Topical nanoparticles interfering with the DNA-LL37 complex to alleviate psoriatic inflammation in mice and monkeys

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Cell-free DNA (cfDNA) released from damaged or dead cells combines with LL37 and is converted into an immune response activator to exacerbate psoriasis. Here, we show that cationic nanoparticles (cNPs) efficiently compete for DNA from the DNA-LL37 immunocomplex and inhibit DNA-LL37-induced cell activation. Using phenotypical images, psoriasis area and severity index scoring, histology, and immunohistochemical analysis, we demonstrate that topical application of cNPs on psoriasiform skin of a mouse model relieves psoriatic symptoms. It is noteworthy that the results were confirmed in a cynomolgus monkey model. Moreover, topicaly administered cNPs showed low in vivo toxicity because of their retention in skin. Mechanistic analyses of cytokine expression in the psoriatic site, cfDNA levels in circulation and inflamed skin, skin permeation, and biodistribution of cNPs also matched the therapeutic outcomes. Therefore, we present a previously unidentified strategy of nanomedicine to treat skin inflammatory diseases, which demonstrates great potential for clinical application.

INTRODUCTION

Psoriasis is an autoinflammatory skin disease affecting 2 to 3% of the worldwide population (1, 2). It is a papulosquamous skin disease with well-recognized morphology of red plaques covered by white or silver scales on skin (3, 4). Histologically, psoriatic lesions have some defined features of epidermal hyperplasia, generation of dermal blood vessels, and leukocyte infiltration into the dermis (5, 6). Choices of treatment strategies for psoriasis depend on the disease status. Topical therapies of glucocorticosteroids and vitamin D derivatives are conventional therapies, but they are only effective for mild disease and have adverse effects such as skin irritation (7, 8). For severe psoriasis, biological agents, such as tumor necrosis factor-α (TNF-α) and interleukin-17 (IL-17) inhibitors, are administered systemically to patients to target specific molecules in psoriasis pathogenesis and inhibit inflammation (9, 10). However, they cause a high risk of infection and cancer owing to long-term chronic immunosuppression (11). In addition, time consumption restricts the use of phototherapy and photochemotherapy, although they are effective for moderate-to-severe psoriasis (6). The limitations of these psoriasis treatments have encouraged further investigation of alternative therapeutic options.

Although the pathogenesis of psoriasis is complicated, it is clearly recognized that cell-free DNA (cfDNA) plays a critical role in psoriasis development. A clinical study showed a significant increase in the serum levels of cfDNA in patients with exacerbated psoriasis (12). The innate tolerance to cfDNA is disrupted by forming an immunocomplex with LL37, an antimicrobial peptide that is highly expressed in psoriatic skin. LL37 delivers cfDNA to plasmacytoid dendritic cells (pDCs) to trigger Toll-like receptor 9 (TLR9) and produce inflammatory cytokines, leading to T cell activation (13, 14). In addition, increasing data suggest that epidermal keratinocytes (KCs) recognize and respond to the DNA-LL37 complex and actively participate in the initiation of the cutaneous inflammatory response (15, 16). These studies suggest that cfDNA may be a target for novel psoriasis medication.

Researchers have recently used cationic polymers to scavenge nucleic acids (NAs) released from dead and dying cells to destroy the immune complexes, providing a new concept to treat sterile inflammation. The proof of principle has been confirmed in animal models of acute toxic shock (17), systemic lupus erythematosus (18), rheumatoid arthritis (19, 20), etc. In these studies, cationic polymers showed an extraordinary effect by blocking cfDNA-induced inflammation through systemic administration. However, systemic administration of cationic materials has a great risk of toxicity. Psoriasis mainly damages the skin, and topical treatment with cationic materials would be preferential and safe for administration (21).

Here, we innovatively applied topical administration of cationic polymers on skin to treat psoriasis (Fig. 1A). On the basis of the pathogenesis of psoriasis related to the DNA-LL37 immunocomplex, we investigated the interaction between cationic polymers and DNA as well as polypeptides. We found that cationic polymers with a high DNA binding affinity effectively pull cfDNA out of the DNA-LL37 complex, which, in turn, efficiently inhibited pDC and primary epidermal cell activation. Topical therapy with cationic polymers on imiquimod (IMQ)–induced psoriatic mice greatly reduced scales and erythema, leukocyte infiltration, and proinflammatory cytokines. More significant therapeutic efficacy was obtained with cationic nanoparticles (cNPs) instead of soluble polycations because of their more favorable retention in psoriatic skin and higher NA-scavenging capacity. It is noteworthy that the therapeutic outcomes of cNPs were confirmed in psoriatic cynomolgus monkeys. In addition, cNPs showed high accumulation only in skin layers with no apparent toxicity in the animals, and both IMQ mice and cynomolgus monkeys maintained normal hepatic and renal functions during cNP treatment. The significant results in this study, especially the data from cynomolgus monkeys, suggest that cNPs demonstrate a great translational potential for clinical application to psoriasis.
RESULTS
cNPs efficiently compete for DNA from its LL37 complex and inhibit inflammation
cNPs with a hydrodynamic diameter of ca. 70 nm were prepared by self-assembly of the diblock copolymer of poly(lactic-co-glycolic acid) (PLGA) and poly(2-(diethylamino)ethyl methacrylate) (PDMA), PLGA-b-PDMA$_{474}$ (fig. S1A), and a homopolymer PDMA$_{480}$ with a similar chain length to the PDMA shell of cNPs was used for comparison (see chemical structure in fig. S1B). Previously, we had observed that PLGA-b-PDMA cNPs have a more positive zeta potential and higher DNA binding efficiency than PDMA in both phosphate-buffered saline (PBS) and 10% fetal bovine serum (FBS) (18). Therefore, we believe that the core-shell nanostructure of cNPs could be beneficial for interfering the DNA-LL37 immune complex of psoriasis. LL37 is a cationic polypeptide that interacts with DNA to form the immune complex. Thus, microscale thermophoresis (MST), a technology to quantify binding events with high sensitivity and broad detection range, was used to determine the binding affinity of DNA with LL37, PDMA, as well as cNPs, respectively (fig. 1B). Cytidine-phosphate-guanosine oligodeoxynucleotide 1826 (CpG 1826), the pattern DNA sequence, was labeled with the fluorescent dye 6-carboxy-fluorescein (FAM) and became an extremely sensitive reporter for all binding interactions. When it was mixed with cationic materials, the binding event caused a change in the MST signal that was detected and translated into quantitative dissociation constant $K_d$ values. The smaller $K_d$ is, the stronger binding between two items is. The MST measurements showed that cNPs had the lowest dissociation constant of 0.13 nM, which was the highest DNA binding affinity of two
cationic materials and LL37. PDMA with a $K_d$ value of 0.98 nM had the second highest affinity for CpG. However, the $K_d$ value between CpG and LL37 was 1.94 µM, indicating that the binding affinity between CpG and cNPs was $\sim 1 \times 10^4$-fold stronger compared with that between CpG and LL37.

