Altered Expressions of CXCR4 and CD26 On T-Helper Lymphocytes in Hereditary Hemorrhagic Telangiectasia

Alexandre GUILHEM (alexandre.guilhem@gmail.com)
CHU Montpellier: Centre Hospitalier Regional Universitaire de Montpellier https://orcid.org/0000-0003-4085-8784
Pierre Portalès
CHU Montpellier: Centre Hospitalier Regional Universitaire de Montpellier
Sophie Dupuis-Girod
Hospices Civils de Lyon
Sophie Rivière
CHU Montpellier: Centre Hospitalier Regional Universitaire de Montpellier
Thierry Vincent
CHU Montpellier: Centre Hospitalier Regional Universitaire de Montpellier

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Abstract

Background: Hereditary hemorrhagic telangiectasia (HHT) is a rare genetic disease characterized by a deregulated neo-angiogenesis. Besides a mainly vascular phenotype (telangiectasia, arteriovenous malformations), patients exhibit a specific infectious risk and a deficit of T and natural killer (NK) lymphocytes. As the CXCR4/CXCL12 chemotactic axis is dysregulated in HHT endothelial cells, we hypothesized that a similar phenomenon could occur on lymphocytes.

Results: Eighteen HHT patients with history of severe infection (HSI) were matched in age and sex with 18 HHT without HSI and 18 healthy control subjects (HC). We assessed the cell count and the surface expression of CXCR4 and CD26 (CXCL12 inactivating peptidase) of circulating helper and cytotoxic T lymphocytes (including naive, memory and activated subsets) and NK cells.

The overall HHT group of 36 patients exhibited a reduction of circulating T-helper lymphocytes compared to HC (median: 517 vs 1026 cells/mm3, $p<0.0001$), correlated with age ($r=-0.46$, $p=0.005$), requirement of intravenous iron or blood transfusions (median: 291 vs 627 cells/mm3, $p=0.03$) and CXCR4 surface expression ($r=0.353$, $p=0.0345$). CXCR4 and CD26 membrane expression were both decreased on HHT T-helper lymphocytes (median MFI ratio: 4.49 vs 5.74 for CXCR4 and 3.21 vs 4.33 for CD26, $p=0.03$ and 0.0018 respectively) with an unchanged CXCR4/CD26 ratio. The HHT group with HSI had a higher CXCR4/CD26 ratio on the naive T-helper lymphocytes (median: 2.34 vs 1.32, $p=0.0002$), also observed on the T and T-helper populations.

Conclusions: Our findings support a dysregulation of the CXCL12/CXCR4 chemotaxis of T-helper lymphocytes in HHT patients, potentially linked to their T-helper lymphopenia and susceptibility to infection.

Background

Hereditary hemorrhagic telangiectasia (HHT) is a vascular genetic disease with an estimated prevalence of 1 in 5000–8000. Mutations in several genes of the BMP9/BMP10 signaling pathway (ENG and ALK1 mainly) result in a deregulated neo-angiogenesis. The inheritance pattern is autosomal dominant, and the penetrance is usually complete after 50 years. The clinical presentation includes spontaneous and recurrent epistaxis (frequently complicated by iron-deficiency anemia), muco-cutaneous telangiectasia (face, oral cavity, hands, digestive tract), and less frequently visceral arteriovenous malformations (AVM) of the lungs, the liver or the central nervous system[1].

Besides this vascular phenotype, about 15% of the patients are affected by severe infectious episodes. The most specific events are cerebral abscess with anaerobic bacteria, generally attributed to septic emboli allowed by right-to-left cardiac shunting due to pulmonary AVM. Patients are also concerned by musculoskeletal infections involving *Staphylococcus aureus*, which may be associated with bacteremia, provoked by prolonged nose packing or/and lesions of the nasal mucosa [2, 3]. In addition to these mechanical factors, innate immunity is suspected to be altered in HHT: functional deficits of neutrophils and monocytes/macrophages (phagocytosis, oxidative burst, NETs formation) have been reported in humans [4, 5] and mice [6], but the underlying mechanism is still unknown. Intriguingly, immunological studies have highlighted a T and NK lymphopenia predominant on naive T-helper cells [5, 7]. Although very low lymphocyte counts were observed in some patients, no clear association with infectious risk has been established to date.

The chemokine CXCL12 (formerly named SDF1) and its cellular receptor CXCR4 constitute a powerful chemotactic axis in many cell types. CD26 is a known CXCL12 inactivating peptidase, functionally associated with CXCR4 on T lymphocytes [8]. This chemotactic axis is involved in T lymphocyte trafficking and homeostasis [9]. In endothelial cells, CXCL12 and CXCR4 expressions are regulated by BMP9 in an endoglin-dependent manner: BMP9 and hypoxia are additive inducers of CXCL12 release while surface CXCR4 is downregulated [10]. Imbalance of this chemotactic axis has also been described on peripheral blood mononuclear cells (MNCs) in one study. The migration capacities of HHT MNCs towards a CXCL12 gradient were reduced as a result of a CD26 hyper-expression, which was overcoming elevated surface levels of CXCR4 [11].

