Response

Granzyme A: cell death–inducing protease, proinflammatory agent, or both?

We recently showed that granzyme A (GzmA) cleaves polyadenosine 5’-diphosphate-ribose polymerase-1 (PARP-1) to promote cell death and inhibit DNA repair.1 PARP-1 cleavage is required for apoptotic cell death, which reduces inflammation, because scavenger macrophages phagocytose apoptotic cells.

Our paper discussed a recent study from Froelich and colleagues2 that asserts that native GzmA from human killer cells is not cytotoxic at nanomolar concentrations, contradicting our results using recombinant GzmA (rGzmA). There is considerable evidence that native GzmA and GzmB both independently activate cell death.3 Mice genetically deficient in either GzmA or GzmB are essentially immunocompetent, whereas mice lacking both Gzms are profoundly immunodeficient. Moreover, as shown in Figure 7 of our paper,1 cytotoxic T lymphocytes (CTLs) lacking GzmA or GzmB induce comparable cytotoxicity.

The Froelich study also could not verify previous work that native GzmA cleaves and activates pro–IL-1β.4 However, they showed that native human GzmA was proinflammatory, because it induced monocytes to secrete IL-1β. Because the GzmA concentration that activates cytokine secretion was approximately 2 to 3 logs less than the cytotoxic concentration, they speculated that GzmA’s primary function is proinflammatory, rather than cytotoxic. However, because Gzms are secreted into a small enclosed space (the immunologic synapse) and only small amounts leak out into extracellular fluids, the local synapse concentration is relatively much higher. During chronic inflammation (rheumatoid arthritis joints, AIDS patient blood), GzmA can reach low-nanomolar extracellular levels (vs <1pM in normal blood).5 Based on the GzmA yield from killer cells (~20 µg/10⁹ cells)⁶ and conservative estimates that approximately one-tenth of CTL granule contents are released into a single synapse with a volume of less than 5 µm³, we estimate that GzmA synapse concentrations are vastly greater (~2 x 10⁻⁹ µg/5 µm³ or ~8µM). Therefore, the local synapse concentration is more than adequate for inducing cytotoxicity, activated at 250nM concentrations (Figure 1).1,7-9

In the absence of a defined mechanism, we are uncertain whether GzmA has additional proinflammatory effects, besides activating pro–IL-1β⁴ (which we recently confirmed using rGzmA). Monocytes/macrophages are exquisitely endotoxin-sensitive. It is impossible to verify endotoxin elimination from GzmA preparations. This is an issue for both recombinant and purified enzymes, considering that buffers, water, and glassware can be contaminated. Commercial kits do not completely remove endotoxin. Moreover, the Limulus clot assay for endotoxin, which measures trypstat activity, cannot be performed on GzmA preparations. Therefore, because there may be residual endotoxin in all GzmA preparations, it will be important to define the molecular basis for additional proinflammatory mechanisms of GzmA.

The Froelich group recently kindly provided us with the native natural killer (NK) cell GzmA that they used. We compared the cytotoxicity of human GzmA from 4 sources⁶: rGzmA,¹ native NK92 cell GzmA purified by us and Froelich,² and native lymphokine-activated killer cell GzmA purified by us. rGzmA induced significant cytotoxicity at approximately 250nM concentrations (consistent with our previous publications¹,7-9), whereas native GzmA had limited cytotoxic activity (consistent with Froelich’s published study²) (Figure 1A). rGzmA was also substantially more active at cleaving SET, the inhibitor of GzmA-activated DNA damage⁷ (Figure 1B). Moreover, rGzmA and native GzmB from YT-Indy NK cells⁶ were active at similar concentrations (~250nM; Figure 1C). Therefore native GzmA from human killer cell granules is less cytolytic than rGzmA.

Further work is needed to understand why native GzmA has reduced cytotoxicity. GzmA is synthesized as an inactive pre-pro-enzyme. One potential explanation could be that GzmA might be stored mostly as the pro-enzyme, which is activated only during CTL degranulation.

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To the editor:

Clonotypic B cells in classic Hodgkin lymphoma

Jones and colleagues described 2 Hodgkin lymphoma (HL) cell lines that contain small subpopulations of aldehyde dehydrogenase (ALDH)\(^+\)CD20\(^-\)CD30\(^-\)IgA\(^+\) B cells, which have clonogenic potential and give rise to the typical CD30\(^+\) Hodgkin and Reed-Sternberg (HRS) cells.\(^1\) Importantly, the authors also reported that in the peripheral blood (PB) of HL patients, ALDH\(^{high}\) cells are detectable, that are often clonally related to the HRS cells. These cells may represent HL-initiating cells, perhaps cancer stem cells.

First indications for the existence of HRS cell clone members among small CD30\(^-\), morphologically normal cells were reported 10 years ago. In several HL the same numerical chromosomal abnormalities were present in HRS cells and in some small (CD30\(^-\)) cells.\(^2,3\) However, such abnormalities are not the best clonal markers in HL.\(^4\) In another study of HL with Epstein-Barr virus (EBV)\(^+\) HRS cells, CD30\(^-\) small EBV\(^+\) lymphocytes in the HL lymph nodes were not (with perhaps rare exceptions) members of the HRS clone.\(^5\) As HRS cells show a clonal EBV infection pattern, putative CD30\(^-\) clone members should also be EBV-infected. Thus, members of the HRS cell clone are usually absent among CD30\(^-\) B cells, at least in EBV\(^+\) cases. Vockerodt and colleagues analyzed multiple PB and bone marrow samples from 2 HL patients, using a highly sensitive HRS cell clone–specific PCR, but HRS cell–specific amplificates were undetectable.\(^6\) Hence, clonotypic cells were very infrequent or absent in the PB of these patients.

Jones and coworkers present 3 pieces of evidence that clonotypic B cells are present in the PB of HL patients among ALDH\(^{high}\)CD27\(^+\)CD19\(^+\) B cells.\(^1\) However, none of these is convincing. First, in the heavy chain fragment length analysis, the isolated HRS cell population surprisingly gave a polyclonal pattern with many fragments of different lengths, so that also seeing 2 bands with the same length as those obtained from the PB B cells does not prove clonal identity. Second, the fragment length analysis of V\(\kappa\) light chain rearrangements is unsuitable to demonstrate clonal identity, because the V-J joints of light chain rearrangements show little length variation; 66% of polyclonal V\(\kappa\)J joints have an identical CDRIII length of 27 bp.\(^7\) Third, sequences of V(D)J joints are principally an ideal clonal marker for B cells. However, the V\(\kappa\)Jk sequences obtained from HRS cells and PB ALDH\(^{high}\)CD27\(^+\)CD19\(^+\) B cells surprisingly stop 5’ of the V-J joint, so that their potential clonal relationship is not demonstrated. The pattern of somatic mutations in the sequences actually argues against a clonal relationship: as V genes in HRS cells show nearly never intraclonal diversity,\(^8\) the finding of different mutations only in the V\(\kappa\) sequences from the HRS and PB B cells suggests that the latter do not carry the clonotypic V gene rearrangement of the HRS cells.

It will also be important in future studies to clarify whether indeed only the CD30\(^-\) cells in HL cell lines have clonogenic potential, because small mononuclear CD30\(^+\) Hodgkin cells were not analyzed separately from large multinuclear Reed-Sternberg cells, and mononuclear Hodgkin cells have a high proliferative potential.\(^9\) Moreover, it will be critical to determine whether B cells in the PB with clonotypic rearrangements—if their existence can be confirmed—are members of the malignant HRS cell clone or represent premalignant B cells that persisted in the patients.

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