We speculated that, with such a strong binding capacity, cNPs had the ability to compete for DNA from its LL37 complex. To test this hypothesis, we conducted competitive binding experiments to analyze the contents of both CpG and LL37 bound to polymers as shown in fig. S2. We first premixed FAM-labeled CpG 1826 with unlabeled LL37 to generate the CpG-LL37 complex. Then, we incubated the complex with PDMA or cNPs for 30 min. The CpG-polymer complex was separated by ultrafiltration centrifugation and the binding efficiency, given as partition CpG (%), was calculated by quantifying the CpG concentration by the intensity of FAM fluorescence. Furthermore, to determine whether polymers could pull CpG out of CpG-LL37 to form a CpG-polymer (hypothesis in fig. S2) or directly form a CpG-LL37-polymer complex without dissociation of CpG-LL37 (hypothesis 2 in fig. S2), LL37 content was evaluated after the polymers were added to the complex of CpG and fluorescein isothiocyanate (FITC)–labeled LL37 by the same procedure used above. High fluorescence intensity of FAM-CpG could be detected in the polymer layers, demonstrating a strong interaction between polymers and CpG (solid line in Fig. 1C). However, we found that LL37 had almost undetectable fluorescent signals in the polymer layers (dash line in Fig. 1C), indicating that cationic polymers dissociated the CpG-LL37 complex to form a CpG-polymer complex. Moreover, compared with PDMA, cNPs had a stronger ability to pull CpG out of the complex even at a concentration as low as 20 µg/ml when the binding efficiency was 60% for cNPs and 40% for PDMA. Moreover, the DNA binding ability was increased with the dose of cNPs (Fig. 1C).

Next, we examined whether cationic polymers inhibited DNA-LL37 complex–mediated activation of cellular inflammation. Although free LL37 was unable to induce TLR9 activation, its DNA complex activated pDCs through TLR9 to secrete inflammatory cytokines (13). cNPs and PDMA inhibited mouse pDC activation induced by the CpG-LL37 complex, which was shown by down-regulation of IL-6 and TNF-$\alpha$ levels (Fig. 1, D and E). These results suggested that cNPs and PDMA could inhibit DNA-LL37 complex–induced pDC activation in psoriasis. Further experiments showed the same effects of cationic polymers on primary epidermal cells from psoriatic mice (Fig. 1F). In addition, cationic polymers alone did not activate inflammation, as manifested by the cytokine levels similar to the control (Fig. 1, D to F).

**Topical treatment with cNPs alleviates psoriatic symptoms of IMQ mice**

To assess the in vivo therapeutic effect, we next assessed topical treatment of the IMQ-induced mouse model with cationic polymers, which is used as an acute psoriasis-inform subjects model because it elicits a dermatitis resembling some aspects of human psoriasis to a certain degree (22). After topically administering IMQ cream for 7 days, IMQ as a TLR7/8 agonist induced a series of skin inflammatory reactions to elicit erythema, scales, and thickening of skin patches, indicating the onset of psoriasis (Fig. 2A). IMQ-induced mice displayed significant higher psoriasis area and severity index (PASI) scores than normal throughout the experiment (Fig. 2B). In the following 5 days, a solution of cNPs or PDMA dispersed in a glycerin-gelatin base was applied daily on the psoriatic skin of IMQ mice at gradient polymer doses of 0.05, 0.25, and 1.25 mg/cm². With the assistance of the base, hydration of the stratum corneum (SC) and the adhesion of polymers on the skin surface were both increased, which benefited the therapeutic effectiveness. The model group applied with only the glycerin-gelatin base was used as a control. During the treatment period from days 9 to 13, both cNPs and PDMA reduced inflammation of IMQ mice in a dose-dependent manner (Fig. 2, B and C). At a dose of 0.25 mg/cm², cNPs had already shown their therapeutic efficacy on mouse psoriatic skin as indicated by the reduction of scales and erythema as well as significant lower PASI scores compared with model. However, PDMA at the same dosage did not result in a significant difference of PASI scores from the model group. At a high dose of 1.25 mg/cm², cNPs even smoothened and thinned the psoriatic skin and brought the appearance much closer to normal skin with barely visible scales, which resulted in the lowest PASI scores of all groups except the normal group. Also, cNPs showed significant lower erythema, thickness, and cumulative scores than PDMA at the high dosage (Fig. 2B).

Psoriasis shows defining histopathological changes. In IMQ model mice, there was increased KC proliferation in the viable epidermis (VE) with psoriatic characteristic features including epidermal acanthosis (see thickening of the VE layer in Fig. 2C), hyperkeratosis, and parakeratosis in the SC (see the SC layer in Fig. 2C). In addition, the epidermal rete ridges became markedly elongated and even formed long and thin undulations that extended down into the dermis [marked as * in the dermal layer (DE) layer in Fig. 2C]. In psoriatic lesions, there was massive infiltration of leukocytes in the dermis, including intermixed T cells and DCs (indicated by arrows in the DE layer in Fig. 2C). After topical treatment by cationic polymers, all of these pathological symptoms of IMQ mice were relieved. After PDMA and cNP treatments at doses of 0.25 or 1.25 mg/cm², the proliferation of KCs was inhibited efficiently, and the epidermis became thinner than that of model mice with a decrease in the elongated epidermal rete (Fig. 2, C and D). However, in 0.25 and 1.25 mg/cm² cNP groups, the thickness of SC layer was even markedly decreased compared with the model group, which was shown by a reduction of scales in the clinical appearance, while all PDMA-treated groups did not show significant thinner SC than the model (Fig. 2, C and D). In addition, after cNP treatment, aggregates of mononuclear leukocytes in the dermis layer were reduced significantly, indicating that cNPs effectively suppressed the inflammation of psoriatic skin (Fig. 2C). Both the analysis of morphology and histology demonstrated that cNPs relieved psoriatic symptoms of IMQ mice better than PDMA.

cNPs more efficiently inhibit immune cell accumulation and expression of multiple cytokines in psoriatic lesions

In the IMQ model, IMQ induces not only phenotypic changes consistent with psoriasis but also changes in multiple cell types, cytokines, and inflammatory pathways involved in loss of homeostasis in the skin (23). The increased numbers of immune cells, especially dendritic cells, T cells, and macrophages, in psoriatic lesions play an important role in the transition from innate to adaptive immunity in psoriasis by producing proinflammatory cytokines and chemokines (2). Immunochemical staining of immune cells showed the quantities of monocytes and macrophages (CD68⁺), T cells (CD3⁺), and dendritic cells recruited to the psoriatic site in the IMQ model (Fig. 3A). However, treatment with cNPs and PDMA efficiently reduced the
infiltration of these immune cells. Quantification of immunostained cells indicated that the numbers of monocytes and macrophages, T cells, and dendritic cells were obviously lower in both cationic polymer groups, while cNPs showed higher inhibition efficiency with the number of immune cells much closer to normal (fig. S3).

We further determined whether the cationic polymers down-regulated proinflammatory cytokines secreted in psoriatic lesions. The cytokine pathway of IMQ-induced psoriasis depends particularly on the IL-17/23 axis, which shares similarities with human psoriasis (22). In addition to IL-23 as well as IL-17 and IL-22, which are relative to effector T helper 17 cells, proinflammatory cytokines such as TNF-α and IL-6 are functionally involved in the pathogenesis of psoriasis. Hence, proinflammatory cytokines, including TNF-α, IL-6, IL-17, IL-22, and IL-23, were measured in psoriatic lesions of IMQ mice. The immunohistochemical staining results showed that the levels of these cytokines were obviously up-regulated by IMQ-induced dermatitis. After treatment with cNPs and PDMA, the cytokine levels of psoriatic skin were all reduced relative to the model group. Quantification showed that cNPs significantly lowered the expression of IL-6 and IL-17 compared with the PDMA group (Fig. 3B and fig. S3). The results were confirmed by real-time polymerase chain reaction (PCR) indicating that the mRNA levels of these key inflammatory cytokines, including TNF-α, IL-6, IL-17A, IL-17F, IL-22, and IL-23, in psoriatic lesions had all been down-regulated after 5 days of treatment with cNPs and PDMA and were even close to normal levels (Fig. 3C). Analysis of both leukocyte infiltration and proinflammatory cytokine levels verified that cNPs demonstrated more efficient inflammatory inhibition in vivo.
various time points. Then, cNP and PDMA accumulation in different skin layers was analyzed by confocal microscopy. In addition, to assess the biodistribution of cationic polymers in vivo, the fluorescence intensity of polymers in organ homogenates was used to quantify the content of polymers in each organ. The results showed that cNPs and PDMA penetrated the skin of both model and normal mice after topical administration (Fig. 4A and fig. S4). However, the accumulation of PDMA and cNPs in the psoriatic skin could peak at 6 hours, while they reached the maximum in the normal skin at least at 24 hours (fig. S4, B and C). Uptake and trafficking of the

