Reviewer’s report

Title: Antagonism of cannabinoid receptor 2 pathway suppresses IL-6-induced immunoglobulin IgM secretion

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Reviewer: Barbara L. F Kaplan

Reviewer’s report:

Feng and colleagues prepared the manuscript entitled “Antagonism of cannabinoid receptor 2 pathway suppresses IL-6-induced immunoglobulin IgM secretion” to be considered for publication in BMC Pharmacology and Toxicology. While the data have potential significance and address important effects of cannabinoid modulation of IgM production in a human B cell line via CB2, the authors should address several issues prior to publication.

Major Compulsory Revisions

1. There is no information provided on statistical analyses performed. In fact, statistics are only provided in Figure 3. For instance, what does “slightly disturbed” mean? Statistical analysis is especially critical for the data presented in Figure 2 in which the authors conclude that the inhibitory effects of SR144528 are reversed by HU308. It is not clear why the authors did not perform a concentration response of reversing SR144528 with HU308, especially since HU308 at concentrations up to 20 µM (seemingly) did not produce any effects on IgM or viability. In addition, it was difficult to determine how many times experiments were replicated in many figures, especially since some data were reported without any error bars.

2. Although the authors reported in the Materials and Methods that vehicle controls were performed, there was only one figure (1E) in which a vehicle control was identified. Are the other figures and legends missing the description of this control? In particular in the studies in which combinations of drugs were used, the vehicle controls are critical to maintain consistent DMSO concentrations across combination treatments.

3. It was not clear why the concentration of IL-6 was different between experiments. In some figures and/or legends, the concentration of IL-6 was not provided. The authors should clarify this.

4. While SR144528 and Bay11-7085 act similarly, this is not evidence that SR144528 inhibits NF-κB. This should be directly measured, especially since several studies have demonstrated that NF-κB is a target of suppression by cannabinoid agonists. Also, can the authors quantify the inhibition of IκB-κB and p-STAT3 in Figure 3B?

Minor Essential Revisions
5. “Concentration” instead of “dose” should be used throughout.
6. “Immunomodulative” should be “immunomodulatory” (Background).
7. “Hu308” should be “HU308” throughout.
8. Use of only SR144528 to determine the effect of “CB2 ligands” isn’t a valid statement since only one ligand was used (Results describing Fig 1E).
9. The data that SR141716A did not suppress IL-6-induced IgM production are sound, but they do not rule out the CB1 pathway entirely. The authors should alter this wording.
10. In Figure 3A, a line indicating that IL-6 was present in all groups should be included.
11. In the Methods, the symbol for µl is incorrect in the “Reagents and cell culture” section.
12. In the Results, “lipopolysaccharides” should be “lipopolysaccharide”.
13. There should be a citation after this sentence in the Results: “CB2 is primarily expressed in B plasma cells.”
14. In Figure legend 1, the authors indicate that the “Cell DNA synthesis was determined with 3H-thymidine assay as in the Materials and Methods.” All assays are reported in the Materials and Methods so the latter part of this sentence is not necessary.

Discretionary Revisions

15. In the regulation of IgM by Bcl-6 and Pax5, Blimp-1 is also an important regulator of this pathway. The authors should provide data on Blimp-1 or state why they only focused on 2 of the 3 proteins in this pathway.

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: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing financial interests. I also publish in the field of cannabinoid effects on B cells.