Delivering Three Punches to Knockout Intracellular Bacteria

Emilie Narni-Mancinelli[1,2,3] and Eric Vivier[1,2,3,4,*]
[1]Centre d'Immunologie de Marseille-Luminy, UM2 Aix-Marseille Université, 13288 Marseille, France
[2]INSERM U1104, 13288 Marseille, France
[3]CNRS UMR7280, 13288 Marseille, France
[4]Service d'Immunologie, Assistance Publique-Hôpitaux de Marseille, Hôpital de la Conception, 13285 Marseille, France
*Correspondence: vivier@ciml.univ-mrs.fr
http://dx.doi.org/10.1016/j.cell.2014.05.023

Cytotoxic lymphocytes kill bacteria-infected cells, but the mechanisms at work remain unclear. Walch et al. show that these lymphocytes deliver a toxic molecular trio in a two-step process, penetrating first the infected cells and then delivering bactericidal granzymes into the intracytoplasmic bacteria.

Killer lymphocytes such as natural killer (NK) cells and $CD8^+$ T cells contribute to the elimination of viruses and intracellular bacteria by inducing the death of infected cells. This killing involves the release of lytic granules containing cytotoxic molecules, including perforin and granzymes in all mammals, and granulysin in humans and some other mammals but not in mice. Perforin binds to cell membranes where it generates pores that allow the delivery of lytic granule content into the target cell cytosol (Figure 1) (Pipkin and Lieberman, 2007). Granzymes are proteases with a broad substrate spectrum; they are not known to play a direct role in the elimination of intracellular pathogens. Granulysin is a saposin-like lipid-binding protein and is toxic for bacteria, parasites, fungi, and tumors (Clayberger and Krensky, 2003; Stenger et al., 1998). At physiological levels, granulysin is not sufficient for permeabilizing target cell membranes, but with the help of perforin, it can be internalized and is thought to exert its bactericidal activity by generating osmotic shock. In this issue of *Cell*, Walch et al. (2014) bring a new and comprehensive view of the bactericidal roles of granulysin and granzymes.

They first show that the combination of granulysin and granzyme at sublytic concentrations induces bacterial growth arrest and death and demonstrate the essential role of granzymes for bacterial elimination. Confocal imaging reveals that granulysin decorates the surface walls of bacteria and allows the entry of granzymes. Importantly, Walch et al. provide several lines of evidence to illustrate that bacterial death does not depend on host cell apoptosis. First, the combination of perforin and granzymes induces host cells apoptosis without directly impairing bacterial survival, whereas the addition of granulysin in the assay is absolutely required for bacterial killing. Second, treatment with a caspase inhibitor or overexpression of an antiapoptotic factor protects the host cell from granzyme-induced apoptosis without affecting bacterial killing. Finally, in the presence of $CD8^+$ T cells, bacterial elimination occurs before the death of infected cells.

By taking advantage of a transgenic mouse expressing human granulysin (Huang et al., 2007), the authors show that granulysin can increase the efficiency of *L. monocytogenes* clearance by 1,000-fold during a primary challenge in vivo. Perforin, but not granzyme B, is absolutely required for the granulysin-mediated effect, suggesting compensation by other granzymes. At 3 days postinfection, the T cell response remains weak, thereby indicating that the benefit of granulysin activity could be attributed to NK cells or other innate lymphocytes. However, the exact identity of the cytotolytic cells involved in the response to primary *L. monocytogenes* infection awaits further investigation. Immune protection against *L. monocytogenes* during a secondary challenge relies on bacteria-specific memory $CD8^+$ T cells. The observation that mice expressing transgenic granulysin are more resistant to this secondary challenge further supports the role of granulysin against bacterial infection. It would be interesting to test whether other saposin-like molecules contribute to bacterial defense in the mouse.

