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Human Infection with Zoonotic Simian Foamy Viruses: Altered CD4+ and CD8+ T lymphocytes

Antoine Gessain[1,2] Thomas Montange[1,2], Edouard Betsem[1,2,3], Chanceline Bilounga Ndongo[4], Richard Njouom[5], and Florence Buseyne[1,2]

[1] Institut Pasteur, Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, Institut Pasteur, Paris, France; [2] CNRS, UMR 3569, Paris, France; [3] Faculté de Médecine et des Sciences Biomédicales, Université de Yaoundé 1, Yaoundé, Cameroun;

[4] Ministère de la Santé Publique du Cameroun, Yaoundé, Cameroun, [5] Centre Pasteur du Cameroun, Yaoundé, Cameroun.

BACKGROUND

A **spillover of simian foamy virus (SFV) to humans**, following bites from infected nonhuman primates (NHPs), is ongoing in exposed populations. These **retroviruses establish persistent infections of unknown physiological consequences to the human host**. Replication-competent virus can be isolated from human blood cells, and SFV DNA has been detected in blood lymphocytes. Human infection with zoonotic SFV is thus a natural model to study the key steps of the emergence of retroviruses.

AIM

We aimed to assess whether **SFV infection** is associated with changes in **the phenotype of peripheral blood mononuclear cells** (PBMCs).

STUDY POPULATION

We performed a **case-control study** to compare **15 Cameroonian hunters infected with gorilla SFV** and **15 controls matched for age and ethnicity**. Participants were recruited among people previously included in epidemiological surveys of SFV infection in rural areas of South and East Cameroon. All participants were men and had been injured by a NHP. SFV infection was defined by positive results for both western blots and polymerase chain reaction assays. All participants tested negative for HIV-1 infection. Ages ranged from 22 to 75 years. The duration of SFV infection ranged from 1 to 45 years. Participants gave written informed consent. Ethics approval was obtained from the relevant national authorities in Cameroon and France.

METHODS

Lymphocyte phenotyping. Blood samples were collected into EDTA. PBMCs were isolated by gradient centrifugation and frozen in liquid nitrogen. After thawing, cells were left to rest overnight before staining. Dead cells were labelled with Live-Dead-Aqua (Life Technologies) before incubation with antibodies of the following specificities: CD3-ECD, CD38-PC5, HLA-DR-FITC (Beckman Coulter), CD279/PD-1-PE, CD4-APC-efluor780 (eBiosciences), CD16-V450, CD19-PerCP-Cy5.5, CD197/CCR7-PC7, CD56-PC7, CD8-Alexa700, (BD Pharmingen), and CD45RA-V450 (BD Biosciences). Data were collected on a LSRII flow cytometer (Becton Dickinson) and analyzed with DIVA and Flow-Jo softwares. Levels of major lymphocytes were expressed as percentage among viable lymphocytes, and naive and memory T cell subsets as percentage among CD4+ or CD8+ T cells, and activated/exhausted cells as percentage among memory (nonCD45RA+CD197+) CD4+ or CD8+ T cells.

Statistics. The levels of lymphocyte subsets were treated as quantitative variables and the paired Wilcoxon signed-rank test was used to compare cases and controls.

Acknowledgments

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RESULTS

❒ **SFV-infected (cases) and uninfected control individuals had similar distribution of T, B and NK lymphocytes. Cases had higher CD8+ T lymphocyte percentages and lower CD4/CD8 ratios than controls.**

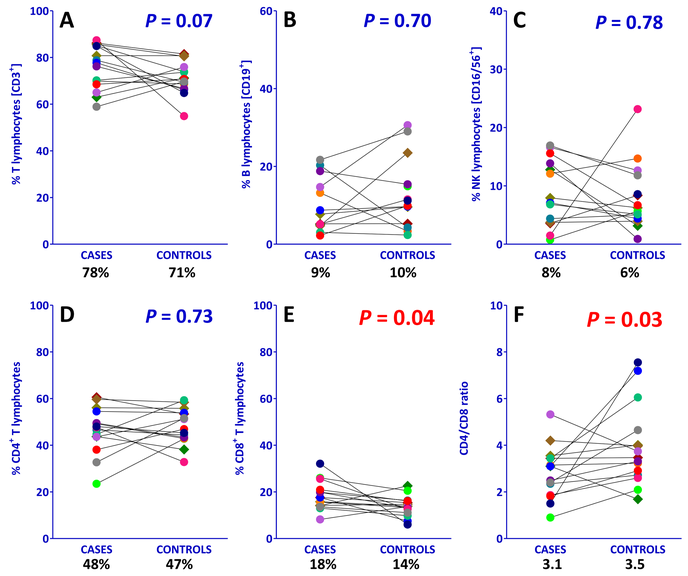

Figure 1: Major lymphocytes subset levels in cases and controls. Frozen PBMCs from 15 SFV-infected (CASES) and 15 matched uninfected (CONTROLS) individuals were analyzed by flow cytometry to quantify major lymphocyte subsets. A. T lymphocytes (CD3+); B. B lymphocytes (CD19+); C. NK lymphocytes (CD56+ and/or CD16+); D. CD4+ T lymphocytes (CD3+CD4+). E. CD8+ T lymphocytes (CD3+CD8+). F. CD4/CD8 ratios. Each pair is represented by a different color. Medians are indicated below the graphs.

