Clinical Implications

- DNA methylation is one of several epigenetic mechanisms that can lead to stable and heritable changes in gene expression without changing the underlying DNA sequence.
- Lesional skin in psoriasis is accompanied by changes in DNA methylation.
- Changes in DNA methylation revert to baseline with treatment, indicating that in psoriasis it is dynamic.

has been shown to be essential for epidermal progenitor cell function and is enriched in undifferentiated cells, where it suppresses differentiation and maintains proliferative properties (Sen *et al.*, 2010). Interestingly, a proportion of genes repressed during differentiation become methylated *de novo,* and some genes lose methylation during this process. Thus, DNA methylation is a dynamic process that occurs during epidermal differentiation (Sen *et al.*, 2010). In agreement with the observed aberrant keratinocyte differentiation and hyperproliferation, we were able to demonstrate upregulation of DNMT1 in psoriasis by approximately 1.5-fold (Gudjonsson, unpublished observation). Interestingly, proinflammatory cytokines such as interleukin-6, which is increased in psoriasis, can induce the expression and activity of DNMT1 (Hodge *et al.*, 2001). Much less is known about the regulation of demethylases in the epidermis; this will be an interesting area for future research.

We have barely scratched the surface of the role of epigenetics in the skin. As technology progresses, and with it our ability to determine other types of epigenetic changes, we predict that a more complete view of the mechanisms operating in psoriatic plaques will emerge. Taken together, data from Roberson *et al.* (2012) indicate that DNA methylation in psoriasis is a dynamic process, influenced—and possibly even driven entirely—by the cytokine environment. These changes in the epigenome are likely to have a major role in orchestrating the altered differentiation and proliferation observed in psoriatic lesions. Characterizing the mechanisms involved and the consequences of these changes are exciting avenues for future research.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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The Three Dimensions of Functional T-Cell Tolerance: From Research to Practice

Christian Hünefeld[1], Markus Mezger[2], Michael Röcken[3,4] and Martin Röcken[1]

In this issue, Paek *et al.* describe two phenomena. First, they show that intermediate concentrations of a "transgenic" autoantigen may cause a lichen planus–like autoimmune disease. Second, and more importantly, they show that high doses of peptide antigen suppress the expression of the T-cell receptor and coreceptors, particularly CD8, and that this suppression improves this T-cell-mediated, destructive inflammatory skin disease that is similar to erosive lichen planus.

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T-cell tolerance resulting from receptor downregulation was first described 20 years ago (Schönrich *et al.*, 1991). The long road between recognizing

[1]*Department of Dermatology, Eberhard-Karls-University, Tübingen, Germany;* [2]*Department of Pediatrics, Eberhard-Karls-University, Tübingen, Germany;* [3]*Veterinary Clinic, Starnberg, Germany and* [4]*Veterinary Clinic, Justus Liebig University, Giessen, Germany*

Correspondence: Martin Röcken, Department of Dermatology, Eberhard-Karls-University, D-72076 Tübingen, Germany. E-mail: mrocken@med.uni-tuebingen.de

this fundamental biological phenomenon and its translation into an inflammatory skin disease (Gutermuth *et al.*, 2009; Paek *et al.*, 2012, this issue) is of considerable scientific and clinical importance. Recent progress in clinical dermatology illustrates the clinical importance of the slow process of translating basic research into appropriate model diseases and then into the development of appropriate therapies. One example is anti-CTLA4 therapy for human melanoma (Robert *et al.*, 2011), initially developed as immune therapy in mice with prostate cancer (Kwon *et al.*, 1999). Other examples include the adoptive transfer of cancer-specific, interferon (IFN)-γ-producing T helper type 1 (Th1) cells, developed as a treatment for model cancers in mice (e.g., Egeter *et al.*, 2000) and now available as therapy for human melanoma (Hunder *et al.*, 2008), and the finding that tumor necrosis factor (TNF) antagonists improve autoimmune diseases in mice (Santambrogio *et al.*, 1993), which has been translated into a therapy for psoriasis (Griffiths and Barker, 2007). Cytokine-induced immune deviation developed to treat inflammatory diseases in mice (Racke *et al.*, 1994) has been translated into interleukin (IL)-4 therapy (Ghoreschi *et al.*, 2003) and anti-IL-12/IL-23p40 therapy for psoriasis (Griffiths *et al.*, 2010). Thus, translational research—the long road from basic descriptions through proof in appropriate models—may ultimately improve human health.

