differences in CD217 expression were observed in relation to other demographic or clinical characteristics, such as IgG levels, respiratory tract colonization, asthma/COPD, chronic rhinosinusitis, chronic diarrhea, malignancy, Chapel phenotypes or particular imaging findings. Taking into consideration the heterogeneous manifestations of CVID, we divided CVID patients in two major clinical groups: (1) patients with immunodysregulatory complications (autoimmunity, malignancy, enteropathy and/or lymphoid hyperplasia – n=16) and (2) patients mainly with infections manifestations

(n=6). No significant differences in CD217 expression of innate cells were observed between the groups.

It should be noted that, even though CVID patients with intestinal microbial colonization had higher expression of CD217 in innate cells, patients without microbial colonization still expressed more CD217 when compared to healthy controls.

Specifically, a higher CD217 expression was observed in the innate cells of CVID patients with a history of gastrointestinal colonization by *Campylobacter jejuni* and *Giardia intestinalis* (Figure 2). More specifically, CD217 expression in all monocyte cell subtypes – classical (controls: 2112 vs CVID patients: 2845, *p=0.007*), intermediate (1658 vs 2524, *p=0.012*) and non-classical (1000 vs 1460, *p=0.026*) – and in both DC types – mDCs (1410 vs 1858, *p=0.019*) and pDCs (476 vs 622, *p=0.056*) – was significantly higher in the 7 patients with a history of *Campylobacter jejuni* colonization. Although only 4 patients had been colonized with *Giardia intestinalis*, this sub-group also achieved a statistically significant higher CD217 expression in non-classical monocytes (1714 vs 1084, *p=0.039*), in comparison to non-colonized patients.

CD217 expression in mDCs (r=0.230, *p=0.042*) and classical monocytes (r=0.627, *p=0.031*) was associated with higher mean fecal calprotectin levels in CVID patients, even after controlling for gastrointestinal colonization history (Table 3).

Discussion:

In summary, our study reveals a significant increase in the expression IL-17RA in innate cells of CVID patients such as neutrophils, non-classical monocytes, and dendritic cells. Moreover, its heightened expression in patients with a history of intestinal colonization suggests a potential association with persistent pro-inflammatory states and microbial infections, hinting at a role in gut inflammation.

Previous published studies reported a decrease in both circulating Th17 cells and IL-17 levels in patients with CVID (15). However, our study shows that the expression of main receptor IL-17RA may follow an opposite trend, with a statistically significant increase in most innate cells – neutrophils, non-classical monocytes and dendritic cells. This is an important finding, as previously described increases in Th17 and IL-17 might not translate into improved IL-17-mediated innate responses in these patients due to lack of IL-17RA signaling.

Patients with CVID are known to present with an altered cytokine profile that is consistent with a persistent activation of monocytic and granulocytic cell lineages (20). This immune activation has been partially explained by chronic microbial translocation, mainly bacterial and fungal, in the respiratory and gastrointestinal tracts of CVID patients (21). IL-17 has a major role in host defense against microbial pathogens by activating IL-17RA and inducing a pro-inflammatory cascade that activates and recruits neutrophils and monocytes for pathogen control. Additionally, the IL-17/IL-17R signaling pathway appears to be stimulated simultaneously, with the blockade of inhibitors such as PI3K or TRAF3 leading to elevated expression of IL-17RA and consequently strengthening IL-17 signaling (22,23).

Therefore, we hypothesized that the increased expression of IL-17RA in the innate cells of CVID patients is associated with a persistent pro-inflammatory state that is observed in these patients. Alternatively, low circulating levels of Il-17 may lead to increased expression of IL-17RA through some unidentified feedback regulation mechanism.

Particularly high levels of IL-17RA were found in patients with a history of intestinal colonization with *Campylobacter* or *Giardia*. IL-17 has an intense activity in intestinal epithelial regeneration and also plays essential roles in host defense against microbial pathogens, including respiratory tract bacteria such as *K. pneumoniae*, as well as gastrointestinal bacteria such as *S. enterica*, fungus like *C. albicans,* and parasites like *Trypanosoma* (24–26). However, we found no studies correlating IL-17 and microbial colonization in CVID, and the specific pattern of pathogen infections in our cohort could explain these results. It should be noted that this study's sample size limits further interpretations. Applying these measurements in a larger cohort could not only help confirming our hypothesis, but also include other pathogens that are commonly found in CVID patients, such as fungi.

