



HAL
open science

Case-control study of the immune status of humans infected with zoonotic gorilla simian foamy viruses

Antoine Gessain, Thomas Montange, Edouard Betssem, Chanceline Bilounga, Ndongo Richard Njouom, Florence Buseyne

► To cite this version:

Antoine Gessain, Thomas Montange, Edouard Betssem, Chanceline Bilounga, Ndongo Richard Njouom, et al.. Case-control study of the immune status of humans infected with zoonotic gorilla simian foamy viruses. 2019. pasteur-02313546v1

HAL Id: pasteur-02313546

<https://pasteur.hal.science/pasteur-02313546v1>

Preprint submitted on 11 Oct 2019 (v1), last revised 18 Dec 2019 (v2)

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The Journal of Infectious Diseases

Case-control study of the immune status of humans infected with zoonotic gorilla simian foamy viruses --Manuscript Draft--

Manuscript Number:	
Full Title:	Case-control study of the immune status of humans infected with zoonotic gorilla simian foamy viruses
Short Title:	Immunology of SFV infection
Article Type:	Major Article
Section/Category:	Viruses
Keywords:	zoonosis; emergence; foamy virus; retrovirus; T lymphocyte; monocyte; immune activation; check-point inhibitor
Corresponding Author:	Florence Buseyne Institut Pasteur Paris, Paris FRANCE
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Institut Pasteur
Corresponding Author's Secondary Institution:	
First Author:	Antoine Gessain
First Author Secondary Information:	
Order of Authors:	Antoine Gessain Thomas Montange Edouard Betsem Chaneline Bilounga Ndongo Richard Njouom Florence Buseyne
Order of Authors Secondary Information:	
Manuscript Region of Origin:	FRANCE
Abstract:	<p>Background. Zoonotic simian foamy viruses (SFVs) establish persistent infections in humans, for whom the long-term consequences for health are poorly described. Here, we aimed to characterize blood-cell phenotypes and plasma biomarkers associated with gorilla SFV infection in humans.</p> <p>Methods. We used a case-control design to compare 15 Cameroonian hunters infected with gorilla SFV to 15 controls matched for age and ethnicity. A flow cytometry-based phenotypic study and quantification of soluble immune biomarkers were carried out on blood samples from all participants. Wilcoxon signed rank tests were used to compare cases and controls.</p> <p>Results. Cases had a significantly higher percentage of CD8 T lymphocytes than controls (median: 17.6% vs. 13.7%, $P = 0.03$), but similar levels of B, NK, and CD4 T lymphocytes. Cases also had a lower proportion of recent CD4 thymic emigrants (10.9% vs. 18.6%, $P = 0.05$), a higher proportion of programmed death receptor 1 (PD-1) expressing memory CD4 T lymphocytes (31.7% vs. 24.7%, $P = 0.001$), and higher plasma levels of the soluble CD163 scavenger receptor (0.84 vs 0.59 $\mu\text{g/mL}$, $P = 0.003$) than controls.</p> <p>Conclusions. We show, for the first time, that chronic infection with SFV is associated with T lymphocyte differentiation and monocyte activation.</p>

Suggested Reviewers:	<p>Martine Peeters martine.peeters@ird.fr Expert in cross-species transmission of retroviruses.</p>
	<p>Arifa Khan arifa.khan@fda.hhs.gov Expert in the threat posed by transmission of retroviruses to humans.</p>
	<p>Edouard Murphy Ed.Murphy@ucsf.edu Leading expert in viral epidemiology.</p>
	<p>Martin Löchelt m.loechelt@dkfz.de Expert in the physiopathology of foamy viruses.</p>
	<p>Marcelo Soares masoares@inca.gov.br Expertise on foamy viruses and other retroviruses.</p>
	<p>Jacek Kuzmak jkuzmak@piwet.pulawy.pl Expert in zoonotic viruses, including bovine foamy viruses</p>



INSTITUT PASTEUR

Florence BUSEYNE, PhD
Institut Pasteur
Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes
UMR CNRS 3569
Bat. Lwoff, 28, rue du Dr. Roux
75724 Paris Cedex 15
Phone: +33 1 45 68 88 99
Fax: +33 1 40 61 34 65
e-mail:florence.buseyne@pasteur.fr

The Journal of Infectious Diseases
Editorial Board

October 9th, 2019

Dear Editors,

I would like to submit our manuscript, entitled "Case-control study of the immune status of humans infected with zoonotic gorilla simian foamy viruses" for publication in *The Journal of Infectious Diseases*. The manuscript is submitted as a major article.

HIV-1 and HTLV-1 have emerged from a simian reservoir with major consequences for human health. Foamy viruses are the third most frequent family of complex retroviruses to infect humans. Our data provide the first evidence of several abnormal immunological markers associated with simian foamy virus infection in humans. Lymphocyte subsets affected in chronic untreated HIV infection were also affected in chronic SFV infection, albeit to a lower extent. We believe that new knowledge concerning simian foamy viruses that infect humans should be widely shared, given the major consequences of the emergence of HIV-1 and HTLV-1 in the human population, for which we chose *The Journal of Infectious Diseases*.

This manuscript has not been submitted or accepted for publication elsewhere. All authors have seen and approved the content of the paper, contributed significantly to the work, and fulfill the criteria for authorship. No writing assistance was provided in the preparation of the manuscript, but the text was edited by a native English speaker.

I would like to suggest the following as possible reviewers for this text:

Martine Peeters, UMI233-TransVIHMI, Institut de Recherche pour le Développement (IRD), INSERM U1175, University of Montpellier, Montpellier, France, martine.peeters@ird.fr. She is an expert in cross-species transmission of retroviruses.

Dr Arifa Khan, Laboratory of Retroviruses, U.S. Food and Drug Administration, Silver Spring, MD 20993, U.S.A., arifa.khan@fda.hhs.gov. She is an expert in the threat posed by transmission of retroviruses to humans.

Pr Edouard Murphy, University of California San Francisco, San Francisco, USA and Blood Systems Research Institute, San Francisco, USA, Ed.Murphy@ucsf.edu. He is a leading expert in viral epidemiology.

Dr Martin Löchelt, Research Program Infection, Inflammation and Cancer, German Cancer Research Center, (DFKZ), Heidelberg, Germany, m.loechelt@dkfz.de. He is an expert in the physiopathology of foamy viruses.

Dr Marcelo Soares, Instituto Nacional de Câncer and Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, masoares@inca.gov.br. He is a virologist with expertise on foamy viruses and other retroviruses.

Dr Jacek Kuzmak, Department of Biochemistry, National Veterinary Research Institute, Puławy, Poland, jkuzmak@piwet.pulawy.pl, He is an expert in zoonotic viruses, including bovine foamy viruses.

On behalf of all authors, I thank you for considering out submission.

Yours sincerely,

F. Busayre

1 Case-control study of the immune status of humans infected
2 with zoonotic gorilla simian foamy viruses
3

4 Antoine Gessain^{a,b}, Thomas Montange^{a,b}, Edouard Betsem^c, Chanceline Bilounga Ndongo^d, Richard
5 Njouom^e, and Florence Buseyne^{a,b,*}

6

7 ^aUnité d'Epidémiologie et Physiopathologie des Virus Oncogènes, Institut Pasteur, 75015 Paris, France

8 ^bUMR CNRS 3569, Paris 75015, France

9 ^cUniversité of Yaoundé I, Yaoundé, Cameroon

10 ^dMinistère de la Santé Publique, Yaoundé, Cameroon

11 ^eCentre Pasteur du Cameroun, Yaoundé, Cameroon

12 *corresponding author

13

14 Running title: Immunology of SFV infection

15

16 Word count: Text, 3434 (max 3500), abstract 199 (max 200)

17

18 Abstract

19 Background: Zoonotic simian foamy viruses (SFVs) establish persistent infections in humans, for whom
20 the long-term consequences for health are poorly described. Here, we aimed to characterize blood-
21 cell phenotypes and plasma biomarkers associated with gorilla SFV infection in humans.

22 Methods: We used a case-control design to compare 15 Cameroonian hunters infected with gorilla SFV
23 to 15 controls matched for age and ethnicity. A flow cytometry-based phenotypic study and
24 quantification of plasma immune biomarkers were carried out on blood samples from all participants.
25 Wilcoxon signed rank tests were used to compare cases and controls.

26 Results: Cases had a significantly higher percentage of CD8 T lymphocytes than controls (median:
27 17.6% vs. 13.7%, $P = 0.03$), but similar levels of B, NK, and CD4 T lymphocytes. Cases also had a lower
28 proportion of recent CD4 thymic emigrants (10.9% vs. 18.6%, $P = 0.05$), a higher proportion of
29 programmed death receptor 1 (PD-1) expressing memory CD4 T lymphocytes (31.7% vs. 24.7%, $P =$
30 0.001), and higher plasma levels of the soluble CD163 scavenger receptor (0.84 vs 0.59 $\mu\text{g/mL}$, $P =$
31 0.003) than controls.

32 Conclusion: We show, for the first time, that chronic infection with SFV is associated with T lymphocyte
33 differentiation and monocyte activation.

34

35 Keywords

36 zoonosis, emergence, foamy virus, retrovirus, T lymphocyte, monocyte, immune activation, check-
37 point inhibitor.

38

39 **Footnotes**

40 Address correspondence to Florence Buseyne, Phone: +33 1 45 68 88 99, Fax: +33 1 40 61 34 65,
41 florence.buseyne@pasteur.fr

42 Disclosure of conflicts of interest: FB, EB, TM, RN, CBN, and AG have nothing to disclose.

43 This work was supported by the Institut Pasteur in Paris, France, the *Programme Transversal de*
44 *Recherche* from the Institut Pasteur [PTR#437] and the *Agence Nationale de la Recherche* [grant ANR-
45 10-LABX-62-IBEID and REEMFOAMY project, ANR 15-CE-15-0008-01]. The funding agencies had no role
46 in the study design, generation of results, or writing of the manuscript.

47 This work was presented at the 26th conference on retroviruses and opportunistic infections (CROI)
48 held in Seattle in 2019.

49 Clinical trials registration. NCT03225794. <https://clinicaltrials.gov/ct2/show/NCT03225794/>

50

51 Introduction

52 Foamy viruses (FV) are complex retroviruses that infect several mammal species, including nonhuman
53 primates (NHPs) [1, 2]. Humans are not natural hosts of FV. However, they are susceptible to infection
54 by zoonotic simian FV (SFV) [2, 3]. Such cross-species transmission occurs mainly through bites [4, 5].
55 Viral exposure to several SFV species originating from apes and African and Asian monkeys leads to
56 lifelong infection, demonstrated by the persistent detection of viral DNA in blood and isolation of
57 replication-competent virus from blood or saliva [4, 6-13].

