intestinal crypts to release antimicrobial substances. The crypt sensors are selective, as fungi and protozoa did not elicit secretion. In agreement with the proposed role of defensins as the predominant local antimicrobials, antibody to mouse Paneth cell defensins neutralized most of the secreted antimicrobial activity. Measurements of the amount of secreted defensins per crypt allowed the authors to estimate the defensin concentration reached within the crypt lumen. The estimated concentration, >10 mg/ml, was comfortably above the range required for an antimicrobial effect. The situation is reminiscent of the phagocytic vacuoles of some leukocytes where the high abundance of defensins[12] and the tight space around engulfed bacteria combine to generate surprisingly high local defensin concentrations.

The findings raise several questions. What receptors and mechanisms allow the crypts to sense bacteria? In Drosophila, Toll and related receptors are essential for the insect's response to microbes including the induction and release of antimicrobial peptides into the insect's blood[13]. However, it now appears unlikely that these receptors are the primary sensors of bacteria. The mammalian Toll-like receptors work similarly to their insect counterparts and require coreceptors such as the lipopolysaccharide-sensing CD14 molecule and the lipopolysaccharide-binding protein of plasma. It is not yet known whether these or related molecules trigger the secretory response of Paneth cells. The most parsimonious scenario would place the receptors directly on the Paneth cells but it is also possible that other crypt cells could sense bacteria and relay the signal to the bottom of the crypt by cell-to-cell communication or via soluble mediators.

How far from the crypt does the antimicrobial shield of Paneth cell secretions extend? Ayabe *et al*. demonstrate that substantial *in vitro* antimicrobial activity remains even when the secretions are diluted a million-fold relative to the estimated concentration in the crypt. Even allowing for some interference with antimicrobial activity due to the presence of food and digestive juices, the Paneth cells may have enough punch left to protect not only the cell hatchery in the crypt but also the more mature cells that have migrated onto the villi. Suddenly, the function of Paneth cells is not so cryptic anymore.

1. Schmidt, G.H., Wilkinson, M.M. & Ponder, B.A. Cell migration pathway in the intestinal epithelium: an in situ marker system using mouse aggregation chimeras. *Cell* **40**, 425–429 (1985).
2. Ayabe, T. *et al.* Secretion of microbial α-defensins by intestinal Paneth cells in response to bacteria. *Nature Immunol.* **2,** 113–118 (2000).
3. Satoh, Y. Effect of live and heat-killed bacteria on the secretory activity of Paneth cells in germ-free mice. *Cell Tissue Res.* **251**, 87–93 (1988).
4. Satoh, Y., Ishikawa, K., Tanaka, H., Oomori, Y. & Ono, K. Immunohistochemical observations of lysozyme in the Paneth cells of specific-pathogen-free and germ-free mice. *Acta Histochem.* **83**, 185–188 (1988).
5. Ouellette, A.J. *et al.* Developmental regulation of cryptdin, a corticostatin/defensin precursor mRNA in mouse small intestinal crypt epithelium. *J. Cell Biol.* **108**, 1687–1695 (1989).
6. Jones, D.E. & Bevins, C.L. Paneth cells of the human small intestine express an antimicrobial peptide gene. *J. Biol. Chem.* **267**, 23216–23225 (1992).
7. Qu, X.D., Lloyd, K.C., Walsh, J.H. & Lehrer, R.I. Secretion of type II phospholipase A2 and cryptdin by rat small intestinal Paneth cells. *Infect. Immunity* **64**, 5161–5165 (1996).
8. Selsted, M.E., Miller, S.I., Henschen, A.H. & Ouellette, A.J. Enteric defensins: antibiotic peptide components of intestinal host defense. *J. Cell Biol.* **118**, 929–936 (1992).
9. Porter, E.M., vanDam, E., Valore, E.V. & Ganz, T. Broad-spectrum antimicrobial activity of human intestinal defensin 5. *Infec. Immunity* **65**, 2396–2401 (1997).
10. Wilson, C.L. *et al.* Regulation of intestinal α-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* **286**, 113–117 (1999).
11. Garabedian, E.M., Roberts, L.J.J., McNevin, M.S. & Gordon, J.I. Examining the role of Paneth cells in the small intestine by lineage ablation in transgenic mice. *J. Biol. Chem.* **272**, 23729–23740 (1997).
12. Ganz, T. Extracellular release of antimicrobial defensins by human polymorphonuclear leukocytes. *Infect. Immunity* **55**, 568–571 (1987).
13. Lemaitre, B., Reichhart, J.M. & Hoffmann, J.A. Drosophila host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. *Proc. Natl Acad. Sci. USA* **94**, 14614–14619 (1997).

