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# Innate Immunity Stimulates Permeability Barrier Homeostasis

Kenneth R. Feingold[1]

**A key function of the skin is to provide a permeability barrier to restrict the movement of water, electrolytes, and other small molecules between the outside environment and the internal milieu. Following disruption of the permeability barrier, there is a rapid restoration of barrier function, and one of the key signals initiating this repair response is a decrease in the concentration of calcium in the outer epidermis. In this issue, Borkowski *et al.* present evidence showing that activation of Toll receptor 3 by double-stranded RNA may be another pathway for activation of permeability barrier repair. These results provide further evidence for a link between innate immunity and the permeability barrier.**

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An important function of the skin is to form a barrier between an outside hostile environment and the internal milieu. One key barrier function is to restrict the movement of water, electrolytes, and other small compounds, that is, the permeability barrier. Without a competent permeability barrier, life outside of the sea would be impossible. Recent studies have linked abnormalities in permeability barrier function to the development and progression of common skin disorders, including atopic dermatitis and psoriasis (Wolf and Wolf, 2012; Wolf *et al.*, 2012).

The permeability barrier is mediated by lipid-enriched lamellar membranes located in the extracellular spaces of the stratum corneum (Feingold, 2007). The membranes contain ceramides, cholesterol, and free fatty acids predominantly. These lipids are delivered to the stratum corneum through the exocytosis of lamellar bodies from stratum granulosum cells (Feingold, 2007). In turn, the lamellar bodies contain glucosylceramides, sphingomyelin, phospholipids, and cholesterol. To form functional lamellar membranes, the glucosylceramides and sphingomyelin are converted to ceramides, a process catalyzed by the enzymes beta-glucocerebrosidase and acid sphingomyelinase, and the phospholipids are converted to free fatty acids, a process catalyzed by PLA2 (Feingold, 2007). The synthesis of lamellar bodies by keratinocytes requires a sufficient pool of cholesterol, sphingolipids, and phospholipids. Fatty acids are required for the synthesis of phospholipids and sphingolipids, and they are derived from either *de novo* synthesis by keratinocytes and/or uptake of exogenous fatty acids by keratinocytes (Feingold, 2007). Cholesterol may also be synthesized in keratinocytes and/or transported into keratinocytes by several uptake pathways (Feingold, 2007). The mechanism by which phospholipids and cholesterol are incorporated into lamellar bodies is not known, but recent studies have demonstrated that the transport of glucosylceramides into lamellar bodies is mediated by ABCA12 (Feingold, 2007).

Following disruption of the permeability barrier, there is a rapid restoration of barrier function (Feingold, 2007). A number of responses occur in the underlying epidermis that facilitate this repair process, including: (1) an almost immediate secretion of a pre-formed pool of lamellar bodies, (2) increased lipid synthesis, (3) an increase in lipoprotein receptors and fatty acid transport proteins that facilitate the

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## Clinical Implications

- A crucial function of the skin is to form a barrier between an outside hostile environment and the internal milieu, and abnormalities in barrier function are linked to common skin disorders, including atopic dermatitis and psoriasis.
- The study by Borkowski *et al.* (this issue) suggests that an increase in double-stranded RNA that occurs with epidermal tissue injury could, via activation of the Toll receptor 3 signaling pathway, stimulate lamellar body formation and permeability barrier repair.
- In combination with earlier studies, these results demonstrate additional links among innate immunity, host defense, and permeability barrier homeostasis.

uptake of exogenous lipids, (4) increased expression of enzymes required for extracellular processing of lipids, and (5) increased formation of new lamellar bodies (Feingold, 2007). One of the key signals that activates this repair program is a change in the level of calcium and other ions in the stratum granulosum (Feingold, 2007; Feingold and Denda, 2012). Under normal conditions, there are high concentrations of calcium in the stratum granulosum. Following permeability barrier disruption, the calcium concentration decreases markedly, and barrier repair ensues. If the decrease in the calcium gradient is inhibited, barrier repair is also inhibited. Conversely, if one lowers the concentration of calcium in the outer epidermis without disrupting the barrier, the secretion and formation of lamellar bodies is stimulated. Thus, the concentration of calcium in the stratum granulosum controls the initiation and/or inhibition of barrier formation (Feingold, 2007; Feingold and Denda, 2012).