58 Thus far, FV are considered to be apathogenic in natural, experimental, and accidental hosts, including
59 humans [1, 2, 14]. However, we recently demonstrated hematological and biochemical alterations in
60 SFV-infected humans relative to matched uninfected controls, including a high prevalence of mild
61 anemia and increased urea and creatinine blood levels [14]. Experimental inoculation of feline FV (FFV)
62 to young, healthy, and specific pathogen-free cats leads to persistent infection, without clinical signs,
63 over the first six months. FFV infection induces mild to moderate blood urea levels, an increased
64 protein:creatinine ratio in urine, and histopathological and ultrastructural changes in the kidneys [15].
65 In NHPs and cats, coinfection by FV is associated with increased viral load of other retroviruses (simian
66 immunodeficiency virus (SIV) and feline leukemia virus (FeLV)) and faster clinical progression in
67 experimentally SIV-infected macaques [16, 17]. Conversely, coinfection with FeLV is associated with
68 higher FFV DNA levels [18], SIV infection with expanded SFV tissue tropism in macaques [19], and
69 simian T-cell leukemia virus type 1 (STLV-1) infection with higher SFV DNA load in blood cells from
70 baboons [20]. In humans, coinfection with SFV and either human immunodeficiency virus type 1
71 (HIV-1) or human T-cell leukemia virus type 1 (HTLV-1) has been reported [5, 21, 22]. Overall, these
72 data support a subclinical impact of FV on host physiology, which is still largely unexplored.

73 As any chronic viral infection, either actively replicating or latent/reactivating, SFV infection may affect
74 the immune system, including pro and anti-inflammatory processes and innate and adaptive immune
75 cells [23]. Indeed, we have documented higher IgG levels in SFV-infected humans than in matched
76 controls [14]. Experimental FFV infection leads to hyperplasia in lymphoid organs and lymphocytic
77 infiltration of several tissues [15]. In addition, blood lymphocytes are infected *in vivo* by SFV [13, 24,
78 25]. Thus, SFV may affect immunity by inducing immune responses and replicating in immune cells.

79 We set-up a case-control study, including 15 gorilla SFV-infected men living in Cameroon and 15
80 controls, to define the impact of chronic SFV infection on the human immune system. We performed
81 a detailed phenotypic study of their blood T, B, and NK lymphocytes. In addition, we quantified immune
82 biomarkers in their plasma samples to gain information on the lymphoid and myeloid activation state.

83 For the first time, we report substantial differences between cases and controls that provide
84 information on the physiological consequences of zoonotic SFV infection for humans.

85 **Materials and methods**

86 **Study design and participants.** The research was conducted in accordance with the Helsinki
87 declaration. Ethics approval was obtained from the relevant authorities in Cameroon (National Ethics
88 Committee and Ministry of Health) and France (*Commission Nationale de l'Informatique et des*
89 *Libertés*, and *Comité de protection des personnes Ile de France IV*). This study was registered at
90 [www.clinicaltrials.gov](https://clinicaltrials.gov), <https://clinicaltrials.gov/ct2/show/NCT03225794/>. All participants gave
91 written informed consent.

92 Participants were Cameroonian men who had been injured by an NHP during hunting. Cases consisted
93 of individuals infected with a gorilla SFV recruited from participants of our former survey [4, 14]. SFV
94 infection was defined by positive results on both western blots (presence of the p70-p74 Gag doublet)
95 and PCR assays (*integrase* gene and/or LTR) [4]. Each case was matched individually for age (\pm 10 years)
96 and ethnicity with one non-SFV-infected control, recruited from hunters who participated in the same
97 survey and who lived in the same or neighboring villages as the cases [14]. All cases and controls tested
98 seronegative for HIV-1 infection (LAV Blot1, Cat No. 72251, Biorad). Six cases and three controls tested
99 seropositive for HTLV-1 infection (HTLV Blot 2.4, MP Diagnostics). All participants of the study were
100 apparently healthy at the time of the analysis.

101 **Biological evaluations.** Blood of the 30 participants was collected into tubes containing EDTA.
102 Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation and stored in
103 liquid nitrogen. Plasma samples were stored at -80°C . Blood tests were carried out by the medical
104 analysis laboratory at the Centre Pasteur du Cameroun (CPC), in Yaoundé [14]. PBMCs were stained
105 with Live-Dead-Aqua (Life Technologies, Saint-Aubin, France) and the following antibodies were used
106 for phenotypic characterization of lymphocyte subsets: CD3-FITC, CD8-Alexa700, CD16-V450, CD19-
107 PerCP-Cy5.5, CD27-V450, CD45RA-V450, CD56-PC7, CD57-APC, CD183(CXCR3)-Alexa 488,
108 CD194(CCR4)-Alexa 647, CD196(CCR6)-PE, CD197(CCR7)-PC7, TCR $\gamma\delta$ 1-FITC, TCR V δ 2-PE (BD, Le Pont
109 de Claix, France), CD3-ECD, CD8 β -ECD, CD10-PE, CD20-ECD, CD21-FITC, CD24-APC, CD27-PE, CD28-
110 FITC, CD28-PC5, CD31-FITC, CD38-PC5, CD127-PE, HLA-DR-FITC (Beckman Coulter, Villepinte, France),
111 CD4-APC-eFluor780, CD8 α -A700, CD25-APC, CD38-PC5, CD161-APC, CD279(PD-1)-PE, and integrin β 7-
112 FITC, (e-bioscience, Paris, France). Data were collected on an LSR II cytometer (BD Biosciences) and
113 analyzed with Flow-Jo software (Treestar, Ashland, USA). Lymphocyte subset definitions are indicated
114 in the figure legends and tables. Measurements of plasma analytes were performed using the human
115 high-sensitivity T-cell 21-plex magnetic milliplex assay (HSTCMAG28PMX21BK, Merck Millipore, saint-

116 Quentin en Yvelines, France), Human Magnetic Luminex Assay (for BAFF, CXCL9, CXCL10, CXCL13 and
117 TRAIL, Biotechne, Lille, France), and the ELISA kits DY383 (sCD14) and DY1607 (sCD163) from
118 Biotechne. The SFV DNA level in buffy coat was quantified by PCR at the time of inclusion in the survey
119 [4].

120 **Statistics.** Wilcoxon signed-rank tests were used to compare the quantitative variables between cases
121 and controls. Fisher's exact test was used to analyze qualitative variables. The power of statistical
122 analyses was calculated for the variables that differed significantly between cases and controls with
123 an α of 0.05 for a two-sided test. Spearman's rank test was used to assess correlations between
124 quantitative parameters.

125 Results

126 Study participants

127 Participants were men living in rural areas of East and South Cameroon who reported injuries, mostly
128 bites inflicted during hunting activities [14], and from whom cryopreserved mononuclear cells were
129 available. The ages ranged from 22 to 75 years, with median values of 45 and 50 years for the cases
130 and controls, respectively (Table 1). The duration of SFV infection ranged from 1 to 45 years, and the
131 median value was 14 years.

132 Cases have a higher percentage of CD8 T lymphocytes than controls

133 We used flow cytometry to quantify T, B, and NK lymphocytes and their major subsets (Figure 1). The
134 cases had a significantly higher percentage of CD8 T lymphocytes and lower CD4/CD8 ratios than
135 controls (median: 17.6% vs. 13.7%, $P = 0.03$ and 2.5 vs. 3.5, $P = 0.04$, respectively, Figure 1A). The
136 statistical power of the test for the percentage of CD8 T lymphocytes and CD4/CD8 ratios were > 0.80
137 and > 0.60 , respectively. The percentage of CD4 T lymphocytes was similar for cases and controls
138 (46.6% vs. 46.9%, $P = 0.73$). The percentage of gamma-delta T ($T\gamma\delta$), B, and NK lymphocytes also did
139 not differ between cases and controls (2.5% vs. 2.8%, $P = 0.86$, 12.3% vs. 10.5%, $P = 0.55$, and 9.6% vs.
140 6.5%, $P = 0.30$, respectively, Figure 1B). Furthermore, the repartition of the major $T\gamma\delta$, B, and NK
141 lymphocyte subsets were similar in the two groups (Supplementary Table 1). In conclusion, SFV-
142 infected men had an expanded CD8 T lymphocyte population relative to matched controls.

143 Cases have more highly differentiated CD4 and CD8 T lymphocytes than controls

144 We then used CD45RA, CCR7, CD31, CD27, CD28 molecules to define the phenotype of CD4 and CD8 T
145 lymphocytes. CD4 and CD8 T lymphocytes were more highly differentiated in cases than in controls.
146 Indeed, the percentage of naive (T_N) and recent thymic emigrants (T_{RTE}) among CD4 T lymphocytes
147 were lower in cases than controls (Figure 2A). Conversely, differentiated CD27⁻ effector memory (T_{EM})
148 cells represented a higher proportion of CD4 T lymphocytes in cases than controls. The differences

149 were statistically significant for CD4 T_{TE} (10.9% vs. 18.6%, $P = 0.05$, Power > .70). Among CD8
150 lymphocytes, we observed a significantly higher percentage of the two T_{EM} subsets lacking CD28
151 expression in the cases than controls (T_{EM}27⁺28⁻: 2.5 vs. 0.9, $P = 0.02$; T_{EM}27⁻28⁻: 16.1 vs. 10.8, $P = 0.02$,
152 Figure 2B). CD8 T_{EM} subset levels showed high interindividual variation and the power of these analyses
153 was < 0.50.

154 We further defined the T-cell phenotype by the quantification of the CXCR3, CCR4, and CCR6
155 chemokine receptors, which reflect their polarization. We also assessed the expression of CD161 and
156 $\alpha 4\beta 7$ molecules which are markers of gut homing capacity. Cases had significantly higher levels of
157 CD161⁺ CD4 T lymphocytes and a higher proportion of CXCR3⁺CCR4⁻CCR6⁺ (Th1/Th17) cells among their
158 CD8 T_{EM} lymphocytes (supplementary Table 2). While the differences in levels of polarized T
159 lymphocytes were modest, they were consistent with a more differentiated T-cell compartment in
160 cases than in controls.

