

Human skin dendritic cells can be targeted *in situ* by intradermal injection of antibodies against lectin receptors

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Abstract: Skin dendritic cells (DC) express C-type lectin receptors for the recognition of pathogens. Langerhans cells (LC) express the receptor Langerin/CD207, whereas DEC-205/CD205 is mainly expressed by dermal DC, but can also be detected at low levels on LC. In this study, we tested an *ex vivo* approach for targeting DC *in situ* with monoclonal antibodies (mAb) against Langerin and DEC-205. The targeting mAb was injected intradermally into human skin biopsies or added to the medium during skin explant culture. Corresponding to the expression patterns of these lectin receptors on skin DC, Langerin mAb was detected merely in LC in the epidermis and DEC-205 mainly in dermal DC in human skin explants, regardless of the application route. Migratory skin DC bound and carried targeting mAb from skin explants according to their lectin receptor expression profiles. In contrast to the very selective transport of Langerin mAb by LC, DEC-205 mAb was more widely distributed on all CD1a⁺ skin DC subsets but almost absent in CD14⁺ dermal DC. As effective vaccination

requires the addition of adjuvant, we co-administered the toll-like receptor (TLR)-3 ligand poly I:C with the mAb. This adjuvant enhanced binding of DEC-205 mAb to all skin DC subsets, whereas Langerin targeting efficacy remained unchanged. Our findings demonstrate that LC can be preferentially targeted by Langerin mAb. In contrast, DEC-205 mAb can be bound by all CD1a⁺ skin DC subsets. The efficacy of DEC-205 mAb targeting strategy can be boosted by addition of poly I:C underlining the potential of this combination for immunotherapeutical interventions.

Abbreviations: DC, dendritic cells; LC, Langerhans cells; mAb, monoclonal antibody; TLR, toll-like receptor; PBS, phosphate-buffered saline.

Key words: dendritic cells – immunotherapy – Langerhans cells – skin

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Introduction

Dendritic cells (DC) are pivotal for the induction of primary immune responses, and hence interesting targets for immunotherapy against cancer. DC-based immunotherapy can be conducted by either non-targeted immunization with tumor antigen (in the form of peptides or RNA) into the skin or by targeted immunization with antigen coupled to DC-specific antibodies. Additionally, *ex vivo* generated tumor antigen-loaded DC are administered to patients via injection into the skin (1,2). For all these approaches, it is essential to understand which DC subsets are best used or targeted to achieve optimal antitumor responses.

Vaccinations, including anticancer immunizations with DC, are commonly administered into (intradermally) or under (subcutaneously, intramuscularly) the skin. In case of immunization with free antigen (i.e. not bound to DC), DC of the skin are responsible for initiating the T cell responses both after intradermal and subcutaneous immunization. Human skin harbours three main DC subsets: CD1a^{high}CD207⁺ Langerhans cells (LC) residing in the epidermis, CD1a^{intermediate} dermal DC and CD14⁺ dermal DC (3–7). An additional subset expressing CD141 (mAb BDCA-3) was recently described as being specialized in cross-presentation and as a possible functional equivalent for Langerin⁺ dermal DC

in the mouse (8,9). When stringently defined as dermal DC expressing CD141 at high levels, CD14 is absent from these cells (9).

LC and dermal DC express different sets of C-type lectins which are preferentially used for antibody–antigen targeted immunotherapy. LC express Langerin/CD207 and DEC-205/CD205, albeit the latter receptor at substantial levels only upon activation. Dermal DC are positive for DEC-205, dectin-1 and many more (10–14). In addition, the various DC subsets in human skin appear to exert different functions. LC are excellent in cross-presenting exogenous antigen to CD8⁺ T cells whereas dermal DC present antigen to CD4⁺ T cells and stimulate humoral responses (15–19). Apart from their pronounced ability to cross-present, CD141⁺ dermal DC are incompletely characterized in this regard.

There is increasing interest in investigating the potential of targeting skin DC for immunotherapy of cancer (20). This can be achieved by addressing and directing antigens to lectin receptors on the surface of DC with the help of antibodies (13,21,22). In the mouse, the proof of principle has been achieved that skin DC can be targeted and immune responses massively boosted (in presence of adjuvant) or dampened (in absence of adjuvant) by anti-DEC-205- and anti-Langerin-antigen complexes (23–25).

However, so far not much is known about the relative targeting potential of DC subsets in the human skin. Thus, we investigated in detail the binding of targeting antibodies to skin DC *in situ* in human skin explants and the transport of antibodies by migratory skin DC.

