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Langerhans cells as targets for immunotherapy against skin cancer

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Abstract

Cancer is the second most common cause of death in the world. Treatment of cancer is very challenging and immunotherapy has been developed as a potential way to fight cancer. The main obstacle with immunotherapy is that cancer cells evolve from healthy body cells in response to an accumulation of genetic mutations. As a consequence, the immune system struggles to detect the abnormal cells as they are mainly recognized as self. This implies that equipping the immune system to eliminate cancer cells is tricky, yet represents a very efficient way to constrain the growth of tumors. We became interested in developing immunotherapeutical strategies against skin cancer in the context of our observations that Langerhans cells (LC) are very potent antigen presenting cells and are able to incorporate protein antigens and present them to CD4⁺ and CD8⁺ T cells in the skin-draining lymph nodes. As a consequence, we developed an immunization strategy through the skin, termed epicutaneous immunization. Protein antigen applied onto barrier-disrupted skin induces long-lasting cytotoxic T-cell responses, potent enough to control and inhibit tumor growth. In this review, we suggest that immunization strategies through the skin could be a promising new approach for the treatment of skin cancer.

Keywords

skin immunology; immunotherapy; Langerhans cells

The skin consists of two compartments, the epidermis and the dermis, each populated by various subsets of dendritic cells (DC). The Langerhans cells (LC) reside in the suprabasal layers of the epidermis surrounded by keratinocytes, the epithelial cells forming the epidermis (Figure 1).¹ In the dermis the DC are distributed throughout the connective tissue and can be subdivided into two subsets.^{2–4} Their special localization in the skin, the physical barrier preventing the invasion of pathogens, makes them the prime antigen presenting cells for any immune response affecting the skin. The specific role of the diverse DC subsets in the skin is currently under investigation and there is some indication that dermal DC and LC might have different roles in the induction of immune responses in skin-draining lymph nodes.

Designing new approaches to vaccinate through the skin are very attractive in regard to simplicity and feasibility. DC are promising candidates for immunotherapy against cancer. Clinical trials in which DC have been used as carriers for tumor antigens have met with some success. In conventional DC immunotherapy, blood monocytes or CD34⁺ precursor cells from patients are differentiated into DC *in vitro*, before they are loaded with tumor

antigen and injected back into cancer patients.⁵ Some cancer patients with solid malignancies, such as renal cell carcinoma and melanoma, showed partial clinical responses or even complete remissions.^{6,7} As this treatment is cumbersome and expensive, we have started investigating simpler and cheaper ways for immunotherapy. LC are potent stimulators of T-cell responses making them optimal targets for immunization strategies through the skin.^{1,7} Hence, we decided to load LC with protein antigen *in situ* by immunizing through the skin, the so-called epicutaneous immunization. When we tested this immunotherapeutical approach for its efficiency, we observed that not just LC but also dermal DC presented topically applied antigen to T cells in the skin-draining lymph nodes and induced long-lasting memory T-cell responses. These CD8⁺ T cells were able to inhibit the growth of transplanted melanoma in a mouse model.⁸ Yet, the efficiency of this new therapeutic approach has to be further improved to render it an alternative treatment for cancer patients. Several different synonyms exist for epicutaneous immunization, such as transcutaneous, transdermal or percutaneous immunization. For the simple reason that antigen is applied onto epidermis, we decided to call it epicutaneous immunization and will use this term throughout this review.

EPICUTANEOUS IMMUNIZATION AS A NEW PROMISING IMMUNOTHERAPY

Immunization strategies topically through the skin offer the advantage of easy-to-use treatments with the possibility of self-medication and the omission of injections for better acceptance by the patients. The skin with its abundance of immune cells and easy accessibility is ideal for such approaches. Moreover, by skin vaccinations divergent immune outcomes can be potentially initiated, as shown by the induction of tolerance inhibiting experimental autoimmune encephalomyelitis,⁹ and of immunity against cancer and infection as demonstrated in several studies that will be outlined here in this review. It is interesting to note that one recent report even gave evidence that skin immunization could be used to decrease the amount of β -amyloid, a peptide contained in plaques of Alzheimer patients, by induction of antibodies against the peptide suggesting its potential to treat Alzheimer's disease.¹⁰

