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running title: Immunology of SFV infection

40 word summary: Zoonotic simian foamy viruses (SFVs) establish persistent infections in humans, for whom the long-term consequences for health are poorly described. We show, for the first time, that chronic infection with SFV is associated with T lymphocyte differentiation and monocyte activation.

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Abstract

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.

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

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%, $P = 0.01$), and higher plasma levels of the soluble CD163 scavenger receptor (0.84 vs 0.59 µg/mL, $P = 0.003$) than controls.

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

Keywords

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

Footnotes

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Introduction

Foamy viruses (FV) are complex retroviruses that infect several mammal species, including nonhuman primates (NHPs) [1, 2]. Humans are not natural hosts of FV. However, they are susceptible to infection by zoonotic simian FV (SFV) [2, 3]. Such cross-species transmission occurs mainly through bites [4, 5]. Viral exposure to several SFV species originating from apes and African and Asian monkeys leads to lifelong infection, demonstrated by the persistent detection of viral DNA in blood, isolation of replication-competent virus from blood or saliva, and high SFV-specific antibodies levels [4, 6-15].

Thus far, FV are considered to be apathogenic in natural, experimental, and accidental hosts, including humans [1, 2, 16]. However, we recently demonstrated hematological and biochemical alterations in SFV-infected humans relative to matched uninfected controls, including an increased prevalence of mild anemia and increased urea and creatinine blood levels [16]. Experimental inoculation of feline FV (FFV) to young, healthy, and specific pathogen-free cats leads to persistent infection, without clinical signs, over the first six months. FFV infection induces mild to moderate blood urea levels, an increased protein:creatinine ratio in urine, and histopathological and ultrastructural changes in the kidneys [17]. Overall, these data support a subclinical impact of FV on host physiology, which is still largely unexplored.

As any chronic viral infection, either actively replicating or latent/reactivating, SFV infection may affect the immune system, including pro- and anti-inflammatory processes and innate and adaptive immune cells [25]. Indeed, we have documented higher IgG levels in SFV-infected humans than in matched controls [16]. In addition, blood lymphocytes are infected *in vivo* by SFV [13, 26, 27]. Thus, SFV may affect immunity by inducing immune responses and replicating in immune cells.

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

For the first time, we report differences between SFV-infected and uninfected controls that provide information on the physiological consequences of zoonotic SFV infection for humans.

Materials and methods

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

Participants were Cameroonian men who had been injured by an NHP during hunting. Cases consisted of individuals infected with a gorilla SFV recruited from participants of our former survey [4, 16]. SFV infection was defined by positive results on both western blots (presence of the p70-p74 Gag doublet) and PCR assays (*integrase* gene and/or LTR) [4]. Each case was matched individually for age (± 10 years) and ethnicity with one non-SFV-infected control, recruited from hunters who participated in the same survey and who lived in the same or neighboring villages as the cases [16]. All participants tested seronegative for HIV-1 infection (LAV Blot1, Cat No. 72251, Biorad). Six cases and three controls tested seropositive for human T-cell leukemia virus type 1 (HTLV-1) infection (HTLV Blot 2.4, MP Diagnostics), no participants tested seropositive for HTLV-2. All participants of the study were apparently healthy at the time of the analysis.

Biological evaluations. Blood of the 30 participants was collected into tubes containing EDTA. Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation and stored in liquid nitrogen. Plasma samples were stored at -80°C. Blood tests were carried out by the medical analysis laboratory at the Centre Pasteur du Cameroun (CPC), in Yaoundé [16]. PBMCs were stained with Live-Dead-Aqua (Life Technologies, Saint-Aubin, France) and the following antibodies were used for phenotypic characterization of lymphocyte subsets: CD3-FITC, CD8-Alexa700 , CD16-V450, CD19-

PerCP-Cy5.5, CD27-V450, CD45RA-V450, CD56-PC7, CD57-APC, CD183(CXCR3)-Alexa 488, CD194(CCR4)-Alexa 647, CD196(CCR6)-PE, CD197(CCR7)-PC7, TCR γδ1-FITC, TCR Vδ2-PE (BD, Le Pont de Claix, France), CD3-ECD, CD8β-ECD, CD10-PE, CD20-ECD, CD21-FITC, CD24-APC, CD27-PE, CD28-FITC, CD28-PC5, CD31-FITC, CD38-PC5, CD127-PE, HLA-DR-FITC (Beckman Coulter, Villepinte, France), CD4-APC-eFluor780, CD8α-A700, CD25-APC, CD38-PC5, CD161-APC, CD279(PD-1)-PE, and integrin β7-FITC, (e-bioscience, Paris, France). Data were collected on an LSR II cytometer (BD Biosciences) and analyzed with Flow-Jo software (Treestar, Ashland, USA). Lymphocyte subset definitions are indicated in the figure legends and tables. Measurements of plasma analytes were performed using the human high-sensitivity T-cell 21-plex magnetic milliplex assay (HSTCMAG28PMX21BK, Merck Millipore, saint-Quentin en Yvelines, France), Human Magnetic Luminex Assay (for BAFF, CXCL9, CXCL10, CXCL13 and TRAIL, Biotechne, Lille, France), and the ELISA kits DY383 (sCD14) and DY1607 (sCD163) from Biotechne. The SFV DNA level in buffy coat was quantified by PCR at the time of inclusion in the survey [4].