Even though levels of IL-17RA were significantly higher in CVID patients with gastrointestinal microbial colonization, the subgroup of patients without microbial colonization still expressed higher IL-17RA levels in innate cells when compared with healthy controls. Besides mucosal barrier protection against microorganisms, IL-17 also has an important function as a mediator in autoimmunity and cancer. About 20% of CVID patients present with autoimmune diseases or other auto-inflammatory manifestations such as lymphoid hyperplasia, granulomatous infiltrations, and "inflammatory bowel disease-like" colitis (14). Therefore, we cannot exclude that other pro-inflammatory factors aside from microbial colonization could lead to increased IL-17RA expression.

Th17 cells play a major role in the development of many autoimmune diseases. Thus, considering the potential impact of pro-inflammation in IL-17RA expression, we compared CVID patients with and without a history of autoimmunity (particularly cytopenia), but no significant differences were found. Interestingly, Il-17RA regulation could be significantly regulated by bowel auto-inflammatory processes – receptor expression in innate immune cells from patients with CVID correlated significantly with higher fecal calprotectin levels, independently of gastrointestinal colonization. Calprotectin is produced mainly by innate immune cells, and its pathophysiological relationship with IL-17RA requires further in-depth studies – there could be a dysregulation of the inflammatory activity in the gut promoted by IL-17; alternatively, IL-17 could also be having a regulatory effect, by promoting mucosal barrier protection in response to inflammatory or infectious mechanisms (27).

This study has some limitations to be taken in consideration. As previously noted, statistical analysis is limited by the small cohort, and there is an absence of cytokine serum measurements, including Il-17, that may hinder our mechanistic understanding of IL-17RA expression. Furthermore, both the distinct immune phenotypes of CVID patients regarding B and T-cell profiles and the heterogeneous genetic background can have a potential correlation with clinical manifestations and complications. As such, despite our small number of CVID patients, this study could have benefited from the inclusion of genetic characterization, as well as immune profiling according to the Euroflow and/or EUROclass phenotypes (4,28,29).

The expression of IL-17RA and IL-17 signaling in the innate immune system of CVID patients might have important implications in infectious and non-infectious inflammatory manifestations of the disease. A better understanding of these observations might be achieved in larger cohorts of patients with detailed clinical, immunological and genetic characterization, paired with the measurement of IL-17 serum levels.

Ethics declarations:

Ethics Approval

This project was approved by the local ethics committee review board. Informed consent for obtaining blood samples for functional assays were taken in accordance with the Declaration of Helsinki.

Consent to Participate

All participants provided written informed consent prior to participation.

Consent for Publication

Not applicable.

Conflict of Interest

No conflicts of interest to declare.

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No sponsorship for additional funding was required for this study.

Availability of data and material

This manuscript has no associated data in a data repository.

Authors' contributions

PBA – conceptualization, data collection, writing

HP – data collection

JC – data collection

IN – data collection

ATB – revision

EF – conceptualization, revision

FR – conceptualization, writing, revision

AP – supervision, conceptualization, writing, revision

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Tables

Variables	CVID (n=22)	Controls (n=14)	*p-value*
Median age at measurement (IQR), years	48 (44-56)	49 (40-58)	*0.602*
Male gender, %	45.5	42.9	*0.878*
Leukocyte count (IQR), n x 10^6/L	6850 (4600-10100)	6200 (4800-7000)	*0.390*
Lymphocyte count (IQR), n x 10^6/L	1521 (1029-2177)	2064 (1688-2359)	*0.049**
Median overall proportion of cell subtypes (IQR), %			
Lymphocytes	24.2 (16.0-30.0)	35.0 (32.9-37.7)	*<0.001**
B-Cells	5.1 (4.0-8.3)	21.2 (15.0-29.8)	*<0.001**
T-Cells	73.1 (64.0-88.2)	71.8 (63.2-78.1)	*0.545*
CD4+	60.8 (48.4-64.2)	64.8 (60.3-72.6)	*0.014**
CD8+	35.4 (31.2-42.0)	26.8 (22.7-32.8)	*0.008**
Gamma-delta	3.2 (1.7-5.6)	3.0 (2.3-5.2)	*0.978*
Triple-negative	1.7 (1.4-3.6)	1.3 (1.0-1.5)	*0.039**
Neutrophils	63.2 (56.0-71.0)	52.4 (47.2-57.9)	*0.003**
Monocytes	6.8 (5.7-9.3)	8.4 (7.3-9.6)	*0.058*
Classic	83.7 (79.6-89.7)	79.0 (73.2-81.1)	*0.014**
Intermediate	7.7 (3.8-12.9)	10.8 (9.0-13.4)	*0.162*
Non-classic	5.0 (3.6-8.6)	11.2 (9.2-12.9)	*<0.001**
Myeloid dendritic cells	0.14 (0.10-0.20)	0.26 (0.23-0.32)	*<0.001**
Plasmacytoid dendritic cells	0.04 (0.02-0.10)	0.14 (0.09-0.20)	*<0.001**
CD217 cell expression by median MFI (IQR), units			
Neutrophils	1908 (1634-2143)	1474 (1182-1807)	*0.003**
Classic monocytes	2295 (1943-2747)	2134 (1714-2368)	*0.289*
Intermediate monocytes	1956 (1629-2313)	2033 (1440-2304)	*0.803*
Non-classic monocytes	1132 (810-1345)	864 (585-995)	*0.012**
Myeloid dendritic cells	1438 (1309-1828)	1135 (987-1282)	*<0.001**
Plasmacytoid dendritic cells	509 (409-633)	344 (213-433)	*<0.001**

