TO THE EDITOR

Psoriasis is a highly visible, chronic, immune-mediated inflammatory skin disorder that affects 2–3% of the US population (Lowes et al., 2014). Tumor necrosis factor-α (TNF-α) and IL-17A synergistically up-regulate the production of other cytokines, chemokines, and antimicrobial peptides from keratinocytes and regional immune cells, initiating and perpetuating the immune activation of psoriasis (Chiricozzi et al., 2011; Di Cesare et al., 2009; Ettehadi et al., 1994; Harden et al., 2015; Lowes et al., 2005). Humanized antibodies and inhibitory fusion proteins inhibiting TNF-α are commercially available for treating moderate to severe psoriasis (Lowes et al., 2007). These systemically administered biologic agents are targeted to disease pathogenesis and have better efficacy and safety than broad immunosuppressants such as cyclosporine and methotrexate. Their high cost and potential adverse effects limit systemic administration for milder disease, but their high molecular weight precludes topical formulation.

We have generated a TNF-α—suppressing antisense spherical nucleic acid (SNA), a promising construct to emerge from the field of nanotechnology (Banga et al., 2014; Cutler et al., 2012; Giljohann et al., 2009; Zheng et al., 2012). These 3-dimensional (3D) arrangements of densely packed and radially oriented oligonucleotides (see Supplementary Figure S1 online) impart properties distinct from linear nucleic acids, especially skin penetration capability without physical or chemical skin disruptors and increased cellular uptake. SNAs use scavenger receptors to enter cells, whereas other oligonucleotide delivery systems (e.g., cationic lipids or polymers) often disrupt anionic cell membranes for delivery (Choi et al., 2013).

Liposomal-cored SNAs (L-SNAs) are physiologically compatible but share characteristics of the early-generation trackable gold-cored SNAs (Banga et al., 2014; Randeria et al., 2015; Zheng et al., 2012). Using both a human 3D cytokine-induced raft model and the mouse imiquimod (IMQ)-generated psoriasis-like model, we found that TNF-targeting L-SNAs prevent the phenotype clinically, histologically, and transcriptionally, suggesting a topical treatment paradigm for psoriasis.

For L-SNA generation, oligonucleotides and 50-nm-diameter liposomes (100:1) were self-assembled to form L-SNAs (see Supplementary Figure S1 and Supplementary Materials online). mRNA expression was assessed by quantitative PCR (see Supplementary Table S1 online). In all studies, data were analyzed by analysis of variance (group) or paired t testing (individual comparisons), with P less than 0.05 considered significant.

Cyanine 5—labeled TNF L-SNAs were taken into normal human epidermal keratinocyte (NHEK) cytoplasm within 15 minutes (Figure 1a) of exposure (keratinocytes for NHEK studies were prepared from otherwise discarded unidentified foreskins, obtained through expedited institutional approval that required no written informed parental consent). TNF L-SNAs knocked down mRNA expression in TNF-α—induced NHEKs by 48 hours (TNF by 93%, DEFB4 (encoding β-defensin 2A) by 62%, S100A7 by 64%; all P < 0.001 vs. scrambled L-SNA control samples [Scr]) (Figure 1b). L-SNAs penetrated human abdominoplasty and psoriatic skin within 24 hours (Figure 1c), suggesting possible translation to human psoriasis. 3D human psoriatic rafts were generated by adding TNF-α, IL-17A, and IL-22 (each at a concentration of 10 ng/ml) to the medium beginning 6 days after NHEK lifting. Histologic (hematoxylin and eosin), immunologic (ELISA and Western blot), and transcriptional alterations in psoriasis markers were present within 3 days of cytokine initiation and were further increased by 6 days (see Supplementary Materials and Supplementary Figure S2a–d online). TNF L-SNAs at 50 nmol/L (Scr and phosphate buffered saline control samples) were applied to the raft center every other day using a ring to prevent leakage, beginning 3 days after cytokine initiation. The rafts were harvested at 7 days after cytokine initiation and 24 hours after the last L-SNA application. TNF L-SNAs improved differentiation (Figure 1d), reduced acanthosis (P < 0.05) (see Supplementary Figure S3a and b online), and normalized psoriatic marker mRNA expression (Figure 1e and f) of the psoriasis raft model to resemble rafts not exposed to cytokines.

The human TNF-targeted oligonucleotide sequence is 89% homologous with mouse Tnf and is able to knock down TNF-α—induced mouse fibroblast Tnf, Defb4, and S100a7a versus Scr by 84%, 56%, and 70%, respectively (all P < 0.001) (Figure 2a). The psoriasis-like model was established in 6-week-old C57BL/6 male mice by daily application of

Abbreviations: 3D, three dimensional; IMQ, imiquimod; L-SNA, liposomal-cored spherical nucleic acid; NHEK, normal human epidermal keratinocyte; Scr, scrambled liposomal-cored spherical nucleic acid control sample; SNA, spherical nucleic acid; TNF-α, tumor necrosis factor-α.

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62.5 mg IMQ cream (5%) for 6 days (van der Fits et al., 2009). Although no ideal psoriatic mouse model exists and the IMQ model has limitations (Hawkes et al., 2016), its reproducible inflammatory skin response simulates psoriasis clinically, histologically, and transcriptionally. Every other day, mice were treated with a template-defined area with topical formulations of 50 μmol/L TNF-L-SNA, 50 μmol/L Scr, Aquaphor (Beiersdorf, Wilton, CT)/phosphate buffered saline (1:1) vehicle, or nothing (untreated). On days with therapy and IMQ application, the IMQ was applied 10 minutes after both therapy and IMQ application, nothing (untreated). On days with buffered saline (1:1) vehicle, or phosphate buffered saline; Scr, scrambled liposomal-cored spherical nucleic acid control sample; SNA, spherical nucleic acid; TNF-α, tumor necrosis factor-α.