Innate immune phagocytes such as neutrophils and macrophages can damage bacteria via the generation of radical oxygen species (ROS). Interestingly, granzymes can also generate ROS and their scavengers block cytolysis by killer lymphocytes (Martinvalet et al., 2005). Walch et al. thus further assess whether granzymes can induce ROS production in bacteria, once delivered by granulysin into the microbe. Indeed, by using a fluorescent reporter, they show that ROS production inside bacteria depends on granulysin-delivered granzymes. Moreover, bacteria overexpressing enzymes involved in ROS degradation, such as superoxide dismutase and catalases, are protected from granzyme-induced damage.

Granzymes A/B generate ROS in mitochondria of mammalian cells by cleaving proteins of the electron transport chain complex I (ETC I) (Martinvalet et al., 2008). In the present report, Walsh et al. show that granzyme B can also cleave the bacterial ETC I complex. In the absence of ETC I, genetically modified bacteria are resistant to granzyme-induced ROS but eventually died from granzymes attack. Further experiments are necessary to define the mechanisms by which these bacteria are killed. Preliminary analysis reveals that granzyme B could target approximately 100 to 200 molecules in *E. coli* and *L. monocytogenes*. Therefore, even if granzymes may degrade oxidoreductases in

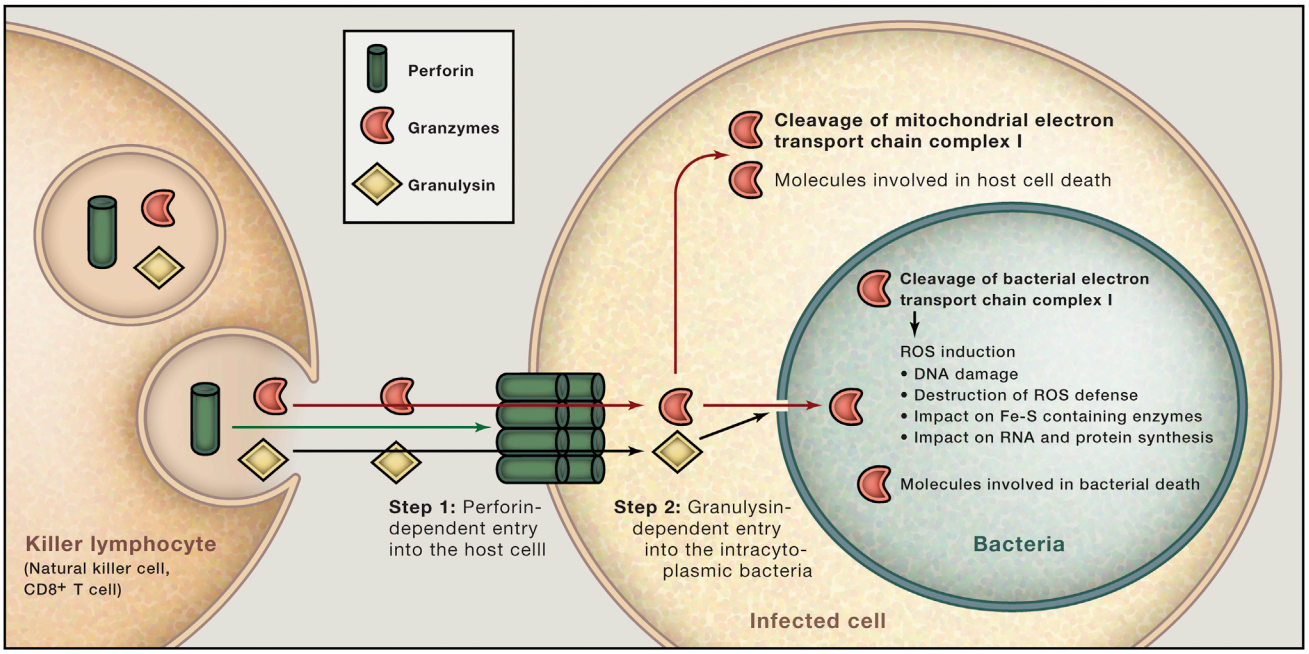

Figure 1. A Two-Step Process for Targeting Intracytoplasmic Bacteria

Step 1. Perforin binds to the cell membrane and generates pores allowing the delivery of lytic granule content into the infected cell. Step 2. Granulysin penetrates the bacterial membrane to facilitate the entry of granzymes into intracytoplasmic bacteria. While granzymes can limit bacterial spreading by inducing apoptosis of infected cells, direct delivery of granzymes into bacteria mediated by granulysin eliminates bacteria independently of host cell death.