Material and methods

Human skin samples and targeting antibodies

Clinically normal appearing skin was derived from plastic surgery for breast or abdominal skin reduction after written patient consent. Ethical approval was granted by the local ethical committee (AN3694 – 279/4.3). Skin samples were trimmed off subcutaneous fat with a scalpel, and 8 mm punch biopsies (Kai Europe, Solingen, Germany) were prepared. The following mAbs were used for targeting DC in human skin: anti-DEC-205/CD205 (five different batches of clone MG38, Serotec, Kidlington, UK; and clone 523203 from R&D Systems, Minneapolis, MN, USA), anti-Langerin/CD207 (clone DCGM4/122D5.03, Dendritics, Lyon, France). Appropriately matched isotype controls (mouse IgG2b and mouse IgG1, respectively, from BioLegend, San Diego, CA, USA) were used.

Culture medium and reagents

Complete medium was prepared by supplementing RPMI1640 (Lonza, Basel, Switzerland) with 10% heat-inactivated foetal calf serum (Lonza), glutamine (Lonza), gentamycin (Gibco-Invitrogen, Carlsbad, CA, USA), and β -mercaptoethanol (Sigma, St. Louis, MO, USA). Phosphate-buffered saline (PBS, Gibco) was used for intradermal injections of targeting mAb. The TLR-3 ligand poly I:C was purchased from Sigma and used for injection at a concentration of 25 μ g/injection.

Administration of targeting antibodies to human skin

An amount of 0.25 μ g of antibody was injected intradermally in a total volume of 50 μ l of PBS into 8 mm skin punch biopsies *ex vivo*, which were then cultured continuously on medium for 4 days to obtain migratory skin DC (26). Alternatively, we took a 'bath' approach where we incubated 8 mm human skin punch biopsies on complete medium containing 5 μ g/ml targeting mAb against Langerin or DEC-205 for 24 h to allow diffusion of mAb into the tissue. Thereafter, the skin biopsies were removed from medium containing targeting mAb, rinsed thoroughly, transferred to fresh medium without mAb and cultured for another 3 days to allow skin DC to emigrate from tissue (scheme of experimental approach depicted in Figure S1).

Immunofluorescence staining

Cryostat skin sections

Frozen skin sections were prepared from 24 h cultured skin biopsies from the 'bath' and injection approach and fixed in acetone (VWR, Darmstadt, Germany) for 10 min at room temperature. The targeting mAbs against Langerin and DEC-205 were visualized with anti-mouse-Ig-Alexa 594 (Invitrogen) followed by blocking residual free anti-mouse binding sites with 100 μ g/ml mouse gamma globulin (Jackson ImmunoResearch Laboratories, Pennsylvania, USA) for 15 min. Skin DC were identified with biotinylated anti-CD1a (clone HI149, BioLegend) and biotinylated anti-HLA-DR (clone L243, BioLegend), followed by Streptavidin-Alexa Fluor 488 (Invitrogen). Appropriate isotype controls were purchased from BioLegend. All staining steps were performed for 30 min at 37°C.

Epidermal sheets

The 24 h cultured skin biopsies from the 'bath' and injection approach were incubated dermal side down on 0.5 M ammonium-isothiocyanate (Merck, Darmstadt, Germany) for 45 min at 37°C. The epidermis was peeled off the dermis and cut into 5 × 5 mm pieces and fixed in acetone for 20 min at room temperature. Targeting mAb was visualized as described above and LC counterstained with biotinylated CD1a (clone HI149, BioLegend), followed by Streptavidin-Alexa-Fluor 488 (Invitrogen). Evaluation was performed on a conventional Olympus epifluorescence microscope.

Flow cytometry of migratory skin DC

Emigrated skin DC were collected 4 days after the start of skin explant cultures. Cells were permeabilized and stained with the BD-Fixation/Perm kit (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instruction. Targeting mAbs against Langerin and DEC-205 were detected with anti-mouse-Ig-APC (BioLegend), followed by blocking with 100 μ g/ml mouse gamma globulin for 5 min (Jackson ImmunoResearch Laboratories) and staining of DC with anti-CD1a-FITC (clone HI149, BD Biosciences), anti-CD163-PerCP-Cy5.5 (clone GHI/61, BioLegend), anti-CD14-PE (clone HCD14, BioLegend), anti-HLA-DR-PE-Cy7 (clone L243, BioLegend) for 10 min at 4°C. Appropriate isotype controls from BioLegend were used for FACS stainings to confirm specific staining. Analyses were performed on a FACS Calibur and Canto II instrument (BD Biosciences). The percentage of targeted skin DC was determined by pregating on viable HLA-DR⁺ cells, followed by gating for the various skin DC subsets with CD1a and CD14. In the three different skin DC subsets, cells positive for the targeting mAb were determined by setting the gate on DC from skin explants injected with isotype controls.