**Fig. 3. cNPs more efficiently reduce inflammatory cells and proinflammatory cytokine expression in IMQ mice.** (A) Immunohistochemical detection of monocyte and macrophage (CD68+), T cell (CD3+), and dendritic cell infiltrates in the psoriatic skin of various groups (×400). The inset indicates the enlarged image of positive staining (×800). (B) Proinflammatory cytokines involved in the pathogenesis of psoriasis, including IL-6, TNF-α, IL-17, IL-22, and IL-23, were analyzed by immunohistochemistry (×400). (C) mRNA levels of cytokines IL-6, TNF-α, IL-17A, IL-17F, IL-22, and IL-23 in skin at day 13 (after 5 days of treatment) (n=5; means ± SEM; **0.001 < P < 0.01 and ***P < 0.001 versus the model group). Statistical significance was calculated by one-way ANOVA with the LSD post hoc test.
materials in psoriatic skin were faster than in normal skin because the permeability was increased by the irregular SC barrier. In addition, cNPs were retained more in the different layers of psoriatic skin than PDMA during the observation times. At 2 hours, cNPs had already accumulated in the epidermis where PDMA showed lower signal, and at 6 hours, more cNPs were dispersed in the dermis than PDMA. Hence, cNPs displayed a more favorable accumulation in both epidermis and dermis as manifested by the significant higher FITC fluorescence intensity at different time points (fig. S4, B and C). In addition, a higher level as well as a longer retention of cNPs in the psoriatic skin could explain their better treatment efficacy.

Next, the distribution of cationic polymers in various organs was quantified by the fluorescence intensity. It was interesting to observe a reverse distribution of the two materials based on the results, although their accumulation in internal organs was quite lower than that in skin (Fig. 4B). In skin, cNP levels were always higher than PDMA levels. However, at 2 hours, PDMA had already distributed in organs especially in liver, and cNP levels in major internal organs, including liver, kidneys, heart, spleen, and lung, were all much lower than PDMA levels during the observation. The biodistribution difference between PDMA and cNPs also led to diverse toxicities. Histological staining of major organs showed that PDMA caused different degrees of lesions in the liver and kidneys, whereas cNP treatment did not cause any obvious damage to the various major organs (fig. S5). In addition, the hepatotoxicity of cNPs reflected by alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST) levels and their nephrotoxicity reflected by creatinine (Cr), uric acid (UA), and urea after treatment were all close to

Fig. 4. cNPs more effectively remain in skin layers with less uptake by internal organs. (A) Confocal images of skin penetration by PDMA and cNPs into IMQ mouse psoriatic skin at 2, 6, and 24 hours after administration (×200). Both PDMA and cNPs were labeled with FITC (green). Cell nuclei were stained with DAPI (blue). HF, hair follicles. (B) Content of FITC-labeled cationic polymers retained in skin and major organs, including the liver, kidneys, heart, spleen, and lung, calculated by fluorescence quantitation (n = 5, means ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001 between two groups and #P < 0.05, ###P < 0.001 versus polymer in the skin of the same group). (C) ALP, ALT, AST, Cr, UA, and urea levels in mouse serum after 5 days of treatment. Mouse sera of normal, model, PDMA (1.25 mg/cm²), and cNP (1.25 mg/cm²) groups were collected at day 13 (after 5 days of treatment) (n = 5; means ± SEM; ###P < 0.001 versus the normal group). In (B) and (C), statistical significance was calculated by one-way ANOVA with the LSD post hoc test.
those in the normal group even at the highest dose of 1.25 mg/cm². In contrast, the PDMA group showed a sixfold increase of the ALT level and a twofold increase of the AST level, implying strong liver injury. In addition, significant increases of UA and urea in the PDMA group indicated disruption of kidney functions (Fig. 4C). Thus, the low accumulation in internal organs of cNPs resulted in low toxicity in contrast to PDMA.

We hypothesized that the biodistribution of PDMA and cNPs may affect cfDNA levels in vivo. Therefore, we measured cfDNA levels in psoriatic lesions and circulation. To detect cfDNA in psoriatic lesions of mice, 6-μm-thick frozen sections of mouse skin were stained with SYTOX Orange, a high-affinity NA stain, which intensely stains extracellular DNAs. These extracellular DNAs can be distinguished from nuclear chromosomes by their structure of entangled strands and barbed wire appearance (24, 25). In psoriatic lesions of IMQ mice, such extracellular DNA with an intertwined strand structure was detected (indicated by arrows in Figs. 5, A and B, and 6A). For verification, another section of the same embedded tissue was used as a control treated with deoxyribonuclease I (DNase I) to degrade extracellular DNA before SYTOX staining. After the treatment with DNase I, the intertwined strand structure was no longer detectable, indicating that the essence of these strands stained with SYTOX Orange was cfDNA (Fig. 5A). Further staining results showed a large amount of cfDNA surrounding the dermis of IMQ model mice at day 11. Three days of treatment with PDMA had some effect on reducing cfDNA level in psoriatic lesions. However, the cNP group showed a significantly lower level of local cfDNA than the model and PDMA groups (Fig. 5B and fig. S6B). At day 13 when IMQ mice had been treated for 5 days, the local cfDNA level in the cNP group was half of that in the PDMA group and markedly lower than that in the model group (fig. S6, A and C). In addition, both cNP and PDMA treatments down-regulated systemic cfDNA to the same level as that in normal mice, whereas the cfDNA level in circulation of model mice was still elevated at day 13 (Fig. 5C). Unlike in psoriatic lesions, cNPs and PDMA made no difference in scavenging systemic cfDNA after 5 days (Fig. 5C). This result was consistent with the distribution of PDMA and cNPs in vivo, in which PDMA dispersed relatively more into circulation to interact with systemic cfDNA, whereas cNPs with more accumulation in psoriatic skin reduced local cfDNA more. Thus, cNPs better alleviated the local inflammatory response and showed no visible toxicity in animal models.

Topical therapy with cNP relieves symptoms of psoriatic cynomolgus monkeys

The above experiments in IMQ-induced murine models demonstrated that cNPs efficiently inhibited DNA-induced inflammation of psoriasis and alleviated psoriatic symptoms. cNPs showed both superior therapeutic efficacy and better biocompatibility than PDMA in IMQ mice, indicating a greater possibility for clinical application. Therefore, to further evaluate cNPs’ translation potential to the clinic, both the efficacy and toxicity of cNPs in a primate animal were assessed in three cynomolgus monkeys (Fig. 6A). Using the same procedure carried out in the mouse experiments, we first applied IMQ cream daily on the cynomolgus monkeys to induce psoriasis-like skin inflammation. In the clinical treatment of patients, topical IMQ as a treatment for basal cell carcinoma causes erythema, erosion, and scabbing or crust ing on the sites of application with a feeling of burning, itching, and pain, which resembles psoriatic symptoms (26, 27). These local skin reactions increase with the dosing frequency (i.e., 2 times/day and 7 times/week) (28). To induce psoriatic lesions in cynomolgus monkeys, we increased the dosage of IMQ cream to 250 mg per application and increased the dosing frequency to twice a day. After 7 days, obvious erythema occurred on the back skin of all monkeys, and the sites of IMQ application became rough and thickened as well as covered with silvery lamellar scales (Fig. 6B for monkey #2 and fig. S7A for monkeys #1 and #3).