161 **Cases express higher levels of check-point inhibitor, PD-1, on memory CD4 T lymphocytes than**
162 **controls.**

163 Then, molecules defining functional capacity (PD-1, CD57), homeostatic proliferation potential (CD127,
164 the high affinity IL-7 receptor) and activation (HLA-DR, CD38) were quantified on memory CD4 and CD8
165 T lymphocytes. PD-1 expression on memory CD4 T lymphocytes was significantly higher in cases than
166 controls (31.7% vs. 24.7%, $P = 0.001$, Figure 3A). The statistical power of the test was > 0.85. The
167 differences were observed in both CD4 T_{CM} and T_{EM} subsets (Supplementary Table 2). In contrast, the
168 percentage of PD-1⁺ CD8 T_M lymphocytes was similar for cases and controls (Figure 3B). The proportion
169 of CD57⁺ cells among total memory CD4 and CD8 T lymphocytes was similar in cases and controls
170 (Figure 3). However, CD57 was expressed by the most highly differentiated CD28⁻ T_{EM} and T_E
171 lymphocytes. Indeed, the percentage of CD57⁺ CD8 T_{EM} among all CD8 T lymphocytes was higher in
172 cases than controls (Supplementary Table 2), a finding consistent with the higher level of total CD28⁻
173 CD8 T_{EM} in cases (Figure 2). Cases and controls expressed similar levels of CD127, HLA-DR, and CD38.
174 We also quantified CD25^{hi}CD127⁺ CD4 regulatory (T_{REG}) lymphocytes, which dampen immune
175 activation. Their percentage (Figure 3A) and differentiation status (Supplementary Table 2) were
176 similar in cases and controls. In conclusion, increased expression of PD-1 molecule, a check-point
177 inhibitor, on CD4 T_M lymphocytes was the most striking difference between cases and controls.

178 **Cases have higher plasma sCD163 levels than controls**

179 We then quantified 27 cytokines, chemokines, and soluble immune mediators in plasma samples using
180 multiplex or ELISA assays (Supplementary Table 3). Cases had significantly higher levels of GM-CSF than
181 controls (64 vs. 46 pg/mL, $P = 0.04$) and tended to have lower IL-8 levels (11 vs. 18 pg/mL, $P = 0.06$).

182 The statistical power was < 0.60 for these analyses. Cases and controls had similar levels of CCL3, CCL4,
183 CCL20, IFN- γ , IL-2, IL-7, IL-10, IL-12, IL-17, IL-21, IL-23, and TNF- α . Cytokines and chemokines associated
184 with B-lymphocyte function and activation, namely CXCL13, BAFF, IL-4, IL-5 and IL-13, were present at
185 comparable levels in cases and controls.

186 Among the plasma molecules associated with inflammation and myeloid cell activation, sCD163 levels
187 were significantly higher in cases than controls (0.84 vs. 0.59 $\mu\text{g/mL}$, $P = 0.003$, Figure 4A); the
188 statistical power of this analysis was > 0.95 . Plasma levels of sCD14, IL-1 β , and IL-6 were similar in the
189 two groups (Figure 4A), as were those of the chemokines and death factors induced by type I and type
190 II IFNs, namely CXCL9/MIG, CXCL10/IP-10, CXCL11/ITAC and TRAIL (Figure 4B). In conclusion, sCD163
191 was the only plasma molecule present at a significantly different level in cases and controls.

192 **Lack of an association between immune parameters, infection status, and haematological variables**

193 Five immune parameters were expressed at significantly different levels between cases and controls,
194 with statistical power of the analyses > 0.70 : percentage of CD8 cells, CD4/CD8 ratio, CD4T_{RTE}, PD-
195 1⁺CD4T_M, and sCD163 levels. The percentage of CD8 cells strongly correlated with the CD4/CD8 ratio
196 in both cases (Spearman's $\rho = -0.835$, $P = 0.0001$) and controls ($\rho = -0.693$, $P = 0.004$). We observed
197 no other correlation between these five parameters (Table 2).

198 Among cases, the five immune parameters were not associated with the duration of infection or SFV
199 DNA load in blood cells. No immune parameter was associated with age in cases or controls. Complete
200 blood counts and biochemistry were available for 13 cases and 15 controls [14]. The immune
201 parameters were not associated with the haematological parameters that differ between the two
202 groups, *i.e.* haemoglobin, urea, creatinine, and lactate dehydrogenase levels. In conclusion, cases and
203 controls had significantly different immune and haematological profiles, without significant
204 correlations between the immune and haematological variables in either group.

205 **Discussion**

206 In a case-control study, we show that humans infected with a zoonotic gorilla SFV have a more highly
207 differentiated T-lymphocyte phenotype and elevated plasma levels of sCD163, a biomarker of
208 monocyte activation. These data are the first description of the blood immune parameters of infected
209 humans and support that SFVs induce an immune reaction common to several chronic viral infections.

210 During acute viral infections, antigen-specific naive T lymphocytes proliferate and differentiate into
211 effectors that lyse infected cells and/or secrete antiviral factors. Following clearance of the virus and
212 the resolution of inflammation, effectors die, and a small subset persist as memory lymphocytes, with
213 reduced effector function and an ability to survive through homeostatic self-renewal driven by

214 cytokines. Persistent antigen simulation during chronic viral infection and cancer promotes another
215 differentiation program called exhaustion [26, 27]. Exhausted lymphocytes have both reduced effector
216 function and proliferative potential. Several mechanisms mediate T-cell exhaustion, including the
217 expression of check-point inhibitors, of which the triggering dampens T-cell activation. The PD-1
218 molecule is one of the most relevant inhibitory receptors and is targeted by antitumoral
219 immunotherapies. Prolonged exposure to chronic viral infection, such as HIV-1, HTLV-1, HBV, HCV, and
220 CMV, is a well-described driver of T-cell exhaustion [26, 27]. In addition, high antigenic levels and
221 exposure to IFNs during T-cell priming may induce CD4 T-cell exhaustion, which is stably maintained
222 by epigenetic reprogramming [26-28].

223 Here, we show that SFV infection is associated with an increased percentage of blood CD8 T
224 lymphocytes, a decreased CD4/CD8 ratio, a reduced proportion of naive CD4 T lymphocytes, and
225 increased expression of the PD-1 molecule on memory CD4 T lymphocytes. These phenotypic changes
226 are consistent with the T lymphocytes responding to a viral infection and overlap those induced by
227 HIV-1, CMV, and ageing [29]. We also observed increased IgG levels, another marker of viral infection
228 [14], in the same population of SFV-infected individuals. Experimental infection of macaques through
229 transfusion leads to a sequential drop in the number of peripheral blood CD4 and CD8 T lymphocytes,
230 followed by their restoration, with an elevated proportion of CD8 T lymphocytes [30]. No change was
231 observed in the peripheral blood of experimentally infected cats, but lymphoid infiltration persisted in
232 tissues [15]. Overall, these data show that chronic SFV infection is associated with phenotypic changes
233 in T lymphocytes.

234 The observed T-lymphocyte phenotype could result from multiple causes, such as ongoing viral
235 replication, infection of immune cells, and coinfections. Direct proof of SFV replication *in vivo* (*i.e.* the
236 presence of SFV RNA, viral proteins, or virions) is currently lacking in humans. Two studies used
237 quantitative RT-PCR to detect SFV RNA in human blood and buccal samples and obtained negative
238 results [12, 31]. These data do not rule out active SFV replication in humans, which may occur at levels
239 below the detection threshold of conventional PCR-based assays or in tissues. Indeed, among patients
240 infected with HIV, HBV, and HCV, some have undetectable viral RNA in blood, despite replication in
241 tissues [32-34]. These situations of HIV control and occult HBV/HCV infection are associated with
242 potent virus-specific responses and a mild disease course relative to infections with detectable viral
243 RNA in blood. Despite apparent viral control, infected individuals show elevated inflammation and
244 some ultimately experience clinical disease or loss of viral control [32-34]. The present data and those
245 obtained on hematological parameters and specific antibodies challenge the proposed SFV latency in
246 humans [2, 14, 35, 36].

247 FV establishes wide tissue tropism after natural or experimental infection in NHPs, cats, and cattle,
248 with consistent detection of viral DNA in blood and lymphoid tissues [15, 19, 37-39]. The separation of
249 lymphocyte subsets from infected individuals showed infection of B and T lymphocytes [13, 24, 25].
250 No human tissue samples have yet been analyzed. SFVs encode a viral transactivator, Tas, which acts
251 on cellular genes [40], and microRNAs that suppress innate immunity [41]. Therefore, SFV can infect
252 immune cells and directly alter their function, similarly to the two human pathogenic retroviruses, HIV-
253 1 and HTLV-1. Immune changes in SFV-infected individuals may be induced by coinfecting pathogens.
254 Indeed, interactions with other retroviruses have been demonstrated in animals [16-20]. Microbial
255 coinfections are a hallmark of infection with the potent immunosuppressive virus HIV-1 [42] and are
256 also common in HTLV-1-infected patients [43]. Whether direct or indirect consequences of SFV
257 infection are related to the immune changes reported here is an open question.

258 We found significantly elevated sCD163 levels in SFV-infected individuals relative to matched controls.
259 The differences were particularly robust, with high statistical power, despite the small size of the study
260 population. CD163 is a transmembrane protein that acts as the hemoglobin scavenger receptor [44].
261 It is expressed by monocytes and alternatively-activated M2 macrophages. These cells are involved in
262 tissue repair and mediate mostly anti-inflammatory functions [45]. Upon inflammatory macrophage
263 activation, the ectodomain is cleaved by matrix metalloproteinases and a soluble form, sCD163, is shed
264 into the plasma [46]. sCD163 is not involved in hemoglobin or iron metabolism.

265 sCD163 is a biomarker of monocyte and/or macrophage activation and its levels are elevated in several
266 immune conditions, including infectious diseases [46]. For example, sCD163 levels are elevated in HIV-
267 1 infected patients and associated with clinical progression [47]. Notably, high sCD163 levels at
268 baseline were associated with incident chronic renal disease with an adjusted odds-ratio > 10, in
269 treated HIV-1-infected patients followed-up for 10 years [48]. This observation is intriguing, as elevated
270 renal markers have been reported in FFV-infected cats and SFV-infected humans [14, 15]. Of eight
271 plasma molecules related to myeloid-cell activation, sCD163 was the only one for which the levels
272 differed between cases and controls. This may result from the activation of a specific pathway or direct
273 infection, as described above. Indeed, one regulatory FFV protein, Bet, was detected by
274 immunochemistry in the cytoplasm of macrophage-like cells from several lymphoid organs (lymph
275 nodes, thymus, tonsil, and spleens) from infected cats [49]. Overall, we document a novel finding in
276 SFV-infected humans, supporting monocyte/macrophage activation.

