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NETs generate structured antimicrobial peptide-nucleosome immune complexes with inter-DNA spacings optimal for TLR9 activation

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The molecular pathogenesis of psoriasis is characterized in part by breakdown of immune tolerance to self-DNA. Recent work demonstrates that the human antimicrobial peptide (AMP) LL37 overexpressed in psoriasis organizes naked DNA into periodic nanocrystals to potently hyperactivate Toll-like receptor 9 (TLR9) in plasmacytoid dendritic cells (pDCs). Interestingly, a subset of self-DNA in psoriatic lesions remain bound to histones as histone-DNA nucleosome core particles (NCPs) released from neutrophil extracellular traps (NETs). At present, it is unknown how NET components like AMPs interact with NCPs, and whether AMP-NCP complexes form structures compatible with TLR9 activation in psoriasis. Here, we combine synchrotron X-ray scattering, cryo-electron microscopy, and computer simulations to demonstrate that under a broad range of conditions, NCPs stack into columns that present periodically arranged dsDNA ligands like threads on a screw, allowing for optimal interdigitation with clusters of TLR9. Remarkably, simulations and electron microscopy indicate that the superhelical pitch of DNA wrapped around the NCP column relaxes to a value that is well-matched with the steric size of TLR9, which predicts strong immune activation. Taken together, our results suggest that AMPs can remodel the structural organization of nucleosomes from NETs into potent amplifiers of inflammation. Preliminary immune activation experiments will be presented.

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Psoriatic regulatory T cells are impaired in their suppressive activity and respond differently to IL-33

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Interleukin 33 (IL-33), initially described as an alarmin released by cells following cell damage, can also act immunosuppressive by inducing regulatory T cells (Treg). Disruption of the skin barrier by tape stripping in mice released IL-33 which induced Treg. Accordingly, contact hypersensitivity (CHS) was suppressed upon barrier disruption. Furthermore, blockade of IL-33 enhanced CHS, whereas injection of IL-33 suppressed CHS via induction of Treg. IL-33 is enhanced in the skin and serum of psoriatic patients. On the other hand, in psoriasis Treg are impaired in their suppressive activity. Thus, we asked whether "psoriatic" Treg are impaired in their response to IL-33 in the imiquimod (IMQ)-induced psoriasis like model. IL-33 was enhanced in skin and serum of IMQ-mice. To study the influence of IL-33 on Treg in IMQ-mice, CD4+CD25+ Treg isolated from IMQ or untreated mice were injected into naïve mice which were sensitized 24 h later. Ear challenge was reduced in mice receiving Treg from untreated donors, whereas injection of Treg from IMQ-mice did not cause suppression, implying impairment of Treg in IMQ-mice. To study the response of Treg to IL-33 in IMQ-mice, Treg from untreated or IMQ-mice were incubated with IL-33 and injected into naïve mice which were sensitized thereafter. The suppressive capacity of Treg from WT mice was further enhanced by IL-33, whereas Treg from IMQ mice did not to respond to IL-33. Treg isolated from blood of psoriatic patients were inhibited in their suppressive activity, when compared to Treg from healthy controls. IL-33 induced the expression of Foxp3 in CD4+ T cells obtained from controls, but not in T cells from psoriatic patients. These data confirm that in psoriasis Treg are functionally impaired which may be associated with an altered response to IL-33. The mechanisms responsible for this altered behavior of Treg in psoriasis remain to be elucidated.

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The adaptor protein Act1 plays a key role in psoriatic inflammation mediated by IL23

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Psoriasis is a debilitating skin disease characterized by epidermal thickening, abnormal keratinocyte differentiation, and pro-inflammatory immune cell infiltrate into the affected skin. The cytokine IL17 has been shown to play a critical role in the etiology of psoriasis. IL17 functions by synergizing with and amplifying the pro-inflammatory functions of TNFa and other cytokines in the skin. Act1 is an intracellular adaptor protein and a putative ubiquitin E3 ligase that is essential for signal transduction downstream of the IL17 receptor. Silencing of Act1 expression by RNA interference or knockout of Act1 by CRISPR blocks pro-inflammatory cytokine secretion induced by IL17 in human keratinocytes and fibroblasts. Thus, IL17 signaling in general, and Act1 specifically, represent attractive targets for the treatment of psoriasis. Act1 knockout mice were resistant to increases in CXCL1 plasma levels induced by subcutaneous injection of recombinant IL17A. Moreover, in a mouse model of psoriasiform dermatitis induced by IL23 injection, Act1 knockout mice were protected against increases in ear thickness, keratinocyte hyperproliferation, expression of genes for antimicrobial peptides and chemokines, and infiltration of immune cells including CD11b+ cells. The L286G mutation was previously suggested to compromise Act1 ubiquitin E3 ligase function and inhibit IL17 signaling. Both IL23- and IL17-mediated proinflammatory effects were similar in our wildtype and Act1 L286G knockin mice. Primary Act1 L286G mouse fibroblasts as well as human Act1 knockout fibroblasts reconstituted with a homologous point mutant responded normally to IL17 stimulation (unchanged from wildtype). Our studies highlight the critical contribution of Act1 to proinflammatory skin changes mediated by the IL23/IL17 signaling axis.