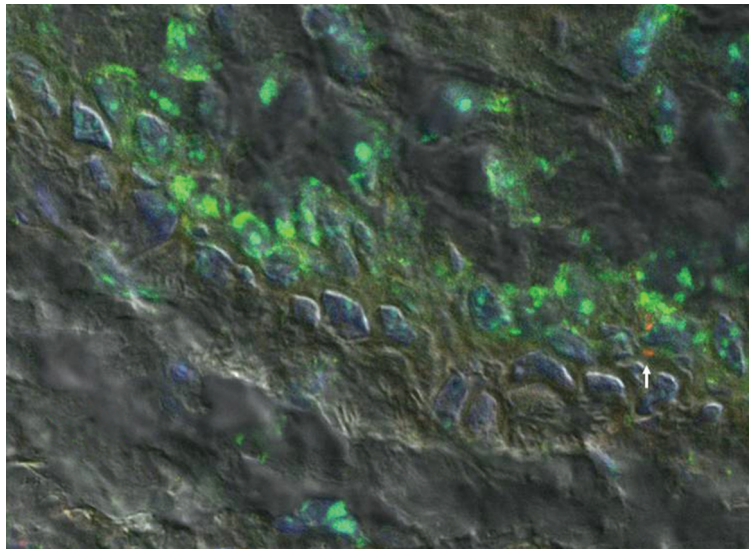

Figure 1. Fluorescent *in situ* hybridization analysis showing single donor-derived male cells in the oral mucosa 6 weeks after transplantation of male bone marrow cells into a C57/BL6 female mouse. White arrow points to donor-derived male cell. Red, Y chromosome; green, X chromosome.

Development of immune responses

Immune responses generally start by alerting the body's immune system through innate signals; an innate alert is translated into an adaptive immune response that protects against viral, bacterial, or fungal infection (Matzinger, 1994). Untreated T-cell-deficient infants with severe combined immune deficiency syndromes die early in life from severe infection (Felgentreff *et al.*, 2011). More frequently, chronic infectious diseases result from defects in the development of appropriate T-cell-adaptive immunity. Disease may also develop in immune-competent individuals when innate or adaptive immune responses fail to neutralize pathogens efficiently; if pathogens persist in significant numbers, the ongoing immune response itself may become harmful. Prominent examples include eczemas or cicatrizing folliculitis resulting from persistent trichophyton infection, liver fibrosis resulting from the persistent interaction between T cells and viruses in chronic hepatitis C, and HIV infection. In these circumstances, disease results from immune-mediated destruction of macrophages and T cells.

A third group of inflammatory diseases results from inappropriate activation of either innate or adaptive immune responses or both. Autoinflammatory diseases are examples of inappropriate activation of innate immunity (Kastner *et al.*, 2010); inappropriate activation of adaptive immunity leads to diseases such as pemphigus (Getsios *et al.*, 2010), lichen planus, and vitiligo (Lang *et al.*, 2001). Both innate and adaptive immune activation are critically involved in psoriasis. Consequently, therapeutically targeting either adaptive T cells or innate immunity ameliorates psoriasis in most patients (Ghoreschi *et al.*, 2003, 2011).

Diseases resulting from aberrant immune activation

Natural barriers and early innate responses routinely cope with most potential threats. Only rarely must incoming threats be recognized as dangerous by the adaptive immune system. In lymph nodes, innate information is translated and amplified through the activation of adaptive T cells and B cells. Highly efficient on a routine basis, this activation process may lead to errors at any one of three important checkpoints:

1. Innate immune activation may be too sensitive to stimuli if either the activation or the silencing pathway includes regulation defects.