277 One limit of our study is that statistically significant associations do not prove causality. Although we
278 observed immune differences between SFV-infected individuals and carefully matched controls, we
279 cannot conclude that they are a direct consequence of SFV infection. However, the experimental

280 infection of cats supports the causal relationship between FV infection, persistent lymphocytic
281 infiltration in peripheral organs, and histological features in kidneys [15]. Importantly, the biological
282 findings in SFV-infected hunters are plausible consequences of viral infection.

283 Obtaining biological samples from hunters living in remote areas of the Cameroonian forest is
284 challenging. The number of participants and quantity of blood drawn are two limiting factors. For
285 example, persisting T-lymphocyte activation drives other conditions, such as anemia or chronic kidney
286 diseases. Correlations between viral parameters and host biomarkers were expected, but not
287 observed; this may result from insufficient power for statistical analyses or from the impact of other
288 health conditions. Importantly, despite the modest size of our study population, we had sufficient
289 statistical power to perform the analyses of several important parameters.

290 Our data need to be interpreted with caution, because biological markers that differ between SFV-
291 infected and noninfected individuals might result from SFV infection or other health conditions. In
292 particular, HTLV-1 prevalence is higher in SFV-infected individuals than in uninfected controls (Table 1
293 and [5]). HTLV-1 induces total CD4 and T_{REG}-cell expansion [43]. Here, we only observed CD8 T-cell
294 expansion. Therefore, HTLV-1 coinfection is a very unlikely driver of the differences between SFV-
295 infected and uninfected individuals reported here. As we previously discussed [14], the similarity of
296 clinical signs and leucocyte subset distributions between cases and controls support adequate
297 matching for health factors.

298 The demonstration of frequent transmission of SFV from NHPs to humans raises the question of
299 whether SFV infection has consequences for human health, as zoonotic agents, and as vectors for gene
300 therapy. This first exploration of blood mononuclear cell phenotypes and plasma biomarkers shows
301 statistically significant differences between SFV-infected individuals and matched controls, supporting
302 a response of T lymphocytes and monocytes. Whether a causal relationship exists between immune
303 activation, anemia, and renal alterations is yet to be established.

304 **Acknowledgements**

305 We thank the participants of the study. We greatly appreciate the *Institut de Recherche pour le*
306 *Développement* (IRD) for their support for the field work. This text has been verified by a native English
307 speaker.

308

309 **References**

- 310 1. Meiering CD, Linial ML. Historical perspective of foamy virus epidemiology and infection. Clin
311 Microbiol Rev **2001**; 14:165-76.
- 312 2. Pinto-Santini DM, Stenbak CR, Linial ML. Foamy virus zoonotic infections. Retrovirology **2017**;
313 14:55.
- 314 3. Gessain A, Rua R, Betsem E, Turpin J, Mahieux R. HTLV-3/4 and simian foamy retroviruses in
315 humans: Discovery, epidemiology, cross-species transmission and molecular virology. Virology **2013**;
316 435:187-99.
- 317 4. Betsem E, Rua R, Tortevoeye P, Froment A, Gessain A. Frequent and recent human acquisition of
318 simian foamy viruses through apes' bites in Central Africa. PLoS Pathog **2011**; 7:e1002306.
- 319 5. Filippone C, Betsem E, Tortevoeye P, et al. A severe bite from a nonhuman primate is a major risk
320 factor for HTLV-1 infection in hunters from Central Africa. Clin Infect Dis **2015**; 60:1667-76.
- 321 6. Schweizer M, Falcone V, Gange J, Turek R, Neumann-Haefelin D. Simian foamy virus isolated from
322 an accidentally infected human individual. J Virol **1997**; 71:4821-4.
- 323 7. Boneva RS, Switzer WM, Spira TJ, et al. Clinical and virological characterization of persistent human
324 infection with simian foamy viruses. AIDS Res Hum Retrovir **2007**; 23:1330-7.
- 325 8. Jones-Engel L, May CC, Engel GA, et al. Diverse contexts of zoonotic transmission of simian foamy
326 viruses in Asia. Emerg Infect Dis **2008**; 14:1200-8.
- 327 9. Rua R, Betsem E, Calattini S, Saib A, Gessain A. Genetic characterization of simian foamy viruses
328 infecting humans. J Virol **2012**; 86:13350-9.
- 329 10. Mouinga-Ondeme A, Caron M, Nkoghe D, et al. Cross-species transmission of simian foamy virus
330 to humans in rural Gabon, Central Africa. J Virol **2012**; 86:1255-60.
- 331 11. Switzer WM, Tang SH, Ahuka-Mundeke S, et al. Novel simian foamy virus infections from multiple
332 monkey species in women from the Democratic Republic of Congo. Retrovirology **2012**; 9:100.
- 333 12. Engel GA, Small CT, Soliven K, et al. Zoonotic simian foamy virus in Bangladesh reflects diverse
334 patterns of transmission and co-infection. Emerg Mic Infect **2013**; 2:e58.

- 335 13. Rua R, Betsem E, Montange T, Buseyne F, Gessain A. *In vivo* cellular tropism of gorilla simian
336 foamy virus in blood of infected humans. *J Virol* **2014**; 88:13429-35.
- 337 14. Buseyne F, Betsem E, Montange T, et al. Clinical signs and blood test results among humans
338 infected with zoonotic simian foamy virus: a case-control study. *J Infect Dis* **2018**; 218:144-51.
- 339 15. Ledesma-Feliciano C, Troyer RM, Zheng X, et al. Feline foamy virus infection: Characterization of
340 experimental infection and prevalence of natural infection in domestic cats with and without chronic
341 kidney disease. *Viruses* **2019**; 11.
- 342 16. Choudhary A, Galvin TA, Williams DK, Beren J, Bryant MA, Khan AS. Influence of naturally
343 occurring simian foamy viruses (SFVs) on SIV disease progression in the rhesus macaque (*Macaca*
344 *mulatta*) model. *Viruses* **2013**; 5:1414-30.
- 345 17. Powers JA, Chiu ES, Kraberger SJ, et al. Feline leukemia virus (FeLV) disease outcomes in a
346 domestic cat breeding colony: Relationship to endogenous FeLV and other chronic viral infections. *J*
347 *Virol* **2018**; 92.
- 348 18. Cavalcante LTF, Muniz CP, Jia H, et al. Clinical and molecular features of feline foamy virus and
349 feline leukemia virus co-infection in naturally infected cats. *Viruses* **2018**; 10.
- 350 19. Murray SM, Picker LJ, Axthelm MK, Linial ML. Expanded tissue targets for foamy virus replication
351 with simian immunodeficiency virus-induced immunosuppression. *J Virol* **2006**; 80:663-70.
- 352 20. Alais S, Pasquier A, Jegado B, et al. STLV-1 co-infection is correlated with an increased SFV
353 proviral load in the peripheral blood of SFV/STLV-1 naturally infected non-human primates. *Plos*
354 *Neglect Trop Dis* **2018**; 12:e0006812.
- 355 21. Switzer WM, Garcia AD, Yang CF, et al. Coinfection with HIV-1 and simian foamy virus in West
356 Central Africans. *J Infect Dis* **2008**; 197:1389-93.
- 357 22. Switzer WM, Tang S, Zheng H, et al. Dual simian foamy virus/human immunodeficiency virus type
358 1 infections in persons from Côte d'Ivoire. *PLoS One* **2016**; 11:e0157709.
- 359 23. Virgin HW, Wherry EJ, Ahmed R. Redefining chronic viral infection. *Cell* **2009**; 138:30-50.

360 24. von Laer D, NeumannHaefelin D, Heeney JL, Schweizer M. Lymphocytes are the major reservoir
361 for foamy viruses in peripheral blood. *Virology* **1996**; 221:240-4.

362 25. Callahan ME, Switzer WM, Matthews AL, et al. Persistent zoonotic infection of a human with
363 simian foamy virus in the absence of an intact orf-2 accessory gene. *J Virol* **1999**; 73:9619-24.

364 26. Cicin-Sain L, Arens R. Exhaustion and inflation at antipodes of T cell responses to chronic virus
365 infection. *Trends Microbiol* **2018**; 26:498-509.

366 27. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T cell exhaustion during chronic viral infection
367 and cancer. *Annu Rev Immunol* **2019**; 37:457-95.

368 28. Crawford A, Angelosanto JM, Kao C, et al. Molecular and transcriptional basis of CD4+ T cell
369 dysfunction during chronic infection. *Immunity* **2014**; 40:289-302.

370 29. Thome JJC, Yudanin N, Ohmura Y, et al. Spatial map of human T cell compartmentalization and
371 maintenance over decades of life. *Cell* **2014**; 159:814-28.

372 30. Brooks JI, Merks HW, Fournier J, Boneva RS, Sandstrom PA. Characterization of blood-borne
373 transmission of simian foamy virus. *Transfusion* **2007**; 47:162-70.

374 31. Rua R, Betsem E, Gessain A. Viral latency in blood and saliva of simian foamy virus-infected
375 humans. *PLoS One* **2013**; 8:e77072.

376 32. Raimondo G, Pollicino T, Romano L, Zanetti AR. A 2010 update on occult hepatitis B infection.
377 *Pathologie Biologie* **2010**; 58:254-7.

378 33. Okulicz JF, Lambotte O. Epidemiology and clinical characteristics of elite controllers. *Curr Opin*
379 *HIV AIDS* **2011**; 6:163-8.

380 34. Vidimliski PD, Nikolov I, Geshkovska NM, Dimovski A, Rostaing L, Sikole A. Occult hepatitis C virus
381 infection: still remains a controversy. *J Med Virol* **2014**; 86:1491-8.

382 35. Lambert C, Couteaudier M, Gouzil J, et al. Potent neutralizing antibodies in humans infected with
383 zoonotic simian foamy viruses target conserved epitopes located in the dimorphic domain of the
384 surface envelope protein. *PLoS Pathog* **2018**; 14:e1007293.

385 36. Lambert C, Batale D, Montange T, et al. An immunodominant and conserved B-cell epitope in the
386 envelope of simian foamy virus recognized by humans infected with zoonotic strains from apes. *J*
387 *Virology* **2019**; 93:e00068-19.

388 37. Falcone V, Leupold J, Clotten J, et al. Sites of simian foamy virus persistence in naturally infected
389 African green monkeys: Latent provirus is ubiquitous, whereas viral replication is restricted to the
390 oral mucosa. *Virology* **1999**; 257:7-14.