Departments of Medicine and Pathology, University of California, Los Angeles, CA 90095-1690, USA.
(TGanz@mednet.ucla.edu)

gp96—The immune system's Swiss army knife

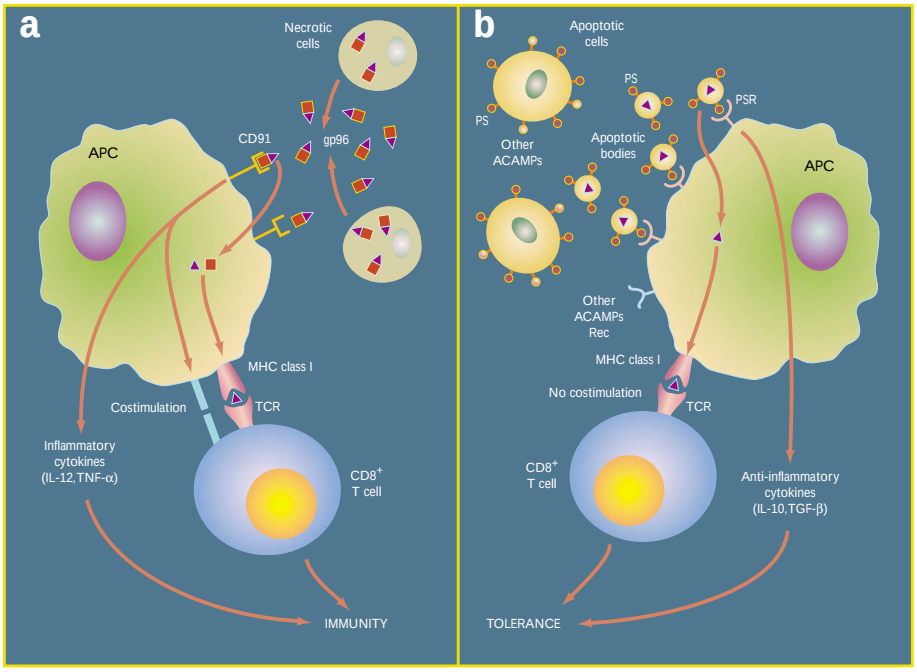
HANSJÖRG SCHILD AND HANS-GEORG RAMMENSEE

Tumor vaccines comprised of the chaperone gp96 can instigate specific immunity to tumor peptides. A receptor on macrophages for the uptake of peptide-loaded gp96 has been identified as CD91, the α_2-macroglobulin receptor.

The cellular heat shock protein gp96 is turning out to be a rather versatile molecule. It has long been known for its ability to induce immunity against antigens from the cell from which it came. For example, gp96 isolated from tumor cells induces tumor-specific immunity in animals that are intraperitoneally injected with it. Also, gp96 from the cells of BALB/c mice primes an anti–minor H cytotoxic T lymphocyte (CTL) response from C57BL/6 mice. One reason for this is the association of gp96 with peptides derived from cellular proteins. This is a strikingly specific reaction: despite the fact that other peptide-binding proteins (for example, protein disulfide isomerase) reside in the endoplasmic reticulum—the same cellular compartment that contains gp96—and that they are loaded with much larger amounts of peptides, they do not induce immunity. Thus, gp96 must possess additional features that account for its immunogenicity.