These psoriasis-like symptoms became increasingly serious with scabbing and crust ing in the following days (Fig. 6C). Histological characteristics also implied that IMQ-induced dermatitis in cynomolgus monkeys closely resembled human psoriasis lesions, including hyperproliferation of KCs in the VE layer, hyperkeratosis, and parakeratotic KCs with retention of nuclei in the SC (Fig. 6D).

We chose a 2 cm by 2 cm area of the lower back skin of three cynomolgus monkeys as the cNP-treated area and a 2 cm by 2 cm area of the upper back of the same monkey treated with only glycerin-gelatin base as self-control. Therefore, there were three samples in each experimental group. To treat IMQ-induced cynomolgus monkeys, we topically applied cNPs (2.5 mg/cm²) with base on the skin twice daily. The skin phenotypic appearance was improved after cNP treatment, and the psoriasis progression was inhibited by cNPs with reduction of erythema, scales, and scabbing (Fig. 6B and fig. S7A). From days 9 to 13, the PASI scores of erythema, scales, and thickness in the cNP group were decreased significantly, although they were still significantly higher than normal (Fig. 6C). In addition, after 5 days of cNP treatment, the psoriatic lesions were desquamated, regenerated, and recovered (indicated by the arrow in Fig. 6B). In contrast, on the upper back skin without cNP treatment, the inflammation symptoms became increasingly serious over time (Fig. 6B and fig. S7A).

Histological characteristics implied that the epidermis in the cNP-treated area was much thinner than that in the model area, demonstrating that cNPs inhibited abnormal epidermal proliferation. Moreover, thickness of SC in the cNP-treated area was decreased obviously with the disappearance of incompletely differentiated KCs, implying that the parakeratosis and hyperkeratosis had been efficiently alleviated after treatment (Fig. 6D and fig. S7B). In addition, immunohistochemistry showed that cNPs were effective to reduce cynomolgus monkey skin inflammation. Expression levels of pro-inflammatory cytokines, including TNF-α, IL-6, IL-17, and IL-23, in psoriasis-like lesions were down-regulated after 5 days of cNP treatment compared with the model (Fig. 6E for monkey #3 and fig. S8A for monkeys #1 and #2), as evidenced by the halved levels of IL-6 and IL-23 and more than 70% decrease in expression of TNF-α and IL-17; among them, the cNP group showed efficient inflammatory inhibition (fig. S8B).

Considering the preclinical safety of topical therapy, we also evaluated the toxicity of cNPs in cynomolgus monkeys. In brief, 2 cm by 2 cm normal back skin of three male cynomolgus monkeys was applied with cNPs at 2.5 mg/cm² with a frequency of twice daily for 7 days. Blood samples were collected for biochemical analysis on days 1 and 8, and their body weight was recorded at the same time (see schedule in fig. S9A). Each animal was also used as its own control. No adverse clinical signs were detected in all animals. Regarding body weight, the recorded fluctuations without marked weight loss were in the normal range of this parameter for 4-year-old monkeys (fig. S9B). Furthermore, no pathological variations of biochemical markers were observed after cNP treatment. ALP, ALT, and AST levels were similar before and after cNP treatment, indicating no
serious liver damage. Furthermore, the normal concentrations of Cr, UA, and urea in blood implied that renal functions were not impaired either (fig. S9C). Together, these results strengthen the potential of cNP as an innovative drug candidate for the topical treatment of inflammatory diseases in the clinic.

DISCUSSION

Self–NAs released from dead or dying cells are recognized as damage-associated molecular patterns (DAMPs) or pattern-associated molecular patterns and trigger activation of endosomal TLRs with the assistance of antimicrobial peptides, leading to autoimmune disorders (29, 30). Therefore, cfdNA as a therapeutic target has been considered to break the cycle of aberrant inflammation. In 2009, Lee et al. (17) first suggested that certain polycations acting as molecular scavengers interact with NAs to inhibit activation of NA-sensing TLRs. The following study further demonstrated that using these NA-binding polymers to limit NA-induced inflammation is effective in some inflammatory and autoimmune diseases (17–20). Considering the toxicity of cationic materials administered systemically, a local (31) or ex vivo method (32) using immobilized polycations to remove DAMP molecules may be a better choice for easy clinical translation.

Encouraged by the high NA-binding capacity and effective inflammatory inhibition of cNPs in a previous study (19), we used cNPs to interfere with the cfdNA-LL37 complex for psoriasis treatment. In particular, the excellent NA-binding capability of cNPs by charge interactions resulted in potent destruction of the NA inflammasome by forming a more compact polymer-NA complex. We have shown that cNPs with ~1 × 10^4-fold stronger DNA binding affinity than LL37 efficiently compete for DNA from the DNA-LL37 complex (Fig. 1B and C). Using a known TLR agonist CpG in complex with LL37 to trigger TLR9, we demonstrated that cNPs are more effective to block the TLR9 activation of mouse primary pDCs and epidermal cells (Fig. 1, D to F), implying the potency of cNPs to reduce DNA-mediated immune stimulatory activities in psoriasis.

In general, topical delivery has significant advantages owing to the minimal first pass of metabolism, avoidance of the direct adverse blood environment, and the ability to provide prolonged and controlled drug release (33). In addition, topical therapy is non-invasive and can be self-administered, showing high patient compliance (34). Hence, topical therapy with cationic materials may be an innovative and safe strategy to treat psoriasis. In IMQ-induced mouse experiments, we confirmed that topically administered cNPs were effective in vivo as supported by skin surface morphologies, PASI scores, histology, and immunohistochemical analysis (Figs. 2 and 3). Next, we extrapolated the concept to a more clinically relevant IMQ model in cynomolgus monkeys. cNP topical treatment recovered psoriatic lesions, renewed the cynomolgus monkey skin (Fig. 6), and was well tolerated without obvious toxicity (fig. S9). Thus, we provide clear clinical evidences of cNPs as a potential treatment for autoimmune diseases.

Fig. 5. cNP treatment better down-regulates the in vivo cfDNA levels in IMQ mice. (A) Confocal images of cfDNA in psoriatic lesions stained with SYTOX Orange (×1890). Enlarged images (×3150) showed the intertwined strand structure of cfDNA (arrow). After treatment with DNase I, the barbed wire–like structure of cfDNA had disappeared, confirming that these strands were extracellular DNA. (B) At day 11, the local cfDNA level was reduced after 3 days of treatment with cationic polymers compared with model mice. Enlarged images showed a more distinct structure of cfDNA as indicated by arrows. (C) Concentration of cfDNA in serum after 5 days of treatment with PDMA and cNPs at day 13 (n = 7; means ± SEM; ***P < 0.001 between the two groups and ###P < 0.001 versus the normal group). Statistical significance was calculated by one-way ANOVA with the LSD post hoc test.
In vivo experiments showed that topical cNPs consistently outperformed the soluble counterpart PDMA, which may be attributed to their more favorable skin retention. The parameters of cationic materials, such as surface charge, size, and lipophilicity, determine their skin deposition/permeation (35–39). Generally, smaller molecules permeate through skin layers more efficiently than larger molecules (38), which could explain the skin diffusion difference of ~70-nm cNPs and soluble PDMA. Moreover, more positive charges of cNPs lead to a stronger interaction with epidermal and dermal cells, and thus, cNPs reside longer. Also, the lipophilicity of cNPs may result in a permeation retardation under the hydrophilic environment existing after the dermoepidermal junction (39). Therefore, cNPs showed higher localization in skin layers, whereas PDMA penetrated more easily into blood and accumulated more in the liver and kidneys (Fig. 4 and fig. S4). These biodistribution differences matched therapeutic performance. cNPs with more retention in skin showed better effectiveness in binding to local cfDNA (Fig. 5 and fig. S6) and thus better inhibition of the local inflammatory response. Moreover, retention of cNPs at the skin site greatly reduced systemic toxicity, unlike PDMA, which penetrated into blood circulation and caused liver and kidney lesions (Fig. 4 and fig. S5). Thus, particulate cationic materials administered topically could retain their inhibitory function for self-DNA–induced psoriasis while avoiding systemic toxicity by retention in the skin.