391 38. Morozov VA, Leendertz FH, Junglen S, Boesch C, Pauli G, Ellerbrok H. Frequent foamy virus
392 infection in free-living chimpanzees of the Tai National Park (Côte d'Ivoire). *J Gen Virol* **2009**; 90:500-
393 6.

394 39. Materniak M, Hechler T, Lochelt M, Kuzmak J. Similar patterns of infection with bovine foamy
395 virus in experimentally inoculated calves and sheep. *J Virol* **2013**; 87:3516-25.

396 40. Wagner A, Doerks A, Aboud M, et al. Induction of cellular genes is mediated by the Bel1
397 transactivator in foamy virus-infected human cells. *J Virol* **2000**; 74:4441-7.

398 41. Kincaid RP, Chen Y, Cox JE, Rethwilm A, Sullivan CS. Noncanonical microRNA (miRNA) biogenesis
399 gives rise to retroviral mimics of lymphoproliferative and immunosuppressive host miRNAs. *mBio*
400 **2014**; 5:e00074.

401 42. Moir S, Chun TW, Fauci AS. Pathogenic mechanisms of HIV disease. *Ann Rev Pathol* **2011**; 6:223-
402 48.

403 43. Bangham CRM. Human T cell leukemia virus type 1: Persistence and pathogenesis. *Annu Rev*
404 *Immunol* **2018**; 36:43-71.

405 44. Kristiansen M, Graversen JH, Jacobsen C, et al. Identification of the haemoglobin scavenger
406 receptor. *Nature* **2001**; 409:198-201.

407 45. Canton J, Neculai D, Grinstein S. Scavenger receptors in homeostasis and immunity. *Nat Rev*
408 *Immunol* **2013**; 13:621-34.

409 46. Zhi Y, Gao P, Xin X, et al. Clinical significance of sCD163 and its possible role in asthma. *Mol Med*
410 *Rep* **2017**; 15:2931-9.

- 411 47. Knudsen TB, Ertner G, Petersen J, et al. Plasma soluble CD163 level independently predicts all
412 cause mortality in HIV-1–infected individuals. *J Infect Dis* **2016**; 214:1198-204.
- 413 48. Kirkegaard-Klitbo DM, Mejer N, Knudsen TB, et al. Soluble CD163 predicts incident chronic lung,
414 kidney and liver disease in HIV infection. *AIDS* **2017**; 31:981-8.
- 415 49. Weikel J, Löchelt M, Truyen U. Demonstration of feline foamy virus in experimentally infected
416 cats by immunohistochemistry. *J Vet Med* **2003**; 50:415-7.
- 417

418 Figure legends

419 **Figure 1. Major lymphocyte subsets among cases and controls.** Frozen PBMCs from cases and controls
420 were stained with a viability marker and antibodies defining major lymphocyte subsets. Results are
421 expressed as the percentage of viable lymphocytes. Wilcoxon signed-rank tests were used to compare
422 cases and controls and *P* values are indicated above the graphs. A: T lymphocytes (CD3⁺), CD4 T
423 lymphocytes (CD3⁺CD4⁺), CD8 T lymphocytes (CD3⁺CD8⁺), and CD4/CD8 ratio; B: $\gamma\delta$ T lymphocytes
424 (CD3⁺TCR $\gamma\delta$ ⁺), B lymphocytes (CD19⁺), and NK lymphocytes (CD3⁻CD16⁺and/orCD56⁺).

425 **Figure 2. T-lymphocyte differentiation among cases and controls.** Frozen PBMCs from cases and
426 controls were stained with a viability marker and antibodies defining T lymphocytes (CD3, CD4, and
427 CD8) and their differentiation phenotype. Results are expressed as the percentage among viable CD4
428 or CD8 T lymphocytes. Wilcoxon signed-rank tests were used to compare cases and controls and *P*
429 values are indicated above the graphs. Naive T cells (T_N) were defined as CD45RA⁺CCR7⁺, CD4 recent
430 thymic emigrant (T_{RTE}) as CD45RA⁺CCR7⁺CD31⁺, central memory (T_{CM}) as CD45RA⁻CCR7⁺, effector
431 memory (T_{EM}) as CD45RA⁻CCR7⁻, and effector (T_E) as CD45RA⁺CCR7⁻. CD4 T_{EM} were further defined by
432 expression of the CD27 molecule, and CD8 T_{EM} by the expression of the CD27 and CD28 molecules. A:
433 CD4 T lymphocytes, B: CD8 T lymphocytes.

434 **Figure 3. T-lymphocyte phenotype and regulatory T cells among cases and controls.** Frozen PBMCs
435 from cases and controls were stained with a viability marker and antibodies defining T lymphocytes
436 (CD3, CD4 and CD8) and their phenotype: PD-1 (CD279), CD57, CD127, HLA-DR, and CD38. Results are
437 expressed as the percentage of viable memory (non CD45RA⁺CCR7⁺) CD4 or CD8 T lymphocytes. Results
438 for CD4 T_{REG} (CD25⁺CD127⁻) are expressed as the percentage among viable CD4 T lymphocytes.
439 Wilcoxon signed-rank tests were used to compare cases and controls and *P* values are indicated above
440 the graphs. A: CD4 T lymphocytes, B: CD8 T lymphocytes.

441 **Figure 4. Plasma molecules associated with myeloid cells among cases and controls.** Frozen plasma
442 samples were used to quantify plasma analytes using multiplex bead-based assays or ELISA. Results
443 are presented as pg/mL, except for sCD14 and sCD163, for which the levels are expressed as μ g/mL.
444 Wilcoxon signed-rank tests were used to compare cases and controls and *P* values are indicated above
445 the graphs.

446

1 **Table 1. Characteristics of study participants**

	Cases	Controls	<i>P</i> ^a
Ethnicity: Bantus/Pygmies	4/11	4/11	1.00
Age at sampling, years	45 [40-68]	50 [39-58]	0.02
Duration of infection, years ^b	14 [12-37]	--	--
HTLV-1 infection, Yes/No	6/9	3/12	0.43

2 Abbreviations: Interquartile range, IQR; Human T lymphotropic virus type 1, HTLV-1.

3 ^aCounts or median [IQR] are indicated; Fisher's exact test and Wilcoxon signed-rank tests were used to
 4 compare cases and controls. ^bThe duration of infection in the case group was estimated as the time
 5 between the reported date of the wound inflicted by the gorilla and the sampling date.

6

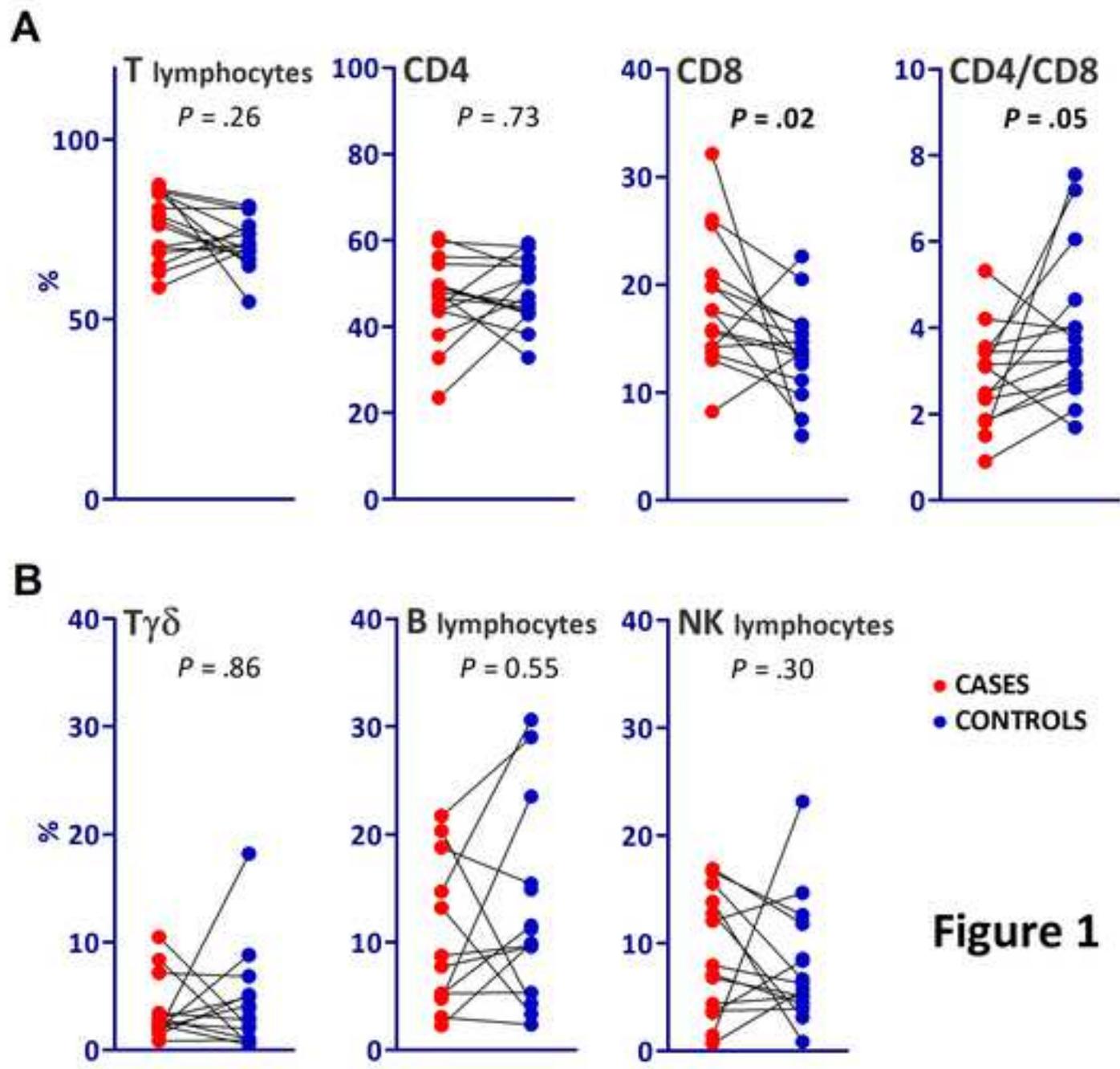
1 **Table 2. Immune parameters that differ between cases and controls are not correlated with**
 2 **parameters of SFV infection and hematological variables.**

