Table 1. Ceftaroline concentrations in the plasma and CSF

Ceftaroline doses prior to sample collection (no.)	Timing of sample collection	CSF (mg/L)	Plasma (mg/L)	CSF:plasma ratio
15	1.5 h after end of infusion of previous dose	0.461	11.323	0.041
17	0.5 h before next scheduled dose	0.190	5.428	0.035

CSF from the external ventricular drain and blood were sent for analysis. CSF samples were cultured an additional seven times during the admission and were negative. On day 14, the patient developed *Serratia marcescens* line-related bacteraemia with a ceftaroline MIC=1.5 mg/L by Etest®. Gentamicin and meropenem were started empirically and streamlined to ciprofloxacin after final susceptibilities were reported. Repeat blood cultures were negative. On day 23 a new VP shunt was placed. The patient received ceftaroline and ciprofloxacin until discharge on day 27, at which point oral linezolid and ciprofloxacin were prescribed for an additional 7 days.

Simultaneous ceftaroline blood and CSF samples were collected 6 and 7 days into therapy. The blood samples were centrifuged and CSF and blood samples were frozen at −70°C. The samples were mailed on dry ice to Keystone Bioanalyticals, which analysed them by liquid chromatography–MS. All storage and handling instructions were strictly followed. Results are shown in Table 1.

By the time of CSF analysis, the patient's symptoms of infection had abated and it is likely that little-to-no meningeal inflammation remained. Thus, the CSF:plasma ratios of 0.035 and 0.041 are consistent with those of other β-lactams in this clinical situation.[8] Although the patient's clinical picture resolved while receiving ceftaroline, we cannot be sure that ceftaroline was the reason since the patient's CSF cultures cleared before it was initiated. While the drug levels from this patient help assess ceftaroline CSF penetration, we believe that ceftaroline levels collected during acute meningitis should be measured to determine the likely role of ceftaroline in the treatment of CNS infections.

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Transparency declarations

J. C. G. is a speaker for Forest, Cubist and Astellas, he is a consultant for Forest and Pfizer, and he has received grant funding from Merck. S. S. K. and M. R.: none to declare.

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SAMHD1 expression in blood cells of HIV-1 elite suppressors and viraemic progressors

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Sir,

Riveira-Muñoz *et al.*[1] recently reported that HIV-1 elite suppressors (ES), who maintain low viral loads without pharmacological intervention,[2] also maintain higher levels of restriction factor SAMHD1 transcript than either healthy donors (HD) or viraemic progressors (VP) in PBMCs. This compelling finding applied to individuals with and without protective HLA-B alleles. As the authors astutely stated, however, increased SAMHD1 'may not be a cause but the consequence' of suppression.[1] Beyond this caveat, it is

also important to determine the extent to which SAMHD1 up-regulation is found in suppressing individuals in disparate cohorts. Indeed, a previous quantification of multiple known or suspected restriction factors did not find different SAMHD1 levels in PBMCs of ES, VP and HD.[3]

We had conducted a similar study with a group of PBMC samples from HD ($n=8$), a cohort of ES ($n=7$) and VP ($n=7$) and would like to add these data to the ongoing discussion. Our samples were described in an earlier publication in the context of microRNA expression.[4] We used essentially the same methods as previously described.[1,5] Total RNA was extracted from PBMCs with the Ambion mirVana microRNA kit and quantitative PCR results for SAMHD1 were normalized to the average of two housekeeping genes: glyceraldehyde 3-phosphate dehydrogenase (GAPDH), as used by Riveira-Muñoz et al.,[1] as well as β-actin. Because SAMHD1 responds to type II and, in some cell types, type I IFNs,[6,7] we also measured MX1, an indicator of type I IFN antiviral response.

All samples used in this study were de-identified prior to transfer, such that this research is not considered human subject research. There are no privacy concerns, as the authors have access to no identifying information. Samples had previously been obtained with appropriate consent under the approval of the Johns Hopkins Institutional Review Board.

Most HD had low baseline levels of MX1, but several exhibited some degree of activation (Figure 1a). Activation was also seen in several ES. However, VP had the highest levels of MX1, on average >3-fold the HD average. Employing a Kruskal–Wallis test on the three groups, the group means were significantly different ($P<0.01$) and post-tests with Bonferroni correction identified the VP–HD difference, but not the ES–HD difference, as significant ($P<0.017$; Figure 1a).

As for SAMHD1 expression, we did not observe the increase in ES PBMCs found in the cohort of Riveira-Muñoz et al.[1] (Figure 1b). No ES had an SAMHD1 level higher than the average of the HD and there was little variability of SAMHD1 expression in the ES group. In fact, both ES and VP registered on average 20% less SAMHD1 transcript than HD. Despite the trend towards lower SAMHD1 expression in infected individuals, there was no significant difference between the groups (Figure 1b).