❒ **Cases and controls had similar levels of naive, memory and effector CD4+ and CD8+ T lymphocytes.**

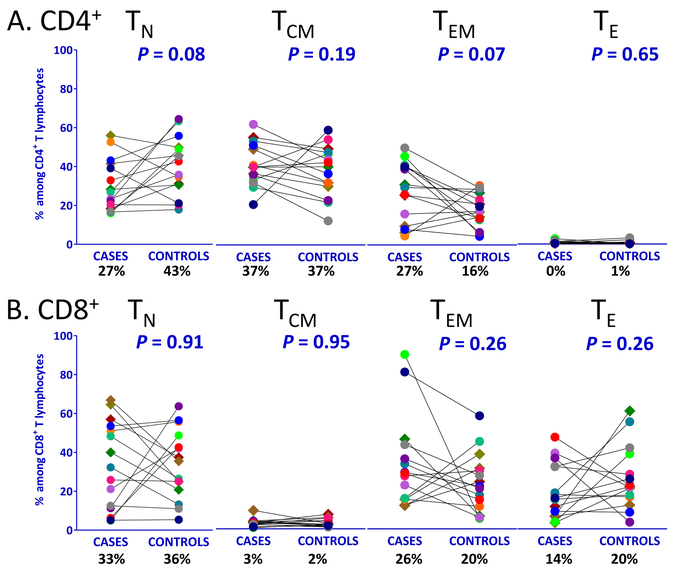

Figure 2: CD4 and CD8+ T lymphocyte differentiation in cases and controls. Expression of CD45RA and CCR7/CD197 was analyzed on peripheral blood CD4+ T lymphocytes (panel A), and CD8+ T lymphocytes (panel B), by flow cytometry. Percentages of naive (T_N, CD45RA+CCR7+), central memory (T_{CM}, CD45RA-CCR7+), effector memory (T_{EM}, CD45RA-CCR7-) and effector (T_E, CD45RA+CCR7-) are presented for cases and controls. The color code is the same as on figure 1. Medians are indicated below the graphs.

❒ **Cases expressed higher levels of the activation/exhaustion marker PD-1 on memory CD4+ T lymphocytes than controls. Cases and controls had similar levels of HLA-DR and CD38 activation molecules on memory CD4+ and CD8+ T lymphocytes.**

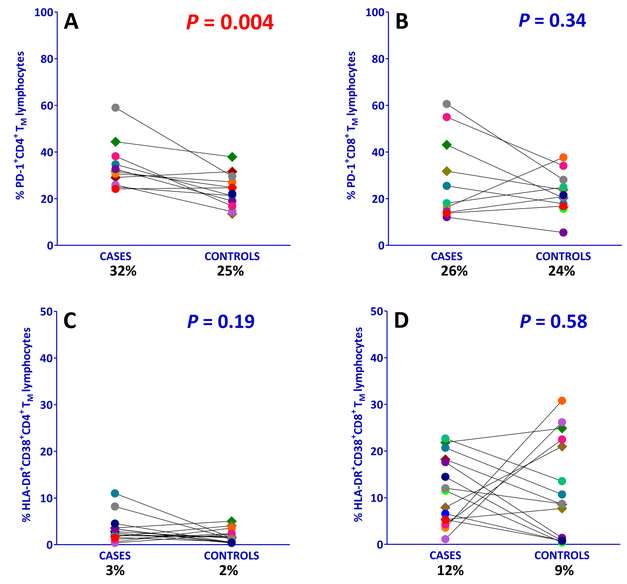

Figure 3: Activation and exhaustion molecules expressed by memory CD4+ and CD8+ T lymphocytes in cases and controls. Percentages of cells expressing of PD-1/CD279 and co-expressing of HLA-DR and CD38 were quantified on memory (T_M, non CD45RA+CCR7+) CD4+ and CD8+ T lymphocytes. A; PD-1+ CD4+ T_M; B. PD-1+ CD8+ T_M; C. HLA-DR+CD38+ CD4+ T_M; D. HLA-DR+CD38+ CD8+ T_M. The color code is the same as on figure 1. Medians are indicated below the graphs.

CONCLUSIONS

This case-control study of apparently healthy SFV-infected Cameroonian hunters showed phenotypic differences among blood T lymphocytes. The **decreased CD4/CD8 ratio and increased expression of the exhaustion marker PD-1** are consistent with a T-cell response against viral infection. Chronic immune activation in SFV-infected individuals is a possible mechanism for reduced hemoglobin levels in SFV-infected individuals *(Buseyne et al., JID, 2018, 218, p144-151)*. Although zoonotic SFV has been reported to be nonpathogenic, our findings of T-lymphocyte activation may have implications for infected individuals.