Statistical analysis

Statistical analyses were performed with GraphPad Software (La Jolla, CA, USA). In Fig. 3, we used ANOVA to compare all data sets and in Fig. 4 one-tailed *t*-test for comparing Langerin mAb with or without poly I:C or DEC-205 with or without poly I:C. If not explicitly indicated, differences were not statistically significant. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Results

Intradermally injected targeting mAb against Langerin binds to LC in epidermis

Immunization approaches with antibody-antigen conjugates would allow to target antigen to a specific subset of DC *in situ*. In this study, we were interested whether we can target skin DC *in situ* with mAb against the C-type lectin receptor Langerin/CD207. For this purpose, we prepared 8 mm punch biopsies of human skin and exposed them to the antibodies in two different ways (scheme of experimental approach depicted in Figure S1). The first approach is the relevant one for vaccination; here, we injected 0.25 μ g of targeting mAb in 50 μ l PBS intradermally into punch biopsies. This formed a blister that disappeared during the subsequent 24 h culture on medium. The other approach we called 'bath', because skin was floated on complete medium containing 5 μ g/ml targeting mAb for 24 h to allow the antibody to diffuse into the tissue. As control, we used an irrelevant antibody of the same isotype as anti-Langerin and applied it in the same way and at the same concentration. After the 24 h culture,

we prepared cryostat sections and visualized the targeting mAb with a secondary antibody against mouse immunoglobulin. Skin DC were identified by staining of the surface molecule CD1a, which stains both LC in the epidermis and a large proportion of the dermal DC (14). Moreover, we used an antibody against HLA-DR to jointly detect LC, dermal DC and macrophages. The targeting mAb against Langerin was found preferentially in LC in the epidermis *in situ* as determined by counterstaining with CD1a and HLA-DR (Fig. 1a). Efficiency of targeting was high as indicated by the fact that we detected Langerin antibody in almost all LC on the skin sections. Complete Langerin targeting of LC became particularly apparent in epidermal sheets that were

prepared at the end of the 24 h culture period (Fig. 1b). These results recapitulate our previous observations in mouse skin, where Langerin antibodies also readily diffuse from the site of injection in the dermis into the epidermis (24). The 'bath' approach was as effective as intradermal injection of the targeting mAb.

Intradermally injected targeting mAb against DEC-205 binds preferentially to dermal cells

When we performed the same experiments with the targeting mAb against DEC-205, the distribution of the antibody was different. HLA-DR⁺ CD1a⁺ LC in the epidermis were very weakly targeted by anti-DEC-205 (Fig. 2a) and this fits with our previous finding of low expression of DEC-205 by immature LC in the

