Reviewer's report

Title: Longitudinal fluctuations in PD1 and PD-L1 expression in association with changes in anti-viral immune response in chronic hepatitis B

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Reviewer: Christoph Neumann-Haefelin

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Wenjin and co-workers study PD1 and PD-L1 expression during flares of chronic HBV infection. They demonstrate that inflammatory flares are associated with a reduction of viral load and HBsAg levels on the one hand, and strongly increased HBV-specific CD8+ T cell responses as well as high PD1 and PD-L1 expression on the other hand. While HBV-specific CD8+ T cell responses as well as PD1 expression decline as the flares resolves, intrahepatic PD-L1 expression remains relatively high. The authors argue that this inhibitory milieu at the site of infection may facilitate persistent HBV infection.

Although generally of interest, there are several issues that need attention prior to publication:

Major compulsory revisions:

1. Quality of the HBV-specific pentamer stainings: HBV-specific CD8+ T cell responses are usually extremely weak in chronic infection, even when tetramers or pentamers are used for detection. Multimer-positive cells, however, usually separate as distinct cell populations. This is definitely not the case in Fig. 1. The FACS blots that are shown produce several concerns: Why are there nearly no CD8+ cells in some samples? Why do the multimer-positive cells (if any!) not separate as distinct cell populations? Are the authors sure that the “positive” fractions are not rather an effect of incorrect compensation (cells with a high FITC signal shifting towards PE)? Importantly, how was the “background” defined, which “never exceeded 0.02%”? The authors need to show a) Background staining without multimer and b) multimer control stainings from healthy controls.

2. The authors argue that the relatively high level of intrahepatic PD-L1 expression during the inflammatory regression period facilitates persistent HBV infection. However, PD-L1 expression declines to a third of its maximum (T2 to T4). How high is the intrahepatic PD-L1 expression in patients without an inflammatory flare? Are the authors sure that it is indeed increased during the regression period compared to time points prior to the inflammatory flare? The lack of these data are a major limitations of the study, and they need to be experimentally addressed and/or carefully discussed.

3. The figures need true legend titles, not just “Representative dot plot from 15 independent experiments” etc. By the way, what does “from 15 independent experiments” mean? I suppose, one out of 15 patients is shown here.
Minor essential revisions:

1. Materials and methods: subjects: “All patients were… with HBV-DNA levels greater than 10x6 copies/ml”. This is incorrect. According to the table, 5 patients have an HBV-DNA level below 10x6 copies/ml.

2. Table in material and methody: units for ALT, HBsAg, HBV-DNA are missing or incomplete. HLA-A2 has been misspelled.

3. Methods and throughout the manuscript: The authors switch from tetramers to pentamers. What kind of multimers was used? From which supplier?

4. Page 5: “The frequency of PD1 expression on pentamers was significantly higher than that among total circulating CD8+ T cells (Fig 2a, Fig 6c)”. The frequency of PD1+ cells in total circulating CD8+ T cells is not shown in the figures stated here.

5. Legend to Fig. 2b: Antibodies against pentamer were definitely not used in this assay.

6. Page 11: “In general, circulating and inta-hepatic PD1 and PD-L1 expression levels were within the normal range before the initiation of inflammation, and were progressively uprgulated at the onset of liver inflammation (T1)…” Intra-hepatic PD1 and PD-L1 expression were not analyzed before the initiation of inflammation and at T1. As discussed above, this is a major limitation of the study and needs to be clearly stated.

7. Language issues:
   - Page 1, abstraction instead of abstract
   - Page 1, hepatitis B infection instead of hepatitis B virus infection
   - Page 2, summarized in follow table instead of summarized in the following table
   - Page 3, anti-CD8-TITC instead of anti-CD8-FITC

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.