We found this lack of increased expression somewhat curious given the significantly higher IFN signalling in VP, as evidenced by increased MX1 levels, and our finding that SAMHD1 tracks with the type I IFN response in the brains of acutely retrovirus-infected animals (E. L. Buchanan, M. A. McAlexander and K. W. Witwer, unpublished results). Accordingly, we examined the relationship of MX1 and SAMHD1 in the three groups. Indeed, in HD and in VP, higher MX1 tended to coincide with higher SAMHD1 despite overall lower levels in VP, with $R=0.73$ (VP) and $R=0.55$ (HD). In contrast, there was no apparent correlation of MX1 and SAMHD1 in PBMCs from ES ($R=0.00$).

Finally, we compared the gene expression data with the CD4+ count and viral load for each infected individual. For ES and VP, SAMHD1 was not correlated with the CD4+ T cell count ($R=0.01$ and $R=0.06$, respectively; data not shown). However, in VP, viral load appeared to be negatively correlated with both MX1 and SAMHD1 ($R=0.58$ and $R=0.69$, respectively; Figure 1c). This result suggests that, regardless of the nature of the link between type I IFN signalling and SAMHD1, increases in both tend to associate with viral control.
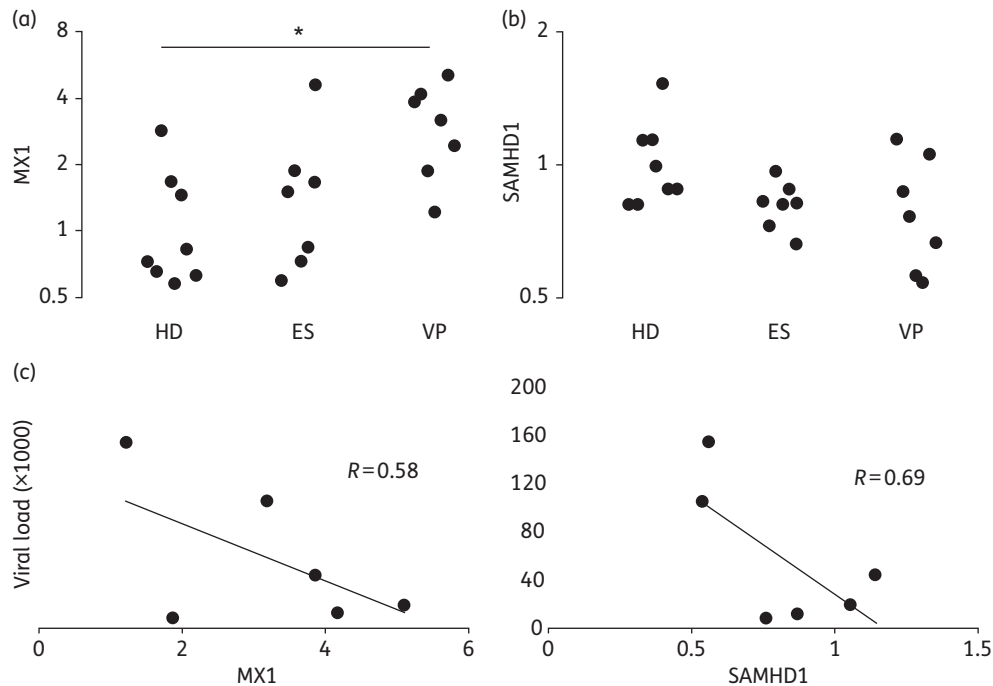

Figure 1. MX1 and SAMHD1 expression and correlation with viral load. HIV-1-positive VP, but not ES, displayed significantly higher levels of the type I IFN-responsive antiviral MX1 according to a Kruskal–Wallis test followed by post-tests (a); *$P<0.05$. No differences were detected for retroviral restriction factor SAMHD1 (b). Within the VP group, there was an apparent trend towards negative correlation of both MX1 and SAMHD1 with viral load (c).

We acknowledge that the small number of samples and the relatively minor variation in the levels of SAMHD1 preclude firm statements about correlation. Nevertheless, we submit that three conclusions are warranted. First, reminiscent of Abdel-Mohsen et al.,[3] the data are inconsistent with increased SAMHD1 mRNA levels in this cohort of ES. Second, it is possible that the lack of apparent correlation of SAMHD1 and IFN response in ES suggests a dampened response of SAMHD1 to IFN in cells from these individuals. Those in other cohorts might have a different response, however. Third, regardless of whether SAMHD1 plays a role in elite suppression, SAMHD1 levels in VP, while often lower than those of HD, appear to retain some sensitivity to IFN signalling and may be associated with lower viral load in VP.

In future research, it will be instructive to examine SAMHD1 and other restriction factors in specific cell types of ES and to include protein and post-translational modification-level tests. To exert an antiviral effect, the SAMHD1 transcript must be present first of all, so quantifying mRNA is certainly important. Beyond transcript and protein abundance, however, multimerization[8] and phosphorylation[9,10] determine SAMHD1 protein activity and the latter is clearly tied to IFN signalling.[10]

Transparency declarations

We have no conflicts of interest to declare. The funding organization played no role in the design, execution, analysis or reporting of this research.

Author contributions

Study conception and design: K. W. W. Data acquisition: E. L. B. and M. A. M. Analysis and interpretation: E. L. B., M. A. M. and K. W. W. Drafting and/or revision of manuscript: K. W. W., E. L. B. and M. A. M.

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