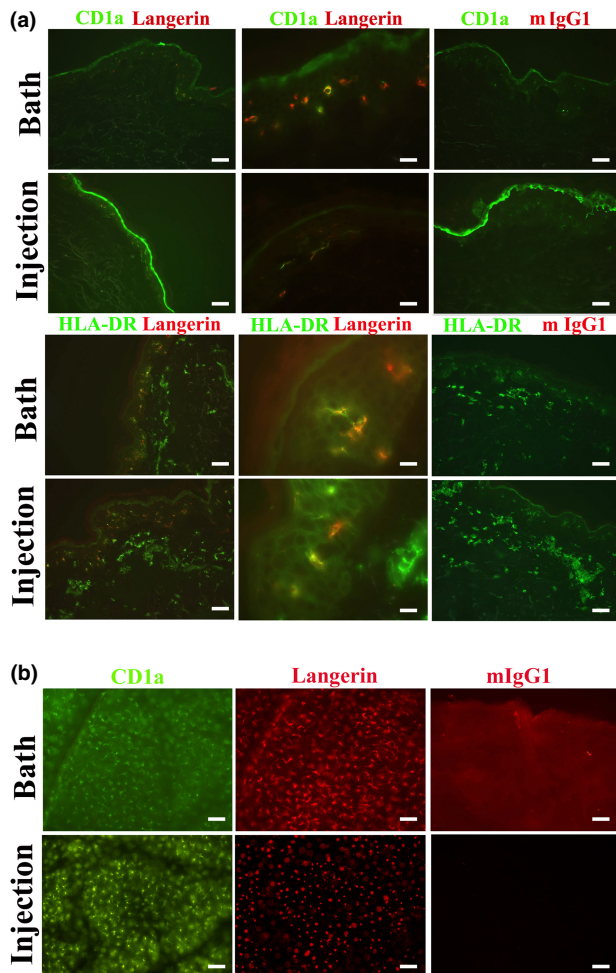


Figure 1. Antibody against Langerin targets LC in the epidermis of human skin. Human skin biopsies were incubated on medium containing 5 $\mu\text{g}/\text{ml}$ antibody against Langerin for 24 h to allow diffusion into the tissue (bath). Alternatively, 0.25 μg of antibody was injected intradermally into skin biopsies that were subsequently cultured for 24 h (injection). (a) Skin sections were analysed for localization of targeting mAb (red fluorescence) in CD1a⁺ LC and dermal DC (green fluorescence, upper panel) and in HLA-DR⁺ LC, DC and macrophages (green fluorescence, lower panel). Specificity of targeting was ascertained by control injection of an irrelevant mouse IgG1. Left and right columns are at lower magnifications (bar represents 20 μm); middle column is at a higher magnification (bar represents 60 μm). (b) Additionally, epidermal sheets were prepared and stained with secondary antibody to visualize the targeting mAb (middle). LC were counterstained with an antibody against CD1a (left). Appropriate isotype control for targeting mAb (mouse IgG1) was used (right), bar represents 60 μm .

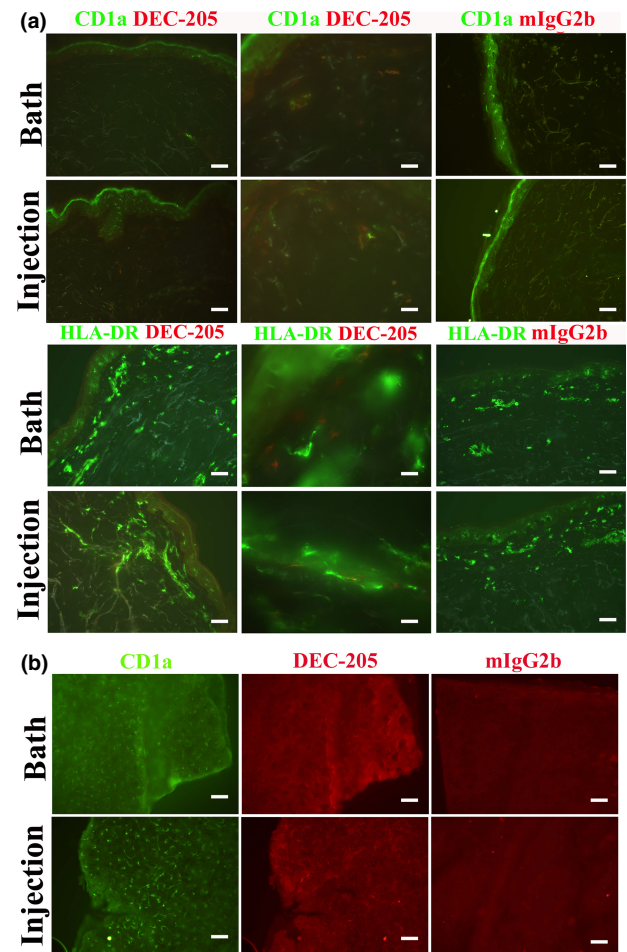


Figure 2. Antibody against DEC-205 targets mainly dermal DC in human skin. Human skin biopsies were incubated on medium containing 5 $\mu\text{g}/\text{ml}$ antibody against DEC-205 for 24 h to allow diffusion into the tissue (bath). Alternatively, 0.25 μg of antibody was injected intradermally into skin biopsies that were subsequently cultured for 24 h (injection). (a) Skin sections were analysed for localization of targeting mAb (red fluorescence) in CD1a⁺ LC and dermal DC (green fluorescence, upper panel) and in HLA-DR⁺ LC, DC and macrophages (green fluorescence, lower panel). Specificity of targeting was ascertained by control injection of an irrelevant mouse IgG2b. Left and right columns are at lower magnification (bar represents 60 μm); middle column is at a higher magnification (bar represents 20 μm). (b) Additionally, epidermal sheets were prepared and stained with secondary antibody to visualize the targeting mAb (middle). LC were counterstained with an antibody against CD1a (left). Appropriate isotype control for targeting mAb (mouse IgG2b) was used (right), bar represents 60 μm .

epidermis (11). This observation was confirmed in epidermal sheets where targeting with the mAb for DEC-205 was virtually undetectable (Fig. 2b). In the dermis, the antibody against DEC-205 was found in cells that were mostly, but not all, CD1a-positive. In contrast, most targeted cells expressed HLA-DR indicative that they were dermal DC or macrophages (Fig. 2a). Overall, the targeting efficiency *in situ* appeared to be lower with a mAb against DEC-205 than with the Langerin antibody. Again no difference was observed between application routes. The bath approach and intradermal injection gave similar results.

