DOI: 10.1111/pde.13516

WILEY Pediatric Dermatology

# Expression of cutaneous immunity markers during infant skin maturation

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## Abstract

**Background/Objectives:** Infant skin undergoes a maturation process during the early years of life. Little is known about the skin's innate immunity. We investigated the dynamics of innate immunity markers collected from the surface of infant skin during the first 36 months of life.

**Methods:** A total of 117 healthy infants aged 3-36 months participated in the study. We extracted human beta defensin-1 and interleukin 1 alpha and its receptor antagonist using transdermal analysis patches from the skin surface of the posterior lower leg area. The extracts were analyzed using a spot enzyme-linked immunosorbent assay.

**Results:** Skin surface human beta defensin-1 levels were higher early in life and decreased with infant age. The ratio of interleukin 1 alpha receptor antagonist to interleukin 1 alpha did not change significantly with age but showed a distinct difference between sexes, with female infants having higher values than male infants.

**Conclusion:** As is the case with skin structure and functional properties, cutaneous innate immunity also appears to undergo a maturation period during infancy, with innate immunity slowly declining as adaptive immunity takes over. Sex differences in immune markers may explain sex-dependent susceptibilities to infection.

KEYWORDS
skin barrier, quality of life, subcutaneous tissue/panniculitis/lipodystrophy, dermatopathology, genetic diseases/mechanisms

## 1 | INTRODUCTION

The skin is the body's first defense mechanism against infection. The structure and biochemical composition of the stratum corneum (SC), the outermost layer of the epidermis, contribute to this barrier function between the body and the environment. The brick-and-mortar structure of the SC provides the physical component of the barrier, and the biochemical component comprises molecules belonging to the innate and adaptive immune systems. Secreted antimicrobial peptides (AMP) and antimicrobial lipids (AML) are present on the SC and constitute the innate immune system of the skin, whereas the viable epidermis just below the SC plays an active role in regulating the adaptive immunologic response (e.g. through the production of interleukins [IL], growth factors).

Epithelial cells secrete a group of AMP molecules called defensins,[1] small cationic peptides that show broad antimicrobial activity against bacteria, fungi, and enveloped viruses by perturbing their membranes.[2] Their expression pattern and regulation in each cell

type is specific for the defensive role that AMP play[3] and as such are classified into three distinctive subfamilies: α-, β-, and θ-defensins.[3] β-defensins are primarily identified in epithelial cells, including keratinocytes, but are also expressed in different cell types, such as peripheral blood mononuclear cells, kidney cells, and cells of other tissues.

During infancy, skin undergoes continuing maturation of its structure[4] and function;[5] the skin microbiome also undergoes significant changes.[6] Expression of skin defensins has been studied in neonates but not in older infants. Dorschner et al[7] showed that skin in the perinatal period constitutively produces AMP. Although certain cathelicidins, a different family of secreted AMP, are significantly higher in the perinatal period than in adult skin,[7] this overexpression is not apparent with β-defensins, which have been found to be present in similar amounts in perinatal and adult skin in mice.[7] Loeffelbein et al[8] documented similar expression profiles of human β-defensin 1 (hBD-1) in the lip vermilion mucosa of neonates as in that of adults. We studied the presence of hBD-1 on the skin surface of infants aged 3-36 months, a period of continuing maturation.

## 2 | MATERIALS AND METHODS

One hundred seventeen healthy infants aged 3-36 months with maternal perceived sensitive skin participated in the study after receipt of signed parental consent. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Samples were collected from the skin surface of the posterior lower leg (calf) area using a transdermal patch (Transdermal Analysis Patch Kit, FibroTX, Tallinn, Estonia) following the manufacturer's directions. The extracted amounts of IL-1 alpha (IL-1α), IL-1 receptor antagonist (IL-1RA), and hBD-1 were visualized using a spot enzyme-linked immunosorbent assay.[9]

