Plasma Levels of Bacterial DNA in HIV Infection: The Limits of Quantitative Polymerase Chain Reaction

To the Editor—In a recent article, Jiang et al [1] showed that blood levels of bacterial DNA, as determined by quantitative polymerase chain reaction (PCR) on 16S ribosomal DNA fragments, were significantly higher in human immunodeficiency virus (HIV)—infected patients than were those in uninfected control patients. Moreover, the level of bacterial DNA in the blood was shown to decrease after antiretroviral treatment. Blood levels of 16S ribosomal DNA could be regarded as an indicator of the translocation of microbial molecules from the gut lumen to other body compartments. This could be responsible for chronic activation of the immune system and could have a role in AIDS progression.

In our laboratories we have applied a different PCR protocol to quantify 16S ribosomal DNA in the blood of 20 HIV-infected subjects, collected into 4 groups: group A, 5 long-term nonprogressors; group B, 5 treatment-naive patients with CD4 cell counts of >350 and <500 cells/µL; group C, 5 patients with a nadir CD4 cell count of <100 cells/µL who after receipt of effective treatment achieved a CD4 cell count of >350 and <500 cells/µL; group D, 5 patients with CD4 cell count nadir of <100 cells/µL who after receiving >2 years of effective treatment did not achieve a CD4 cell count of >200 cells/µL. Five healthy uninfected individuals were also examined (group E). Total DNA from blood samples was extracted using a commercial kit (DNeasy blood and tissue kit; Qiagen), following the pretreatment steps for bacteria. A further pretreatment of Qiagen columns was performed to wash away contaminating bacterial DNA [2, 3].

A spike of exogenous DNA, a quantified plasmid with a known insert, was added to each sample to normalize the efficiency of DNA extraction. Eubacterial broad range PCR primers were chosen: 314F, 5′-CCTACGGGAGGCAGCAG; 518R, 5′-ATTACCGCGGTGTGCTGG [4, 5]. Amplification profiles were set according to manufacturer’s indications (Applied Biosystems). Samples were run in triplicate and reaction volume (25 µL) contained 1× power SYBR green master mix (Applied Biosystems), 100 nmol/L of each primer, and 1 µL of the extracted DNA solution. After a step at 95°C for 5 min, the steps repeated for 40 cycles were 95°C for 15 s and 60°C for 1 min. Melting curve analyses were performed after amplification cycles to assess specificity. Amplification efficiencies were measured and compared for both target and endogenous control. Two empty tubes (control samples) were processed as biological samples to quantify contamination.

To normalize the samples, for a relative quantification assay, we performed a PCR targeted on the exogenous DNA that was added to the blood samples before DNA purification. One control sample was chosen as the reference sample. For absolute quantification, a quantified plasmid with known insert was used to establish the standard curve, on the basis of a series of dilutions. The results were calculated as the number of copies of 16S ribosomal DNA per microliter of DNA.

Amplification efficiency between 98% and 102% was reached for primers 341F–518R and for the primers targeting the endogenous control. No secondary peaks were observed. The level of bacterial DNA was normalized for DNA extraction efficiency. Only 1 patient (in group D) showed a bacterial DNA amount similar to that of the control samples. Relative and absolute quantifications showed consistent results (see Figure 1), but no significant difference was observed by Student t test between the HIV-negative group and each one of the 4 HIV-positive groups.

In our study we could not observe any significant correlation between HIV serostatus and blood levels of 16S ribosomal DNA (Figure 1). Moreover, we showed that HIV seronegative subjects have detectable 16S ribosomal DNA in the blood, at a level 1 log greater than that of the control samples, for which the signal is likely due to contamination. These results are in agreement with other previous studies [6, 7]. In contrast, Jiang et al [1] found no 16S ribosomal DNA in blood from healthy subjects. This discrepancy could result from differences in PCR sensitivity. First, the method used by Jiang et al [1] involves an assay that was not designed for high sensitivity [8]; it uses very distant primers and a probe with a deoxyguanosine base in 5′ linked to FAM (6-carboxyfluorescein) reporter, which can reduce the detection of fluorescence [9, 10]. Furthermore, the TaqMan probe shows a melting temperature of 64.5°C, which is too similar to the melting temperature of primers (57.6°C and 63.3°C); as a consequence, altered sensitivity and efficiency are expected [9]. Third, the absolute quantification performed by Jiang et al [1] targets only 1 gene and shows the results as the number of copies per volume of extracted DNA. This does not allow one to take into account the different efficiencies of DNA extraction between samples.

In conclusion, we do not question the possibility that bacterial translocation plays a role in the pathogenesis of AIDS, and we believe that Jiang et al [1] have published a seminal article in this field and have opened a new line of research. Rather, we would emphasize that this new re-
Figure 1. Absolute (black bars) and relative (white bars) quantification values of 16S ribosomal DNA in blood samples. On the X axis, cs indicates the control samples; a, b, c, and d indicate samples from human immunodeficiency virus (HIV)–infected patients; e indicates samples from healthy, HIV-uninfected blood donors. The scale on the Y left axis indicates the number of copies of 16S ribosomal DNA per microliter of blood; the scale on the right Y axis indicates the relative quantification (see text).

search area deserves additional work with different methodologies.

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