IQR - interquartile range; MFI - mean fluorescence intensity; *- statistically significant (*p<0.050*)

Table 1 - Comparison between both groups - demographic characteristics, cell counts and CD217 expression

Clinical variables	Yes	No	Unknown
History of microbial colonization, n (%)			
Total	14 (63.6)	7 (31.8)	1 (4.6)
Respiratory agents	11* (50.0)	9 (40.9)	2 (9.1)
Gastrointestinal agents	11** (50.0)	11 (50.0)	0 (0.0)
Chapel phenotype characteristics, n (%)			
Autoimmunity	11 (50.0)	11 (50.0)	0 (0.0)
Unexplained enteropathy	6 (27.3)	16 (72.7)	0 (0.0)
Polyclonal lymphoproliferation	10 (45.5)	12 (54.5)	0 (0.0)
Other clinical characteristics, n (%)			
Hepatomegaly	5 (22.7)	17 (77.3)	0 (0.0)
Splenomegaly	9 (40.9)	13 (59.1)	0 (0.0)
Bronchiectasis	14 (63.6)	6 (27.3)	2 (9.1)
Asthma/COPD	7 (31.8)	4 (18.2)	0 (0.0)
Chronic rhinosinusitis	13 (59.1)	9 (40.9)	0 (0.0)
Chronic diarrhea	12 (54.5)	10 (45.5)	0 (0.0)
History of pneumonia	12 (54.5)	10 (45.5)	0 (0.0)
History of malignancy	3 (13.6)	19 (86.4)	0 (0.0)
Laboratory variables, median (IQR)			
Pre-treatment IgG levels, g/L	2.4 (0.8-4.1)		
Current IgG levels, g/L	8.0 (7.1-9.9)		
Faecal calprotectin levels, µg/g	330 (230-781)		

IQR - interquartile range; IgG - Immunoglobulin G

**- H. influenzae = 10; P. aeruginosa = 2; M. catarrhalis = 2; S. pneumoniae = 2; S. aureus = 1; Aspergillus niger = 1*

***- H. pylori = 10; C. jejuni = 7; S. entérica =1; Giardia intestinalis = 4*

Table 2 – Clinical characterization of CVID patients (n=22)

Variable	*Regression coefficient*	*Standard-error*	*p-value*
Neutrophils - CD217 expression by MFI, units			
Faecal calprotectin	0.185	0.144	*0.228*
Gastrointestinal colonization	-148.0	199.2	*0.475*
Classic monocytes - CD217 expression by MFI, units			
Faecal calprotectin	0.627	0.249	*0.031**
Gastrointestinal colonization	-142.4	345.4	*0.689*
Intermediate monocytes - CD217 expression by MFI, units			
Faecal calprotectin	0.251	0.243	*0.326*
Gastrointestinal colonization	102.1	336.7	*0.768*
Non-classic monocytes - CD217 expression by MFI, units			
Faecal calprotectin	0.192	0.152	*0.240*
Gastrointestinal colonization	155.7	222.2	*0.501*
Myeloid DC - CD217 expression by MFI, units			
Faecal calprotectin	0.230	0.097	*0.042**
Gastrointestinal colonization	167.5	141.2	*0.266*
Plasmacytoid DC - CD217 expression by MFI, units			
Faecal calprotectin	-0.078	0.091	*0.416*
Gastrointestinal colonization	259.1	133.2	*0.084*

DC - dendritic cells; MFI - mean fluorescence intensity; *- statistically significant (*p<0.050*)

Table 3 - Linear regression analysis of CD217 cell expression, with calprotectin and gastrointestinal colonization as co-variates

