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March 27, 2013

Dear Editor:

Please find enclosed the edited manuscript in Word format (file name: 2287-review.doc).

Title: Caspase-1 activation and mature IL-1β release are uncoupled events in monocytes

Author: Amy J. Galliher-Beckley, Liqiong Lan, Shelly Aono, Lei Wang, Jishu Shi

Name of Journal: *World Journal of Biological Chemistry*

ESPS Manuscript NO: 2287

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated according to editor's comments.
Comment #1- We have reworded the results section so that results are not discussed.
Comment #2-We reorganized the discussion section to include a section highlighting the main conclusions and future direction.

2 Revision has been made according to the suggestions of the reviewers

Referee #1 (00504335)

"In Fig. 1, second vertical line (i.e., cells treated with HD-5, ATP and LPS)- there is no band for proIL-1beta in lysate. In the same setting in Fig. 2(the third vertical line), there is a strong band for proIL-1beta in lysate. The authors should explain the difference between experiments in Fig. 1 and Fig. 2, and/or to explain the difference in the expression of proIL-1beta in Figs. 1 and 2."

We agree with the referee that this difference should be explained. Therefore, we added the following text to the manuscript (lines 136-139) "Interestingly, we also observed that freshly isolated monocytes had enhanced cellular processing of pro-IL-1β to IL-1β (Figure 1, lower panel, lanes 1-2 vs 3-4) suggesting that increased time in culture can decrease the ability of monocytes to process IL-1β"

Referee #2 (00227418)

Suggestion 1- "The rationale for having performed experiments on both freshly isolated and overnight incubated monocytes should be explained."

We agree with the referee that this difference should be explained. Therefore, we added the following text to the manuscript (lines 132-134) "Since culturing monocytes *in vitro* can lead to further differentiation to macrophage-like cells, we determined whether the subcultured monocytes behaved similarly to freshly isolated cells."

Suggestion 2 - "The authors should explain why in Figure 1 intracellular proIL-1β is not detected in freshly isolated monocytes"

We agree with the referee that this difference should be explained. Therefore, we added the following text to the manuscript (lines 136-139) "Interestingly, we also observed that freshly isolated monocytes had enhanced cellular processing of pro-IL-1β to IL-1β (Figure 1, lower panel, lanes 1-2 vs 3-4) suggesting that increased time in culture can decrease the ability of monocytes to process IL-1β"

Suggestion 3 -"The faint band in the first lane of the lower panel is not aligned to mature IL-1β. Why?"

In a paper by Laliberte, Eggler, and Gabel in 1999 entitled "ATP Treatment of Human Monocytes Promotes Caspase-1 Maturation and Externalization" they show that Il-1β can be processed to include several slightly larger (20kD) polypeptides. We had also observed this in our past studies (**Shi J**, Aono S, Lu W, Ouellette AJ, Hu X, Ji Y, Wang L, Lenz S, van Ginkel FW, Liles M, Dykstra C, Morrison EE and Elson CO. A novel role for defensins in intestinal homeostasis: regulation of IL-1beta secretion. J Immunol 2007).

Suggestion 4- "The results shown in Figure 2 were obtained in freshly isolated or O/N cultured monocytes?"

Overnight, and this fact was clarified in the text as well as the figure legend so readers with not confused.

Suggestion 5- "To the benefit of non-specialist readers, the authors should specify that nigericin is an inflammasome agonist."

We agree with the referee that this should be explained. Therefore, we added the following text to the manuscript (lines 147-149) "Similar to ATP, nigericin is a microbial toxin that acts as an inflammasome inducer, leading to caspase-1 maturation and IL-1β processing and release."

Suggestion 6- "Indication of molecular weights in Figure 1 and 2 (as already shown in Fig 3) would be helpful"

Molecular weights have been added to figures 1 and 2. Thanks for the suggestion.

Suggestion 7- "The authors should check the manuscript for a few typographical errors."

The authors have re-read the manuscript and changed all typographical errors seen.

3 References and typesetting were corrected.
Editor's comment #3 – Per his/her suggestion, references were expanded to include 26 citations.
Editor comment #4- Figures will be provided as Powerpoints files as requested.

Thank you again for publishing our manuscript in the *World Journal of Biological Chemistry*.

Sincerely,

Jishu Shi, D.V.M., PhD.
Professor of Immunophysiology