0031-3998/03/5302-0203
PEDIATRIC RESEARCH
Vol. 53, No. 2, 2003
Printed in U.S.A.

DOI: 10.1203/01.PDR.0000047470.04132.87

COMMENTARY

Vernix, the Newborn, and Innate Defense

Commentary on the article by Yoshio *et al.* on page 211

MICHAEL ZASLOFF

Georgetown University Medical Center, Department of Pediatrics, Washington, DC 20007, U.S.A.

Considering the importance of the fetus to our survival as a species, it is surprising that we know so little about what protects it from microbial assault. The literature teaches us that what separates the fetus from the microbes that pass from the mother's rectum or inhabit her genitourinary tracts is a simple physical barrier, "a mucous plug." The fetus itself is immunologically naïve. We are told that the fetus exists in a state of "immunologic" privilege relative to its mother, because otherwise it would be regarded as "foreign" and destroyed no differently than an unwelcome cancer cell or a transplanted organ from its father or a nonidentical sibling. Indeed, if microbes were to invade the amniotic sac and begin an assault on the fetus, we have virtually no body of knowledge available to guide us as to how the fetus, living in its privileged enclave, could defend itself.

Recent discoveries in immunology suggest that powerful innate systems of defense, previously unrecognized, actually defend the fetus in its uterine home with a potency and certainty that begins to explain the paradox (1–4). The report by Yoshio *et al.* in this month's issue discusses the nature of the antimicrobial defenses provided to the newborn by the vernix caseosa in the context of innate immunity.

Multicellular animals evolved in a world first inhabited by microbes and emerged before the evolution of adaptive immunity. The ancient system of defense, called "innate immunity," can be found in creatures that appeared millions of years before the first vertebrate and persist in their modern counterparts as the principal mode of antimicrobial defense. The insect, for instance, does not produce an antibody or a lymphocyte (5). Neither does the octopus, like all invertebrates.

The innate system utilizes molecules such as lysozyme, first discovered by Alexander Fleming; complement; and lectins, such as the mannose binding protein (1). Cellular elements, such as the types of phagocytic cells that migrate to sites of injury, such as neutrophils and macrophages, are part of this ancient system.

Recently, a new arm of the innate system has been discovered. The weapons are antimicrobial peptides (2–4). Every living organism produces them. In multicellular organisms, these peptides are generally gene encoded, processed from precursors, and produced by both epithelial tissues and cells that circulate through the organism's vascular network. The antimicrobial peptides are diverse in structure, broad spectrum, and disinfectant-like in their mode of action. In general, they target a microbe's cytoplasmic membrane, damaging it fatally and in many cases actually also permeating the membrane to inactivate further the intracellular targets. Bacteria, fungi, protozoa, and even viruses are commonly susceptible. The nature of the microbial target, being the design and composition of the microbial membrane, results in the low probability that a microbe can evolve a defense against this type of weapon (4).

In humans, two major classes of antimicrobial peptide have been described: the defensins and the cathelicidins. Defensins fall into two major subgroups, alpha and beta. Only one cathelicidin exists in humans. The alpha defensins are produced in leukocytes, such as neutrophils. They are also produced by Paneth cells, the granule-rich cells that lie at the base of the crypts of the gastrointestinal tract. In the fetus, Paneth cell defensins are synthesized by the 25th week of gestation and continue to increase in abundance as term approaches (5). In white cells, defensins are responsible for nonoxidative microbial killing. Paneth cells secrete defensins into the bowel lumen, where they serve to control the growth of microbial populations in a nutrient-rich incubator-like environment of the small bowel. At least four beta defensins are produced in humans, all principally by epithelial tissues and differing from alpha defensins in the manner in which they are folded. HBD1 is constitutively synthesized and produced in early fetal life in tissues such as the urinary tract. The others are inducible. (The beta defensin story is clearly still incomplete, because >20 new genes have been discovered in our genome and the expression and tissue distribution of these gene products have yet to be fully explored (6).) Cathelicidins represent another family, and humans possess only one, LL-37 (7). This antibiotic peptide can be secreted from leukocytes, after exposure to microbes, and will accumulate in both the cerebrospinal fluid and blood. LL-37 is strongly induced on epithelial surfaces after an injury, microbial invasion, or a stimulus that generally provokes inflammation.

Received October 7, 2002; accepted October 7, 2002.
Correspondence: Michael Zasloff M.D, Ph.D., Georgetown University Medical Center, Department of Pediatrics, 3900 Reservoir Road NW, Washington, DC 20007, U.S.A.; e-mail: Maz5@georgetown.edu

Beta defensins and LL-37 protect our epithelial surfaces. On dry epithelia, they function as "preservatives." On mucosal surfaces, they can be secreted into the biofilm that covers the epithelial surface, creating an environment that is chemically hostile for microbial survival. Cystic fibrosis is associated with chronic infection in part because the genetic defect impairs the body's capacity to control the precise milieu required for optimal functioning of the resident antimicrobial peptides. In CF, *Staphylococcus aureus* and *Pseudomonas aeruginosa* proliferate, inducing a futile and destructive inflammatory response that ultimately destroys the functionality of the lung (4).