Motivated by the finding that self-DNA coupled with LL37 activates an abnormal immune response in psoriasis, we demonstrated...
that cNPs bound to proinflammatory NAs and interrupted DNA-LL37 immunocomplexes to treat experimental psoriasis. Topical administration of cNPs successfully achieved unification of efficacy and safety in both IMQ mice and cynomolgus monkeys. Because of the genetic and physiological similarities of nonhuman primates to humans, the results obtained from cynomolgus monkeys suggest a previously unidentified strategy for clinical application of cationic polymers. As an innovative trial, this study greatly increases the translational potential of cNPs as a promising treatment option for inflammatory diseases initiated by inappropriate activation of TLR pathways.

MATERIALS AND METHODS

Study design

The objective of the study was to capitalize on cDNA scavenging by topical cNPs to inhibit psoriatic inflammatory response in murine and cynomolgus monkey models and compare the therapeutic efficacy and in vivo toxicity with the soluble counterpart PDMA. The effects of both cationic polymers on inflammation inhibition in psoriasis were evaluated by a series of experiments including mouse primary pDC and epidermal cell stimulation in vitro and phenotypical photographs, PASI scoring, histology, and cytokine expression in the psoriatic site in the animal study. In vivo behavior difference between cNPs and PDMA was further explored by mechanistic analyses of skin penetration and biodistribution, as well as the measurement of cytokine expression in the psoriatic site and cDNA levels in circulation and skin lesions.

Throughout the study, neither animals nor samples were excluded from the study. For in vitro experiments, the stimulation of primary cells was performed in triplicate. For in vivo study, the sample size of animals was estimated after consultation with the biostatistics service at Sun Yat-sen University following the standard of the animal uses. Mouse study included 10 mice per treatment group, and cynomolgus monkey study included three monkeys. All animals were randomly allocated to the individual treatment groups, and the investigators were blinded to the group allocation when analyzing all samples. Mouse study was repeated three times to verify the reproducibility. The experimental end point for the IMQ mouse model of psoriasis was determined to be at least 13 days after the first IMQ administration of cNPs successfully achieved unification of efficacy and safety in both IMQ mice and cynomolgus monkeys. Because of the genetic and physiological similarities of nonhuman primates to humans, the results obtained from cynomolgus monkeys suggest a previously unidentified strategy for clinical application of cationic polymers. As an innovative trial, this study greatly increases the translational potential of cNPs as a promising treatment option for inflammatory diseases initiated by inappropriate activation of TLR pathways.

Bioreagents

PBS (pH 7.4), Dulbecco’s modified Eagle medium (DMEM), Roswell Park Memorial Institute 1640 medium (RPMI 1640), 0.25% trypsin-EDTA, and FBS were purchased from Gibco. Calf thymus DNA was purchased from Sigma-Aldrich, and its stock solution, 1 mg/ml, was prepared in distilled water. Nonlabeled CpG 1826 and FAM-labeled CpG 1826 were purchased from GenScript China, and their stock solutions in distilled water were prepared as 1 mg/ml. Nonlabeled LL37 and FITC-labeled LL37 were purchased from Invivogen and GenScript China, respectively, and their stock solutions in distilled water were prepared as 5 mg/ml. Anti–TNF-α antibody (catalog no. ab6671), anti–IL-6 antibody (catalog no. ab9324), anti–IL-17 antibody (catalog no. ab79056), anti–IL-23 antibody (catalog no. 45420), anti–IL-22 antibody (catalog no. ab18499), anti CD68 antibody (catalog no. ab955), anti CD3 antibody (catalog no. ab16669), goat anti-mouse immunoglobulin G (IgG) H&L [horseradish peroxidase (HRP)] (catalog no. ab6789), and goat anti-rabbit IgG H&L (HRP) (catalog no. ab6702) were purchased from Abcam. Dendritic cells antibody (NB100-64608) was purchased from Novus Biologicals LLC, and goat anti-rat IgG HRP (HAF005) was purchased from R&D Systems.

MiniMACS Starting Kit (MS), autoMACS Running Buffer, anti–mPDCA-1 MicroBeads, and a mouse epidermis dissociation kit were purchased from Miltenyi Biotec. DNase I (Roche), murine Fms-related tyrosine kinase 3 ligand (FLT3-L) (PeproTech), mouse TNF-α ELISA Kit (BioLegend), mouse IL-6 ELISA Kit (BioLegend), Aldara cream (5% IMQ; 3M Pharmaceuticals), Zoetelit 50 (mg/ml; Virbac), isofurane (RWD Life Science, China), hematoxylin (Jiangyuan, China), Eosin Y (Aladdin), 4’,6-diamidino-2-phenylindole (DAPI; Invitrogen), Tissue-Tek optimal cutting temperature compound (O.C.T. Compound, Sakura), diaminobenzidine (DAB) plus kit (Maxin Biotechnologies), Quant-iT PicoGreen dsDNA Assay Kit (PicoGreen, Invitrogen), SYTOX Orange NA stain (Invitrogen), Dynabeads SILANE Viral NA Kit (Invitrogen), TRIZol reagent (Invitrogen), RNasy Universal Tissue Kit (Qiagen), PrimeScript RT reagent Kit with gDNA Eraser and oligo (dT) (Takara), and SYBR Premix Ex Taq II kit (Takara Bio, China) were purchased from companies.

Cells

Mouse pDCs were generated by mouse bone marrow cells (BM cells) as described in literature (40). BM cells were isolated and cultured in RPMI 1640 with murine FLT3-L (100 ng/ml) and 10% heat-inactivated FBS at 37°C in a humidified atmosphere with 5% CO2. After 4 days, 50% of the culture medium was replenished. At day 8, pDCs were isolated with anti–mPDCA-1 MicroBeads using the MiniMACS Starting Kit. Mouse primary epidermal cells were enriched from mouse epidermal tissue using the mouse epidermis dissociation kit. Briefly, epidermal cells were collected as following steps. First, mouse epidermis tissue of IMQ mouse was separated from dermis by overnight enzymatic hydrolysis at 4°C using the mouse epidermis dissociation kit. The next day, the epidermis was peeled off from the dermis using curved tweezers and transferred into the gentleMACS C Tube containing the enzyme mix. The dissociation of epidermis was carried out using the gentleMACS Dissociator. Then, the debris were removed by filtration through 200-mesh screen cloth, and the epidermal cells were enriched after centrifuge with 800g and cultured with completed DMEM in a humidified incubator at 37°C under 5% CO2.

Animals

Female BALB/c mice (8 weeks) were purchased from the laboratory animal center of Sun Yat-sen University. All mice were bred, housed, and used under specific pathogen–free conditions in the animal facility of the School of Life Science, Sun Yat-sen University. The mouse studies were performed with the approval of the Ethics Committee of the School of Life Science, Sun Yat-sen University.