Here, we describe the CXCR4 and CD26 expression levels in different lymphocyte subsets of HHT patients with history of severe infection (HSI), in comparison with HHT patients without HSI and with healthy control subjects (HC) matched in age and sex. We provide several elements supporting an alteration in the CXCL12/CXCR4 axis on T-helper lymphocytes in HHT patients, possibly related to their specific infectious risk and T-helper lymphopenia.

Results

Demographics and clinical characteristics

The study included 54 subjects: 36 HHT patients and 18 HC. The main characteristics are detailed in Table 1. With a median age of 60 years and a male predominance, the HHT group was well-balanced in term of causative mutation and epistaxis intensity during the last month before inclusion. One third of the group had at least one pulmonary AVM. A majority of patients (56%) required intravenous (IV) treatments for iron-deficient anemia (IV iron or blood transfusion), and 4 (11%) were under bevacizumab.

Table 1
Demographics and clinical characteristics of the subjects

	HHT (n = 36)	Controls (n = 18)	
Age (mean, SD)	60.2 (10.4)	59.7 (10.2)	p = 0.868
Sex (males, %)	26 (72%)	13 (72%)	p = 1
History of severe infection (n, %)	18 (50%)	0 (0%)	
Mutation (n, %) :			
ENG	18 (50%)		
ACVRL1	16 (44%)		
SMAD4	0 (0%)		
Not identified	2 (6%)		
Visible telangiectasia (median, min-max)	70 (0-242)		
Epistaxis frequency* (n, %)			
daily	13 (36%)		
weekly	13 (36%)		
Monthly or more rarely	10 (28%)		
Arteriovenous malformations (n/nt, %)			
lung	12/35 (34%)		
digestive tract	19/26 (73%)		
liver	15/34 (44%)		
brain	6/24 (25%)		
Treatment (n, %)			
IV iron or blood transfusion (at least 5)	20 (56%)		
tranexamic acid	6 (17%)		
bevacizumab	4 (11%)		
n: number of patients			
nt: number of tested patients			
* during the last month before blood sampling			

Thirty episodes of severe infection were identified in the medical history of 18 HHT patients (1.67 episodes/patient): 7 cerebral abscesses, 8 osteoarticular infections (including 6 cases of spondylodiscitis), 5 cutaneous or soft tissue infections, 3 cases of bacteremia, 2 cases of pneumonia (including one with pulmonary abscess), 2 cases of pyelonephritis, 1 case of prostatitis, 1 case of sigmoiditis and 1 case of bartholinitis. The infectious agents were: *Staphylococcus aureus* in 10 cases, anaerobic bacteria from the oral cavity in 9 cases, 4 cases of *Enterobacteriaceae*, and 2 anaerobic bacteria from the digestive tract (no microbe identified in 5 cases). The median age at the first episode was 46 years (min-max: 21–67 years). Two episodes were classified as septic shock and one as sepsis according to the Sepsis-3 consensus definitions [12].

Surface expression of CXCR4 and CD26 on T and NK lymphocytes

By comparing all the 36 HHT patients (with or without HSI) to the 18 HC in Fig. 1, we observed a significant decrease in total T lymphocyte count in the HHT group (median: 833 vs 1436 cells/mm3, $p = 0.0009$), linked to a reduction of the T-helper sub-population (median: 517 vs 1026 cells/mm3, $p < 0.0001$) with a more pronounced impact on the naive cells (median: 254 vs 577 cells/mm3, $p < 0.0001$). The NK lymphocytes were equally diminished (median: 184 vs 284 cells/mm3, $p = 0.048$).

CXCR4 and CD26 had both a lower mean fluorescence intensity (MFI) on T-helper lymphocytes in the HHT group (median MFI ratio: 4.49 vs 5.74 for CXCR4 and 3.21 vs 4.33 for CD26, $p = 0.03$ and 0.0018 respectively). Decreased membrane expression was also significant for CD26 on naive and memory subsets (median MFI ratio: 4.96 vs 7.26 for naive and 2.48 vs 3.08 for memory, $p = 0.01$ and 0.0022 respectively). T-cytotoxic lymphocytes and their subsets exhibited no significant difference in CXCR4 and CD26 MFI ratio (data not shown). The CXCR4/CD26 ratio was not altered in the NK and the different T lymphocyte subsets.