In this issue, Borkowski *et al.* (2013) describe another potential mechanism for the activation of permeability barrier repair. Epidermal injury leads to an increase in double-stranded RNA (dsRNA) that can bind to Toll receptor 3 (TLR3) (Lai *et al.*, 2009; Bernard *et al.*, 2012). Toll receptors are well known to recognize pathogen-associated molecular patterns and TLR3 recognizes dsRNA, which is a key pathway by which the host recognizes and combats viral infections (Aderem and Ulevitch, 2000). Borkowski *et al* demonstrate that treatment of keratinocytes with dsRNA stimulates the expression of several genes that have key roles in permeability barrier repair. Specifically, they demonstrate the upregulation of ABCA12, glucocerebrosidase, acid sphingomyelinase, serine palmotyltranferase (the first enzyme in the synthesis of ceramides), glucosylceramide synthase, and transglutaminase 1. They further show that many of these changes induced by dsRNA are mediated by TLR3 by demonstrating that, when TLR3 is knocked down using siRNA or the intracellular signaling pathways for TLR3 are blocked, the upregulation of many of these proteins by dsRNA fails to occur. Notably, the increase in enzymes involved in ceramide and glucosylceramide synthesis is not inhibited, indicating that recognition pathways in addition to TLR3 may have important roles. Furthermore, the authors show that dsRNA treatment leads to an increase in oil red O staining in keratinocytes, and lipid analysis revealed an increase in certain sphingolipids, without changes in cholesterol. The marked increase in oil red O staining is unlikely to be accounted for by the relatively small increase in sphingolipids. Unfortunately, triglyceride levels were not determined, but it is likely that an increase in triglycerides could account for the increase in oil red O staining. Finally, and most importantly, Borkowski *et al.* demonstrate that treatment with dsRNA increases the number of lamellar bodies in skin constructs and that this increase is dependent on TLR3. Taken together, these results suggest that the increase in dsRNA that occurs with epidermal tissue injury could stimulate lamellar body formation and permeability barrier repair. These results suggest that activation of innate immunity in the epidermis stimulates permeability barrier homeostasis. It should be recognized that dsRNA and TLR3 activation appears to stimulate only certain aspects of the permeability barrier repair program. For example, cholesterol and fatty acid synthesis do not appear to be enhanced. Future studies determining the ability of TLR3-deficient mice to restore permeability barrier function following barrier perturbation will be required to understand the role and significance of the TLR3 signaling pathway more fully. Whether the activation of other Toll receptors or the nucleotide-binding oligomerization domain-like receptor (NLR) family of proteins stimulates permeability barrier homeostasis also needs to be addressed.

That there may be links between innate immunity and the permeability barrier should not be surprising. Providing both a permeability barrier and a barrier to microorganisms are key functions of the skin. Moreover, earlier studies have shown that these functions are linked. For example, disruption of the permeability barrier has been shown to increase the synthesis of anti-microbial peptides, whereas the production of anti-microbial peptides has been shown to have an important role in the formation of the permeability barrier (Aberg *et al.*, 2008; Ahrens *et al.*, 2011; Rodriguez-Martin *et al.*, 2011). Thus, it is easy to visualize that the activation of pathogen-associated molecular pattern receptors (TLRs) by either microbial products or endogenous compounds could stimulate permeability barrier formation. A competent permeability barrier limits the ability of microorganisms and toxic products to enter the host.

Finally, it should be recognized that there is a link between activation of TLRs and lipid metabolism that extends beyond keratinocytes and the epidermis. Our laboratory and others have shown

that LPS, a TLR4 activator, stimulates cholesterol ester and triglyceride storage in macrophages (Lopes-Virella *et al.*, 1987; Oiknine and Aviram, 1992; Funk *et al.*, 1993). When macrophages were incubated with cholesterol ester-rich lipoproteins, LPS-stimulated cholesterol ester accumulated, whereas in the absence of cholesterol ester-enriched lipoproteins there was an increase in triglyceride accumulation (Lopes-Virella *et al.*, 1987; Oiknine and Aviram, 1992; Funk *et al.*, 1993). In the presence of triglyceride-enriched lipoproteins or free fatty acids, the increase in macrophage triglyceride storage was markedly enhanced by LPS (Funk *et al.*, 1993; Feingold *et al.*, 2010, 2012). Activation of TLR2 by zymosan or TLR3 by dsRNA has also been shown to increase lipid accumulation in macrophages (Feingold *et al.*, 2010, 2012). In addition, incubation of bacteria with macrophages results in lipid accumulation, which is dependent on TLR signaling (Nicolaou and Erridge, 2010; Nicolaou *et al.*, 2012). Finally, lipid-filled macrophages have been observed during chronic inflammatory conditions such as parasitic infections and leprosy (D'Avila *et al.*, 2008; Mattos *et al.*, 2010; Nicolaou and Erridge, 2010). This increase in lipid storage is thought to have a role in the ability of macrophages to fight infection. The lipolysis of triglycerides has a key role in macrophage phagocytosis and the increase in fat droplets may have a role in sequestering intracellular microorganisms and preventing their replication (Chandak *et al.*, 2010). It is also possible that the large quantities of triglycerides or perhaps metabolic products of triglycerides (for example, free fatty acids) may be toxic to microorganisms and have a role in killing bacteria, viruses, or fungi (Drake *et al.*, 2008). Thus, one could speculate that the stimulation of lipid metabolism induced by TLR3 activation, leading to an increase in lipids in keratinocytes, may have a role not only in facilitating permeability barrier repair but perhaps also in enhancing host defense.

A very recent study has linked activation of TLR2 with skin barrier repair. In TLR2 KO mice there is a delay in the repair of the permeability barrier (Kuo *et al.*, 2013).

## CONFLICT OF INTEREST

The author states no conflict of interest.

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