Topical immunization through the skin elicits humoral and cellular immune responses against cancer antigens (for example, melanoma antigens), and pathogens, such as influenza virus and *Escherichia coli* (*E. coli*). The first attempts at epicutaneous immunization against cancer were carried out by a Japanese group a few years ago. In their study in mice, they disturbed the skin barrier by tape stripping, which is the repeated application of sticky tape onto the skin to remove the stratum corneum. The applied major histocompatibility complex (MHC) class I restricted peptides from melanoma antigens induced cytotoxicity that could hamper the growth of transplanted tumors.¹¹ In a study with melanoma patients conducted some time later the same authors observed stimulation of cytotoxic T cells after immunization with melanoma peptides through barrier-disrupted skin in four out of six patients. Moreover, T cells infiltrated regressing tumor lesions.¹² In support of these findings another group showed that topical vaccination with peptides derived from the papilloma-virus antigen, E7, could inhibit the growth of transplanted E7-transfected tumor cells.¹³ In our own work we demonstrated that the use of whole protein antigen (ovalbumin which we used as a tumor model antigen) induced potent CD4⁺ and CD8⁺ T-cell responses with long-lasting cytotoxic memory responses. As a result, the growth of transplantable B16-melanoma cells expressing ovalbumin was inhibited in prophylactic and therapeutical settings. The substitution of standard cream (Ultrasicc a basic cream consisting of a water:oil emulsion) with Aldara cream that contains the toll-like receptor (TLR)-7 ligand imiquimod improved anti-tumor responses.⁸ Another study reported recently that topical application of the melanoma peptide gp100 together with Aldara cream primed antigen-

specific T cells in a comparable way than immunization with peptide loaded DC.¹⁴ These results point at the potential to use epicutaneous immunization with protein or peptide antigen in combination with barrier disruption and the TLR ligand imiquimod as a treatment against skin cancer.

Another promising application for epicutaneous immunization is the treatment or prevention of infections. Glenn *et al.*¹⁵ started to develop vaccination with toxins, such as cholera toxin and tetanus toxoid, which could protect mice from a subsequent challenge with the toxin.¹⁶ These data were underscored by a report showing that immunization with heat-labile enterotoxin of *E. coli* through bare skin protected mice against lethal challenge with the toxin.¹⁷ As a result of these promising findings the group of Gregory M Glenn (formerly Iomai, Gaithersburg, MD, USA, now Intercell, Vienna, Austria) developed patches containing heat-labile toxin from *E. coli* against travellers' diarrhea. In a phase II trial, participants were either protected against diarrhea or experienced shorter and milder courses of illness.¹⁸ In another study with volunteers, Aggripal, a commercial vaccine against influenza was applied onto barrier-disrupted skin and compared to intramuscular immunization. Both routes of vaccination induced interferon- γ production in the activated CD4⁺ T cells; however, CD8⁺ T-cell responses were only detected after immunization through the skin and not the muscle.¹⁹ It is interesting to note that epicutaneous immunization could also be a new interesting option for vaccination to protect against HIV as shown recently.²⁰ In conclusion, epicutaneous immunization represents a new technique to treat and/or prevent cancer and infection. First results are promising, yet the outcome in patients has to be improved to render it an alternative to other immunotherapies.

EVIDENCE OF THE SUITABILITY OF LC AS TARGETS FOR IMMUNOTHERAPY

LC have been described as potent antigen presenting cells stimulating T-cell proliferation.^{21, 22} Several studies indicate that LC are prime candidates for immunotherapy:

Evidence from human LC-like DC

Human LC generated from CD34⁺ precursors were able to capture apoptotic tumor cells and stimulate autologous CD8⁺ T-cell responses *in vitro*.^{23, 24} In one of these studies, LC-like cells proved to be superior to *in vitro* generated interstitial dermal DC in the induction of cytotoxic T-cell responses.²³ In a series of clinical trials in melanoma patients, CD34-derived DC that contain LC-like DC were capable of priming immune responses against tumor antigen.²⁵