	Group	CD8% ^a	CD4/CD8	CD4 T _{RTE}	PD-1 ⁺ CD4 T _M	sCD163
CD8%	Cases	-.835***				
	Controls	-.693**				
CD4/CD8	Cases	-.111	.233			
	Controls	.111	.104			
CD4 TRTE	Cases	.027	-.283	-.036		
	Controls	.196	.007	-.139		
PD-1+CD4 T _M	Cases	-.297	.207	.130	.182	
	Controls	.039	-.050	.269	.000	
Duration of infection	Cases	.139	-.142	-.360	.320	.009
SFV DNA	Cases	.050	-.316	-.168	-.417	.218
Age	Cases	.150	-.288	-.154	.091	.037
	Controls	-.307	.041	-.200	-.433	.160
Hemoglobin	Cases	.108	-.008	.083	-.400	.523
	Controls	.307	-.222	.222	-.031	.052
Urea	Cases	.054	-.169	.204	.633	.137
	Controls	-.366	.470	.045	-.375	.061
Creatinine	Cases	.327	-.252	.128	.092	-.231
	Controls	.044	.013	.118	.179	.387
LDH	Cases	-.041	.146	-.017	.383	-.105
	Controls	.250	-.286	.243	.103	-.214

3

4 ^aFive immune parameters differed significantly between cases and controls. CD8%: percentage of CD8
 5 T lymphocytes among lymphocytes; CD4/CD8: ratio of CD4 and CD8 T lymphocytes; CD4 T_{RTE}:
 6 percentage of CD31⁺CD45RA⁺CCR7⁺ recent thymic emigrants among CD4 T lymphocytes; PD-1⁺CD4T_M:
 7 percentage of PD-1⁺ cells among memory (nonCD45RA⁺CCR7⁺) CD4 T lymphocytes; sCD163: plasma
 8 level of soluble CD163 (µg/mL). ^bSpearman's rank test was used to assess correlations between
 9 parameters. Spearman's correlation coefficients are indicated. ** $P < .01$; *** $P < .001$.

10



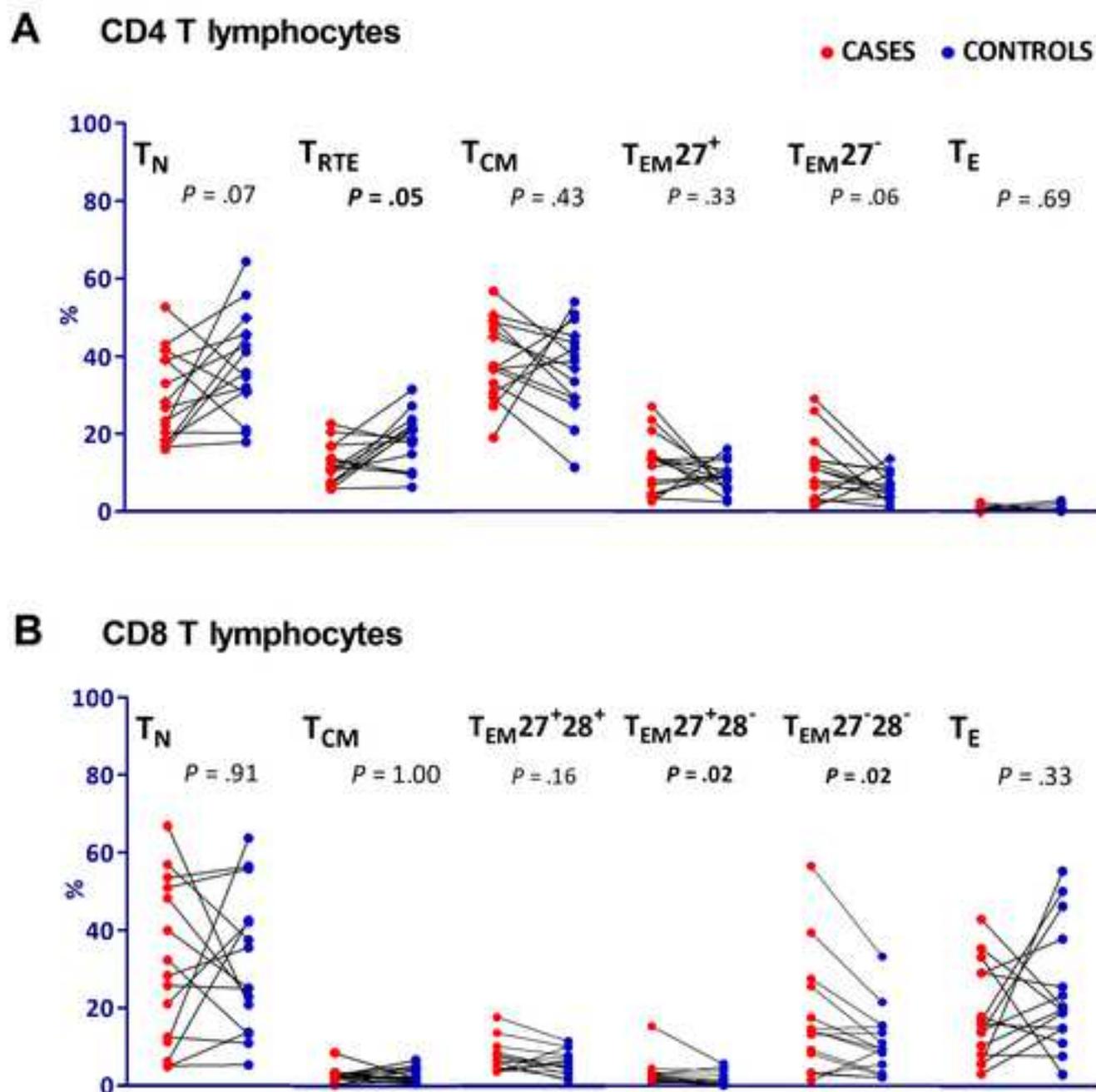
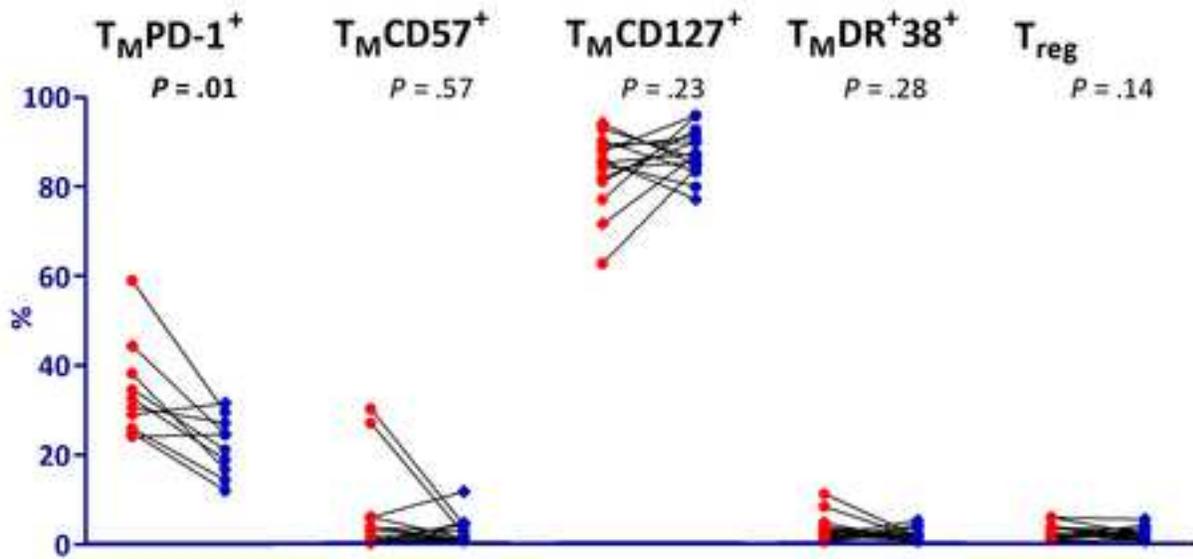
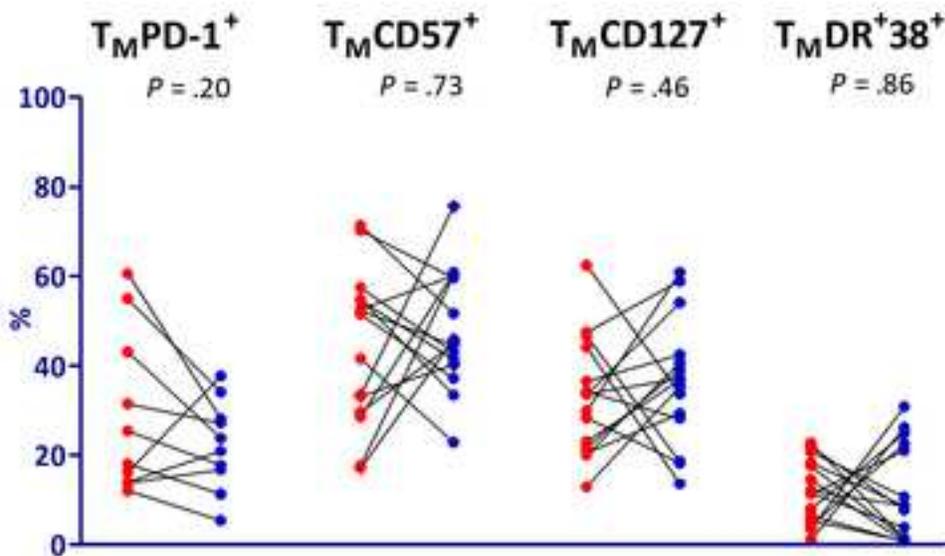
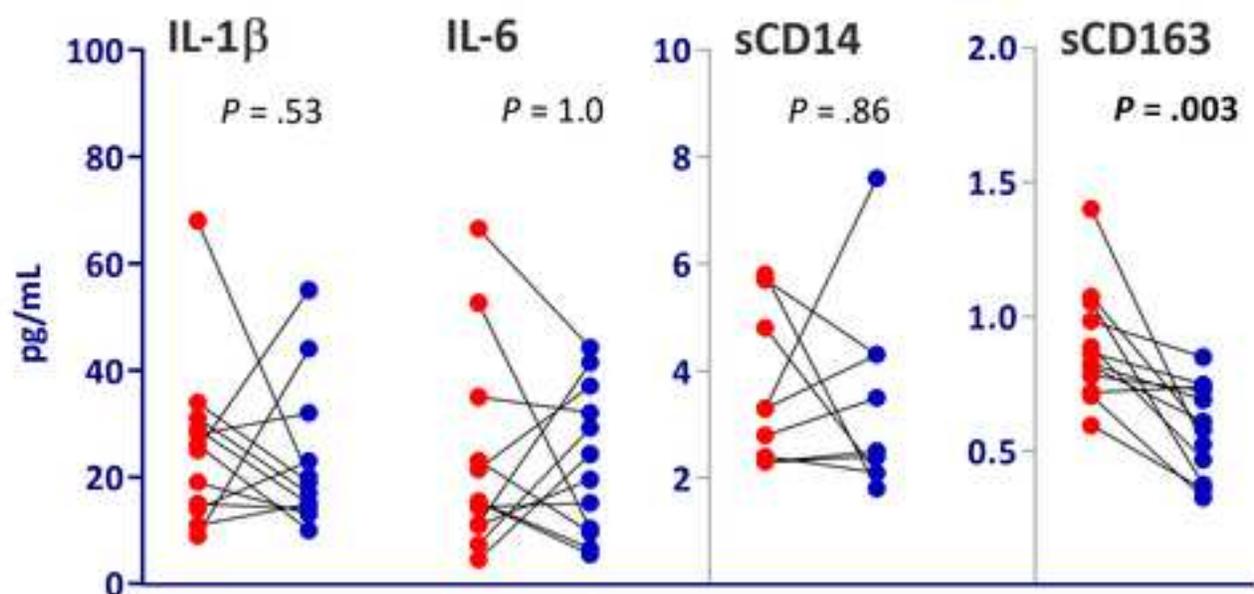
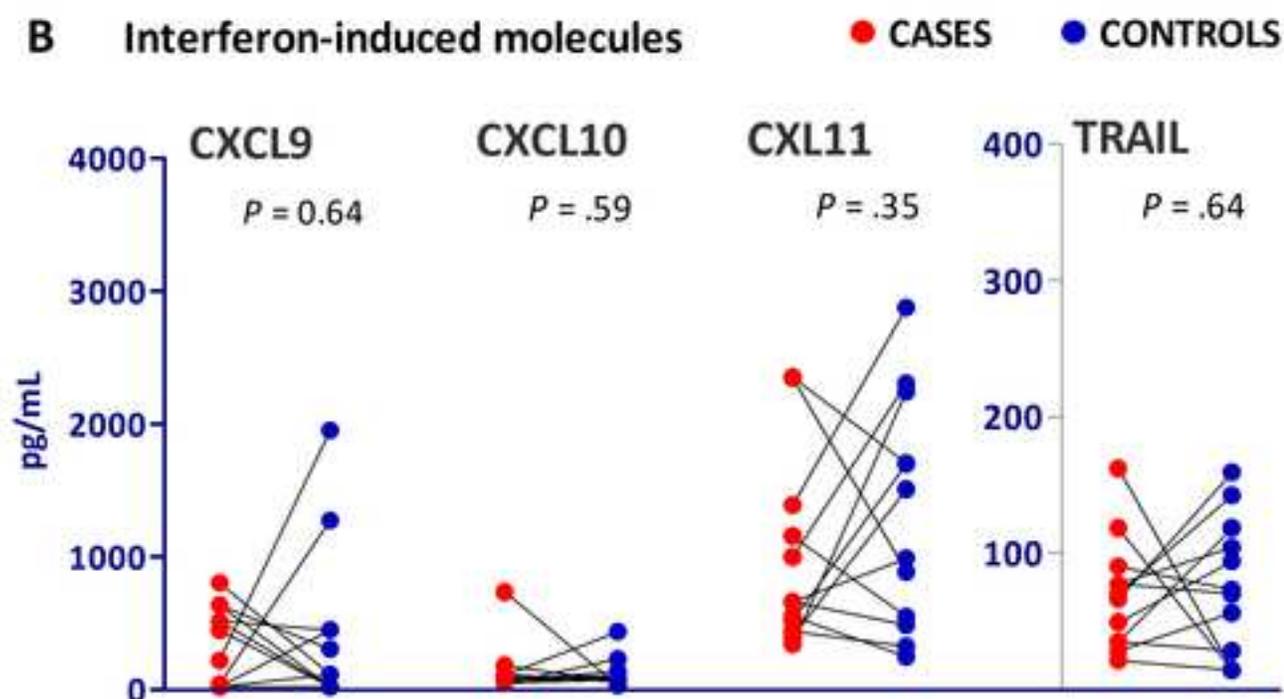