Migratory skin DC transport targeting mAb out of skin explants

For vaccination approaches, it is mandatory that skin DC incorporate the antibody-antigen complexes and process the antigen on their way to the skin-draining lymph nodes. Thus, in the next set of experiments, we examined the ability of skin DC to transport targeting mAb out of the skin tissue. For this purpose, we made use of the fact that DC emigrate spontaneously from skin explants without addition of cytokines or chemokines when cultured for several days (27,28). In the approach in which targeting mAb were injected into the skin biopsies, skin explants were continuously cultured for 4 days to allow binding of targeting mAb to DC and emigration of DC into the culture medium. In the bath approach, we cultured the skin for 24 h on the medium containing the targeting mAb, rinsed thoroughly, and then transferred the skin onto fresh medium without mAb (scheme of experimental approach depicted in Figure S1). Migratory skin DC should be able to bind antibodies, that have diffused into the skin, and then transport them out of the tissue during 3–4 days of skin explant culture, similar to our observations in the mouse (24). Migratory DC from human skin can be subdivided into three main HLA-DR⁺ subsets: CD14⁻ CD1a^{high} LC, CD14⁻ CD1a^{inter} dermal DC and CD14⁺ CD1a^{+/-} dermal DC (Fig. 3a, upper row). Interestingly, most of the CD14⁺ dermal DC co-expressed CD163, a macrophage marker (29), and also CD141/BDCA-3, that was recently described to identify a novel cross-presenting dermal DC subset (8,9) (Fig. 3a, middle row; Figure S2a). CD1a^{high} LC showed the highest levels of DEC-205 and Langerin surface expression, whereas CD1a^{inter} dermal DC displayed lower DEC-205 and virtually no Langerin staining. The CD14⁺ dermal DC were negative for both DEC-205 and Langerin (Fig. 3a, bottom row; Figure S2b). The efficacy of targeting with antibodies correlated with lectin receptor expression. The CD1a^{high} LC and CD1a^{inter} dermal DC transported targeting mAb out of the skin. Langerin mAb was found mainly in CD1a^{high} LC. In contrast, DEC-205 mAb targeted CD1a^{high} LC and CD1a^{inter} dermal DC. CD14⁺ dermal DC were not efficiently targeted by DEC-205 antibody and not at all by Langerin antibody (Fig. 3b and c). In this set of experiments, the intradermal injection showed slightly more targeted skin DC than the bath approach (Fig. 3d); however, differences were not statistically significant. The incorporation of the targeting mAbs was tested in a separate series of experiments in which we compared unfixed/non-permeabilized (reflecting only surface binding) and fixed/permeabilized (reflecting surface binding plus internalization) migratory skin DC side-by-side to determine how much of the targeting mAb was found inside the cells or was bound to the cell surface. Langerin mAb was predominantly incorporated into the emigrated LC as indicated by a large increase in mean fluorescence intensity upon