Evidence from studies with mouse LC

In an early study it was shown that LC were responsible for the induction of protective immunity in a mouse model of sarcoma.²⁶ Likewise, LC can be used to treat transplantable B16 melanoma when loaded with melanoma-specific peptides comparable with bone-marrow derived DC.²⁷ We reported recently that LC isolated from epidermis or emigrated from epidermal skin explants cross-presented soluble protein or cell-associated antigen on MHC-class I molecules to CD8⁺ T cells. Activated CD8⁺ T cells exerted effector function, such as cytokine production and cytotoxic activity.²⁸ The discovery of Langerin as a marker for LC,²⁹ allowed for the first time to visualize and characterize LC^{30, 31} and develop mice in which LC are absent.^{32, 34} Mice, in which LC can either be depleted by injection of diphtheria toxin or isolated by an EGFP molecule linked to the Langerin molecule (Langerin-DTREGFP mice^{32, 33}), were the crucial step to carry out *in vivo* experiments and explore the function of LC. In another mouse model, LC never develop and these mice are constitutively devoid of LC (Langerin-DTA mice³⁴). In spite of some conflicting results

about the involvement of LC in the induction of contact hypersensitivity responses, it became clear that LC are important in the immune response when limiting amounts of antigen are applied onto the skin. However, when large amounts of contact allergen are applied, dermal DC can substitute for the missing LC and mediate contact hypersensitivity (Noordegraaf M, in revision, *J Invest Dermatol*). These findings in the inducible mouse models^{32,33} are in contrast to the mouse model in which LC are absent throughout life.³⁴ The mice that constitutively lack LC, show enhanced contact hypersensitivity reactions regardless of the amount of contact sensitizer used.³⁵ The explanation for this discrepancy is still uncertain. This topic will be discussed in more detail in the reviews by Björn E Clausen and Dan Kaplan in this Special Feature on LC. Yet, so far it seems that the skin DC possess redundant roles and their functional involvement *in vivo* depends critically on their ability to gain access to the antigen and to present antigen in draining lymph nodes rather than on their cell-intrinsic properties.

Do LC or dermal Langerin⁺ DC present epicutaneously applied antigen?

The recent discovery of a new dermal DC subset that expresses Langerin complicates the story even more.^{2,4} The Langerin⁺ dermal DC subset makes up to 30–60% of all the Langerin⁺ DC in the skin-draining lymph nodes although they are very rare in the dermis and hard to spot by FACS analysis and in skin sections. These cells represent a distinct DC subset from LC and seem to fulfill a lot of the functions which have been attributed to LC.³⁶ Several reports indicate that these dermal Langerin⁺ DC are mediating immune responses in the skin, such as contact hypersensitivity^{4,37} and cross-presentation of antigen expressed in the keratinocytes of the epidermis as well as viral antigens.³⁸ So far, the question which skin DC subset mediates the T-cell response after epicutaneous immunization has not been adequately addressed. Still, there is some indication that LC participate in the antigen presentation of topically applied antigen. When a peptide against β -amyloid was used for immunization, LC could be visualized in the dermis that contain antigen.¹⁰ Moreover, LC from tape-stripped skin were efficient in the stimulation of cytotoxicity against a melanoma antigen.¹¹ In our own work we demonstrated that skin-derived Langerin⁺ DC sorted from skin-draining lymph nodes were able to present epicutaneously applied ovalbumin protein to CD8⁺ T cells. In addition, depletion of Langerin⁺ DC around the time of immunization with low doses of protein antigen reduced the immune responses against transplantable melanoma indicating an important role of LC for this kind of vaccination.⁸ So far, from our results we cannot conclude which Langerin⁺ DC subset, the LC or dermal Langerin⁺ DC, or if both populations together present epicutaneously applied antigen to CD8⁺ T cells. That both Langerin⁺ subsets might be important for epicutaneous immunization was reported by Wang *et al.*³⁷ Protein antigen applied on ear skin was presented independently of Langerin⁺ DC. In contrast, immunization through the flank skin required the presence of Langerin⁺ DC. This can be explained by the fact that flank skin is thicker and contains more Langerin⁺ DC. In our study higher amounts of ovalbumin protein applied epicutaneously induced anti-tumor responses partly independent of Langerin⁺ DC, but lower amounts were only presented when Langerin⁺ DC were present. Thus, antigen dose, application site and thickness of skin, which limits diffusion of antigen, are important factors that will determine the efficiency of epicutaneous immunization and which skin DC subset will induce the immune response.