Statistics. Wilcoxon signed-rank tests were used to compare the quantitative variables between cases and controls. Fisher's exact test was used to analyze qualitative variables. The Mann-Withney test was used to compare HTLV-1 seronegative (HTLV-1^{neg}) and seropositive (HTLV-1^{pos}) individuals. The power of statistical analyses was calculated for the variables that differed significantly between cases and controls with an α of 0.05 for a two-sided test. Spearman's rank test was used to assess correlations between quantitative parameters.

Results

Study participants

Participants were men living in rural areas of East and South Cameroon who reported injuries, mostly bites inflicted during hunting activities [16], and from whom cryopreserved mononuclear cells were available. The ages ranged from 22 to 75 years, with median values of 45 and 50 years for the cases

and controls, respectively (Table 1). The duration of SFV infection ranged from 1 to 45 years, and the median value was 14 years.

SFV-infected individuals have a higher percentage of CD8 T lymphocytes than controls

We used flow cytometry to quantify T, B, and NK lymphocytes and their major subsets (Figure 1). The cases had a significantly higher percentage of CD8 T lymphocytes and lower CD4/CD8 ratios than controls (median: 17.6% *vs.* 13.7%, $P = 0.03$ and 2.5 *vs.* 3.5, $P = 0.04$, respectively, Figure 1A). The statistical power of the test for the percentage of CD8 T lymphocytes and CD4/CD8 ratios were > 0.80 and > 0.60, respectively. The percentage of CD4 T lymphocytes was similar for cases and controls (46.6% *vs.* 46.9%, $P = 0.73$). The percentage of gamma-delta T (Tγδ), B, and NK lymphocytes also did 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.* 6.5%, $P = 0.30$, respectively, Figure 1B). Furthermore, the repartition of the major Tγδ, B, and NK lymphocyte subsets were similar in the two groups (Supplementary Table 1). In conclusion, SFV-infected men had an expanded CD8 T lymphocyte population relative to matched controls.

SFV-infected individuals have more highly differentiated CD4 and CD8 T lymphocytes than controls

We then used CD45RA, CCR7, CD31, CD27, CD28 molecules to define the phenotype of CD4 and CD8 T lymphocytes. CD4 and CD8 T lymphocytes were more highly differentiated in cases than in controls. Indeed, the percentage of naive (T_N) and recent thymic emigrants (T_{RTE}) among CD4 T lymphocytes were lower in cases than controls (Figure 2A). Conversely, differentiated $CD27^-$ effector memory (T_{EM}) cells represented a higher proportion of CD4 T lymphocytes in cases than controls. The differences were statistically significant for CD4 T_{RTE} (10.9% *vs.* 18.6%, $P = 0.05$, Power > .70). Among CD8 lymphocytes, we observed a significantly higher percentage of the two T_{EM} subsets lacking CD28 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, $P = 0.02$, Figure 2B). CD8 T_{EM} subset levels showed high interindividual variation and the power of these analyses was < 0.50.

We further defined the T-cell phenotype by the quantification of the CXCR3, CCR4, and CCR6 chemokine receptors, which reflect their polarization. We also assessed the expression of CD161 and α4β7 molecules which are markers of gut homing capacity. Cases had significantly higher levels of $CD161^+$ CD4 T lymphocytes and a higher proportion of $CXCR3^+CCR4^-CCR6^+$ (Th1/Th17) cells among their CD8 T_{EM} lymphocytes (supplementary Table 2). While the differences in levels of polarized T lymphocytes were modest, they were consistent with a more differentiated T-cell compartment in cases than in controls.

SFV-infected individuals express higher levels of check-point inhibitor, PD-1, on memory CD4 T lymphocytes than controls.

Then, molecules defining functional capacity (PD-1, CD57), homeostatic proliferation potential (CD127, the high affinity IL-7 receptor) and activation (HLA-DR, CD38) were quantified on memory CD4 and CD8 T lymphocytes. The percentage of $PD\text{-}1^+$ memory CD4 T lymphocytes ($PD\text{-}1^+CD4T_M$) was significantly higher in cases than controls (31.7% *vs.* 24.7%, $P = 0.01$, Figure 3A). The statistical power of the test was > 0.85. The differences were observed in both CD4 T_{CM} and T_{EM} subsets (Supplementary Table 2). In contrast, the percentage of $PD\text{-}1^+CD8T_M$ lymphocytes was similar for cases and controls (Figure 3B). The proportion of $CD57^+$ cells among total memory CD4 and CD8 T lymphocytes was similar in cases and controls (Figure 3). However, CD57 was expressed by the most highly differentiated $CD28^-T_{EM}$ and T_E lymphocytes. Indeed, the percentage of $CD57^+$CD8 T_{EM} among all CD8 T lymphocytes was higher in cases than controls (Supplementary Table 2), a finding consistent with the higher level of total $CD28^-$ $CD8T_{EM}$ in cases (Figure 2). Cases and controls expressed similar levels of CD127, HLA-DR, and CD38. We also quantified $CD25^{hi}CD127^+$ CD4 regulatory (T_{REG}) lymphocytes, which dampen immune activation. Their percentage (Figure 3A) and differentiation status (Supplementary Table 2) were similar in cases and controls. In conclusion, increased expression of PD-1 molecule, a check-point inhibitor, on CD4 T_M lymphocytes was the most striking difference between cases and controls.