## 3 | RESULTS AND DISCUSSION

Skin surface hBD-1 levels were found to be higher early in life and decrease with age (Figure 1). Previously reported levels for the apparent concentration of hBD-1 on the skin surface of adults were approximately 0.2 ng/mL,[9] which is similar to levels we found for the children at 36 months old. According to Dorscher et al,[7] newborns have little ability to initiate an appropriate immune response to bacterial infection, which suggests that innate immunity early in life may in part compensate for the developmental immaturity of the adaptive immune system[3] and that the innate antimicrobial peptide defense mechanism is a means of antimicrobial protection in newborns.[7]

According to Capone et al,[6] microbial colonization of the human skin begins immediately after birth and is not fully established until after the first year of life. It was found that the number of genera does not significantly change over the first year of life; rather there was evidence of greater diversity and evenness in the microbial

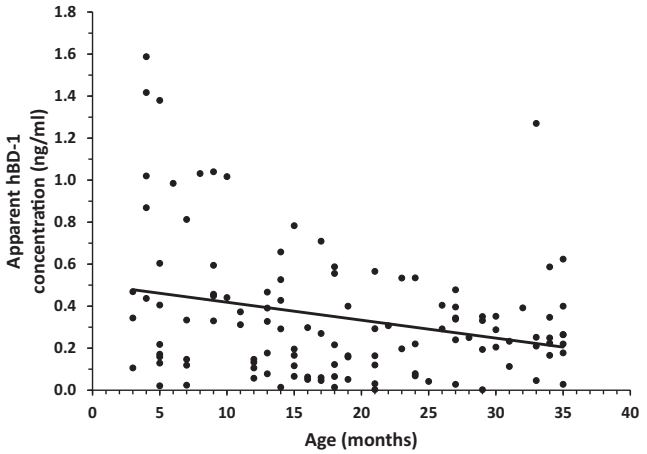


**FIGURE 1** Skin surface concentration of human β-defensin 1 (hBD-1) is higher early in life and decreases with age ($P < .05$ for the linear regression)

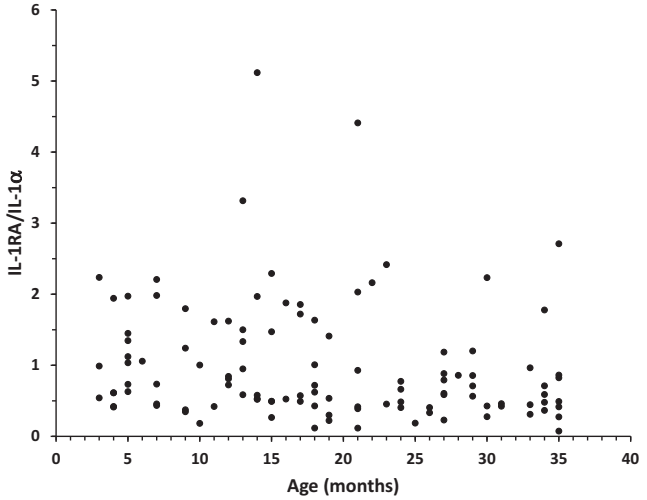


**FIGURE 2** Skin surface levels of the ratio of interleukin (IL)-1 receptor antagonist (RA) to IL-1α are independent of infant age

populations of the skin sites sampled.[6] This initial exposure to microbial diversity may be critical to the education of the immune system and development of proper function. In the absence of mature adaptive immunity, an innate immune system is the only defense mechanism against bacterial infection. According to Dorscher et al,[7] defensins were found in similar concentrations when perinatal skin was with adult skin in their murine model. Their study examined mouse β-defensin expression and used only hBD-2 immunostaining. The presence of antimicrobial peptides is not unique to infant skin. However, the observation that hBD-1 shows a higher concentration on the infant skin surface compared to adult implies that innate immunity is strong early in life compensating for the weaker adaptive immune defense system that requires longer time to mature.