Influence of migratory properties of skin DC on immunization

An important point one should keep in mind is that dermal DC migrate quicker to the draining lymph nodes and also localize more around B cell follicles, making them prime antigen presenting cells for induction of humoral responses.³³ This topic will be covered in more detail by Hideki Ueno in this Special feature on LC. LC need 2–4 days to reach the lymph nodes and end up in the T-cell area, thereby enabling them to induce T-cell

responses.^{31, 39-41} Antigen injected into the dermis is incorporated and presented as early as 30 min by lymph node resident DC followed by a second wave of antigen presentation by migratory skin DC that was mandatory to sustain a T-cell response.⁴² Lymph node resident DC might help migratory skin DC in antigen presentation by retaining antigen-specific CD4⁺ T cells in the lymph node, thus, facilitating the interaction of skin DC with T cells.⁴³ From our own work we know that intradermal immunization with ovalbumin protein and the synthetic glycolipid antigen α -galactosylceramide, which is presented on CD1d molecules to invariant natural killer T cells, works independent of migratory skin DC. However, α -galactosylceramide is a very potent adjuvant that exerts its effects systemically after intradermal injection. Furthermore, we did not investigate the sustainability of the T-cell response.⁴⁴ This leads to the concept that fast migrating dermal DC, together with lymph node resident DC, might be the initiators of humoral and cellular immune responses and later arriving LC can prolong antigen presentation and might even drive memory T-cell development and imprinting of tissue homing receptors on T cells, yet more work has to be done to confirm this concept.

WHAT IS MANDATORY TO MAKE EPICUTANEOUS IMMUNIZATION WORK?

There are several points that need to be addressed if epicutaneous immunization is to be made optional for the treatment of cancer patients.

Generation of long-term memory

One important point is the induction of long-lasting memory cytotoxic T-cell responses, which requires CD4⁺ T cell help to develop. Most studies so far have been carried out with repeated applications of high doses of MHC class I restricted peptides which excluded the activation of antigen-specific CD4⁺ T cells together with CD8⁺ T cells.^{11, 14, 45} When we carried out our first attempts of epicutaneous immunization, we used whole ovalbumin protein in combination with contact allergen to activate skin DC and to achieve antigen presentation on MHC class I and II molecules by migratory skin DC in the lymph node. Ovalbumin protein applied onto untreated skin stimulated some proliferation of CD4⁺ T cells *in vivo* and *in vitro*, whereas vaccination through skin pretreated with contact sensitizer dramatically improved the T-cell responses.⁴⁶ When we further refined the system we applied ovalbumin protein in standard cream (Ultrasicc) onto tape-stripped skin. We generated long-lasting memory CD8⁺ T-cell responses with one single application of antigen,⁸ in contrast to most of the other studies, in which repeated applications were required to generate T-cell responses. Thus, protein antigen could have an advantage over peptides, as less immunization cycles are required to achieve similar potent memory cytotoxic T-cell responses. For clinical trials it will be difficult to get sufficient amounts of protein with good manufacturing practice standards, thus, either synthetic long peptides containing epitopes for presentation on MHC class I and II¹³ or mixes of MHC class I and II peptides might be favorable. Moreover, smaller molecules will gain access to deeper regions of the skin more easily than large molecules.

Modulation of lymphocyte homing properties

Another important point that has to be considered for effective epicutaneous immunization is the induction of homing receptors on activated T cells. Homing of cytotoxic CD8⁺ T cells to the tumor site is mandatory to control the disease as shown in long-term melanoma survivors. Tumor-specific cytotoxic CD8⁺ T cells were detected in metastases and lymph nodes even after the disappearance of tumor cells as a local defense against recurrence.⁴⁷ In early studies it became clear that lymphoid organs draining different tissues contain T cells with variable adhesion molecules. T cells activated in the skin-draining lymph nodes expressed P-selectin, in contrast to T cells in mesenteric lymph nodes which upregulated