The receptors that are linked to the expression of the inducible antimicrobial peptides expressed by our epithelial surfaces have recently been described (8). On the basis of analogous receptors found in insects, they have been termed toll-related receptors (TLR). At least 10 different receptors have been described. Each TLR seems to recognize a specific microbial component. TLR4, for example, responds to lipopolysaccharide. The current view is that both epithelial tissues and circulating cells designated to guard the "front line" separating ourselves and the microbes in the environment are equipped with TLR linked through intracellular networks that converge on specific batteries of antimicrobial genes.

Another interesting twist on our relationship between microbes and ourselves has come from studies on the relationship between microbes that we have come to regard as beneficial "commensals" and the innate immune system (3, 9). In general, commensals, such a Lactobacilli, are resistant to endogenous antimicrobial peptides. However, they themselves induce expression of antimicrobial peptides. Their presence can keep the epithelial defense system chronically on guard. The microbes that initially populate the epithelial surfaces in newborns might play a role in stimulating the innate system.

The report by Yoshio *et al.* explores the antimicrobial properties of vernix caseosa, the creamy white substance that covers the skin of term babies. Vernix is a greasy hydrophilic substance that has long been regarded as a protective barrier of some sort, notably as an emollient-like agent. Recent studies have suggested that vernix has antimicrobial activity and could be providing a fetus with a "protective shield" while *in utero* or in preparation for its eventual entry into a microbially hostile world.

In this study, Yoshio *et al.* collected vernix, extracted the constituent peptides, and identified the presence of antimicrobial peptides. The unfractionated proteinaceous extract of vernix exhibited activity against a Gram-positive microbe and a fungal microbe, *Candida albicans*. Antimicrobial peptides identified in vernix included alpha defensins and LL-37. Yoshio *et al.* suggest that vernix provides a layer of antimicrobial defense for the infant after delivery.

Several intriguing issues are provoked by this study. Does the vernix protect the fetus from subclinical intrauterine infections, while *in utero*? Which tissue contributes to the antimicrobial peptides present in vernix? Does variation in the expression of these peptides occur normally, and is it correlated with susceptibility to intrauterine or perinatal infection? It is curious that the mucous plug that forms at the os of the cervix contains many of the same peptides present in vernix (10), suggesting that both vernix and the mucous plug have been designed to defend against specific microorganisms. What might these organisms be? Can the physical and chemical properties of the vernix be duplicated in the synthetic laboratory and serve a therapeutic purpose in the treatment or prevention of disease? What is the physical structure of the vernix on the surface of the fetus? Is it a stratified, physically structured and organized covering, with peptides highly concentrated within a narrow discrete layer? Vernix itself has potent inflammatory properties, as evidenced by numerous reports of vernix-associated maternal peritonitis (11). Might vernix have a proinflammatory role in the fetus, acting as an inducer of fetal epithelial defensin expression before birth? Indeed, recent studies suggest that the newborn's skin expresses high levels of beta defensins and LL-37, at levels much higher than found in normal healthy adult skin (R. Gallo, personal communication). Many wonderful questions are provoked by this simple study of a film that we wipe from the skin of the newborn baby. Like birth itself, vernix is another miracle.

REFERENCES

1. Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA 1999 Phylogenetic perspectives in innate immunity. Science 284:1313–1318
2. Huttner KM, Bevins CL 1999 Antimicrobial peptides as mediators of epithelial host defense. Pediatric Res 45:785–794
3. Boman HG 2000 Innate immunity and the normal microflora. Immunol Rev 173:5–16
4. Zasloff M 2002 Antimicrobial peptides of multicellular organisms. Nature 415:389–395
5. Salzman NH, Polin RA, Harris MC, Ruchelli E, Hebra A, Zirin-Butler S, Jawad A, Martin Porter E, Bevins CL 1998 Enteric defensin expression in necrotizing enterocolitis. Pediatr Res 4:20–26
6. Schutte BC, Mitros JP, Bartlett JA, Walters JD, Jia HP, Welsh MJ, Casavant TL, McCray Jr PB 2002 Discovery of five conserved beta-defensin gene clusters using a computational search strategy. Proc Natl Acad Sci U S A 99:2129–2133
7. Gennaro R, Zanetti M 2000 Structural features and biological activities of the cathelicidin-derived antimicrobial peptides. Biopolymers 55:31–49
8. Akira S, Takeda K, Kaisho T 2001 Toll-like receptors: critical proteins linking innate and adaptive immunity. Nat Immunol 2:675–680
9. Krisanaprakornkit S, Kimball JR, Weinberg A, Darveau RP, Bainbridge BW, Dale BA 2000 Inducible expression of human beta-defensin 2 by Fusobacterium nucleatum in oral epithelial cells: multiple signaling pathways and role of commensal bacteria in innate immunity and the epithelial barrier. Infect Immun 68:2907–2915
10. Hein M, Valore EV, Helmig RB, Uldbjerg N, Ganz T 2002 Antimicrobial factors in the cervical mucus plug. Am J Obstet Gynecol 187:137–144
11. Davis JR, Miller HS, Feng JD 1998 Vernix caseosa peritonitis: report of two cases with antenatal onset. Am J Clin Pathol 109:320–323