The IL-1 family of pro-inflammatory cytokines, which consists of two agonists, IL-1α and IL-1ß, and a specific receptor antagonist (IL-1RA) is also a component of the skin immune barrier.[10] IL-1 is

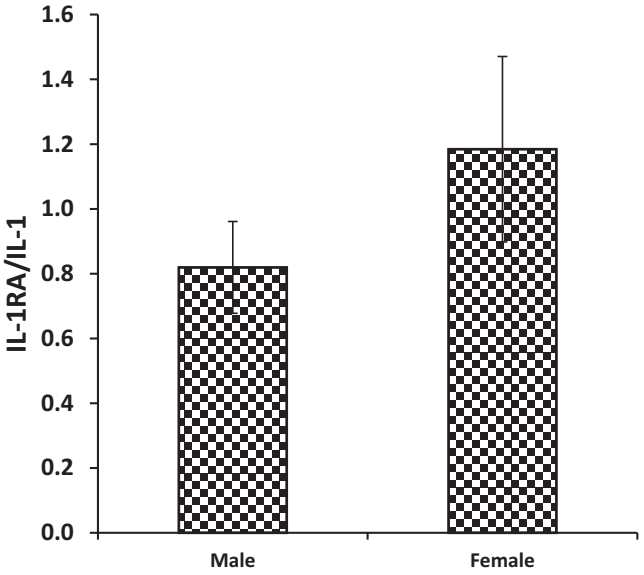


**FIGURE 3** Skin surface levels of the ratio of interleukin (IL)-1 receptor antagonist (RA)/IL-1α is higher in female than male infants ($P < .05$). Error bars indicate 95% confidence intervals for the mean

associated with the host defense against microorganisms that proliferate inside the mammalian cells.[10] Keratinocytes and other epithelial cells secrete IL-1α, which has been shown to be associated with keratinocyte differentiation, as well as upregulation of certain AMP associated with cutaneous immunity.[11] Nevertheless, according to Bando et al,[11] although IL-1α selectively modulates the expression of specific cutaneous AMP, IL-1α does not affect or regulate hBD-1 expression. hBD-1 is shown to be constitutively expressed in healthy and inflamed skin tissue.[11] It is likely that IL-1α expression does not regulate hBD-1 levels. Instead, our observation that hBD-1 levels are higher early in life and decrease with infant age supports the notion of an enhanced innate immune barrier early on in infant development.

Keratinocytes and other epithelial cells also secrete IL-1RA, which when bound to IL-1 receptors competitively antagonizes the inflammatory effects of IL-1α and IL-1ß.[12] Skin reactivity, or the ability of the skin to react to external factors, can be measured by assessing the relationship between IL-1RA and IL-1α.[13] The ratio of IL-1RA to IL-1α increases in response to certain environmental conditions such as ultraviolet exposure, temperature, and contact because IL-RA has been shown to modulate the inflammatory response in skin.[13] The balance between IL-1RA and IL-1α also modulates susceptibility to infection; a higher ratio of IL-RA to IL-1α is associated with greater susceptibility to infection, whereas a lower ratio is associated with greater resistance to infection.[10]

The quality of skin barrier function improves over the first years of life.[5] Nevertheless, although molecular diffusion in the SC and therefore barrier quality may affect measurements of skin surface levels of IL-1α and IL-1RA, taking their ratio is expected to normalize such effects. On the other hand, there is no evidence of any diffusion process for hBD-1, since there is no observation of a concentration gradient in the SC, which shows strong immunostaining for hBD-1 throughout its thickness.[14] It is likely that hBD-1 is packaged in lamellar bodies, as hBD-2 is.[15] Therefore, the quality of the skin barrier is not expected to affect the surface concentration of hBD-1 strongly.

Data from the present study show that the skin surface ratio of IL-1RA to IL-1α does not change significantly over the first 36 months of life (Figure 2). Female infants have a significantly higher ratio of IL-1RA to IL-1α than male infants (Figure 3), which may imply sex differences in capacity to elicit an immune response, for example, after onset of an infection. Robinson et al[16] showed that females experience more severe disease and have worse outcomes from an influenza virus infection than males. Infections related to diseases caused by highly pathogenic influenza viruses initiate pro-inflammatory responses by the host,[16] and it is likely that multiple factors, including sex hormones, indirectly connected sex differences in disease outcome.[16] Breaking the collected data into groups defined according to sex or ethnicity did not result in any statistically significant differences for the apparent concentration of hBD-1. There was no effect of ethnicity on the ratio of IL-1RA to IL-1α (data not shown).