SFV-infected individuals have higher plasma sCD163 levels than controls

We then quantified 27 cytokines, chemokines, and soluble immune mediators in plasma samples using multiplex or ELISA assays (Supplementary Table 3). Cases had significantly higher levels of GM-CSF than controls (64 *vs.* 46 pg/mL, $P = 0.04$) and tended to have lower IL-8 levels (11 *vs.* 18 pg/mL, $P = 0.06$). The statistical power was < 0.60 for these analyses. Cases and controls had similar levels of CCL3, CCL4, CCL20, IFN-γ, IL-2, IL-7, IL-10, IL-12, Il-17, IL-21, IL-23, and TNF-α. Cytokines and chemokines associated with B-lymphocyte function and activation, namely CXCL13, BAFF, IL-4, IL-5 and IL-13, were present at comparable levels in cases and controls.

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

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

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

Among cases, the five immune parameters were not associated with the duration of infection or SFV DNA load in blood cells. No immune parameter was associated with age in cases or controls. Complete blood counts and biochemistry were available for 13 cases and 15 controls [16]. The immune parameters were not associated with the haematological parameters that differ between the two groups, *i.e.* haemoglobin, urea, creatinine, and lactate dehydrogenase levels. In conclusion, cases and

controls had significantly different immune and haematological profiles, without significant correlations between the immune and haematological variables in either group.

Immune parameters and SFV infection: no obvious effect of age and viral coinfections

HTLV-1 is a possible confounder, as this infection is found more frequently in SFV-infected individuals than in matched controls [5]. In our study, 40% of cases and 20% of controls were seropositive for HTLV-1, and none were seropositive for HTLV-2. The percentage of PD-1^{+}CD4T$_{M}$ was significantly higher in HTLV-1^{pos} than in HTLV-1^{neg} participants, whereas the percentage of CD8 cells, the CD4/CD8 ratio, and CD4T$_{RTE}$ and sCD163 levels were similar in both groups (Table 3). Among HTLV-1^{neg} participants, we found trends or significant differences between cases and controls for the percentage of CD8 cells, the CD4/CD8 ratio, and CD4T$_{RTE}$, and sCD163 levels. The percentage of PD-1^{+}CD4T$_{M}$ was higher in cases than in controls, but the difference was not significant. Both the case and control were HTLV-1^{pos} for a single pair of participants, precluding further analysis. Overall, HTLV-1 coinfection cannot account for all the differences observed between cases and controls.

The immune parameters that differ between cases and controls can be modified by several other chronic viral infections and ageing. After exclusion of participants older than 65 years, differences in the percentage of CD8 cells and the CD4/CD8 ratio between cases and controls were observed but were not statistically significant. Differences in CD4T$_{RTE}$, PD-1^{+}CD4T$_{M}$, and sCD163 levels remained significant or closed to significance. CMV and HIV-1 are major drivers of immune activation and ageing. CMV seroprevalence is close to 100% in the Sub-Saharan African population [28] and all participants were seronegative for HIV-1 infection. All cases and controls were infected with HBV and two cases tested positive for HBs antigen [16]. Our recent nationwide survey showed an HCV prevalence of approximately 5% in men from the southern and eastern regions of Cameroon [29]. The HCV seroprevalence was 0.6% in Pygmies included in our epidemiological survey [30]. Among the eight Bantus included in the study, only one control was born before 1960 and thus at high risk for HCV infection through the iatrogenic route [31]. Furthermore, no liver function anomaly was shown by

blood analysis [16]. Overall, CMV, HIV-1, HBV, and HCV coinfection should not account for differences observed between cases and controls.

Discussion

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

Here, we show that SFV infection is associated with an increased percentage of blood CD8 T lymphocytes, a decreased CD4/CD8 ratio, a reduced proportion of naive CD4 T lymphocytes, and increased expression of the PD-1 molecule on memory CD4 T lymphocytes. These phenotypic changes are consistent with the T lymphocytes responding to a viral infection and overlap those induced by HIV-1, CMV, and ageing [32]. We also observed increased IgG levels, another marker of viral infection [16], in the same population of SFV-infected individuals. Experimental SFVmfa infection of macaques through transfusion leads to a sequential drop in the number of peripheral blood CD4 and CD8 T lymphocytes, followed by their restoration, with an elevated proportion of CD8 T lymphocytes [33]. Overall, these data indicate that chronic SFV infection is associated with phenotypic changes in T lymphocytes.

Our data need to be interpreted with caution, because biological markers that differ between SFV-infected and noninfected individuals may result from SFV infection or other health conditions. Here, we observed that differences in the percentage of CD8 cells and the CD4/CD8 ratio between cases and controls were less marked in the youngest participants than in the entire group (Table 3). Similarly, the differences in the percentage of PD-1^{+}CD4T_{M} were not significant in participants not infected by HTLV-1. Age and HTLV-1 infection may be true confounders or stratified analysis may lead to spurious results due to a small sample size. Importantly, neither age nor HTLV-1 infection modified the five immune parameters. HTLV-1 induces total CD4 and T_{REG}-cell expansion [34]. Here, we only observed CD8 T-cell

252 expansion. As we previously discussed [16], the similarity of clinical signs and leucocyte subset
253 distributions between SFV-infected individuals and controls support adequate matching for health
254 factors.

255 The observed T-lymphocyte phenotype could result from multiple causes, such as ongoing viral
256 replication, infection of immune cells, and coinfections. Direct proof of SFV replication *in vivo* (*i.e.* the
257 presence of SFV RNA, viral proteins, or virions) is currently lacking in humans. Two studies used
258 quantitative RT-PCR to detect SFV RNA in human blood and buccal samples and obtained negative
259 results [12, 35]. These data do not rule out active SFV replication in humans, which may occur at levels
260 below the detection threshold of conventional PCR-based assays or in tissues. Indeed, among patients
261 infected with HIV, HBV, and HCV, some have undetectable viral RNA in blood, despite replication in
262 tissues [36-38]. These situations of HIV control and occult HBV/HCV infection are associated with
263 potent virus-specific responses and a mild disease course relative to infections with detectable viral
264 RNA in blood. Despite apparent viral control, infected individuals show elevated inflammation and
265 some ultimately experience clinical disease or loss of viral control [36-38]. The present data and those
266 obtained on hematological parameters and specific antibodies challenge the proposed SFV latency in
267 humans, simians and felines [2, 14-17, 39].