A weak but significant positive correlation was observed in the HHT group between the surface expression of CXCR4 on T-helper lymphocytes and their absolute count ($r = 0.353$, $p = 0.0345$). A stronger positive correlation was present between the CXCR4 and the CD26 expression on this subset ($r = 0.561$, $p = 0.0004$).

The influence of the main HHT characteristics on T-helper count, the CXCR4 MFI ratio and the CD26 MFI ratio was tested in Fig. 2. Patients receiving IV iron or blood transfusions showed a significant reduction of T-helper count (median: 291 vs 627 cells/mm3, $p = 0.03$). The T-helper count was also correlated with the age of the patients ($r=-0.46$, $p = 0.005$). The decrease of the CD26 MFI ratio followed the same tendencies for age and IV treatment of anemia, without reaching the statistical threshold. Another positive tendency was found between CXCR4 MFI ratio and hemoglobin level.

A multivariate analysis was carried out to assess the independence of the correlations between T-helper absolute count, age, requirement of IV treatment for iron-deficiency anemia and CXCR4 MFI ratio on T-helper cells, in the HHT group. The results of the multivariable linear regression are shown in table SI

(Supplementary Information). Only the age of the HHT patients remained significantly related to the T-helper cell count (p = 0.042).

Clinical and biological parameters associated with history of severe infection

By comparing the clinical characteristics of the 18 HHT patients with HSI to the 18 without HSI (Table 2), we observed a higher frequency of pulmonary AVM (50% vs 18%, p = 0.044). The comparison of the immunological parameters revealed a higher expression of CXCR4 on naive T-helper lymphocytes (median MFI ratio: 9.65 vs 7.01, p = 0.03) and a lower expression of CD26 on naive T-helper lymphocytes (median MFI ratio: 4.59 vs 5.73, p = 0.03) in the HSI group. Consequently the CXCR4/CD26 ratio of this subset was higher in the HSI group (median: 2.34 vs 1.32, p = 0.0002). Similarly, we observed in the HSI group an increase of the CXCR4/CD26 ratio on the T-helper (median: 1.58 vs 1.37, p = 0.01) and the total T lymphocyte populations (median: 1.63 vs 1.32, p = 0.04). This ratio was not significantly different between patients with and without pulmonary AVM (median: 2.15 vs 1.51, p = 0.71).

Table 2

Comparisons of clinical and biological characteristics (a) and lymphocyte parameters (b) between HHT patients with or without history of severe infection

(a)	**HHT with HSI**	**HHT without HSI**	**p**
Age (mean, SD)	60.0 (10.3)	60.4 (10.5)	0.91
Sex (male, %)	13 (72%)	13(72%)	1
Mutation (n, %) :			
ENG	11 (61%)	7 (39%)	0.49
ACVRL1	7 (39%)	9 (50%)	
Epistaxis frequency (n, %)			
daily	6 (34%)	7 (39%)	0.56
weekly	8 (44%)	5 (28%)	
Monthly or more rarely	4 (22%)	6 (34%)	
Visible telangiectasia (median, min-max)	58 (0-242)	90 (0-200)	0.39
Arteriovenous malformations (n/nt, %)			
lung	**9/18 (50%)**	**3/17 (18%)**	**0.044**
digestive tract	7/11 (63%)	12/15 (80%)	0.35
liver	8/18 (44%)	7/16 (44%)	0.97
brain	5/17 (29%)	1/7 (14%)	0.78
Treatment (n, %)			
IV iron or blood transfusion (at least 5)	8/18 (44%)	12/18 (67%)	0.14
tranexamic acid	2/18 (11%)	4/18 (22%)	0.37
bevacizumab	1/18 (6%)	3/18 (17%)	0.29
Basic biological parameters (median, min-max) :			
Hemoglobin (g/l)	135 (80–188)	126 (78–157)	0.16

Statistically significant parameters are in bold
n: number
nt: number tested
HSI: history of severe infection
MFI: mean fluorescence intensity