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Brunsting-Perry pemphigoid after remission of previous bullous pemphigoid related to epitope spreading from BP180 to BP230

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Brunsting–Perry pemphigoid is a localized form of cicatricial pemphigoid. Here, we report a case of Brunsting–Perry pemphigoid after remission of previous bullous pemphigoid. A 63-year-old Chinese male presented to our department with a three-month history of pruritic lesions in August 2013. Tense vesicles and bullae appeared on his hands, upper limbs and trunk. The skin biopsy showed subepidermal bulla with eosinophil infiltrations in upper dermis. The enzyme-linked immunosorbent assay (ELISA) for IgG antibodies against the BP180 NC16A domain was 90.44 and for the BP230 was 33.75. The patient was diagnosed as BP and treated with prednisone 30mg per day. The lesions improved quickly. Then the dose of prednisone was tapered down and kept at 5mg daily. Four years later, he revisited our department with a seven-month history of head lesions. Clinical examination revealed crusts, and trophic scars on the parietal scalp region. IIF using 1 mol L^{-1} NaCl-split healthy human skin sections revealed that the anti-basement membrane zone (BMZ) antibodies bound to the roof of the split. The ELISA index for the BP180 NC16A was 25.45 and for the BP230 was 110.82. From these findings, we diagnosed this patient as Brunsting–Perry type pemphigoid. He received oral prednisone (10 mg/d) but the improvement was limited. He later had the crusts debrided and 0.1mg mL^{-1} dexamethasone wet packing. The lesions healed gradually. The patient continuously had oral prednisone 10 mg daily in the following 9 months and was in stable condition. In this case, we observed a rare transition to Brunsting–Perry type pemphigoid from previous BP. As far as we know, it is firstly reported. And the dominant IgG anti-BMZ antibody switched from anti-BP180 NC16A to anti-BP230. But the mechanism still need further study.

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Immunization of dermatomyositis-specific autoantigen transcriptional intermediary factor (TIF1)-γ induces experimental myositis in mice

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A number of myositis-specific autoantigens have been identified in dermatomyositis patients. While murine models of experimental myositis have been established using immunizations of muscle-specific proteins like myosin and C protein, they are not targeted in actual diseases. Here we established a new murine model of experimental myositis inducible with immunizations of TIF1-γ, one of self-antigens for myositis-specific autoantibodies. We purified recombinant whole human TIF1-γ protein using baculovirus expression systems, and immunized wild-type C57BL/6J (B6) mice with subcutaneous injections of emulsions containing the protein and complete Freund's adjuvant four times. Myositis was observed in bilateral muscles of the immunized mice 14 days after the last immunizations. The lymph node T cells from the mice proliferated when stimulated with TIF1-γ, and IgG reacting TIF1-γ were detected in the sera of the mice. Adoptive transfer of the T cells from the mice stimulated with TIF1-γ could cause myositis in naïve B6 mice. Moreover, transfer of the purified $CD8^+$ T cells, but not the purified $CD4^+$ T cells, caused define myositis in naïve B6 mice (the incidences: 90 % and 0 %, respectively). β2 microglobulin-deficient mice, which lack major histocompatibility complex I, and perforin-deficient mice, in which $CD8^+$ T cells lack cytotoxicity, developed significantly weaker myositis than wild-type mice (myositis scores: 0.13 ± 0.23, 0.30 ± 0.48, and 0.91 ± 0.70, respectively). Transfer of IgG collected from TIF1-γ-immunized mice could not cause myositis in naïve B6 mice, moreover, μMT (B cell-deficient) mice developed myositis after TIF1-γ immunizations with no inferiority compared to wild-type mice. Collectively, TIF1-γ is an autoantigen with especial immunogenicity to induce experimental myositis, in which the muscle injury is directly mediated by $CD8^+$ T cells, but not $CD4^+$ T cells or antibodies. TIF1-γ-induced experimental myositis would be an useful tool to investigate pathology of anti-TIF1-γ antibody-associated dermatomyositis.

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Langerhans cells suppress CD8 T cells *in situ* during acute graft-versus-host disease-like autoimmune mucocutaneous disease

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Previous studies revealed that cutaneous manifestations of acute graft-versus-host disease (aGVHD) patients, in which donor immune cells react against host tissues after allogeneic hematopoietic stem cell transplant, are mediated by CD8 T cells. Cutaneous aGVHD is histologically characterized by reduced number of epidermal Langerhans cells (LCs). To investigate the roles of LCs, we analyzed a murine model using transgenic mice that express membrane-bound chicken ovalbumin under control of a keratin 14 promoter (K14-mOVA Tg mice), which develop aGVHD-like mucocutaneous disease after OVA-specific CD8 T cell (OT-I cell) transfer. LCs did not disappear from the epidermis in this model, unlike in aGVHD induced by major histocompatibility complex (MHC)-mismatched bone marrow (BM) transplant. Langerin-diphteria toxin receptor (DTR)/K14-mOVA double Tg mice treated with DT developed exacerbated aGVHD-like mucocutaneous disease (mean of skin scores [MSS]: 4.18 ± 2.08), in which more OT-I cells infiltrated into the skin with reduced number of apoptotic cells, as compared to control mice (MSS: 2.10 ± 1.14, $p<0.05$). Irradiated Langerin-DTR/K14-mOVA double Tg mice transplanted with BM from K14-mOVA Tg mice also developed exacerbated mucocutaneous disease after DT treatment and OT-I cell transfer. WST-1 assay revealed that the number of proliferating OT-I cells stimulated with OVA CD8 epitope peptide was decreased when co-cultured with LCs isolated from the epidermis (optical density [OD]: 1.04 ± 0.02) as compared to when co-cultured with splenic dendritic cells (OD: 1.79 ± 0.26, $p<0.05$). Blockade of the B7 family proteins, B7-H3 and B7-H4 expressed on activated LCs, partially canceled apoptosis of OT-I cells. Collectively, LCs negatively regulate aGVHD-like autoimmune mucocutaneous disease by *in situ* inhibiting the number of infiltrating CD8 T cells.