268 FV establishes wide tissue tropism after natural or experimental infection in NHPs, cats, and cattle,
269 with consistent detection of viral DNA in blood and lymphoid tissues [17, 21, 40-42]. The separation of
270 lymphocyte subsets from infected individuals showed infection of B and T lymphocytes [13, 26, 27].
271 No human tissue samples have yet been analyzed. SFVs encode a viral transactivator, Tas, which acts
272 on cellular genes [43], and microRNAs that suppress innate immunity [44]. Therefore, SFV can infect
273 immune cells and directly alter their function, similarly to the two human pathogenic retroviruses, HIV-
274 1 and HTLV-1. Immune changes in SFV-infected individuals may be induced by coinfecting pathogens.
275 Indeed, interactions with other retroviruses have been demonstrated in animals [18-22]. Microbial
276 coinfections are a hallmark of infection with the potent immunosuppressive virus HIV-1 [45] and are

277 also common in HTLV-1-infected patients [34]. Whether direct or indirect consequences of SFV
278 infection are related to the immune changes reported here is an open question.

279 We found significantly elevated sCD163 levels in SFV-infected individuals relative to matched controls.
280 The differences were particularly robust, with high statistical power, despite the small size of the study
281 population. CD163 is a transmembrane protein that acts as the hemoglobin scavenger receptor [46].
282 It is expressed by monocytes and alternatively-activated M2 macrophages. These cells are involved in
283 tissue repair and mediate mostly anti-inflammatory functions [47]. Upon inflammatory macrophage
284 activation, the ectodomain is cleaved by matrix metalloproteinases and a soluble form, sCD163, is shed
285 into the plasma [48]. sCD163 is not involved in hemoglobin or iron metabolism.

286 Of eight plasma molecules related to myeloid-cell activation, sCD163 was the only one for which the
287 levels differed between SFV-infected individuals and controls. This may result from the activation of a
288 specific pathway or direct infection, as described above. Indeed, one regulatory FFV protein, Bet, was
289 detected by immunochemistry in the cytoplasm of macrophage-like cells from several lymphoid organs
290 (lymph nodes, thymus, tonsil, and spleens) from infected cats [49]. Overall, we document a novel
291 finding in SFV-infected humans, supporting monocyte/macrophage activation.

292 One limit of our study is that statistically significant associations do not prove causality. Although we
293 observed immune differences between SFV-infected individuals and carefully matched controls, we
294 cannot conclude that they are a direct consequence of SFV infection. However, the experimental
295 infection of cats supports the causal relationship between FV infection, persistent lymphocytic
296 infiltration in peripheral organs, and histological features in kidneys [17]. Importantly, the biological
297 findings in SFV-infected hunters are plausible consequences of viral infection.

298 Obtaining biological samples from hunters living in remote areas of the Cameroonian forest is
299 challenging. The number of participants and quantity of blood drawn are two limiting factors. For
300 example, persisting T-lymphocyte activation drives other conditions, such as anemia or chronic kidney
301 diseases. Correlations between viral parameters and host biomarkers were expected, but not

observed; this may result from insufficient power for statistical analyses or from the impact of other health conditions. Importantly, despite the modest size of our study population, we had sufficient statistical power to perform the analyses of several important parameters.

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

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424

Figure legends

Figure 1. Major lymphocyte subsets among cases and controls. Frozen PBMCs from cases and controls were stained with a viability marker and antibodies defining major lymphocyte subsets. Results are expressed as the percentage of viable lymphocytes. Wilcoxon signed-rank tests were used to compare cases and controls and *P* values are indicated above the graphs. A: T lymphocytes ($CD3^+$), CD4 T lymphocytes ($CD3^+CD4^+$), CD8 T lymphocytes ($CD3^+CD8^+$), and CD4/CD8 ratio; B: γδ T lymphocytes ($CD3^+TCR\gamma\delta^+$), B lymphocytes ($CD19^+$), and NK lymphocytes ($CD3^-CD16^+$and/or$CD56^+$).

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

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

Figure 4. Plasma molecules associated with myeloid cells among cases and controls. Frozen plasma samples were used to quantify plasma analytes using multiplex bead-based assays or ELISA. Results

450 are presented as pg/mL, except for sCD14 and sCD163, for which the levels are expressed as µg/mL.
451 Wilcoxon signed-rank tests were used to compare cases and controls and *P* values are indicated above
452 the graphs.