(a)	HHT with HSI	HHT without HSI	p
Platelets (G/l)	245 (178–388)	262 (236–488)	0.15
Leucocytes (G/l)	5.44 (3.00-8.92)	5.23 (3.02–10.23)	0.95
Neutrophils (G/l)	4.06 (1.73–7.65)	4.02 (1.94–8.19)	0.67
Lymphocytes (G/l)	0.92 (0.3–2.4)	1.02 (0.4–2.34)	0.89
C reactive protein (mg/l)	2.63 (0.2–8.4)	1.85 (0.2–7.3)	0.63
Ferritin (ng/ml)	76 (9-158)	57 (7-221)	0.43
Creatinine clearance (MDRD, ml/min)	85 (36–107)	86.5 (42–140)	0.52
Immunoglobulin G (g/l)	8,73 (2.7–16.8)	9.16 (3.16–16.9)	0.87
Immunoglobulin A (g/l)	2.10 (1.04–4.3)	2.23 (1.07–4.06)	0.64
Immunoglobulin M (g/l)	0.69 (0.36–1.79)	0.72 (0.19–1.9)	0.97
(b) (median, min-max)	**HHT with HSI**	**HHT without HSI**	**p**
T lymphocytes (cells/mm3)	841 (219–2116)	829 (214–1831)	0.44
T-helper lymphocytes (cells/mm3)	517 (96-1499)	550 (123–1061)	0.83
T-cytotoxic lymphocytes (cells/mm3)	339 (90–848)	187 (51-1110)	0.12
NK lymphocytes (cells/mm3)	211 (12–989)	170 (55–946)	0.64
Naive T-helper lymphocytes (cells/mm3)	273 (8-978)	201 (14–630)	0.55
Memory T-helper lymphocytes (cells/mm3)	236 (79–519)	280 (109–798)	0.60
Activated T-helper lymphocytes (cells/mm3)	59 (19–151)	58 (30–215)	0.78
CXCR4 MFI on T lymphocytes	3,87 (2.17–18.36)	3.74 (2.20–14.30)	0.80
CXCR4 MFI on T-helper lymphocytes	4.80 (2.91-14.00)	4.17 (2.40–8.20)	0.48
CXCR4 MFI on naive T-helper lymphocytes	**9.65 (4.48-35.00)**	**7.01 (3.34–14.20)**	**0.03**

Statistically significant parameters are in bold
n: number
nt: number tested
HSI: history of severe infection
MFI: mean fluorescence intensity

(a)	HHT with HSI	HHT without HSI	p
CXCR4 MFI on memory T-helper lymphocytes	3.06 (2.28–9.79)	3.33 (1.91-6.00)	0.64
CXCR4 MFI on activated T-helper lymphocytes	2.40 (1.71–8.15)	2.49 (1.63-4.00)	0.97
CD26 MFI on T lymphocytes	2.71 (1.41–5.13)	2.90 (2.21–6.54)	0.13
CD26 MFI on T-helper lymphocytes	3.01 (1.72–5.75)	3.26 (2.17–6.92)	0.19
CD26 MFI on naive T-helper lymphocytes	**4.59 (1.65–9.33)**	**5.73 (4.17–14.1)**	**0.03**
CD26 MFI on memory T-helper lymphocytes	2.27 (1.58–3.48)	2.58 (2.00-5.08)	0.09
CD26 MFI on activated T-helper lymphocytes	1.63 (1.32–2.95)	1.57 (1.13–2.77)	0.29
CXCR4/CD26 ratio on T lymphocytes	**1.63 (0.52–5.45)**	**1.32 (0.45–4.38)**	**0.04**
CXCR4/CD26 ratio on T-helper lymphocytes	**1.58 (0.86–3.41)**	**1.37 (0.62–2.91)**	**0.01**
CXCR4/CD26 ratio on naive T-helper lymphocytes	**2.34 (0.87–5.68)**	**1.32 (0.51–3.01)**	**0.0002**
CXCR4/CD26 ratio on memory T-helper lymphocytes	1.38 (0.79–2.82)	1.39 (0.71–2.50)	0.25
CXCR4/CD26 ratio on activated T-helper lymphocytes	1.70 (0.70–2.96)	1.63 (0.99–2.65)	0.93
Statistically significant parameters are in bold			
n: number			
nt: number tested			
HSI: history of severe infection			
MFI: mean fluorescence intensity			

Discussion

CXCR4 is the G protein-coupled receptor of the pleiotropic chemokine CXCL12. This chemotactic axis is fundamental during organogenesis, angiogenesis and homeostasis of the hematopoietic and immune systems. CXCL12 is highly expressed in bone marrow and is locally upregulated via HIF-1 after tissue injury to recruit stem cells and progenitors for damage repair [13, 14]. On T lymphocytes, CXCR4 is co-localized with CD26 (dipeptidylpeptidase IV), and both proteins are co-internalized after activation by CXCL12. They form a functional unit participating in chemotaxis towards bone marrow and lymph nodes [8]. This pathway is involved in several diseases, including cancer [15], rheumatoid arthritis [16] and HIV [17]. Here, we report for the first time elements supporting a dysregulation of this axis on T lymphocytes in HHT.