From the values of IL-1α and IL-1RA reported previously in adults,[9] we can infer that the ratio of IL-1RA to IL-1α ranges from 1 to 2, which is the same range we report in Figure 3.

In conclusion, we present for the first time data relating to innate skin immunity that contribute to our understanding of skin maturation processes during the first years of life. Innate immunity appears to be active early in life and decreases as the adaptive immune system matures. The data show that infant skin maturation affects expression of the innate immunity marker hBD-1 but not the immune reactivity marker of the ratio of IL-1RA to IL-1α. It is tempting to hypothesize that our observations may be the first early-life connection between sex hormones and immune response and may provide evidence of the reported greater susceptibility to infection of female infants.

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## REFERENCES

1. Nemes Z, Steinert PM. Bricks and mortar of the epidermal barrier. *Exp Mol Med*. 1999;31:5-19.
2. Parham P. *The immune system*, 3rd edn. New York, KY: Garland Science; 2009.
3. Bardan A, Nizet V, Gallo RL. Antimicrobial peptides and the skin. *Expert Opin Biol Ther*. 2004;4:543-549.
4. Stamatas GN, Nikolovski J, Luedtke MA, et al. Infant skin microstructure assessed in vivo differs from adult skin in organization and at the cellular level. *Pediatr Dermatol*. 2009;27:125-131.
5. Nikolovski J, Stamatas GN, Kollias N, Wiegand BC. Barrier function and water-holding and transport properties of infant stratum corneum are different from adult and continue to develop through the first year of life. *J Invest Dermatol*. 2008;128:1728-1736.

6. Capone KA, Dowd SE, Stamatas GN, Nikolovski J. Diversity of the human skin microbiome early in life. *J Invest Dermatol*. 2011;131:2026-2032.
7. Dorschner RA, Lin KH, Murakami M, Gallo RL. Neonatal skin in mice and humans expresses increased levels of antimicrobial peptides: innate immunity during development of the adaptive response. *Pediatr Res*. 2003;53:566-572.
8. Loeffelbein DJ, Steinstraesser L, Rohleder NH, et al. Expression of host defense peptides in the lip vermilion mucosa during early infancy. *J Oral Pathol Med*. 2011;40:598-603.
9. Orro K, Smirnova O, Arshavskaja J, et al. Development of TAP, a non-invasive test for qualitative and quantitative measurements of biomarkers from the skin surface. *Biomark Res*. 2014;2:20.
10. Arend WP. The balance between IL-1 and IL1RA in disease. *Cytokine Growth Factor Rev*. 2002;13:323-340.
11. Bando M, Hiroshima Y, Kataoka M, et al. Interleukin-1α regulates antimicrobial peptide expression in human keratinocytes. *Immunol Cell Biol*. 2007;85:532-537.
12. Perrier S, Darakhshan F, Hajduch E. IL-1 receptor antagonist in metabolic diseases: Dr Jekyll or Mr Hyde? *FEBS Lett*. 2006;580:6289-6294.
13. Hirao T, Aoki H, Yoshida T, et al. Elevation of interleukin 1 receptor antagonist in the stratum corneum of sun-exposed and ultraviolet B-irradiated human skin. *J Invest Dermatol*. 1996;106:1102-1107.
14. Ali RS, Falconer A, Ikram M, et al. Expression of the peptide antibiotics human β defensin-1 and human β defensin-2 in normal human skin. *J Invest Dermatol*. 2001;117:106-111.
15. Oren A, Ganz T, Liu L, Meerloo T. In human epidermis, beta-defensin 2 is packaged in lamellar bodies. *Exp Mol Pathol*. 2003;74:180-182.
16. Robinson DP, Lorenzo ME, Jian W, Klein SL. Elevated 17β-estradiol protects females from influenza A virus pathogenesis by suppressing inflammatory responses. *PLoS Pathog*. 2011;7:e1002149.

**How to cite this article:** Kirchner F, Capone KA, Mack MC, Stamatas GN. Expression of cutaneous immunity markers during infant skin maturation. *Pediatr Dermatol*. 2018;35:468–471. https://doi.org/10.1111/pde.13516