453

Table 1. Characteristics of study participants

	Cases	Controls	*P* [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

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

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

Table 2. Immune parameters that differ between cases and controls are not correlated with 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 TM	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

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

Table 3. Analyses stratified on the basis of HTLV-1 infection and age

	All [a]			HTLV-1^{neg} [b]			< 65 yrs [c]		
	HTLV-1^{neg}	HTLV-1^{pos}	*P*	Cases	Controls	*P*	Cases	Controls	*P*
CD8 T lymphocytes	15.6 [13.1;18.3]	14.1 [13;15.4]	0.56	18.3 [17.6;25.6]	13.5 [9.4;15.4]	0.09	16.6 [14.1;18.3]	13.8 [11.3;15.4]	0.20
CD4/CD8 ratio	3.2 [2.4;4.0]	3.1 [2.5;3.5]	0.94	2.4 [1.8;3.2]	3.5 [3.1;5.6]	0.09	3.1 [2.4;3.4]	3.6 [3.2;4.0]	0.33
CD4 T_{RTE}	16.6 [10.3;21.3]	10.4 [6.4;17.0]	0.15	10.9 [7.5;13.1]	19.9 [16.1;23.1]	0.06	12.0 [7.5;17.0]	19.7 [17.5;21.3]	0.07
CD4 T_M PD1$^+$	**24.8 [21.2;27.2]**	**32.2 [27.3;40.0]**	**0.03**	27.5 [25.6;30.7]	22.1 [14.4;3]	0.28	29.1 [25.6;34.7]	23.0 [14.4;27.2]	**0.05**
sCD163, µg/mL	0.71 [0.58;0.85]	0.72 [0.53;0.82]	0.94	**0.82 [0.70;0.97]**	**0.59 [0.52;0.74]**	**0.04**	0.80 [0.70;1.05]	0.55 [0.42;0.67]	**0.02**

Abbreviations: Interquartile range: IQR, human T lymphotropic virus type 1: HTLV-1, HTLV-1^{neg}: participants who tested seronegative for HTLV-1 infection, HTLV-1^{pos}: participants who tested seropositive for HTLV-1 infection. [a]n = 21 HTLV-1^{neg} and n = 9 HTLV-1^{pos}: participants were compared using the Mann-Whitney test, [b]cases and controls were compared using the Wilcoxon signed-rank test for the seven pairs in which both participants were seronegative for HTLV-1 infection, [c]cases and controls were compared using the Wilcoxon signed-rank test for the ten pairs in which both participants were less than 65 years old. Median [IQR] and *P* values are presented.