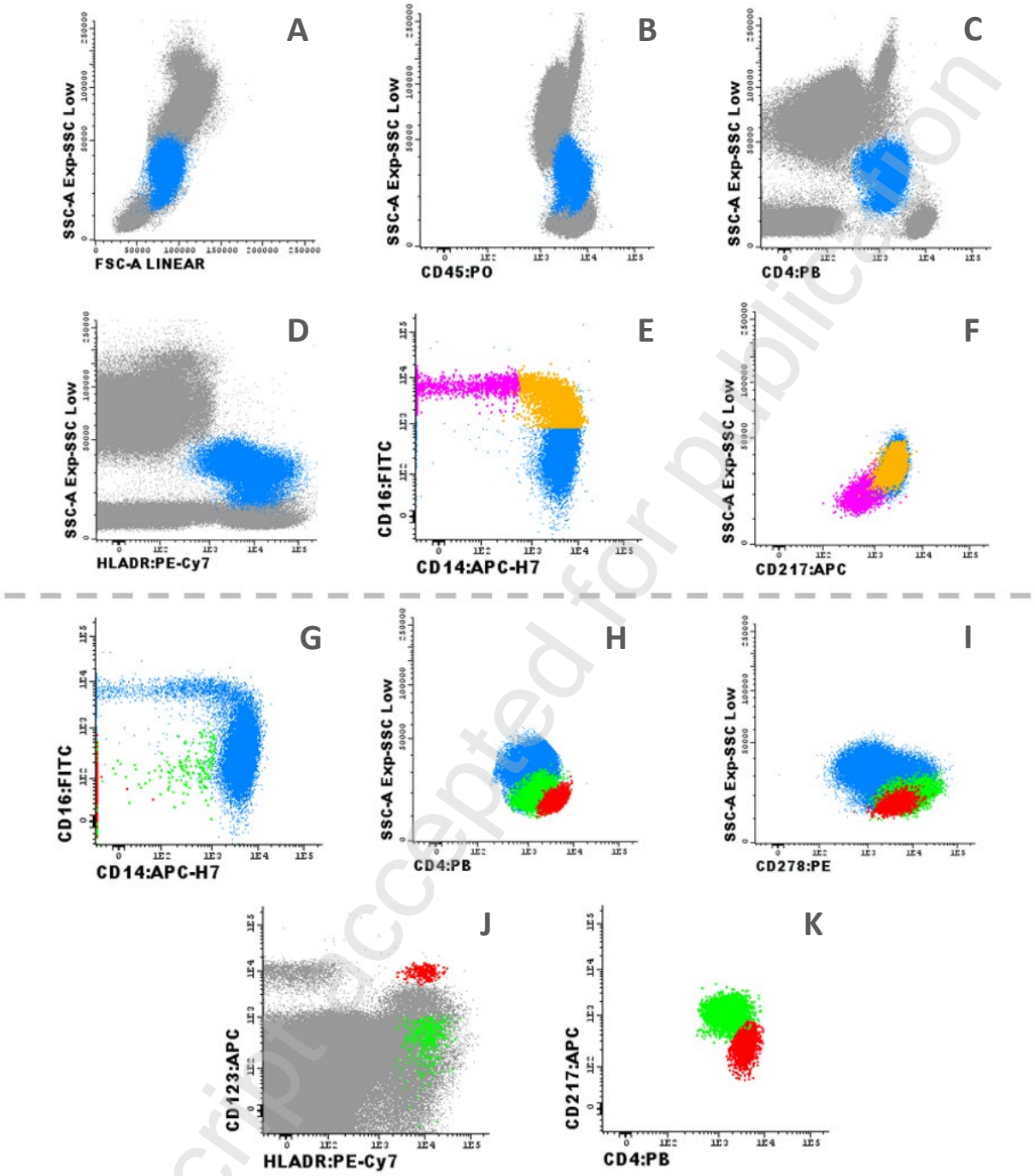
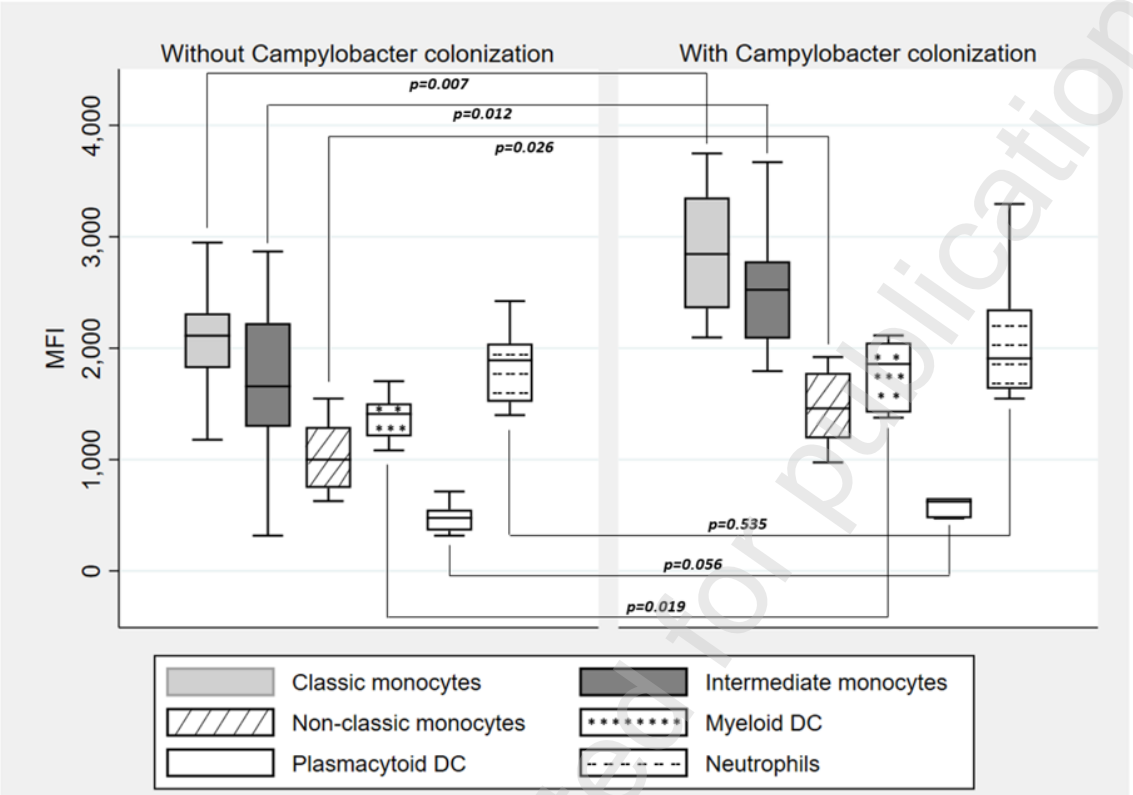