Three male cynomolgus monkeys (4.4 to 4.8 years) purchased from Guangdong Blooming-Spring Biological Technology Development Co. Ltd. were involved in the study. The study monkeys were housed alone in stainless steel cages and acclimated to adaptive feeding for at least 2 weeks before the first day of the study. They were body-tattooed and on–cage–identified. All monkeys were bred, housed, and used under conventional conditions in the experimental animal center in Guangdong Blooming-Spring Biological Technology Development Co. Ltd. The monkey studies were performed with the approval of the Ethics Committee of the Zhongshan Ophthalmic Center, Sun Yat-sen University.
Binding affinity between DNA and cationic materials or LL37

The binding affinity of PDMA, cNPs, and LL37 with DNA was measured by MST (Monolith NT.115, NanoTemper). First, the stock solutions, 200 µl of 100 nM FAM-labeled CpG 1826 and 30 µl of 3.2 µM cationic material or LL37 solution, were prepared, respectively. After a serial dilution of cationic materials or LL37 was prepared using PBS in tubes 1 to 16, the same volume of FAM-labeled CpG was added to each tube and mixed by pipetting. Then, a Monolith NT.115 Standard Treated Capillary was dipped into each tube from 1 to 16 and put in positions 1 to 16 of the device tray to start the measurement. The excitation color was blue with 50% excitation power. The MST data were analyzed by MO.Affinity Analysis software, and dissociation constant $K_d$ values was calculated using a $K_d$ model.

Competing for DNA from DNA-LL37 complex

To evaluate the competitive binding efficiency of cationic polymers with CpG from the CpG-LL37 complex, first, the FAM-labeled CpG 1826 solution and unlabeled LL37 solution were mixed for 30 min with the mass ratio of 1:5 to form the CpG-LL37 complex according to the method in literature (13). To which, different volume of the stock solutions of the cationic polymers were added. After incubation at 37°C for 30 min, the mixture was transferred to a ultrafiltration tube to be washed and concentrated by a centrifuge, and 100 µl of supernatant containing the remaining CpG-polymer complex of each dose was transferred to a 96-well plate. The fluorescence intensity of the complex at wavelength of 590 nm was measured by excitation at a wavelength of 485 nm with a multiwall plate reader (BioTek Synergy2 Gen5). Meanwhile, the standard curve of the fluorescence intensity of different concentration of FAM-labeled CpG was evaluated for converting the fluorescence intensity into CpG concentration. The NA-binding efficiency with cationic polymers was evaluated by $C_1/C \times 100\%$, where $C_1$ was the content of FAM-labeled CpG in the supernatant after centrifuge and wash and C was the total content of FAM-labeled CpG in the supernatant before centrifuge. To verify whether LL37 was still combined with CpG after the interaction of CpG and polymers, the LL37 content of intercepted polymer layer was measured by the same method, whereas unlabeled CpG 1826 and FITC-labeled LL37 were used.

Blocking DNA-LL37–activated cells with cationic polymers

For evaluation of the cationic polymers inhibiting the DNA-LL37 stimulation of mouse pDCs, $5 \times 10^5$ cells in 200 µl of complete medium per well were plated in a 48-well plate. After 12-hour culture, the medium was replaced with 200 µl of medium containing the premixed complex of CpG 1826 (10 µg/ml) and LL37 (50 µg/ml). Cationic polymers (25 µg/ml) were added at the same time. After incubation for 48 hours, the supernatants were collected, and both IL-6 and TNF-α expression levels were determined by ELISA Kit.

Cytokine concentration analysis with enzyme-linked immunosorbent assay

The concentration of IL-6 and TNF-α in the culture supernatants of mouse pDC cells was determined with ELISA Kit using mouse IL-6 ELISA Kits and mouse TNF-α ELISA Kits. Also, the concentrations of TNF-α in the culture supernatants of mouse primary epidermal cells from psoriatic skin were determined with ELISA Kits using mouse TNF-α ELISA Kits.

Mouse model induction and treatment

BALB/c mice were under the anesthesia of 1.5 to 2% isoflurane during the psoriasis induction and topical treatment with cationic polymers. The psoriasis-like mouse model was induced by IMQ cream, called IMQ-induced mouse model (IMQ mouse model) (23). First, a 2 cm by 2 cm area of back skin of BALB/c mice was shaved. Then, this skin area of back-shaved mice received a daily topical dose of 62.5 mg of the Aldara cream containing 3.125 mg of IMQ. After 7 days, the psoriasiform skin inflammation was successfully induced with the symptoms of erythema and scale, indicating the onset of psoriasis. The IMQ mice were evenly divided into model and treatment groups randomly, with 10 mice per group, and 10 back-shaved mice without Aldara cream administration were taken as the normal group.

The mouse treatment groups contained six groups: PDMA (0.05 mg/cm²), PDMA (0.25 mg/cm²), PDMA (1.25 mg/cm²), cNP (0.05 mg/cm²), cNP (0.25 mg/cm²), and cNP (1.25 mg/cm²). The cationic polymer solution with different concentration was dispersed into glycerin-gelatin base (volume ratio 1:1) before use, containing 15% gelatin and 10% glycerin. Then, from days 8 to 12, 200 µl of glycerin-gelatin base with cationic polymer was daily topically administered onto the psoriatic skin on the back, which was then immobilized with gauze and bandages. For the model group, 200 µl of glycerin-gelatin base alone was applied on the back skin of each IMQ mouse. At day 13 (after 5 days treatment), peripheral blood sample of mice of different groups were taken from the eye socket. Back skin and major internal organs were collected after mice sacrifice under excess isoflurane.

IMQ induction and therapy of cynomolgus monkey

The psoriasis induction and cNP topical administration of cynomolgus monkeys were all conducted under the fixation of animals. The back skin of three male cynomolgus monkeys was shaved before model induction. Then, a 2 cm by 2 cm area of the lower back skin of each cynomolgus monkey was chosen as cNP-treated area, and a 2 cm by 2 cm area of the upper back of model skin served as self-control. Therefore, there were three samples in each group. For the induction of psoriatic symptom of cynomolgus monkey, the same procedure was performed, whereas 250 mg of IMQ cream was applied on each skin area twice a day.

At day 8, after the onset of psoriasis occurred, 100 µl of the glycerin-gelatin base with 10 mg of cNPs was applied on the cNP-treated area of back skin slowly until absorption twice a daily. The same volume of the glycerin-gelatin base alone was applied on model skin area as the control. After 5 days of treatment, small areas (0.5 cm by 1 cm) of cNP-treated skin and model skin were collected, respectively, after the cynomolgus monkeys after anesthesia using intramuscular injection of Zoletil 50 (4 to 6 mg/kg). Besides, a small area (0.5 cm by 1 cm) of normal skin was also collected as control. Then, the back skin was disinfected with medical iodine and sewn up.

For evaluating in vivo toxicity of cNPs, three cynomolgus monkeys were topically applied with cNPs (2.5 mg/cm²) twice daily for 7 days. A peripheral blood sample of cynomolgus monkey was collected by venipuncture from the femoral vessel before cNP treatment (day 1) and after 7-day treatment of cNPs (day 8), and the body weight of cynomolgus monkeys was also recorded at these days. Animals
were fasted for at least 8 hours before blood collection for biochemical analysis.

**Scoring severity of skin inflammation**
Skin inflammation severity of back skin of mice and cynomolgus monkey was daily scored using an objective scoring system developed on the basis of the clinical PASI. Erythema, scaling, and thickening were scored independently on a scale between 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked. The cumulative score of erythema, scaling, and thickening on a scale of 0 to 12 served as a measure of the severity of inflammation.

**Photograph**
For evaluating the skin inflammation, the back skin of mice from different groups was photographed by camera at day 11 when psoriatic symptoms became most marked. The model back skin and cNP-treated back skin of cynomolgus monkey were photographed by camera at day 1 (before model establishment), day 8 (after model establishment), and days 9 to 13 (during treatment).