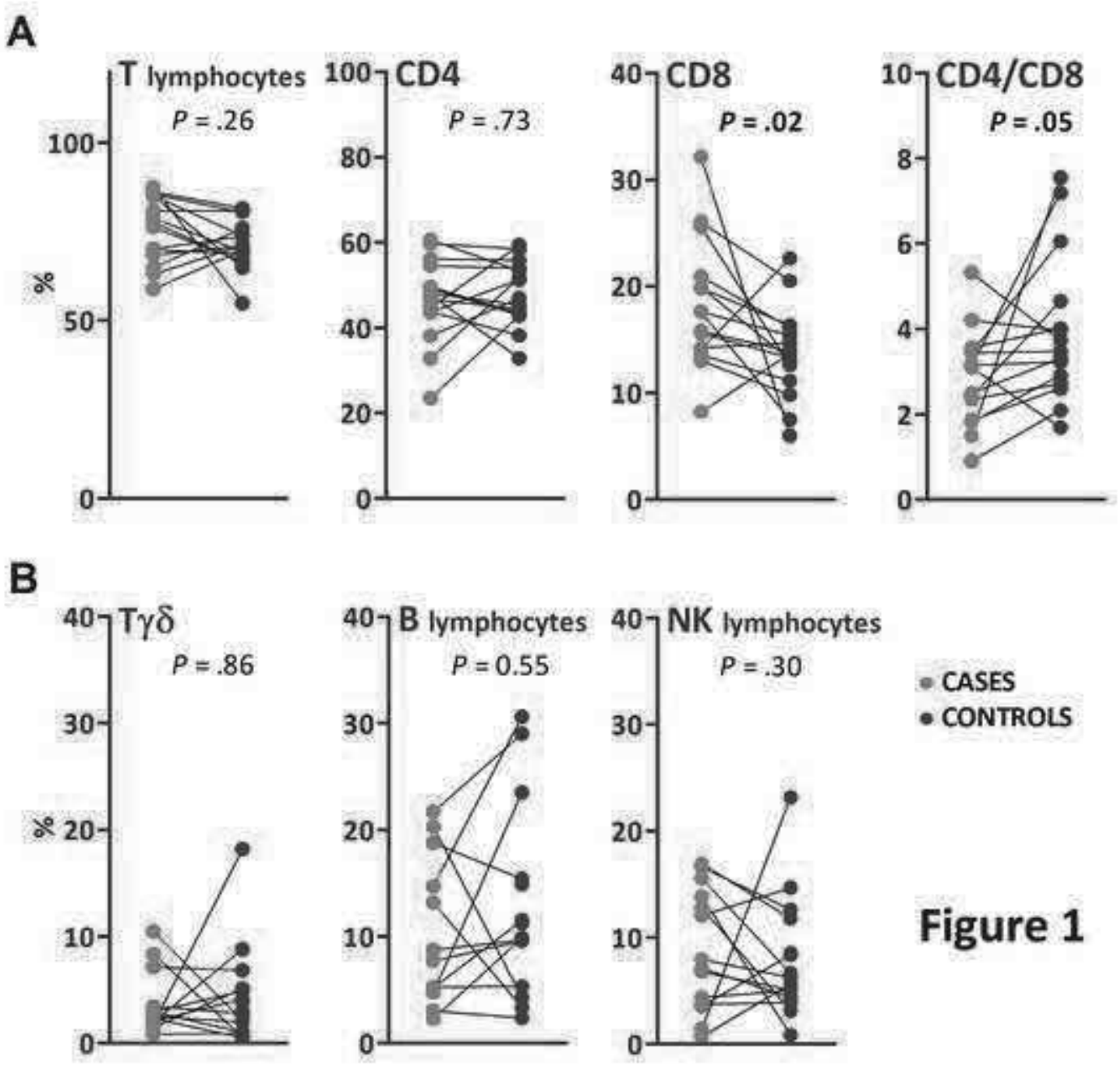


Figure 1

Figure

Click here to access/download;Figure;Gessain_Figure2.tif

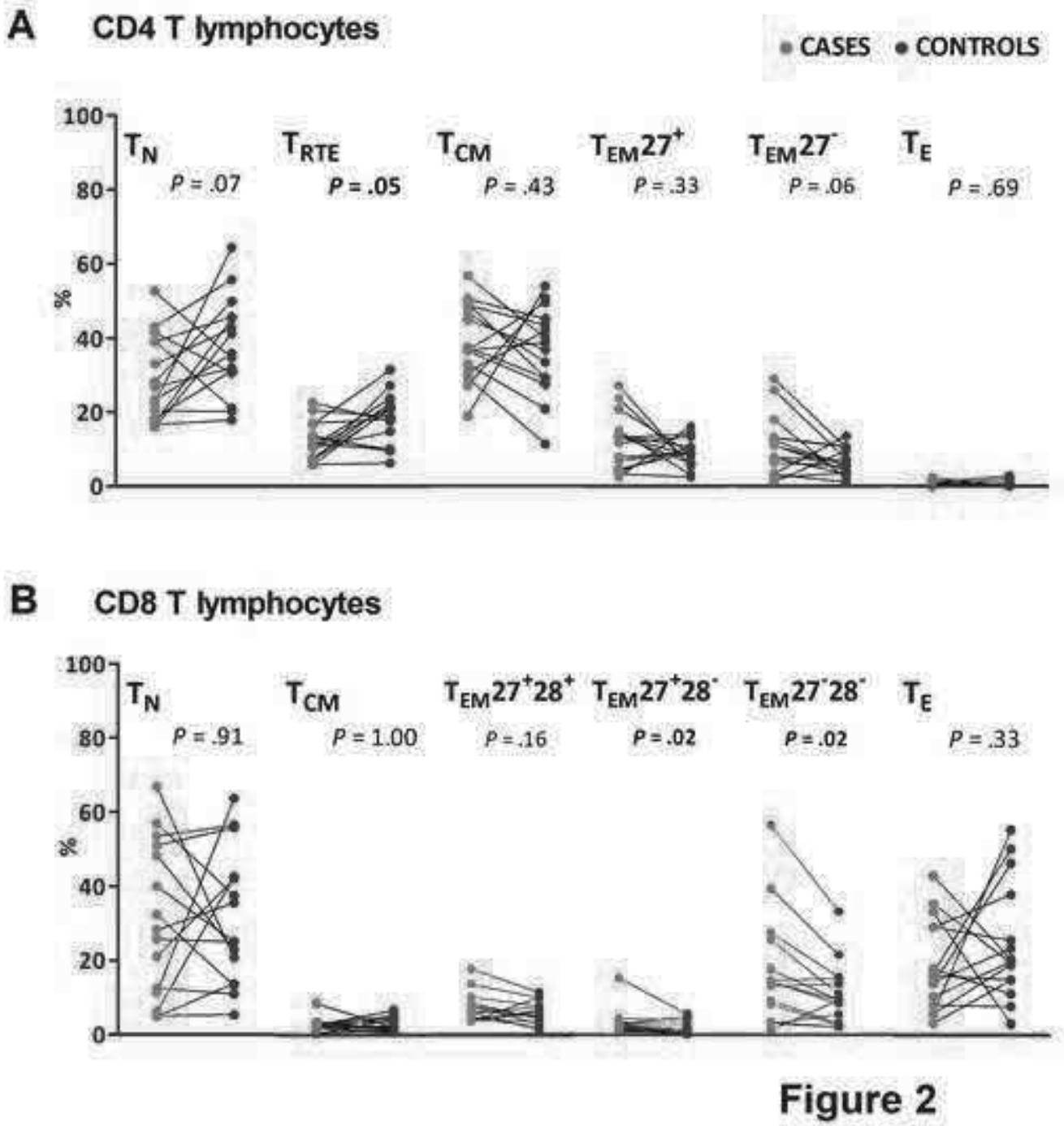
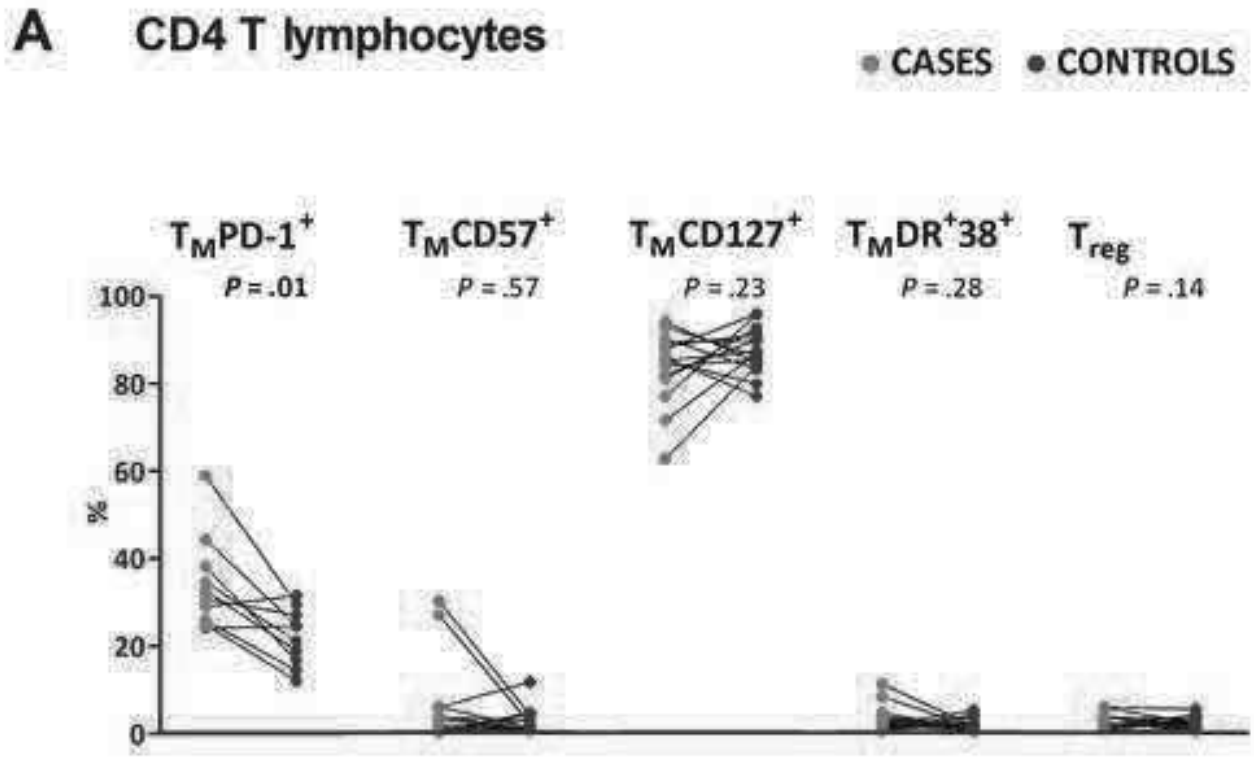