One of these features, which prods the immune system to wake up and take notice, is the recently demonstrated receptor-mediated uptake of gp96 by dendritic cells (DCs); the identity of the gp96 receptor(s), however, remained elusive. Now, in this issue of *Nature Immunology*, Srivastava's group—who discovered the immunogenicity of gp96 many years ago[1]—report the identification of CD91 as such a receptor. In this paper, Binder *et al*. tagged radiolabeled gp96 with a cross-linker capable of photoactivation and used this to extract an 80-kD molecule from the membrane of macrophages. Protein sequencing identified the molecule as CD91, which is known as the α_2-macroglobulin (α_2M) receptor and is expressed on phagocytes. The presentation of a gp96-associated peptide by antigen presenting cells (APCs) is indeed blocked by α_2-macroglobulin.

In addition to the identification of a receptor for gp96, Binder *et al*. incorporated this observation into an intriguing model for the general role of HSPs in the regulation of APC function. The finding that the gp96-mediated re-presentation of associated peptides can be inhibited by α_2M offers a mechanism for regulating antigen uptake by macrophages. In the blood, uptake of gp96-associated antigens by phagocytes is inhibited by the α_2M present in the serum. In tissues, however, CD91 becomes accessible to gp96 molecules, possibly



Bob Crimi

Figure 1. Necrotic cells and apoptotic cells exert opposite effects upon antigen presenting cells. **(a)** Necrotic cells release gp96 (and possibly other stress proteins) that carry peptides derived from the cells' proteins. gp96 is bound by CD91 on DCs and internalized. Peptides associated with gp96 are transferred to MHC class I molecules (by a so far unknown pathway) and, at the same time, gp96 induces the expression of costimulatory molecules and the release of interleukin 12 (IL-12) and tumor necrosis factor α (TNF-α) by the APC. Thus, the T cell receives both signals 1 and 2 and is thereby activated. Because cell necrosis only takes place as a result of nonphysiological assault such as injury, infection, or neoplasia, T cells specific for foreign or altered antigens are likely to be activated. **(b)** Apoptotic cells and the derived apoptotic bodies expose apoptotic cell–associated ligands (ACAMPS)[8] at the surface, one of which is phosphatidylserine (PS), which, as part of the inner membrane leaflet, faces towards the cytosol in intact cells and is flipped to the outside during apoptosis. PS is recognized by the 80-kD phosphatidylserine receptor (PSR) expressed on macrophages[4]. This leads to internalization of the apoptotic material, the re-presentation of antigen from the apoptotic cell on the APC's MHC class I molecules and the release of anti-inflammatory cytokines. Costimulatory molecules (such as B7) are not induced. Thus, the T cell recognizing the APC only receives signal 1, which is tolerogenic and leads either to anergy or deletion. As apoptotic cell death is the fate of many normal cells not only during development, but also at a later point, the antigens presented are usually self antigens. Thus, the whole process leads to permanent peripheral removal of self reactive T cells that have not undergone central deletion in the thymus. This drawing is based on a published illustration [3].

released from necrotic cells (during a viral infection, for example), and the associated peptides can then be presented in an immunogenic context to the adaptive immune system by macrophages and DCs.

Thus, the gp96-CD91 interactions may act as a sensor for tissue damage. Furthermore, the identification of CD91 as a gp96 receptor sheds new light on the dichotomy of the APC reaction to apoptotic *versus* necrotic cell death[3–7] (**Fig. 1**). 'Silent' cell death by apoptosis provides a tolerogenic cytokine milieu and induces APCs that lack costimulatory molecules, whereas necrotic cell death induces inflammatory cytokines and the expression of costimulatory molecules on APCs. Apoptotic cells do not release their contents as soluble molecules into the extracellular space; instead, the cells are fragmented into apoptotic bodies. These bodies as well as the apoptotic cells themselves expose phosphatidylserine on the outer leaflets of their membranes; this is sensed by APCs via their phosphatidylserine receptor and leads to the production of anti-inflammatory cytokines[4]. Other receptors, such as CD14 and CD36, recognize other apoptotic cell-associated ligands (ACAMPS)[8] and transmit similar tolerogenic signals to the APC[8,9].