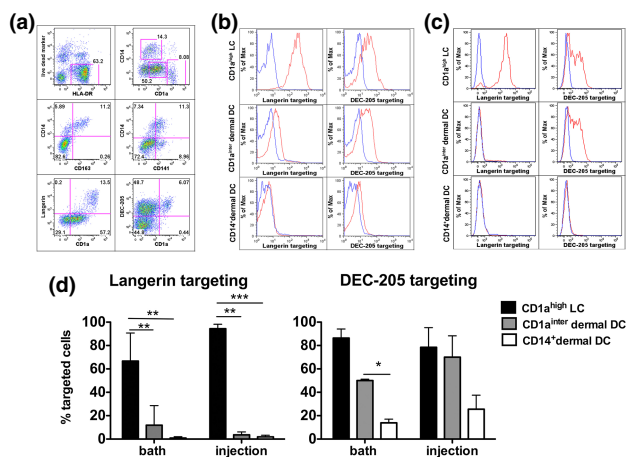


Figure 3. Targeting antibody against Langerin is transported by LC only and DEC-205 by dermal DC and LC. (a) Human skin explants without pretreatment were cultured for 4 days, and migratory DC were collected for flow cytometry. Viable HLA-DR⁺ cells were subdivided into CD1a^{high} LC, CD1a^{inter} dermal DC and CD14⁺ dermal DC. The expression of CD163, CD141, Langerin and DEC-205 was analysed on the various skin DC subsets. One representative experiment of 3 is shown. (b–d) Human skin biopsies were incubated on medium containing 5 μ g/ml antibody against Langerin or DEC-205 for 24 h to allow diffusion into the tissue. Skin was then transferred to fresh medium and cultured for another 3 days to allow emigration of skin DC (bath). Alternatively, 0.25 μ g of antibody was injected intradermally into skin biopsies that were then cultured continuously for 4 days (injection). After prepping on viable HLA-DR⁺ cells, the various migratory skin DC subsets as defined above were analysed by flow cytometry for transport of targeting mAbs. Representative FACS histograms for one bath experiment (b) and one injection approach (c) are shown. Isotype antibody, blue; targeting antibody, red. (d) Summary of three experiments for each condition is shown as the percentage of skin DC of each subset positive for targeting mAb, mean \pm SEM.

permeabilization (Figure S2c). Interestingly, with DEC-205 the comparison of fixed/permeabilized and unfixed/non-permeabilized cells revealed no differences in staining intensity. We ascertained this unexpected observation with two different anti-DEC-205 antibodies. This would suggest that little if no antibody is taken up into the cell or it has already been degraded within the 4 day culture (Figure S2c).

The TLR-3 ligand poly I:C increases targeting efficacy of DEC-205 mAb

The addition of adjuvant to vaccines is mandatory to augment the efficacy of immunization. We tested the TLR-3 ligand poly I:C for its ability to enhance targeting with mAbs against Langerin and DEC-205. For this purpose, we co-injected 25 μ g poly I:C together with the targeting mAb into skin biopsies and examined the emigrated skin DC after 4 days of explant culture with flow cytometry. The migration of DC was not significantly altered by the poly I:C injection (Figure S3a) as had been reported before (30). In line with this report, we also observed a shift in skin DC subsets in that slightly more CD1a^{high} LC and CD1a^{inter} dermal DC emigrated after poly I:C injection (Figure S3b). Most importantly, the addition of poly I:C to the injection of targeting mAb significantly increased the binding of the DEC-205 mAb to CD1a^{high} LC and CD1a^{inter} dermal DC as we detected a larger percentage of targeted cells (Fig. 4a and b). Moreover, the mean fluorescence intensity of the targeting anti-DEC-205 mAb was also higher, albeit not significantly increased, indicating that slightly more mAb was captured by skin DC (Fig. 4a and c). In the next set of experiments, we co-injected poly I:C with anti-Langerin mAb to examine, if we