Figure 2


A
CD4 T lymphocytes
CASES
CONTROLS
$T_M PD\text{-}1^+$
P = .01
$T_M CD57^+$
P = .57
$T_M CD127^+$
P = .23
$T_M DR^+38^+$
P = .28
T_{reg}
P = .14
100
80
60
40
20
0
%

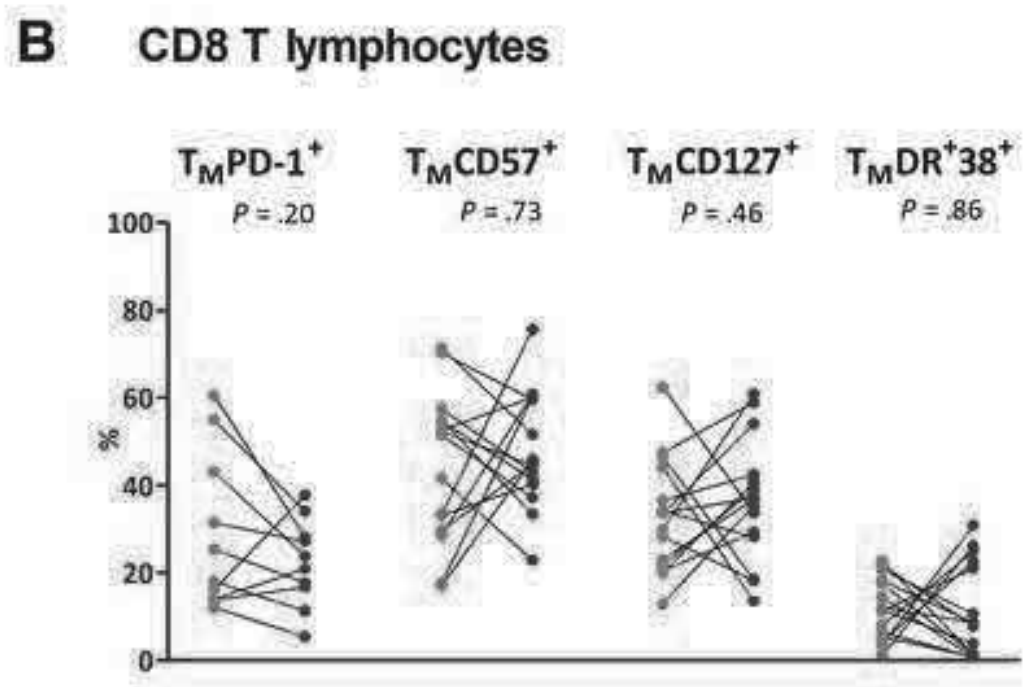

B
CD8 T lymphocytes
$T_M PD\text{-}1^+$
P = .20
$T_M CD57^+$
P = .73
$T_M CD127^+$
P = .46
$T_M DR^+38^+$
P = .86
100
80
60
40
20
0
%