In contrast, the contents of necrotic cells are released into the surrounding fluid and somehow trigger APCs to release pro-inflammatory cytokines and to express costimulatory molecules[5]. Recently, gp96 and HSP70 were was identified to be the first heat shock proteins to activate DCs[10]. It is likely, therefore, that both gp96 and HSP70 serve as messengers for necrotic cell death, and CD91 and other HSP-receptors on DCs as sensors for necrosis, which is equivalent to danger.

Thus, multifunctional gp96 can be viewed as combining a number of features. It carries peptides that represent cellular proteins, it transfers these peptides to the MHC class I molecules of DCs and other APCs via binding to CD91 and receptor-mediated endocytosis[2,12], it behaves like a cytokine in that it induces DCs to express costimulatory molecules such as B7[10] and to produce IL-12 and TNF-α[10], and, finally, it turns down the expression of its receptor on DCs[10]. Thus, gp96 molecules combine nonspecific signals for the innate immune system and specific signals for the adaptive immune system–they not only report the murder, they also provide a description of the suspect, thereby helping the immune system to discriminate between self and nonself, or between self and altered-self, respectively. On top of this, by binding to platelets as well (N. Hilf *et al*., unpublished data), gp96 might also be a messenger for the initiation of wound healing. Taken together, gp96 has at least as many functions as a mid-size pocket knife.

1. Srivastava, P.K. & Udono, H. Heat shock protein-peptide complexes in cancer immunotherapy. *Curr. Opin. Immunol.* **6**, 728–732 (1994).
2. Binder, R. J., Han, D. K. & Srivastava, P. K. CD91: a receptor for heat shock protein gp96. *Nature Immunol.* **2**, 151–155 (2000).
3. Green, D. R. & Beere, H. M. Apoptosis. Gone but not forgotten. *Nature* **405**, 28–29 (2000).
4. Fadok, V. A. *et al.* A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* **405**, 85–90 (2000).
5. Sauter, B. *et al.* Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J. Exp. Med.* **191**, 423–434 (2000).
6. Gallucci, S., Lolkema, M. & Matzinger, P. Natural adjuvants: endogenous activators of dendritic cells. *Nature Med.* **5**, 1249–1255 (1999).
7. Steinman, R.M., Turley, S., Mellman, I. & Inaba, K. Human CD14 mediates recognition and phagocytosis of apoptotic cells. *J. Exp. Med.* **191**, 411–416 (2000).
8. Devitt, A. *et al.* Human CD14 mediates recognition and phagocytosis of apoptotic cells. *Nature* **392**, 505–509 (1998).
9. Gregory, C. D. CD14-dependent clearance of apoptotic cells: relevance to the immune system. *Curr. Opin. Immunol.* **12**, 27–34 (2000).
10. Singh-Jasuja, H., *et al.* The heat shock protein gp96 induces maturation of dendritic cells and down-regulation of its receptor. *Eur. J. Immunol.* **30**, 2211–2215 (2000).
11. Moroi, Y. *et al.* Induction of cellular immunity by immunization with novel hybrid peptides complexed to heat shock protein 70. *Proc. Natl Acad. Sci. USA.* **97**, 3485–3490 (2000).
12. Singh-Jasuja *et al.* Cross-presentation of glycoprotein 96-associated antigens on major histocompatibility complex class I molecules requires receptor-mediated endocytosis. *J. Exp. Med.* **191**, 1965–1974 (2000).

University of Tübingen, Institute for Cell Biology, Department of Immunology, Auf der Morgenstelle 15, D-72076 Tübingen, Germany. (rammensee@uni-tuebingen.de)