As already published [5, 7], HHT is associated with a T and NK lymphopenia despite the absence of any comorbidities or treatment known to cause lymphopenia [18]. The decrease of the T-helpers could be a consequence of a dysfunctional CXCL12/CXCR4 axis since we observed a weak but significant correlation between their CXCR4 surface expression and their absolute counts in the blood. This could be due to a direct effect of CXCL12 on naive T lymphocyte survival [19] or through disturbance in the T lymphocyte recirculation in lymph nodes and bone marrow, in which the CXCR4/CXCL12 axis is known to be important [9, 20]. The link between age and T-helper lymphopenia could also occur through this chemotactic pathway since altered regulation of CXCR4 on T-helper cells has been described during human aging [21]. The association between iron treatment and T-helper lymphopenia is more difficult to explain. It could be due to a direct effect of the treatment, maybe by increasing the oxygen reactive species in peripheral blood [22]. It also could be a consequence of the iron deficiency, which is known to induce a reduction in peripheral T cells and atrophy of the thymus [23]. As a third hypothesis, it could exist a confounding factor, such as a subpopulation of patients with greater neo-angiogenesis, impacting on both iron requirements and lymphocyte recruitment. Interventional studies are necessary to differentiate these hypotheses, especially since a link between the risk of cerebral abscess and IV iron loading has been reported [24].

We observed a correlated decrease in CXCR4 and CD26 expressions on T-helper lymphocytes of HHT patients without modification of the CXCR4/CD26 ratio. This is in apparent contradiction with a previous report of an over-expression of CXCR4 and CD26 on mononuclear cells with a decreased CXCR4/CD26 ratio associated with chemotactic impairment [11]. This discrepancy could be due to a different pattern of CXCR4 and CD26 surface expression between lymphocytes and monocytes. The monocytes have never been specifically studied in human HHT but are known to express these markers [25, 26]. On T-helper lymphocytes, CXCR4 under-expression seems to be a central phenomenon in the idiopathic CD4 lymphopenia [27], but the infectious profiles are quite different. An alternative explanation could be a chronic elevation of the CXCL12 plasma level in HHT leading to continuous CXCR4 and CD26 internalization [8, 28].

We describe for the first time a significantly higher CXCR4/CD26 ratio on naive T-helper lymphocytes of HHT patients that have experienced at least one serious infectious event. This ratio could constitute an infectious risk factor in the same range as pulmonary AVM, previously reported [2] and confirmed here. Since CXCR4 and CD26 are functionally linked, it is logical to suspect a higher chemotaxis of naive T-helper lymphocytes in HHT patients with HSI. Elevated chemotaxis of leukocytes towards CXCL12 is a central phenomenon in the physiopathology of the WHIM syndrome, a rare genetic immunodeficiency characterized by Warts, Hypogammaglobulinemia, Infections, and Myelokathexis [29]. In this disease, most of the infectious phenotype seems to be due to the chemotactic dysfunction of the myeloid lineage, but patients also exhibit a decrease of NK and naive T lymphocytes [30]. This disease model does not perfectly fit with our observations since neutrophils and B lymphocyte counts and immunoglobulin levels are not correlated with HHT infectious history. A chemotactic dysfunction concerning the Th17 subtype could alternatively be suspected since these cells are known to express CXCR4 [31] and CD26 [32] and participate in the mucosal antibacterial immunity [33]. The high number of cases (n = 10) of

Staphylococcus aureus involvement is concordant with a Th17-deficient phenotype, but we did not observe a clinical vulnerability to candidiasis.

Our study suffered from several limitations. The first is the low number of subjects, limiting the statistical analyses. This fact is inherent to studies on rare diseases. Nevertheless, the ability to identify pulmonary AVM as an infectious risk in HHT allows us to think that our population size was large enough to spot clinically relevant parameters. Plasma levels of chemotactic agents (especially CXCL12) and functional assessment of T lymphocyte chemotaxis could have brought more interpretability in our results. These investigations should be conducted in future studies on this topic, as well as a more detailed evaluation of the Th1, Th2 and Th17 lymphocyte sub-populations.

Conclusions

We report several findings suggesting that HHT is associated with an alteration of the CXCL12/CXCR4 chemotactic axis on T-helper lymphocytes. They exhibit a decrease of their total count in the blood correlated with a decrease of CXCR4 MFI ratio, a decrease of CXCR4 and CD26 surface expressions and a higher CXCR4/CD26 ratio on the naive subset in the group with a history of severe infection. As the CXCL12/CXCR4 chemotactic axis is important in many parts of the immune system, it may contribute to the greater susceptibility to infection observed in HHT.

Methods

Selection of participants and collection of baseline characteristics

Patients and HC were prospectively recruited between January and October 2012 in two French HHT centers (Montpellier and Lyon). Subjects were excluded if they were under 18 years of age or had any active or recent (< 3-month) conditions known to alter the immune system (infection, surgery, pregnancy, solid cancer or lymphoma, autoimmune disease, immunosuppressant or systemic corticosteroid therapy).

All patients included in the study fulfilled 3 or 4 of the Curaçao criteria and had undergone genetic analysis. A thoracic computed tomography scan and an echographic liver assessment were systematically proposed to all patients, according to the French guidelines for HHT diagnosis and treatment. Cerebral or gastrointestinal tract investigations were proposed only according to the clinical context, including symptoms, clinical signs and personal or familial histories.