$\alpha 4\beta 7$ upon intraperitoneal immunization with antigen.⁴⁸ The immunization route determines which homing receptors are upregulated on activated T cells. Intracutaneous injection of DC induced E-selectin ligand expression and allowed effector cells to enter the skin, in contrast to intraperitoneal injection of DC which caused upregulation of the gut-homing integrin $\alpha 4\beta 7$.⁴⁹ In a model of self-antigen expression in the skin, antigen-specific T cells upregulated E-selectin in skin-draining but not in mesenteric lymph nodes and spleen, indicating the imprinting of skin tissue homing receptors by a skin self antigen.⁵⁰ Our own observations with intradermal immunization with ovalbumin protein together with the synthetic glycolipid antigen α -galactosylceramide revealed proliferation of antigen-specific CD8⁺ T cells in the lymph node draining the skin immunization site. In contrast intravenous immunization caused proliferation of T cells preferentially in the spleen.⁴⁴ These findings suggest that the immunization route will be important to prime T cells specific for melanoma antigens that are able to home to tumors and metastases growing in the skin or in other locations. It is interesting to note that two reports indicate that epicutaneous immunization might also lead to antigen presentation in Peyer's patches and mesenteric lymph nodes, and as a result to activation of mucosal cytotoxic T-cell responses and production of IgA antibodies. The explanation, that skin-derived DC can migrate to gut mucosa to present antigen there, is quite unlikely; however, Langerin⁺ DC might differentiate from bone marrow-derived cells and infiltrate mesenteric lymph nodes. How the antigen makes it to the distal mesenteric lymph nodes is unclear, yet topically applied antigen could either be orally ingested by mice through licking the immunization site or the cholera toxin used as adjuvant increases permeability of the blood vessels and allows diffusion of antigen into the blood stream.^{20,51} All in all, the systemic induction of T-cell responses is desirable and will allow using epicutaneous immunization for the treatment of tumors growing in other locations than the skin.

Modulation of skin barrier

Skin barrier-disruption is needed to improve antigen diffusion into the skin and incorporation by skin DC. Antigens can penetrate across bare skin through the stratum corneum with a patch;⁵² however, appendages like hair follicles and sebaceous or sweat glands can also serve as a portal for antigen entry.^{19,53} Skin barrier disruption can be achieved in many different ways. Repeated tape stripping with a sticky tape was developed to remove the stratum corneum and to activate the migration of LC.^{54,55} This is most probably caused by the production of proinflammatory cytokines, such as tumor necrosis factor- α and interleukin-1, in response to the barrier-disruption as could be shown for murine and human skin.^{56,57} Excessive repetitions of tape stripping (up to 30 times) leads to the removal of the LC by pulling them out of the epidermis, whereas moderate tape stripping (up to 12 times) leads to the induction of inflammation in the skin followed by emigration of LC within the next few days (Figure 2). Full recovery of LC numbers is achieved within a month after tape stripping. Ear and body skin of mice react in a different way to tape stripping, that is, shaved body skin needs less repetition of tape stripping than unshaved ear skin (Tripp CH and Stoitzner P, unpublished data). The idea is that the inflammation in the skin activates DC to develop into potent antigen presenting cells. This is the case as was shown by analyzing LC from tape-stripped skin since they expressed more CD40 and CD86 and were better in priming the development of cytotoxic T-cell responses (Stoitzner P, unpublished data).^{11,58} Other groups have tried to develop devices that allow more controlled removal of the stratum corneum or improved delivery of antigen into the skin, such as skin prep system,⁵⁹ propelled microparticles applied with a PowderJet device,⁶⁰ microneedle arrays^{61,62} and electroporation.⁶³ All of these variants for barrier-disruption allowed the stimulation of either antibody secretion or cytotoxic T-cell responses accompanied by the induction of LC emigration from the epidermis.