Figure 1 - Representative dot plots illustrating the identification of monocytes (A-F) and dendritic cells (G-K) subtypes, in peripheral blood samples, using a combination of eight-colour mouse anti-human antibodies.

- Monocytes (E): classical monocytes in blue (based on the positive expression of CD14 and negative expression of CD16), intermediate monocytes in yellow (based on the positive expression of both CD14 and CD16) and non-classical monocytes in pink (based on the positive expression of CD16 and dim/negative expression of CD14);
- Dendritic cells (J, K): defined by HLA-DR and CD4 expression in the absence of CD14 and CD16. Plasmacytoid dendritic cells exhibit a higher expression of CD4 when compared with myeloid dendritic cells, as well as a dim expression of CD217 (confirmed with the specific

combination of CD123 and HLA-DR that allows for a better definition of these two subsets): plasmacytoid dendritic cells (pDC) in red and myeloid dendritic cells (mDC) in green.



Cell subtype	*Campylobacter jejuni*		p-value	*Giardia intestinalis*		p-value
	Colonized (n=14)	Non-colonized (n=7)		Colonized (n=4)	Non-colonized (n=17)	
	CD217 cell expression by median MFI (IQR), units					
Neutrophils	1908 (1634-2350)	1891 (1518-2042)	*0.535*	2048 (1610-2822)	1908 (1634-2042)	*0.635*
Classic monocytes	2845 (2358-3353)	2112 (1820-2314)	*0.007**	2724 (1637-3550)	2295 (1943-2474)	*0.635*
Intermediate monocytes	2524 (2085-2780)	1658 (1293-2225)	*0.012**	2430 (1606-3223)	1794 (1629-2308)	*0.462*
Non-classic monocytes	1460 (1189-1779)	1000 (745-1293)	*0.026**	1714 (1291-1850)	1084 (773-1281)	*0.039**
Myeloid dendritic cells	1858 (1421-2051)	1410 (1207-1506)	*0.019**	2051 (1376-2114)	1429 (1299-1704)	*0.146*
Plasmacytoid dendritic cells	622 (473-654)	476 (363-551)	*0.056*	553 (472-654)	489 (389-622)	*0.546*

IQR - interquartile range; MFI - mean fluorescence intensity; *- statistically significant ($p<0.050$)

Figure 2 - CD217 expression in innate cells - comparison according to *Campylobacter* and *Giardia* colonization