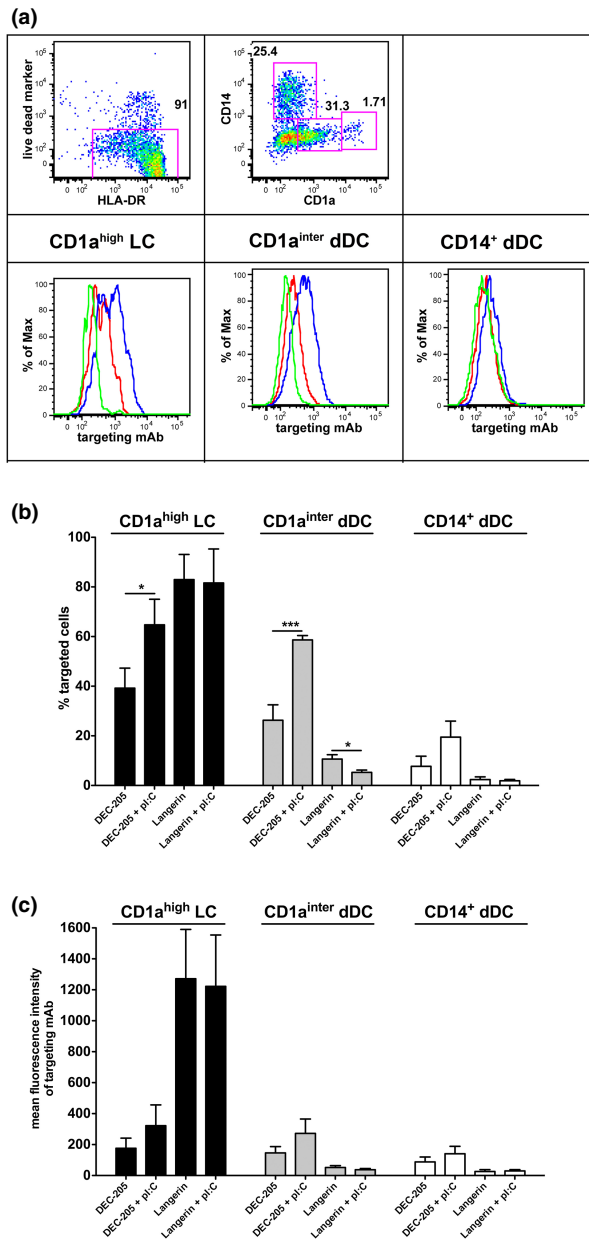


Figure 4. Addition of poly I:C improves targeting efficiency for DEC-205 on LC and dermal DC. Human skin biopsies were injected with 0.25 μ g DEC-205 or Langerin targeting mAb plus/minus 25 μ g poly I:C and cultured for 4 days. Migratory skin DC were analysed by flow cytometry for binding of targeting mAb in the absence and presence of poly I:C. (a) One representative experiment of 5 is shown. Viable HLA-DR⁺ cells were subdivided into the various skin DC subsets. Lower row depicts the binding of anti-DEC-205 targeting antibody to the indicated skin DC subsets; irrelevant control antibody (green histogram), anti-DEC-205 (red histogram), anti-DEC-205 + poly I:C (blue histogram). (b) The targeting efficacy was determined by the percentages of targeted LC and dermal DC. (c) The geometric mean for the mean fluorescence intensity indicates the amount of bound targeting mAb. A summary of five separate experiments (i.e. with skin from different donors) for each targeting mAb (DEC-205 or Langerin) in the various skin DC subsets is shown in b and c, mean \pm SEM.

can also boost the targeting efficacy of the Langerin mAb. In contrast to DEC-205, the TLR-3 ligand had no effect on Langerin targeting, neither on percentages of targeted LC nor on fluorescence intensity (Fig. 4b and c).

Discussion

The first indication that antigen can be more efficiently delivered into DC when coupled to a DC-specific mAb was published over 10 years ago. In that study, the T cell responses could be augmented 100-fold when the antigen was targeted to the DEC-205 cell surface C-type lectin receptor (31). The proof of principle that this concept can be used to induce either immunity or tolerance depending on the co-administration of adjuvant came out of Ralph Steinman's lab and opened the way for the development of novel therapeutic strategies by *in situ* targeting of patient's DC (32). Whereas much work has been carried out in mouse models, little is known about targeting DC in human skin. Thus, we used an *ex vivo* approach with human skin explants to examine targeting of two important C-type lectin receptors, Langerin and DEC-205. We observed that intradermal injection of a mAb against Langerin allows efficient targeting of LC, whereas DEC-205 targets LC plus dermal DC. The co-injection of the TLR-3 ligand poly I:C improved targeting efficacy for DEC-205, holding promise for using this TLR-3 ligand as an adjuvant (33,34).

For the successful establishment of *in situ* targeting of skin DC, it is necessary to investigate in detail which DC subset is optimal as a target. Several studies demonstrated that human LC are potent inducers of cytotoxic T cell responses, and CD14⁺ dermal DC are more suited to stimulate humoral responses (15–17,35). Thus, dependent on the disease to be treated, different DC subsets of the skin may need to be targeted. In the case of cancer immunotherapy, the induction of cytotoxicity is essential; hence, efficient targeting of LC with Langerin might be the appropriate approach. Interestingly, the functional properties of human and murine LC might differ in that human LC induce cytotoxic T cell responses, whereas in the mouse LC appear to be more involved in regulatory immune responses (4,36). Murine studies on DEC-205-mediated delivery of protein antigen showed that many different DC subsets can be targeted including LC, dermal DC and the lymph node resident CD8⁺ DC, however, just the latter two subsets are essential to induce cytotoxicity in CD8⁺T cells (25,37). Regarding Langerin-mediated delivery of antigen, experiments in the murine system showed targeting of LC and Langerin⁺ dermal DC (24,38), albeit their T cell stimulatory capacity turned out to be different (37). Nevertheless, the various C-type lectin receptors can be harnessed for immunotherapeutic interventions (38,39). In the human system, efficient targeting of monocyte-derived DC could be achieved by DEC-205 mAb conjugated to the EBNA-1 antigen and induced protective CD4⁺ and CD8⁺T cell responses (40). Human monocyte-derived DC can also be efficiently targeted by anti-DEC-205 single chain fragments, and the presented epitopes are subsequently recognized by MAGE-3-specific CD4⁺ T cells (41).

Due to the fact that little is known about targeting of human skin DC, we studied this issue by injecting human skin explants with mAbs against Langerin and DEC-205. We observed that Langerin specifically targets LC, which is in contrast to the murine system where also a dermal DC subset is targeted (25). Thus, Langerin would allow to narrow the targeting approach to one specific DC subset in human skin. In contrast, DEC-205 is expressed by LC and dermal DC which allows to target overall the CD1a⁺ LC and a large fraction of the dermal DC. The functional

outcome of this broader DC spectrum needs to be examined to make sure that an optimally suited T cell response is induced.