Application of specific adjuvants

Various adjuvants have been used to enhance humoral and cellular immune responses after epicutaneous immunization. For a start, toxins like cholera toxin and heat-labile enterotoxin of *E. coli* were used to boost humoral and cellular responses.^{17,64} This was partly achieved by increased migration of LC as could be shown for cholera toxin.^{10,65} These toxins function as adjuvants and antigens at the same time; however, they might pose some problems for approval in clinical trials with patients because of their toxicity. Thus, several groups have tested TLR ligands as potential adjuvants for epicutaneous immunization. Most skin cell subsets express a panel of TLRs. Keratinocytes express TLRs 1–9, except TLR 8, though the expression of TLR 4 and TLR 7 on keratinocytes is still disputed and might depend on culture conditions.^{66,67} Human LC express TLRs 1, 2, 3 and 6, however, TLRs 4, 7 and 8 are absent from this cell type and expression of the intracellular TLR 9 was inconsistently demonstrated.^{67–69} Mitsui *et al.*⁷⁰ reported that murine LC express TLR 2, 4 (but not the TLR 4 coreceptor CD14) and 9, whereas TLR 7 was absent. Moreover, dermal cells respond to many different TLR ligands, with dermal DC being shown to express TLR 1–8, skin-derived mast cells being found to express TLRs 1–4, 6, 7 and 9.^{71,72} And dermal endothelial cells, fibroblasts or macrophages responding to TLR 4 activation.^{73,74} It is interesting to note that tape stripping can induce the expression of TLR 9 in the skin which improved migration of DC, suggesting that tape stripping and CpG in combination could work very efficiently for epicutaneous immunization.⁷⁵ That this is the case was shown in a recent report in which the oligonucleotide CpG that binds to TLR 9 increased the number of interferon γ -producing CD8⁺ T cells.⁷⁶ However, CpG could not stimulate a potent antibody response comparable with heat-labile enterotoxin.^{63,77} Several other studies showed that Aldara cream containing the TLR 7 ligand imiquimod could further enhance the induction of cytotoxicity.^{8,45} This mainly works through the activation and induction of emigration of LC, most probably caused by increased cytokine production in the skin.⁷⁸ In our own work we observed, that Aldara cream enhances migration of antigen-loaded LC and dermal Langerin⁺ DC to skin-draining lymph nodes; dermal Langerin⁺ DC arrive within the first 2 days and LC mainly by day 4 (Flacher V, unpublished data). It is interesting to note that an MHC class I-binding peptide from an adenovirus protein induced equal cytotoxic T-cell responses when applied epicutaneously with Aldara cream or injected intradermally followed by topical Aldara cream treatment. Both, epicutaneous and intradermal immunization strategies were as potent as vaccination with peptide loaded DC.⁴⁵ A follow up of this study revealed that dermal mast cells express TLR 7 and are critically involved in the emigration of LC in response to Aldara cream. In the absence of mast cells cytotoxic T-cell responses after epicutaneous immunization were diminished indicating an important role of mast cells in the early response to the adjuvant imiquimod.⁷⁹ Thus, so far barrier-disruption in combination with Aldara cream seems to be an efficient way of inducing T-cell responses. Nevertheless, the many different TLRs expressed by the skin cells permit the testing of various ligands for their ability to improve the efficiency of epicutaneous immunization. This is of importance as the application of a potent adjuvant or even combinations of several during epicutaneous immunization might allow for substitution or boosting of CD4⁺ T cell help.⁸⁰

Targeting of antigen to subsets of DC

A powerful means of potentiating epicutaneous immunization would be to conjugate antigens to antibodies that specifically bind to the cell surface of DC.⁸¹ Interesting candidates for targeting approaches are members of the C-type lectin receptor family, such as DEC-205/CD205, langerin/CD207 and dectin-1, as they allow targeting of LC and dermal DC.^{82,85} Antigens targeted to these receptors were presented much more efficiently to T cells than unconjugated soluble antigen, and in the presence of DC-activating stimuli strong immunity ensued (Flacher V, Tripp CH and Stoitzner P, unpublished data).^{86,87} What needs

to be tested is the ability of targeting complexes to cross the skin barrier and reach skin DC. Antigen targeting will be discussed in more detail in a companion article by Nikolaus Romani.