Figure 1. TNF L-SNAs prevent the psoriatic phenotype in 3D raft models. (a) Time-dependent uptake of 6 nmol/L cyanine 5–L-SNA in NHEKs, DAPI-stained nuclei. Scale bars = 20 μm. (b) Quantitative PCR of NHEKs pretreated with 10 ng/ml human TNF-α or PBS for 24 hours and incubated with L-SNAs or PBS (0 nmol/L/L-SNA) for 48 hours. Cytomix 5–L-SNA (30 μmol/L) in Aquaphor/PBS (1:1) was applied to normal or psoriatic human skin explants in lifted cultures and imaged after 24 hours. Scale bars = 50 μm. (d) Hematoxylin and eosin staining of untreated (NT) and cytokine-treated (cytomix) psoriasis-like rafts after two topical treatments with 50 nmol/L TNF-L-SNAs or controls. Scale bars = 50 μm. (e, f) Quantitative PCR analysis of NT or cytokine-treated rafts. Mean ± standard error of the mean, n = 3/group, three runs. Asterisks over columns are in comparison with TNF/PBS (lilac) in b and versus cytomix/burgundy columns in e and f; other comparisons are defined by the ends of the horizontal bars with asterisks (***P < 0.01, **P < 0.001). 3D, three dimensional; Exp, expression; hr, hour; L-SNA, liposomal-cored spherical nucleic acid; M, mol/l; min, minute; NHEK, normal human epidermal keratinocyte; NT, not treated; PBS, phosphate buffered saline; Scr, scrambled liposomal-cored spherical nucleic acid control sample; SNA, spherical nucleic acid; TNF-α, tumor necrosis factor-α.

The ability of TNF L-SNA to penetrate human psoriatic skin and completely reverse the development of 3D human psoriasis mouse model phenotypes suggests the therapeutic potential for topically applied SNA-mediated antisense therapy. Although TNF-α was chosen for this proof-of-concept study, the ease of altering the molecular target by changing the oligonucleotide sequence emphasizes the broad potential applicability of SNA topical therapeutics.

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The ability of TNF L-SNA to penetrate human psoriatic skin and completely reverse the development of 3D human and IMQ-treated mouse model phenotypes suggests the therapeutic potential for topically applied SNA-mediated antisense therapy. Although TNF-α was chosen for this proof-of-concept study, the ease of altering the molecular target by changing the oligonucleotide sequence emphasizes the broad potential applicability of SNA topical therapeutics.

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Figure 2. Topically applied TNF L-SNAs prevent development of the imiquimod-induced psoriasis-like phenotype in mice. (a) Mouse J2 fibroblasts were pretreated with or without 10 ng/ml mouse TNF for 24 hours and then with 1.5 nmol/L or 6 nmol/L TNF L-SNAs, 6 nmol/L Scr L-SNAs, or PBS for 48 hours. (b) Application schedule. (c, d) Skin was assessed clinically by modified Psoriasis Area Severity Index score; all data shown are with IMQ treatment, with modified Psoriasis Area Severity Index scores for untreated mice all equal to 0. (e) Hematoxylin and eosin and immunohistochemical staining was performed (scale bars = 50 μm), and (f) the extent of epidermal hyperplasia, hyperproliferation, and T-cell infiltration was quantified. (g) Real-time quantitative polymerase chain reaction analysis of mouse skin. Mean ± standard error of the mean, n = 12 mice/group. Asterisks over columns are (a) versus TNF/0 nmol/L-treated NHEK or (f, g) IMQ-treated mice controls; others as indicated (**P < 0.001). Epi, epidermal; H&E, hematoxylin and eosin; IMQ, imiquimod; L-SNA, liposomal-cored spherical nucleic acid; M, mol/L; NT, not treated; PBS, phosphate buffered saline; Scr, scrambled liposomal-cored spherical nucleic acid control sample; TNF, tumor necrosis factor-α; Veh, vehicle.
TO THE EDITOR

The bacterial pathogen *Staphylococcus aureus* frequently colonizes patients with atopic dermatitis (AD), a skin disease associated with loss of barrier function (Boguniewicz and Leung, 2011). Elevated levels of staphylococcal lipoteichoic acid (LTA) are found on the skin of patients affected by severe AD (Travers et al., 2010); however, the relative contributions of this product to keratinocyte differentiation and skin barrier formation are unknown.

To identify gene expression pathways altered by staphylococcal LTA, we performed gene microarray analysis on RNA isolated from primary human keratinocytes treated with media control or with LTA. We found that keratinocytes are highly responsive to this bacterial product, with 302 genes being either repressed or activated at least 2-fold (see Supplementary Table S1 online). The biological process most significantly affected by LTA was that of epidermal development (Figure 1a), with a 14.6-fold change. Other processes affected included the response to wounding, keratinocyte proliferation, negative regulation of cell differentiation, and changes in the Notch signaling pathways. We focused our studies on examination of genes involved in the keratinocyte differentiation process. Figure 1b and Supplementary Table S2 online show that staphylococcal LTA down-regulated a number of genes essential for keratinocyte differentiation, including KRT-1, KRT-10, and DSC-1 (Candi et al., 2005). The Notch ligands Jag-2 and Dll-1 were similarly repressed. Several genes involved in cell proliferation, including ID-1, FOX, and Cyclin A1 (CCNA1) were prominently up-regulated by LTA. To validate the results of the microarray, we measured expression of genes by real-time PCR, primarily focusing on genes known to play a critical role in the keratinocyte differentiation process and barrier formation. Real-time PCR

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