**Histological analysis and immunohistochemical staining**
The skin samples of mice and cynomolgus monkey as well as the heart, liver, spleen, lung, and kidneys of mice were previously fixed in 10% formalin. After being paraffin-embedded, 2-µm paraffin sections were cut by microtome (Leica) and then stained with hematoxylin and eosin, respectively. The inflammatory cell accumulation of different skin layers skin as well as the tissue morphology of organs were evaluated by the Vectra Automated Quantitative Pathology Imaging System (PerkinElmer), and the thickness of different skin layers was measured using ImageJ software. Average thickness of SC, epidermis, and dermis was calculated from three randomly selected sites of the skin sections of each animal.

For immunohistochemical staining, after deparaffinization and rehydration, the slices of mice and cynomolgus monkey skin were immersed into 0.01 M sodium citrate buffer at 125°C for 30 s, followed by 10 s at 90°C for antigen recovery. Then, slices were covered with 3% hydrogen peroxide for 5 min to inactivate endogenous peroxidase. After the nonspecific binding sites were blocked by 10% goat serum in PBS, the slices were incubated with different primary antibody respectively at 4°C for 24 hours. Then, the slices were incubated with secondary HRP-conjugated antibody at 37°C for 1 hour. Sections were developed using freshly prepared DAB substrate, then counterstained with hematoxylin, dehydrated, and mounted. The primary antibodies included anti–TNF-α antibody, anti–IL-6 antibody, anti–IL-17 antibody, anti–IL-22 antibody, anti–IL-23 antibody, anti–CD68 antibody, anti–CD3 antibody, and dendritic cell antibody. The images were captured and analyzed by the Vectra Automated Quantitative Pathology Imaging System. The positive results (brown staining) were evaluated using Nuance 3.0.2 and inForm 2.1.1 software. Sections were scanned and analyzed by Vectra Automated Quantitative Pathology Imaging System, and the positive results (brown staining) were evaluated using Nuance 3.0.2 and inForm 2.1.1 software.

**Staining of extracellular DNA in skin layers**
The extracellular DNA of psoriatic skin was stained according to the literature (24). At day 11 (after 3 days of treatment) and day 13 (after 5 days of treatment), the mouse back skin of various groups was collected and immersed in O.C.T. Compound, and then snap-frozen in liquid nitrogen. Six-micrometer cryosections of snap-frozen skin were prepared using a clinical cryostat (Leica) and fixed in 4% paraformaldehyde for 30 min. Slides were stained with SYTOX Orange NA stain (1:3000) for 15 min and then washed with PBS three times, and coverslips were mounted. Besides, another slide of the same embedded tissue without fixation was treated with DNase I (1 mg/ml) for 30 min before SYTOX Orange staining, which was served as control according to the literature (24). Slides were imaged using confocal microscopy (Leica SP8), and cfDNA of skin quantitatively was analyzed by ImageJ software.

**DNA extraction and measurement**
For extraction of cfDNA in mouse serum, peripheral blood sample of mice were first centrifuged at 300g for 10 min, and then the fraction was recentrifuged at 12,000g for 10 min to remove cell debris, which was stored at ~80°C before analysis. The cfDNA from 100 µl of serum was extracted with the Dynabeads SILANE Viral NA Kit, and its concentration was determined using the Quant-iT PicoGreen dsDNA Assay Kit.

**Real-time PCR**
At day 13, the skin of sacrificed mice was cut off and homogenized in a TRIzol reagent with a homogenizer (T 25 digital ULTRA-TURRAX, IKA), and the total RNA of the skin was extracted using the RNeasy Universal Tissue Kit. For RNA reverse transcription into complementary DNA (cDNA), 2 µg of RNA was treated with the PrimeScript RT reagent Kit with gDNA Eraser and oligo (dT) in a total volume of 20 µl. Quantitative real-time PCR of cDNA levels was performed by SYBR Green real-time in the Lightcycler (Applied Biosystems quantitative PCR), and cytokine mRNA levels were corrected with their glyceraldehyde-3-phosphate dehydrogenase mRNA levels to normalize RNA input. The relative mRNA levels of cytokines in the normal group were set as 1. The sequences of the primers were as follows: qGADPH FP: 5′-TGTGTCGTCGATGATG-3′ and qGADPH AP: 5′-TTGCTGTGAAGTGCGAGGAG-3′; qTNF-α FP: 5′-ACTCCACGGCCTGCTATGTTG-3′ and qTNF-α AP: 5′-GTGAGGTCTGTGGCCATAGAA-3′; qIL-6 FP: 5′-CCACATTCACAAGTCCGGAGGTT-3′ and qIL-6 AP: 5′-TCAAGTGCATCTCAGTTGTC-3′; qIL-17A FP: 5′-GAAGGCCCCTCAGACTACTCAA-3′ and qIL-17A AP: 5′-TCTAGTGTTGTTGGGCCATTTGTC-3′; qIL-17F FP: 5′-TGTCCCACGGAATTTCCGA-3′ and qIL-17F AP: 5′-CATTGATGCAAGCCTGAGTGC-3′; qIL-22 FP: 5′-TCCACGAGCCATACATC-3′ and qIL-22 AP: 5′-CTTCCAGGATGGAAGTCAGCA-3′.

**Skin penetration and biodistribution of cationic polymers**
At day 11, both normal mice and IMQ mice were topical administered with FITC-labeled PDMA or cNPs in glycerin-gelatin base. At 2, 6, and 24 hours, the back skin of the mice treated with FITC-labeled cationic polymers was cut off and embedded in O.C.T. Compound. After being snap-frozen in liquid nitrogen, the tissue was sectioned at 6 µm. After the fixation with 4% paraformaldehyde for 30 min, the slice was stained with DAPI (1:1000) for 20 min and washed with PBS three times. The penetration of cationic polymers of different skin layers was evaluated by confocal microscopy, and the fluorescence intensity of polymers in the epidermis and dermis of normal and model mice was manifested by average optical density quantified by ImageJ software. For the quantification, the cationic...
polymers of skin and major organs, back skin, heart, liver, spleen, lung, and kidney of IMQ mice treated with FITC-labeled polymers were collected and homogenized in PBS with a homogenizer. The fluorescence intensity of the supernatant was evaluated with a multiwall plate reader with the excitation light at a wavelength of 485 nm. The standard curve of the fluorescence intensity of different concentration of FITC-labeled polymers was evaluated and the amount of cationic polymers in different organs was calculated. The percentage of the polymer retained in different organs was evaluated by $A_1/A \times 100\%$, where $A_1$ was the amount of the polymer in the organ and $A$ was the initial amount of the polymers applied on the skin.

Analysis of ALP, ALT, AST, Cr, urea, and UA in serum
Peripheral blood samples of mice collected at day 13 were centrifuged at 300g for 10 min, and the plasma fraction was recentrifuged at 12,000g for 10 min again to obtain cell-free serum. Then, 100 μl of the mouse serum was diluted to 300 μl with PBS. The levels of ALP, ALT, AST, Cr, urea, and UA in mouse sera were measured by the Automatic Biochemical Analyzer (FAITH-1000, Nanjing Laola Electronics Co., Ltd.) and the serum levels of ALP, ALT, AST, Cr, urea, and UA in cynomolgus monkeys before cNP treatment and after cNP treatment were analyzed by Guangdong Blooming-Spring Biological Technology Development Co. Ltd.

Statistics
Statistical differences of PASI sores were evaluated using one-way analysis of variance (ANOVA), followed by least significant difference (LSD) post hoc test with GraphPad Prism 7.0 software. Statistical analysis of other experimental data was performed by one-way ANOVA with LSD post hoc test using IBM SPSS Statistic 22. $P < 0.05$ was considered statistically significant.

SUPPLEMENTARY MATERIALS
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/31/eabb5274/DC1

View/request a protocol for this paper from Bio-protocol.

