To the editor:

Granzyme A is a proinflammatory protease

We have read with great interest the paper by Zhu et al[1] that suggests poly ADP ribose polymerase 1 (PARP-1) is cleaved when granzyme A (gzmA) enters target cells. This new finding may have implications for the proinflammatory effects of this protease.[2-4] PARP-1, which has proinflammatory properties, is cleaved in response to a variety of apoptotic stimuli, which would lessen inflammation during cell death.[5-7]

The authors dismiss our finding[2] that gzmA lacks cytotoxic activity and rather induces human and mouse monocyte/macrophages to express proinflammatory cytokines. Although data are not presented to the contrary, they state in their discussion that endotoxin contamination of recombinant gzmA is responsible.[1]

To minimize misunderstanding, we reiterate the crucial issues that surround the controversy.

1. Foremost, native or bacterial, human or mouse gzmA do not display cytotoxic activity against several targets delivered by several agents (human perforin, streptolysin, or adenoviral particles) when used at concentrations known to cause death by granzyme B.

2. Native and recombinant human gzmA do not cross-compete in their binding and internalization suggesting the former is preferred for biologic analysis. In this regard, native human and mouse gzmA were used for the bulk of the studies designed to show these proteases induce proinflammatory cytokines. The proteases were isolated, respectively from a human NK-cell line (NK92) and a mouse Tc-cell line (1.3E6SN) and documented to be virtually endotoxin-free with levels below the stimulatory threshold necessary to induce the proinflammatory cytokines in the indicator cells. The proteases were identified by N-terminal sequencing, by specific gzmA antibodies and by predicted enzymatic activities.[2] Notably, human gzmA isolated from primary LAK cells[8] and the NK92 cell line both induce proinflammatory cytokines from freshly isolated human monocytes[2] and native mouse gzmA induces release of active IL-β from ex vivo presensitized mouse peritoneal cells.

3. We used recombinant human and mouse gzmA expressed in bacteria for select studies, primarily the cytotoxicity experiments. The endotoxin contamination is eliminated by isolating these proteins via cation-exchange chromatography. Although lipopolysaccharide contamination is a theoretical risk, inactive recombinant granzymes used as controls contained similar level of the contaminant but did not induce proinflammatory cytokines. Only a single lot of recombinant human gzmA was contaminated with endotoxin. This material, acquired commercially, was rendered endotoxin-free by the endo-trap technique and was presented to demonstrate our capacity to minimize this fundamental concern. In comparison, the proteins expressed in-house displayed levels below stimulatory threshold.

4. Finally, we have confirmed our findings using intact cytotoxic cells that secrete gzmA. Here, a human NK-cell line induced the proinflammatory effect in human monocytes and mouse ex vivo virus immune Tc cells stimulated a response in mouse peritoneal macrophages.

Our primary conclusion that gzmA has proinflammatory activity is supported by a wide variety of replicated studies in mice and humans. Importantly, the proinflammatory effect of gzmA seems to be quite restricted favoring primarily monocyte/macrophage cells, which apparently express the necessary inflammasome components. It seems hardly serendipitous that most laboratories working in the mouse, including our own, use ex vivo–derived peritoneal macrophages, sensitized with thioglycolate in vivo and with LPS in vitro, as one appropriate source of inflammasome components.

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Conflict-of-interest disclosure: The authors declare no competing financial interests.

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blood 2009 114: 3968
doi:10.1182/blood-2009-07-231027

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