Response to: 'Total adenosine deaminase highly correlated with adenosine deaminase 2 activity in serum' by Gao *et al*

We thank Gao and colleagues for their interest in our recent study on adenosine deaminase 2 (ADA2) as a biomarker of macrophage activation syndrome.[1] In their letter, the authors demonstrated a strong correlation between total adenosine deaminase (tADA) and ADA2 levels in the peripheral blood of healthy controls and patients with immune-mediated diseases.[2] They suggest that, as it is easier to measure tADA than ADA2 activity, tADA activity alone would be suitable as a diagnostic marker. Because tADA is the sum of ADA1 and ADA2 activity, the biology of both ADA isoforms should be considered in evaluating this claim.

Among several differences between ADA1 and ADA2, two are relevant to their assay in clinical laboratories: the affinity of ADA1 for adenosine is 100-fold greater than that of ADA2, and ADA1 is inhibited by the analogue erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), but ADA2 is not.[3] Plasma ADA2 activity is therefore performed at a saturating adenosine concentration that is ~10 000-fold higher than physiological, and in the presence of EHNA to inhibit ADA1. Under these in vitro conditions, ADA2 accounts for the majority of measured ADA activity in plasma. The correlation between ADA2 and tADA activity observed by Gao *et al* has previously been recognised.[4] We observed a similar association in our measurement of 294 healthy individuals comprised 174 children and 120 adults (figure 1A). As expected, ADA2 activity was generally higher than ADA1 activity (median 11.8 U/L (IQR 9.5–15.4) vs 3.6 U/L (1.9–4.9); figure 1B), although considerable variability among individuals was seen in both parameters. We observed that ADA1 levels were twofold higher in children compared with adults (median 4.3 U/L (IQR 3.4–5.5) vs 2.1 U/L (1.0–3.4), p<0.0001; figure 1C). Taking into account the higher ADA2 levels in children we previously described,[1] the fraction of tADA represented by ADA1 remained elevated in children compared with adults (median 25% (IQR 18–33) vs 17% (8 – 24), p<0.0001; figure 1D).

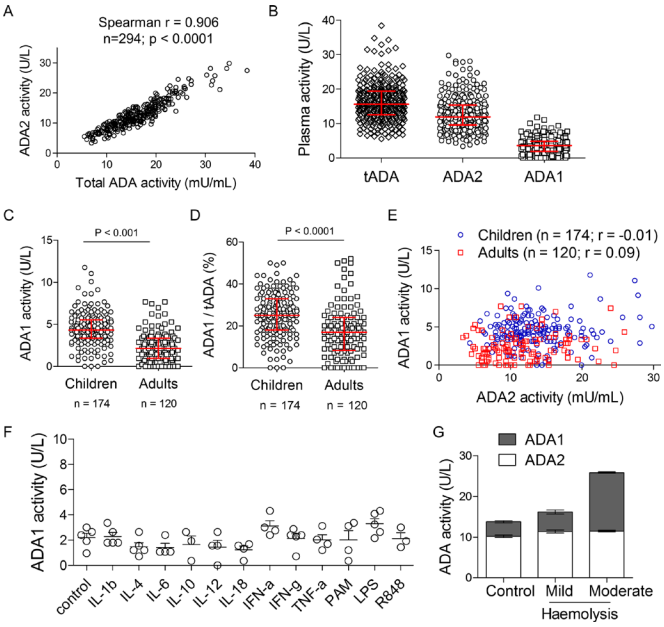

Figure 1 Evaluation of ADA1, ADA2 and tADA in healthy subjects. (A) Correlation between plasma ADA2 activity and tADA activity in 294 healthy individuals (174 children under age 18 years and 120 adults). (B) Comparison of ADA1, ADA2 and tADA in 294 healthy individuals. Median and IQR are displayed in scatter dot plots. (C) Comparison of plasma ADA1 levels and (D) fraction of ADA1 in tADA (ADA1/tADAx100%) in children and adults. Median and IQR are displayed in scatter dot plots. (E) Correlation between plasma ADA1 activity and ADA2 activity in children (blue; n=174) and adults (red; n=120). (F) ADA1 activity in the supernatant of healthy donor PBMC stimulated with cytokines or Toll-like receptor ligands for 5 days. Dots represent results from three to five healthy donors per condition. (G) Impact of haemolysis on plasma ADA1, ADA2 and tADA levels. Bars represent mean and error bars present SD of duplicate samples. ADA, adenosine deaminase; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; PAM, Pam3CSK4; PBMC, peripheral blood mononuclear cells; tADA, total adenosine deaminase; TNF, tumour necrosis factor.

How ADA1 levels are regulated has not been well studied. Whereas ADA2 is primarily secreted by monocytes/macrophages, ADA1 is an intracellular enzyme present in all cells including erythrocytes.[3] The ADA isoforms have distinct physiological functions; genetic deficiency of ADA1 results in severe combined immunodeficiency while deficiency of ADA2 causes systemic vasculitis and bone marrow failure.[5] In line with this view, ADA1 and ADA2 levels did not correlate in either the paediatric group or adult group (figure 1E). Cytokines that induce ADA2 secretion in vitro, including interleukin (IL)-12, IL-18 and interferon-γ, did not affect ADA1 activity (figure 1F).

Importantly, we have observed that blood samples with visible haemolysis tend to have higher levels of ADA1. As haemolysis during phlebotomy is more common in young children,[6] the release of ADA1 from damaged erythrocytes may explain higher ADA1 levels in children. Indeed, when we simulated haemolysis by passing whole blood through a 30-gauge needle (one passage for mild haemolysis, two for moderate haemolysis), plasma ADA1 activity and tADA activity showed a stepwise increase, while ADA2 activity remained stable (figure 1F).

We agree with Gao and colleagues that tADA activity provides a reasonable proxy for ADA2 activity under some conditions. However, intrinsic differences in ADA1 levels among individuals (including disease-related changes still to be defined) and extrinsic factors such as haemolysis can sometimes give rise to substantial discrepancy between these values. Specific measurement of ADA2 is particularly important in the paediatric population where haemolysis associated with phlebotomy is more common. The confounding effects of ADA1 can be eliminated by simply adding the inhibitor EHNA to the assay without other changes to the protocol, which is neither technically challenging nor costly. Therefore, given the incomplete understanding of ADA biology, we suggest the use of ADA2 activity as a more informative and specific biomarker compared with tADA.

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Contributors PYL and PAN conceived and designed the study. PYL and ZH performed the experiments and acquired data. MSH provided the method for measuring ADA2 activity. PYL, ZH, MSH and PAN analysed the data. PYL drafted and all authors edited the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

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