Patients with a scheduled routine visit in the HHT centers were screened for inclusion during the medical consultation. They were considered to have a HSI if the infectious episode required at least two days of hospitalization (appendicitis excluded). For each patient included with at least one HSI, a HHT patient without any HSI and a HC were included, respecting a matching in sex and age (+/- 2 years).

Data regarding HHT symptoms, complete medical history, treatments and clinical status at the time of the study were prospectively collected. Concerning pulmonary AVM, only those large enough to be considered for treatment were taken into account. Blood samples were collected after the medical assessment. Standard biological measurements (blood cell count, C-reactive protein, ferritin, creatinine, immunoglobulins G-A-M) were made in each center according to their usual procedures.

CXCR4 and CD26 surface expressions on lymphocytes subsets

The phenotypic characterization of lymphocyte subsets was performed using whole blood and standard immunofluorescence / flow cytometry technology. All the analyses were performed on EDTA-collected blood samples, in the department of immunology of the Saint Eloi University Hospital (Montpellier - France).

The following monoclonal antibodies were used for staining: CD3-Krome Orange, CD4-PC7, CD8-APC-Alexa Fluor 700, CD56-PC5.5, CD45RA-ECD, HLA-DR-Pacific Blue (Beckman-Coulter), CXCR4-APC and CD26-FITC (BD biosciences), IgG1-APC and IgG1-FITC (Beckman-Coulter).

Analyses were realized on whole blood. Erythrocyte lysis was realized with the Immunoprep solution on TQ-prep automat (Beckman-Coulter), after antibody fixation. Cells were analyzed by a Navios flow cytometer with the Kaluza software (Beckman Coulter).

The gating strategy is detailed in Additional file 1. Total lymphocytes were identified based on morphological properties. T, T-helper, T-cytotoxic and natural killer (NK) lymphocytes were defined according to the following phenotypes: CD3+, CD3 + CD4+, CD3 + CD8 + and CD56 + CD3-. Naive T-helper and T-cytotoxic lymphocytes were defined as CD45RA + CD4 + and CD45RA + CD8+, respectively. CD4 + HLA-DR + and CD8 + HLA-DR + cells were considered as activated T-helper and T-cytotoxic lymphocytes.

The CXCR4 and CD26 expressions were measured on each subset by calculating the ratio of their MFI to the MFI of their isotype counterparts and subtracting the nonspecific antibody labelling and the cell's auto-fluorescence.

The B lymphocyte population was simply estimated by subtracting T and NK numbers from the total lymphocytes count.

Statistical analysis

Clinical and biological characteristics are presented in tables. Immunological results are presented in bar charts with median and interquartile ranges or as individual values with Spearman r coefficient.

Comparisons between groups were made using the chi-squared, the Kruskal-Wallis or the Mann–Whitney tests, as appropriate. Correlations between variables were tested with the Spearman's rank test.

All the univariate analyses were done with PRISM version 6.01 (GraphPad Software).

For the multivariate analysis, we used the website https://www.pvalue.io [34] to perform a linear regression. As the distribution of residuals did not follow a normal distribution, confidence intervals and p-values were calculated by bootstrap (1000 iterations).

A p-value < 0.05 was considered as statistically significant.

Abbreviations

HHT
hereditary hemorrhagic telangiectasia
NK lymphocytes
natural killer lymphocytes
HSI
history of severe infection
HC
healthy control
AVM
arteriovenous malformation
MNCs
mononuclear cells
IV
intravenous
MFI
mean fluorescence intensity

Declarations

Ethics approval and consent to participate

The protocol was approved by our local Ethics Committee (Montpellier-Nimes Institutional Reviewing Board, registration number: IRB 12/07-01). All subjects gave a written informed consent in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' contributions

AG, TV, PP and SR conceived the study and analyzed data. SR and SDG provided samples. AG and PP performed the experiments. AG wrote the manuscript. SR, TV, PP and SDG corrected the manuscript. All authors reviewed and approved the manuscript.