Figure 3

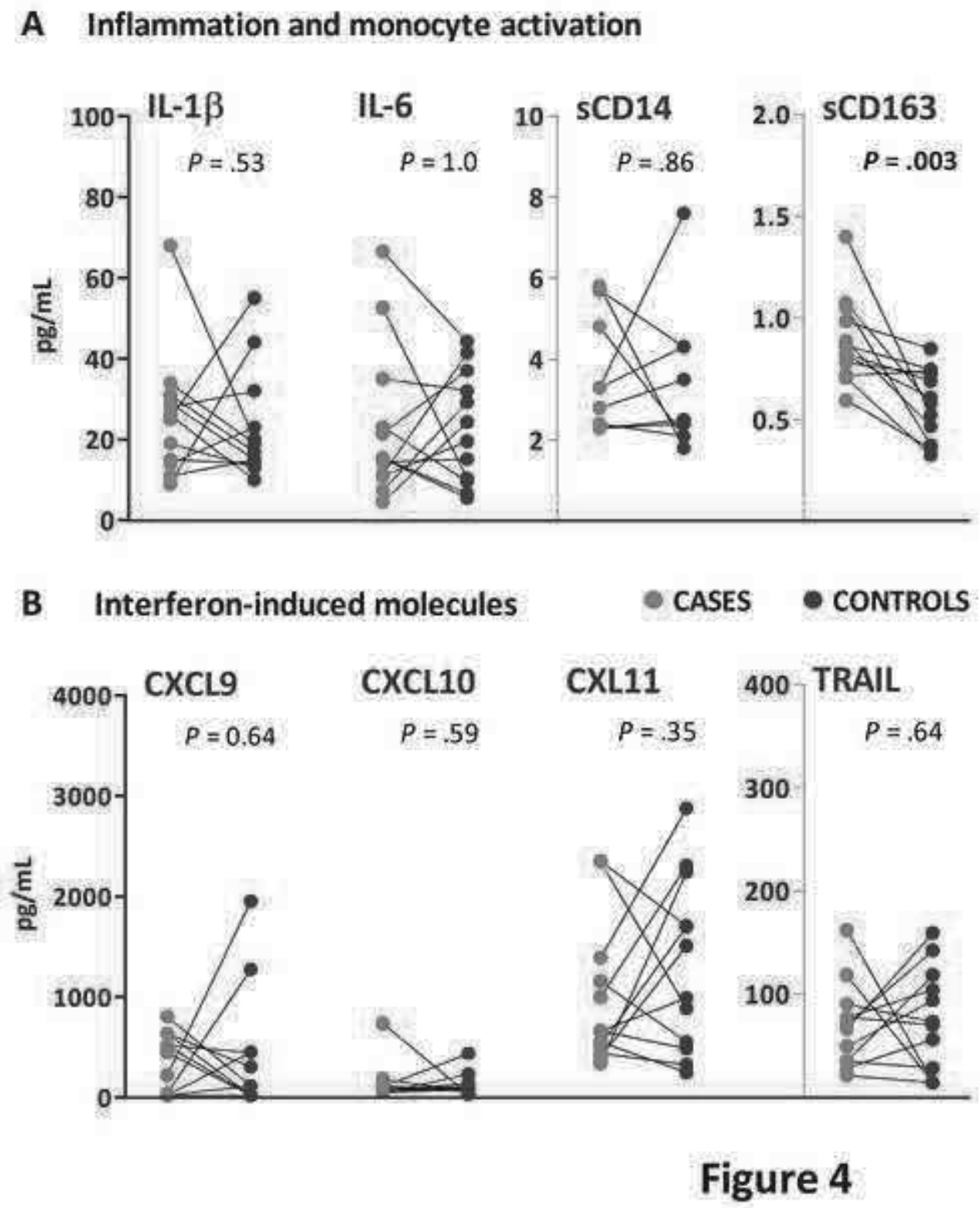

A Inflammation and monocyte activation
IL-1β
P = .53
IL-6
P = 1.0
sCD14
P = .86
sCD163
P = .003
pg/mL
100
80
60
40
20
0
10
8
6
4
2
2.0
1.5
1.0
0.5
B Interferon-induced molecules
CASES
CONTROLS
CXCL9
P = 0.64
CXCL10
P = .59
CXL11
P = .35
TRAIL
P = .64
pg/mL
4000
3000
2000
1000
0
400
300
200
100

Figure 4

Supplementary Tables 1 to 3.

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

Lymphocyte subsets	Cases[a]	Controls[a]	*P*[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
$V\delta1^+$ T lymphocytes [c]	63.6 [53.0;73.6]	67.2 [40.2;85.7]	0.75
$V\delta2^+$ 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
$CD56^{brigth}$ 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

[a]Medians [interquartile range] are indicated; the Wilcoxon signed rank test was used to compare cases and controls. [b]expressed as the percentage among viable lymphocytes. [c]expressed as the percentage among γδ, B, or NK lymphocytes.

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_ECD57+	CD45RA+CCR7-CD27-CD28-CD57+	CD8	15	13.0 [5.5;22.8]	15.3 [9.7;21.8]	0.50
CD4 T_MDR+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_MDR+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_MCD127+	Non(CD45RA+CCR7+)CD127+	CD4 T_M	15	85.0 [80.8;89.1]	86.6 [83.6;90.9]	0.23
CD8 T_MCD127+	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_MCD57+	Non(CD45RA+CCR7+)CD57+	CD4 T_M	15	1.8 [0.4;5.8]	1.2 [0.9;4.0]	0.57
CD8 T_MCD57+	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_ETc1	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}Tc17	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

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

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

[b]Medians [interquartile ranges] are indicated; the Wilcoxon signed rank test was used to compare cases and controls.

Supplementary table 3. Plasma analytes among cases and controls

Plasma analyte	Cases [a]	Controls [a]	*P* [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-1β, pg/mL	26 [15;30]	18 [15;28]	0.53
IL-6, pg/mL	15 [11;29]	22 [10;34]	1.00
sCD14, µg/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

[a]Medians [interquartile range] are indicated; the Wilcoxon signed rank test was used to compare cases and controls.