2. Adaptive immunity may have silencing defects that result from either absent T-cell silencing or absent regulatory T cells (Tregs) (Sakaguchi, 2005; Shevach, 2011).

3. Perpetuation of cytokine production may cause self-destruction, as in the case of delayed-type hypersensitivity responses (DTHRs) that are associated with IFN-γ-producing Th1 or IL-17-producing Th17 cells.

Protection from autoimmune disease: three dimensions of functional T-cell tolerance

The thymus protects against autoimmune diseases by deleting potentially harmful, autoreactive T cells (Kisielow

et al., 1988). Similarly, strong stimulation in the periphery may cause T-cell deletion (Paek *et al*., 2012; Röcken *et al*., 1992). Other peripheral mechanisms leading to T-cell tolerance are functional T-cell silencing, the induction of Tregs, and functional immune deviation (a treatment already in practice through the use of anti-IL-12/IL-23 monoclonal antibodies).

> Peptide-induced tolerance may be most useful as preparation before organ transplantation.

Functional T-cell silencing. Functional T-cell silencing may result from T-cell "over"-activation or from activation in the absence of appropriate costimulation. Thus, *in vivo* overactivation, sometimes also called high-zone tolerance, may lead to the failure of T cells to respond, neither proliferating nor producing cytokines in response to antigenic stimuli (Moskophidis *et al*., 1993; Röcken *et al*., 1992). Frequently, functional T-cell tolerance is determined by the inability of T cells to produce IL-2 or IFN-γ or to proliferate in response to appropriate antigenic stimulation. Anergy and exhaustion can be explained in part by defects in the T-cell receptor (TCR) signaling cascade (Saibil *et al*., 2007). Alternatively (or, more likely, in addition), T-cell anergy results from suppression in the expression of the TCR and coreceptors, such as CD4 and CD8, which are required for T-cell activation (Schönrich *et al*., 1991). In this issue, Paek *et al*. (2012) demonstrate that continuous application of high concentrations of specific peptides results in TCR suppression and T-cell anergy. More importantly, they also demonstrate that intermediate concentrations of the peptides may cause severe, destructive inflammatory skin inflammation. Increasing the antigen concentrations via continuous peptide therapy functionally silences T cells by suppressing TCR and CD8 expression, leading to the generation of T cells that are no longer capable of damaging skin. High-dose peptide therapy may thus be a valuable therapeutic approach for T-cell-mediated diseases. Yet, this sort of therapy will have several critical prerequisites, one of which is the identification of the key peptide that is recognized by the T cells that may cause human autoimmune disease.

Moreover, the disease should be strictly T-cell mediated, such as in lichen planus, alopecia areata, and vitiligo. Successful treatment of lichen planus would still require the identification of the relevant peptides. Vitiligo appears to be a candidate because data suggest that it is initiated and caused by melan A–specific CD8 T cells (Lang *et al*., 2001). Yet it remains uncertain whether suppression of melan A–specific Th1 or Tc1 cells increases the risk of melanoma (Müller-Hermelink *et al*., 2008; Ziegler *et al*., 2009). In humans, B-cell-mediated autoimmune diseases, particularly pemphigus vulgaris and pemphigus foliaceus, share many similarities. This is especially true for the endemic Brazilian disease pemphigus foliaceus. T- and B-cell epitopes depend on autoreactive T cells capable of stimulating B cells for the production of disease-causing autoantibodies (Getsios *et al*., 2010).

Unfortunately, T-cell anergy establishes labile equilibrium because it may be overcome by infections that cause general T-cell expansion (Röcken *et al*., 1994). Another risk is inappropriate activation of potentially harmful T cells (Bielekova *et al*., 2000).