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Figures

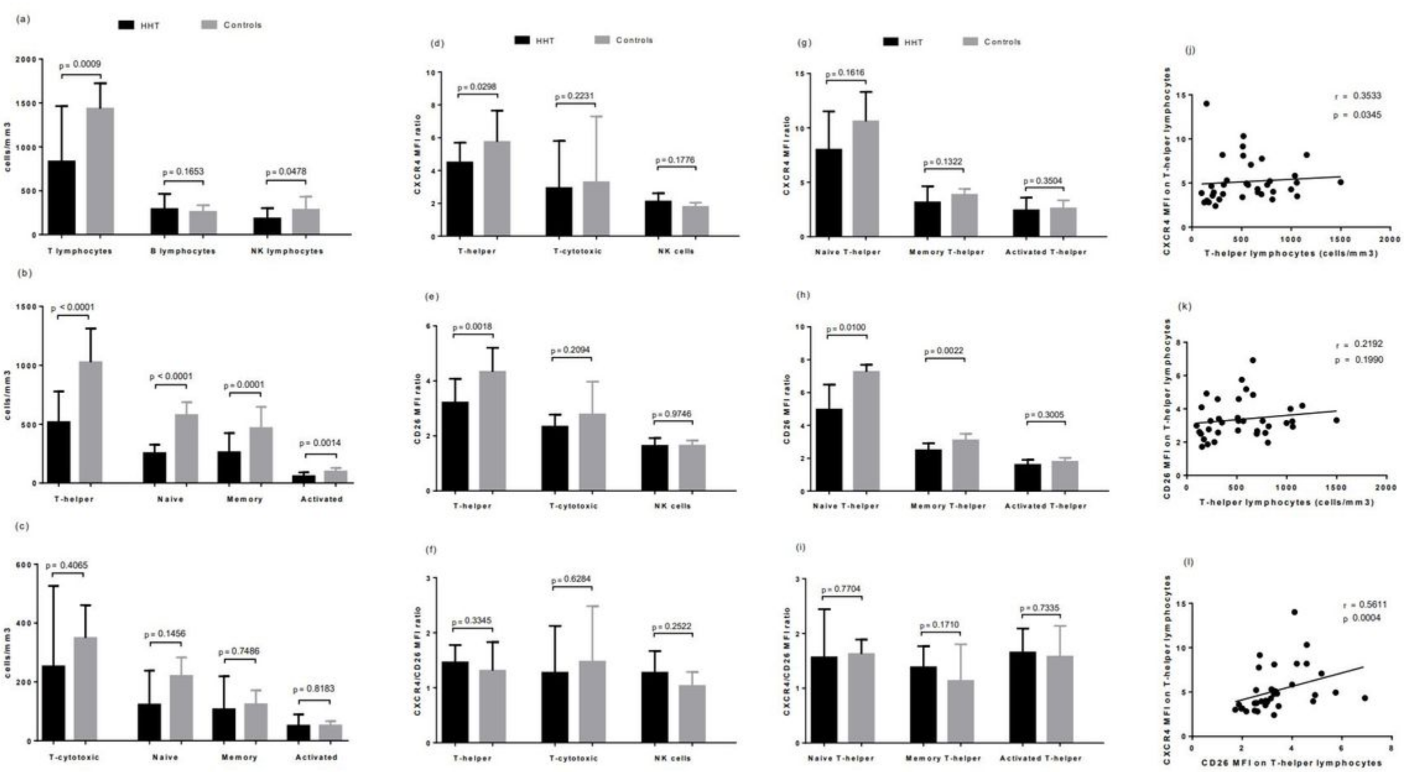

Figure 1

Lymphocyte subpopulations and CXCR4-CD26 surface expressions In 36 HHT patients (black bar) and 18 healthy control subjects (grey bar) matched by age and sex: (a) absolute counts of T, B and NK lymphocytes (b) absolute counts of T-helper lymphocytes (total, naive, memory and activated) (c) absolute counts of T-cytotoxic lymphocytes (total, naive, memory and activated) (d) CXCR4 expression on T-helper, T-cytotoxic and NK lymphocytes (e) CD26 expression on T-helper, T-cytotoxic and NK lymphocytes (f) ratio of CXCR4/CD26 expressions on T-helper, T-cytotoxic and NK lymphocytes (g) CXCR4 expression on naive, memory and activated T-helper lymphocytes (h) CD26 expression on naive, memory and activated T-helper lymphocytes (i) ratio of CXCR4/CD26 expressions on naive, memory and activated T-helper lymphocytes (j) correlation between absolute counts of T-helper lymphocytes and their expression of CXCR4 in the HHT group (k) correlation between absolute counts of T-helper lymphocytes and their expression of CD26 in the HHT group (l) correlation between CXCR4 and CD26 surface expression on T-helper lymphocytes in the HHT group MFI: mean fluorescence intensity Results are presented in bar charts with median and interquartile ranges (for comparisons) or individual values with Spearman r coefficients (for correlations). Comparisons between groups were performed using the Mann–Whitney U-test. Correlations between variables were performed using the Spearman's rank test. P-values are shown.