REFERENCES AND NOTES
1. M. A. Lowes, A. M. Bowcock, J. G. Krueger, Pathogenesis and therapy of psoriasis. *Nature* **445**, 866–873 (2007).
2. F. O. Nestle, D. H. Kaplan, J. Barker, Mechanisms of disease: Psoriasis. *N. Engl. J. Med.* **361**, 866–873 (2009).
3. T. E. Christensen, K. P. Callis, J. Papenfuss, M. S. Hoffman, C. B. Hansen, B. Wong, F. O. Nestle, D. H. Kaplan, J. Barker, Mechanisms of disease: Psoriasis. *Nature* **449**, 564–566 (2007).
4. F. O. Nestle, C. Conrad, A. Tun-Kyi, B. Homey, M. Gombert, O. Boyman, G. Burg, Y. J. Liu, M. Gilliet, Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* **499**, 654–656 (2005).

5. J. E. Kim, B. J. Kim, M. S. Jeong, S. J. Seo, M. N. Kim, C. K. Hong, B. I. Ro, Expression and modulation of LL-37 in normal human keratinocytes, HaCaT cells, and inflammatory skin diseases. *J. Korean Med. Sci.* **20**, 649–654 (2005).
6. S. Morizane, K. Yamazaki, B. Muehleisen, P. F. Kotol, M. Murakami, Y. Aoyama, K. Iwatsuki, T. Hata, R. L. Gallo, Cathelicidin antimicrobial peptide LL-37 in psoriasis enables keratinocyte reactivity against TRAIL ligands. *J. Invest. Dermatol.* **132**, 135–143 (2012).
7. J. Lee, J. W. Sohn, Y. Zhang, K. W. Leong, D. Pisetsky, B. A. Sullenger, Nucleic acid-binding polymers as anti-inflammatory agents. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 14055–14060 (2011).
8. E. K. Holl, K. L. Shumansky, L. B. Borst, A. D. Burnett, C. J. Sample, E. A. Ramsburg, B. A. Sullenger, Scavenging nucleic acid debris to combat autoimmunity and infectious disease. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 9728–9733 (2016).
9. H. Liang, B. Peng, C. Dong, L. Liu, J. Mao, S. Wei, X. Wang, H. Xu, J. Shen, H.-Q. Mao, X. Gao, K. W. Leong, Y. Chen, Cationic nanoparticle as an inhibitor of cell-free DNA-induced inflammation. *Nat. Commun.* **9**, 4291–4304 (2018).
10. B. Peng, H. Liang, Y. Li, C. Dong, J. Shen, H.-Q. Mao, K. W. Leong, Y. Chen, L. Liu, Tuned cationic dendrimerized polymer: Molecular scavenger for rheumatoid arthritis treatment. *Angew. Chem. Int. Ed.* **58**, 4254–4258 (2019).
11. M. Lebwohl, Psoriasis. *Lancet* **361**, 1197–1204 (2003).
12. L. van der Fitis, S. Mörts, J. S. A. Voerman, K. M. L. Doom, C. Fornellisen, A.-M. Mus, E. Florenca, E. Prens, E. Lubberts, Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the TLR-23/IL-17 axis. *J. Immunol.** **182**, 5836–5845 (2009).
13. B. Flutter, F. O. Nestle, TLRs to cytokines: Mechanistic insights from the imiquimod mouse model of psoriasis. *Eur. J. Immunol.* **43**, 3138–3146 (2013).
14. F. Wen, G. Curylango-Rivera, D. A. Husky, Z. Xiong, M. C. Hawes, Visualization of extracellular DNA released during border cell separation from the root cap. *Am. J. Bot.* **104**, 970–978 (2017).
15. C. Schorhn, C. Janko, V. Krenn, Y. Zhao, L. E. Munoz, G. Schett, M. Herrmann, Bonding the foe - NETing neutrophils immobilize the pro-inflammatory monosodium urate crystals. *Front. Immunol.* **3**, 376 (2012).
16. P. S. Cannon, B. O’Donnell, S. C. Huilgol, D. Selva, The ophthalmic side-effects of imiquimod therapy in the management of periocular skin lesions. *Br. J. Ophthalmol.* **95**, 1682–1685 (2011).
17. J. Leppala, K. Kaamiranta, H. Uusitalo, M. Kontkanen, Imiquimod in the treatment of eyelid basal cell carcinoma. *Acta Ophthalmol. Scand.* **85**, 566–568 (2007).
18. J. Geisse, I. Caro, J. Lindholm, L. Gollitz, P. Stampone, M. Owens, Imiquimod 5% cream in the treatment of superficial basal cell carcinoma: results from two phase III, randomized, vehicle-controlled studies. *Am. Acad. Dermatol.* **50**, 722–733 (2004).
19. S. K. Chauhan, V. V. Singh, R. Rai, M. Rai, G. Rai, Distinct autoantibody profiles in systemic lupus erythematosus patients are selectively associated with TLR3 and TLR9 upregulation. *J. Clin. Immunol.* **33**, 954–964 (2013).
20. T. Celhar, R. Magalhes, A. M. Fairhurst, TLR7 and TLR9 in SLE: When sensing self goes wrong. *Immunol. Res.* **53**, 58–77 (2012).
21. J. G. Jackman, H. Juwarker, L. P. Poveromo, H. Levinson, K. W. Leong, B. A. Sullenger, Polycationic nanofibers for nucleic acid scavenging. *Biomacromolecules* **17**, 3706–3713 (2016).
22. J. Lee, J. G. Jackman, J. Kwun, M. Manoak, A. Moreno, E. A. Elster, A. D. Kirk, K. W. Leong, B. A. Sullenger, Nucleic acid scavenging microfiber mesh inhibits trauma-induced inflammation and thrombosis. *Biomaterials* **120**, 94–102 (2017).
23. A. C. Watkinson, Transdermal and Topical Drug Delivery Today (John Wiley & Sons Ltd., 2012).
24. M. R. Prausnitz, R. Langer, Transdermal drug delivery. *Nat. Biotechnol.* **26**, 1261–1268 (2008).
25. V. V. K. Venuganti, O. P. Perumal, Poly(lamidoamine) dendrimers as skin penetration enhancers: Influence of charge, generation, and concentration. *J. Pharm. Sci.* **98**, 2345–2356 (2009).
26. Y. Yang, S. Sunoqrot, C. Stowell, J. Ji, C.-W. Lee, J. W. Kim, S. A. Khan, S. Hong, Effect of size, surface charge, and hydrophobicity of poly(lamidoamine) dendrimers on their skin penetration. *Biomacromolecules* **13**, 2154–2162 (2012).
37. S. P. Hong, A. U. Bielinska, A. Mecke, B. Keszler, J. L. Beals, X. Y. Shi, L. Balogh, B. G. Orr, J. R. Baker Jr., M. M. B. Holl, Interaction of poly(amidoamine) dendrimers with supported lipid bilayers and cells: Hole formation and the relation to transport. Bioconjug. Chem. 15, 774–782 (2004).

38. P. Karande, A. Jain, K. Ergun, V. Kispersky, S. Mitragotri, Design principles of chemical penetration enhancers for transdermal drug delivery. Proc. Natl. Acad. Sci. U.S.A. 102, 4688–4693 (2005).

39. S. Khandavilli, R. Panchagnula, Dermal drug delivery: Revisited. Drug Discov. Ther. 2, 64–73 (2008).

40. E. Janssen, K. Tabeta, M. J. Barnes, S. Rutschmann, S. McBride, K. S. Bahjat, S. P. Schoenberger, A. N. Theofilopoulos, B. Beutler, K. Hoebe, Efficient T cell activation via a Toll-interleukin 1 receptor-independent pathway. Immunity 24, 787–799 (2006).

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