Figure 2

A CD4 T lymphocytes

● CASES ● CONTROLS

**B** CD8 T lymphocytes**Figure 3**

A Inflammation and monocyte activation**B Interferon-induced molecules****Figure 4**

Supplementary Tables 1 to 3.

1

2

3 **Supplementary table 1. T, B, and NK lymphocytes subsets among cases and controls**

Lymphocyte subsets	Cases ^a	Controls ^a	<i>P</i> ^a
T lymphocytes ^b	76.2 [66.4;85.1]	69.4 [66.2;75.9]	0.26
CD4 T lymphocytes ^b	46.6 [43.6;49.5]	46.9 [42.9;53.4]	0.73
CD8 T lymphocytes ^b	17.6 [14.1;20.9]	13.3 [11.1;15.4]	0.02
CD4/CD8 ratio	2.5 [1.9;3.4]	3.5 [2.9;4.7]	0.05
γδ T lymphocytes ^b	2.5 [2.0;3.4]	2.8 [1.0;5.1]	0.86
Vδ1 ⁺ T lymphocytes ^c	63.6 [53.0;73.6]	67.2 [40.2;85.7]	0.75
Vδ2 ⁺ T lymphocytes ^c	24.1 [9.8;35.1]	30.4 [11.8;39.8]	0.70
NK lymphocytes ^b	9.6 [3.7;15.6]	6.5 [3.9;12.6]	0.30
CD56 ^{brigh} NK lymphocytes ^c	1.3 [0.6;3.3]	1.4 [0.5;2.4]	0.55
CD16 ⁺ CD56 ⁺ NK lymphocytes ^c	70.1 [61.3;77.4]	57.5 [36.1;77.9]	0.27
CD16 ⁺ CD56 ⁻ NK lymphocytes ^c	26.6 [19.7;37.9]	40.1 [21.0;61.7]	0.20
B lymphocytes ^b	12.3 [5.2;18.8]	10.5 [9.6;23.5]	0.55
CD21 ⁺ CD27 ⁻ naive B lymphocytes ^c	29.7 [22.9;39.8]	39.1 [24.3;51.1]	0.55
CD10 ⁺ immature B lymphocytes ^c	1.3 [0.9;2.1]	1.6 [1.0;2.4]	0.86
CD21 ⁺ CD27 ⁺ resting memory B lymphocytes ^c	11.0 [8.9;15.3]	10.0 [9.4;12.8]	0.65
CD21 ^{lo} CD27 ⁺ activated mature memory B lymphocytes ^c	8.8 [3.3;11.6]	5.0 [1.5;9.8]	0.65
CD21 ^{lo} CD20 ^{hi} CD27 ⁻ tissue like memory B lymphocytes ^c	50.4 [32.7;54.3]	43.1 [34.0;56.8]	0.75
CD20 ⁻ CD38 ⁺ plasmablasts ^c	16.6 [6.2;22.9]	20.2 [11.5;25.9]	0.25

4 ^aMedians [interquartile range] are indicated; the Wilcoxon signed rank test was used to compare cases
5 and controls. ^bexpressed as the percentage among viable lymphocytes. ^cexpressed as the percentage
6 among γδ, B, or NK lymphocytes.