various bacteria, it is important to note that granzyme-mediated bacterial death is a complex program of damage that disrupts key pathways involved in bacterial survival. This wide target spectrum of granzymes indicates that multiple mechanisms could be employed to eliminate diverse bacteria strains growing under various conditions, including both aerobic and anaerobic strains. These results thus illustrate the strength of these bactericidal mechanisms for combating bacterial evasion. Notably, there are other serine proteases and saposin-like molecules present in macrophages and granulocytes, raising the intriguing question of whether these innate phagocytes use comparable mechanisms for bacterial defense.

NK cells express high levels of granulysin. Although NK cell deficiencies have not been associated with susceptibility to bacterial disease (Jouanguy et al., 2013), findings in this paper (Walch et al., 2014) prompt investigators to revisit their role in antibacterial defense. It will also be interesting to look at granulysin expression in other innate lymphocytes such as NKT cells, mucosa-associated invariant T cells, and innate lymphoid cell subsets.

Previous works have reported the involvement of granulysin in host defense against tuberculosis, leprosy, and malaria (Clayberger and Krensky, 2003). Granulysin also participates in the elimination of *C. neoformans*, which can cause fungal meningitis and encephalitis in AIDS patients (Clayberger and Krensky, 2003). Along this line, granulysin production has been shown to be defective in patients with HIV (Zheng et al., 2007). Recently, genetic polymorphims in granulysin have been associated with differential abilities to clear hepatitis B virus-infected cells (Park et al., 2012). Future experiments will thus be crucial to fully appreciate the biological relevance of the perforin/granulysin/granzymes trio in host defense against bacteria, parasites, fungi, viruses, and possibly even tumor cells.

ACKNOWLEDGMENTS

E.V. laboratory is supported by the European Research Council (THINK Advanced Grant), by Equipe Labellisée La Ligue and by institutional grants from INSERM, CNRS, and Aix-Marseille University to CIML. E.V. is a scholar of the Institut Universitaire de France. E.V. is a cofounder and shareholder in InnatePharma.

REFERENCES

Clayberger, C., and Krensky, A.M. (2003). Curr. Opin. Immunol. *15*, 560–565.

Huang, L.P., Lyu, S.C., Clayberger, C., and Krensky, A.M. (2007). J. Immunol. *178*, 77–84.

Jouanguy, E., Gineau, L., Cottineau, J., Béziat, V., Vivier, E., and Casanova, J.L. (2013). Curr. Opin. Allergy Clin. Immunol. *13*, 589–595.

Martinvalet, D., Zhu, P., and Lieberman, J. (2005). Immunity *22*, 355–370.

Martinvalet, D., Dykxhoorn, D.M., Ferrini, R., and Lieberman, J. (2008). Cell *133*, 681–692.

Park, G.H., Kim, K.Y., Cheong, J.Y., Cho, S.W., and Kwack, K. (2012). DNA Cell Biol. *31*, 1492–1498.

Pipkin, M.E., and Lieberman, J. (2007). Curr. Opin. Immunol. *19*, 301–308.

Stenger, S., Hanson, D.A., Teitelbaum, R., Dewan, P., Niazi, K.R., Froelich, C.J., Ganz, T., Thoma-Uszynski, S., Melián, A., Bogdan, C., et al. (1998). Science *282*, 121–125.

Walch, M., Dotiwala, F., Mulik, S., Thiery, J., Kirchhausen, T., Clayberger, C., Krensky, A.M., Martinvalet, D., and Lieberman, J. (2014). Cell *157*, this issue, 1309–1323.

Zheng, C.F., Ma, L.L., Jones, G.J., Gill, M.J., Krensky, A.M., Kubes, P., and Mody, C.H. (2007). Blood *109*, 2049–2057.