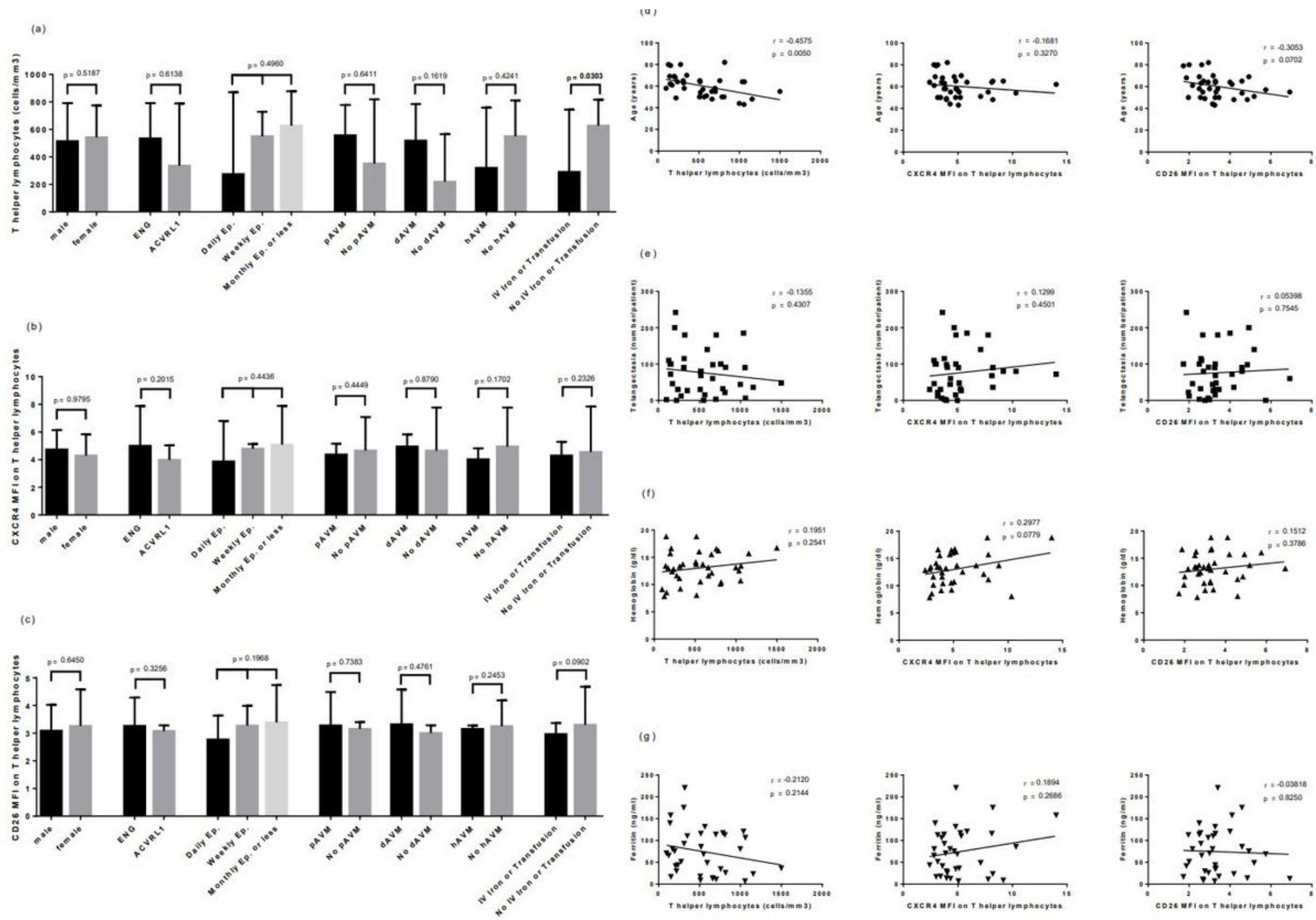

Figure 2

Influence of main HHT characteristics on T-helper count and their CXCR4 and CD26 surface expressions Among the 36 subjects of the HHT group, comparisons of the T-helper count (a), the CXCR4 MFI ratio (b) and the CD26 MFI ratio (c) according to sex (male: 26, female: 10), mutations (ENG: 18, ACVRL1: 16), frequency of epistaxis (daily: 13, weekly: 13, month or less: 10), pulmonary AVM (with: 12, without: 23), digestive AVM (with: 19, without: 7), hepatic AVM (with: 15, without: 19), IV iron or transfusion (with: 20, without: 16). Correlations between T-helper count, CXCR4 expression, CD26 expression and age (d), number of telangiectasia per patient (e), hemoglobin (f), ferritin (g). MFI: mean fluorescence intensity Ep.: epistaxis pAVM: pulmonary arteriovenous malformation dAVM: digestive arteriovenous malformation hAVM: hepatic arteriovenous malformation Results are presented in bar charts with median and interquartile ranges (for comparisons) or individual values with Spearman r coefficient (for correlations). Comparisons between groups were performed using the Kruskal-Wallis or the Mann–Whitney U-test. Correlations between variables were performed using the Spearman’s rank test. P-values are shown.

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