Tregs. Besides achieving a state of anergy or unresponsiveness, activated T cells may achieve a state in which they actively silence neighboring T cells. Such Tregs express the IL-2 receptor CD25, reflecting their activated status. The most important characteristic of Tregs is their expression of the transcription factor FoxP3. Tregs suppress coactivated T cells through contact-dependent mechanisms and through the production of IL-10 or transforming growth factor-β (TGF-β). The functional importance of Tregs was demonstrated either by deleting FoxP3-expressing T cells with anti-CD25 antibodies or by genetic *Foxp3* knockout (Sakaguchi, 2005; Shevach, 2011). Animals devoid of Treg cells develop severe autoimmune diseases, with inflammatory destruction of multiple organs and severe wasting (Lahl *et al*., 2007). Because Tregs are difficult to generate *in vivo* through antigen-specific stimulation or to expand *in vitro* in sufficient amounts for clinical use, their therapeutic potential remains uncertain.

Immune deviation. From the therapeutic point of view, immune deviation (in which IFN-γ-dominated Th1 or IL-17-mediated Th1 responses are deviated into IL-4-dominated Th2 responses that fail to cause efficient DTHRs) is the most advanced mode of functional T-cell silencing. This was originally shown in mice developing immunity to *Leishmania major*, in which immune deviation caused susceptibility to the parasite (Sadick *et al*., 1990). It was subsequently demonstrated that *in vivo* activation of $CD4^+$ T cells in the presence of IL-4 deviates the developing Th1/Th17 responses into Th2 responses and thus abrogates T-cell-mediated control of parasite infections and endogenous cancer (Biedermann *et al*., 2001; Müller-Hermelink *et al*., 2008; Röcken *et al*., 1994). Deviation of Th1/Th17 into Th2 responses requires IL-4 and allows the prevention, and even treatment, of harmful DTHRs. This was shown for experimental encephalitis in mice (Racke *et al*., 1994; Ghoreschi *et al*., 2011), psoriasis in humans (Ghoreschi *et al*., 2003, 2011; Griffiths *et al*., 2010), and probably also multiple sclerosis (Kappos *et al*., 2008).

Although peptide-mediated suppression of T-cell function, as shown by Paek *et al*. (2012) and in a model of diabetes, may help in some individuals whose response is strongly restricted to either CD4 or CD8 T cells, such therapies bear the risk of inappropriate immune activation, seem to have a short window of opportunity as diabetes develops, and require an individualized peptide vaccine for each patient (Paek *et al*., 2012; Bielekova *et al*., 2000). Thus, therapeutic immune deviation that primarily targets T cells (Biedermann *et al*., 2001; Ghoreschi *et al*., 2003) or mononuclear and dendritic cells (Ghoreschi *et al*., 2011; Hoetzenecker *et al*., 2012) may represent a safer therapy that also can be standardized.

New clinical fields for functional T-cell tolerance

The most obvious example of functional T-cell tolerance in medical practice occurs after bone marrow or—less frequently—solid-organ transplantation. In this newly developing field, tissues will be replaced by the transplantation of either somatic cells generated *in vitro* from pluripotential precursors or cells expressing a missing neo-antigen. Although somatic cells, generated *in vitro* from adipocytes, express neo-antigens at the time of transplantation, preliminary data from veterinary medicine suggest that they can serve appropriately in replacement therapy (Richardson *et al*., 2007). Hosts may even tolerate real neo-antigens. Thus, in mice, transplantation of various precursor cells allows long-lasting engraftment, even of cells that express immunogenic peptides, such as those derived from the HY gene (Figure 1) or enhanced green fluorescent protein. The data described by Paek *et al*. (2012) suggest that induction of peptide-specific tolerance should allow tolerance induction toward highly immunogenic protein antigens. Because, in this case, the neo-antigen is well known, it is suitable for specific desensitization. Therefore, peptide-induced tolerance as presented in this report may be most relevant for the immunological preparation of recipients for replacement therapy in patients with defined genetic defects.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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