Modulation of epicutaneous antigen application

The way epicutaneous immunization is carried out determines the function and cytokine production of T cells as well as antibody secretion. Epicutaneous immunization, with or without patches, on normal or tape-stripped skin in the absence of adjuvant induces predominately Th2 responses and after repeated application the development of allergic contact dermatitis.⁸⁸⁻⁹⁰ Moreover, a systemic production of interleukin-17 was observed that could elicit airway inflammation in response to inhalation of the same antigen highlighting a link between atopic dermatitis and allergic asthma.⁹¹ In contrast, antigen plus adjuvant on barrier-disrupted skin stimulates Th1 and cytotoxic T-cell responses.^{8, 11, 45, 58} Besides immunity, tolerance can be induced by repeated immunization onto bare skin that could suppress autoimmunity.⁹ Repeated UV irradiation before epicutaneous immunization generated the development of regulatory T cells and a robust antigen-specific tolerance.⁹² Thus, epicutaneous immunization with patches on bare or UV-irradiated skin may be used to induce tolerance to treat autoimmunity. In contrast, immunization on barrier-disrupted skin together with adjuvant elicits strong cytotoxic Th1 responses against tumors and infection. All in all, this new immunotherapy can have manifold effects and be an interesting alternative for the treatment of cancer, infection and autoimmunity.

CONCLUSION

The design of new immunization strategies is crucial in the light of the failure of existing therapeutic approaches.⁹³ Some very aggressive forms of cancer, such as melanoma, renal and pancreatic cell carcinoma are resistant to common treatments with radiation and chemotherapeutics. Besides, these treatments cause severe side effects that pose additional health risks in cancer patients. Melanoma is a highly immunogenic tumor as can be evidenced by the spontaneous remissions in some patients. These observations and the fact that tumors inhibit the proper function of the patients' immune system underline the importance of boosting the immune system with immunotherapy. Adoptive immunotherapy with DC has proven to be safe and well-tolerated with barely any side effects.⁷ Nevertheless, modifications such as targeting resident DC in the cancer patients with epicutaneous immunization could increase efficiency and replace the laborious and expensive preparations of the DC vaccine outside the patient. Non-melanoma skin cancers of the epithelial cells of the epidermis such as squamous cell carcinoma and basal cell carcinoma are very common. Conventional treatment includes surgical excision, photodynamic therapy, radiation and topical application of the TLR 7 ligand containing cream Aldara. These tumors are recurrent, especially in immunosuppressed patients which also indicates that they are immunogenic. Even though basal cell carcinomas hardly metastasize, they can cause problems when they start to grow in several locations at the same time or in areas that are difficult to access, like the oral cavity. In these cases treatment with Aldara cream or surgery cannot be utilized, thus, immunotherapeutical strategies with DC would be desirable. Moreover, a prophylactic treatment of patients with immunotherapy to strengthen the immune system could help to prevent recurrence of tumors. Epicutaneous immunization would offer a number of advantages, listed below, over conventional vaccination schemes including vaccination with *in vitro* generated and injected DC. (1) Even though injecting an immunogen is not a complicated procedure, epicutaneous application of antigen would still be simpler and would even allow self medication. (2) Given the problems with impaired migration of DC injected into the skin to the draining lymph nodes, it seems conceivable that targeting skin DC on a large area of skin may lead to a larger influx of immunogenic

DC into the nodes and, as a consequence, to better immunity.^{7,81} (3) The preferential selection of LC as antigen presenting cells in epicutaneous immunization may result in more robust cytotoxic anti-tumor responses.^{8,23,94,95} (4) Antigen uptake and presentation in the absence of inflammation leads to peripheral tolerance.⁹⁶ It may be envisaged that LC might also be particularly suited to induce tolerance when targeted with autoantigens. (5) Finally, one should also not forget that the epicutaneous approach is certainly less costly than other immunization schemes. All these facts demonstrate that there is the need for alternative therapies, and enhancing the immune system against tumors and pathogens seems to be a promising approach.

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Figure 1.

Several layers of keratinocytes build up the epidermis as can be seen in the left hand panel. Two strands of hair with attached follicles are still protruding from the epidermis. In skin explant cultures, LC emigrate from the suprabasal layers of the epidermis and squeeze through gaps between keratinocytes (right hand panel). In both panels the view is from the dermal side of the epidermis.

Figure 2.

Tape stripping of mouse ear skin activates Langerhans cells in the epidermis. They express more MHC class II, as indicated by immunofluorescent staining with a MHC class II antibody, are enlarged in size and start to leave the epidermis.