7

8 Supplementary table 2. T-lymphocyte subsets among cases and controls

Variable ^a	Phenotype	Expressed as % among	n	Cases ^b	Controls ^b	P ^b
CD4 T _N	CD45RA+CCR7+	CD4	15	26.8 [18.4;39.1]	35.8 [30.6;45.7]	0.07
CD4 T_{RTE}	CD45RA+CCR7+CD31+	CD4	15	10.9 [7.0;16.6]	18.6 [10.0;22.5]	0.05
CD4 T _{CM}	CD45RA-CCR7+	CD4	15	34.1 [27.9;41.5]	34.9 [27.2;42.5]	0.36
CD4 T _{TM}	CD45RA-CCR7-CD27+	CD4	15	14.1 [5.0;16.1]	9.7 [6.3;11.5]	0.33
CD4 T _{EM} CD28+	CD45RA-CCR7-CD27-CD28+	CD4	15	6.7 [3.4;9.6]	4.4 [3.2;9.0]	0.26
CD4 T _{EM} CD28-	CD45RA-CCR7-CD27-CD28-	CD4	15	2.22 [0.30;4.37]	1.42 [0.54;2.47]	0.26
CD4 T _{EM} CD28 ⁺ CD57+	CD45RA-CCR7-CD27-CD28-CD57+	CD4	15	0.93 [0.21;3.11]	0.53 [0.33;1.64]	0.31
CD4 T _E	CD45RA+CCR7-CD27-CD28-	CD4	15	0.47 [0.17;0.99]	0.49 [0.20;0.91]	0.69
CD8 T _N	CD45RA+CCR7+	CD8	15	28.3 [11.3;51.0]	25.1 [13.7;42.6]	0.91
CD8 T _{CM}	CD45RA-CCR7+	CD8	15	2.8 [2.0;3.3]	2.2 [1.5;4.4]	1.00
CD8 T _{TM} CD28+	CD45RA-CCR7-CD27+CD28+	CD8	15	6.9 [4.7;9.8]	6.6 [4.1;8.8]	0.16
CD8 T_{TM}CD28-	CD45RA-CCR7-CD27+CD28-	CD8	15	2.45 [1.21;3.42]	0.89 [0.39;3.12]	0.02
CD8 T _{TM} CD28-CD57+	CD45RA-CCR7-CD27+CD28-CD57+	CD8	15	1.37 [0.54;1.94]	0.30 [0.05;1.05]	0.10
CD8 T_{EM}CD28-	CD45RA-CCR7-CD27-CD28-	CD8	15	16.1 [4.0;29.2]	10.8 [3.6;17.4]	0.02
CD8 T_{EM}CD28-CD57+	CD45RA-CCR7-CD27-CD28-CD57+	CD8	15	13.4 [3.5;23.6]	8.9 [2.9;14.3]	0.01
CD8 T _E	CD45RA+CCR7-CD27-CD28-	CD8	15	16.1 [8.3;29.2]	19.8 [14.9;37.9]	0.33
CD8 T _E CD57+	CD45RA+CCR7-CD27-CD28-CD57+	CD8	15	13.0 [5.5;22.8]	15.3 [9.7;21.8]	0.50
CD4 T _M DR+38+	Non(CD45RA+CCR7+)HLA-DR+CD38+	CD4 T _M	15	2.5 [1.2;3.6]	1.4 [0.6;2.4]	0.28
CD8 T _M DR+38+	Non(CD45RA+CCR7+)HLA-DR+CD38+	CD8 T _M	15	11.5 [5.3;18.2]	8.6 [1.3;22.5]	0.86
CD4 T _M CD127+	Non(CD45RA+CCR7+)CD127+	CD4 T _M	15	85.0 [80.8;89.1]	86.6 [83.6;90.9]	0.23
CD8 T _M CD127+	Non(CD45RA+CCR7+)CD127+	CD8 T _M	15	33.5 [21.5;44.0]	36.7 [28.1;42.3]	0.46
CD4 T _{REG}	CD25+CD127-	CD4	15	2.5 [1.5;3.7]	1.9 [0.9;3.1]	0.14
T _N	CD45RA+CCR7+	CD4 T _{REG}	15	33.0 [26.2;53.7]	45.4 [39.9;59.4]	0.13
T _{CM}	CD45RA-CCR7+	CD4 T _{REG}	15	12.3 [8.5;25.0]	9.1 [5.5;13.7]	0.17
T _{EM}	CD45RA-CCR7-	CD4 T _{REG}	15	34.4 [25.7;39.4]	27.4 [19.8;34.7]	0.19
T _E	CD45RA+CCR7-	CD4 T _{REG}	15	0.7 [0.4;2.1]	1.4 [0.5;2.5]	0.36
T _{REG} DR+38+	HLA-DR+CD38+	CD4 T _{REG}	15	5.4 [2.2;10.9]	3.1 [1.9;5.5]	0.09
CD4 T_M PD1+	Non(CD45RA+CCR7+)CD279+	CD4 T_M	10	31.7 [25.9;38.2]	23.0 [16.8;27.2]	0.01
CD4 T_{CM} PD1+	CD45RA-CCR7+CD279+	CD4 T_{CM}	10	19.0 [14.6;23.2]	12.7 [7.9;17.7]	0.04
CD4 T _{EM} PD1+	CD45RA-CCR7-CD279+	CD4 T _{EM}	10	38.8 [33.6;44.9]	31.7 [28.6;33.8]	0.06
CD8 T _M PD1+	Non(CD45RA+CCR7+)CD279+	CD8 T _M	10	21.8 [14.1;43.1]	22.4 [16.8;28.1]	0.17
CD8 T _{CM} CD28+PD1+	CD45RA-CCR7+CD28+CD279+	CD8 T _{CM}	10	9.0 [7.1;17.2]	11.6 [11.1;12.3]	0.58
CD8 T _{EM} CD28+PD1+	CD45RA-CCR7-CD28+CD279+	CD8 T _{EM}	10	14.7 [11.2;18.8]	10.8 [8.0;17.2]	0.24
CD8 T _{EM} CD28-PD1+	CD45RA-CCR7-CD28-CD279+	CD8 T _{EM}	10	16.1 [10.1;21.1]	17.9 [9.6;28.1]	0.51
CD8 T _E PD1+	CD45RA-CCR7-CD28-CD279+	CD8 T _E	10	11.2 [6.4;30.3]	16.9 [9.7;23.1]	0.80
CD4 T _M CD57+	Non(CD45RA+CCR7+)CD57+	CD4 T _M	15	1.8 [0.4;5.8]	1.2 [0.9;4.0]	0.57
CD8 T _M CD57+	Non(CD45RA+CCR7+)CD57+	CD8 T _M	15	51.5 [29.6;54.9]	44.8 [40.1;60.2]	0.73
CD4 Th1	Non(CD45RA+CCR7+)CXCR3+CCR4-CCR6-	CD4 T _M	13	1.5 [0.6;3.7]	1.3 [0.5;1.9]	0.55
CD4 Th2	Non(CD45RA+CCR7+)CXCR3-CCR4+CCR6-	CD4 T _M	13	5.4 [1.2;12.0]	2.8 [1.0;7.4]	0.20
CD4 Th17	Non(CD45RA+CCR7+)CXCR3-CCR4+CCR6-	CD4 T _M	13	21.3 [16.9;23.8]	20.8 [15.7;24.2]	0.81
CD4 Th1/2	Non(CD45RA+CCR7+)CXCR3+CCR4+CCR6-	CD4 T _M	13	0.1 [0.0;0.2]	0.0 [0.0;0.1]	0.35
CD4 Th1/17	Non(CD45RA+CCR7+)CXCR3+CCR4-CCR6+	CD4 T _M	13	0.3 [0.1;0.9]	0.1 [0.1;0.2]	0.46

CD4 Th2/17	Non(CD45RA+CCR7+)CXCR3-CCR4+CCR6+	CD4 T _M	13	2.5 [0.7;6.3]	1.4 [0.5;4.6]	0.25
CD8 T _{CM} Tc1	CD45RA-CCR7+CXCR3+CCR4-CCR6-	CD8 T _{CM}	13	11.7 [1.4;18.0]	5.1 [4.4;16.4]	0.42
CD8 T _{EM} Tc1	CD45RA-CCR7-CXCR3+CCR4-CCR6-	CD8 T _{EM}	13	5.2 [0.8;8.1]	2.2 [1.0;2.9]	0.55
CD8 T _E Tc1	CD45RA+CCR7-CXCR3+CCR4-CCR6-	CD8 T _E	13	3.0 [1.3;8.7]	2.0 [0.8;4.0]	0.35
CD8 T _{EM} Tc1/17	CD45RA-CCR7-CXCR3-CCR4-CCR6+	CD8 T _{EM}	13	7.2 [4.4;11.0]	5.6 [4.2;9.1]	0.51
CD8 T _{CM} Tc1/17	CD45RA-CCR7+CXCR3+CCR4-CCR6+	CD8 T _{CM}	13	2.5 [1.9;3.6]	1.4 [0.7;1.8]	0.11
CD8 T_{EM}Tc1/17	CD45RA-CCR7-CXCR3+CCR4-CCR6+	CD8 T_{EM}	13	0.7 [0.5;1.2]	0.4 [0.1;0.6]	0.02
CD4 CD161+	CD161+	CD4	14	12.9 [10.9;18.0]	10.4 [8.8;13.2]	0.05
CD8αβ CD161+	CD8α+CD8β+CD161+	CD8	14	6.0 [2.2;9.0]	3.1 [2.2;7.3]	0.73
CD8αα CD161+	CD8α+CD8β-CD161+	CD8	14	31.3 [12.6;39.6]	19.8 [4.4;54.2]	0.88
CD4 T _M α4β7+	Non(CD45RA+CCR7+)CD49d+integrinβ7+	CD4 T _M	11	19.0 [12.2;22.7]	15.1 [12.5;20.4]	0.42
CD8 T _M α4β7+	Non(CD45RA+CCR7+)CD49d+integrinβ7+	CD8 T _M	11	31.0 [21.7;38.7]	30.6 [19.6;48.0]	0.66

9

10 Abbreviations: CM, central memory; EM, effector memory; E, effector; M, memory, N, naive; Tc, T
 11 cytotoxic; Th, T helper; T_M, memory.

12 ^aFrozen PBMCs from cases and controls were stained with a viability marker and antibodies defining T
 13 lymphocyte phenotypes. The distinction between CD27⁺ (T_{TM}) and CD27⁻ (T_{EM}) CD45RA⁻CCR7⁻ T
 14 lymphocytes was made when anti-CD27 antibodies were present in the mix, otherwise T_{EM} refers to
 15 CD45RA⁻CCR7⁻ T lymphocytes. When a molecule was expressed at comparable levels on T_{CM}, T_{EM}, and
 16 T_E, we merged the 3 subsets into a single one (T_M) for data presentation and analysis.

17 ^bMedians [interquartile ranges] are indicated; the Wilcoxon signed rank test was used to compare
 18 cases and controls.

19

20 **Supplementary table 3. Plasma analytes among cases and controls**

Plasma analyte	Cases ^a	Controls ^a	<i>P</i> ^a
IFN- γ , pg/mL	122 [83;157]	123 [76;181]	0.78
IL-10, pg/mL	164 [60;290]	163 [66;179]	0.69
IL-12, pg/mL	68 [44;92]	47 [28;90]	0.39
IL-17, pg/mL	63 [53;103]	80 [52;95]	0.94
IL-2, pg/mL	17 [12;22]	15 [11;18]	0.18
IL-21, pg/mL	388 [172;606]	344 [267;578]	0.91
IL-23, ng/mL	1.9 [1.8;9.9]	2.3 [1.1;3.3]	0.24
IL-7, pg/mL	64 [39;83]	70 [41;94]	0.53
IL-8, pg/mL	11 [7;20]	18 [10;26]	0.06
GM-CSF, pg/mL	64 [47;101]	46 [38;62]	0.04
TNF- α , pg/mL	42 [34;66]	45 [32;56]	0.22
CCL3, pg/mL	68 [45;102]	65 [46;70]	0.11
CCL4, pg/mL	35 [29;44]	46 [38;61]	0.21
CCL20, pg/mL	691 [101;1 276]	451 [44;795]	0.08
IL-4, pg/mL	415 [342;488]	453 [294;600]	0.53
IL-5, pg/mL	16 [8;45]	23 [13;50]	0.69
IL-13, pg/mL	30 [20;51]	40 [27;52]	0.69
CXCL13, pg/mL	29 [9;74]	33 [23;52]	0.91
BAFF, ng/mL	1.5 [1.2;1.7]	1.6 [1.2;1.9]	0.41
IL-1 β , pg/mL	26 [15;30]	18 [15;28]	0.53
IL-6, pg/mL	15 [11;29]	22 [10;34]	1.00
sCD14, μ g/mL	3.3 [2.4;4.8]	2.5 [2.1;4.3]	0.86
sCD163, μg/mL	0.84 [0.75;1.02]	0.59 [0.42;0.73]	0.003
CXCL9, pg/mL	449 [32;577]	115 [21;451]	0.64
CXCL10, pg/mL	76 [50;110]	83 [60;133]	0.59
CXCL11, pg/mL	651 [474;1 244]	1222 [513;1 928]	0.35
TRAIL, pg/mL	68 [35;84]	72 [21;111]	0.64

21

22 ^aMedians [interquartile range] are indicated; the Wilcoxon signed rank test was used to compare cases
 23 and controls.

24