The small population of CD14⁺ dermal DC we observed in the migratory DC was inefficiently targeted with mAb against DEC-205. This is most likely due to the fact that these DC, which in part co-expressed the macrophage marker CD163, do not express DEC-205 (11). Dermal macrophages are numerous in human skin. However, they are sessile cells, firmly attached to the connective tissue. They can be isolated only by a harsh additional collagenase treatment of the dermis (29). Moreover, they are situated in a dermal layer beneath the majority of dermal DC, and they do not migrate out of the skin (42). In the standard skin explant culture method, that we used here, the majority of dermal macrophages was therefore not included.

Potent antigen cross-presenting DC were recently discovered in the human blood, their defining marker being CD141 (as recognized by the BDCA-3 antibody) (43–46). Their comparably high expression of DEC-205 was shown at the levels of mRNA (46) and protein (47). The latter was also ascertained by flow cytometry in a humanized mouse model: human CD141-expressing cells in the mouse spleen were distinctly DEC-205-positive on their cell surfaces. When immunizing these mice with an antigen coupled to anti-human DEC-205 antibodies, potent clonal T cell responses ensued (48). A likely counterpart of CD141⁺ blood DC, that was also able to cross-present, was recently described in human dermis (8). However, no Langerin nor DEC-205 expression was found on CD141⁺ dermal DC. In another report, in which this DC subset was defined as CD141^{high} and CD11c^{low-inter}, DEC-205 expression was not studied and the cells were negative for Langerin (9). Even if this subset would express low levels of DEC-205 (or even Langerin), perhaps only after stimulation with a TLR ligand such as poly I:C, its contribution to an immune response would presumably be minor simply due to the fact that they are so rare: only about 1% of all dermal mononuclear migratory CD45⁺ cells are CD141-high (9). For comparison, consistently more than 50% are CD1a⁺ and CD14⁺ DC. A word of caution may be raised regarding this minute subset of skin DCs and its importance for vaccination. In the mouse, the functionally equivalent population of Langerin⁺ dermal DCs is missing in certain locations of the body (footpad and tail skin), as recently described (49). Similar considerations are therefore warranted for human skin as well.

Induction of immunity is achieved when a maturation signal is provided as has been demonstrated in the murine system. Targeting DC with DEC-205-antigen complexes led to immunity upon administration of adjuvant, for example CD40 agonistic mAb,

whereas tolerance occurred without stimulation (50,51). Protective immunity can be achieved against infection and cancer (32). We chose the TLR-3 ligand poly I:C due to the fact that it is clinically approved (Hiltonol[®], Oncovir Inc.) and has been described to be a potent adjuvant for induction of Th1 responses (52–54). In this study, here, we show that poly I:C boosts targeting efficiency of DEC-205 but not Langerin which most probably is caused by upregulation of DEC-205 on the surface of LC and dermal DC. This increase in DEC-205 expression upon activation of skin DC has previously been demonstrated in skin explant culture (11).

Our results have implications for vaccinations against skin cancer that are currently being explored. Targeting antigens via DEC-205 into DC showed promising results in various mouse melanoma models in that melanoma-associated antigens conjugated to anti-DEC-205 slowed down tumor growth (55,56). In the case of tumor immunity, the targeting of human DC with conjugates of DEC-205 and MAGE-3 melanoma antigen allowed to stimulate CD4⁺ T cell responses (40). Finally, a recent report describes the first step into the clinics: Melanoma patients vaccinated with anti-DEC-205-conjugated melanoma antigen NY-ESO1 mounted cellular and humoral responses and, remarkably, did better when subsequently treated with checkpoint inhibitor drugs (34). This first-in-human study (i) emphasizes the potential to combine immunization strategies with checkpoint control (57,58) and (ii) it warrants future studies where we will examine the functional consequences of targeting LC by Langerin-antigen conjugates alone or together with dermal DC by DEC-205-antigen complexes.

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Author contribution

P.S. and N.R. designed the research study and wrote the paper. S.S., C.H.T., D.R., K.K. and S.E. performed the research. B.D.F. and G.D. contributed materials and expertise.

Conflict of interest

The authors have declared no conflicting interests.

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Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Figure S1. Figure depicting schematically the bath and injection treatment of skin biopsies and the subsequent analysis.

Figure S2. Surface expression of CD141, DEC-205 and internalization efficacy of targeting mAbs in skin DC subsets.

Figure S3. Effect of poly I:C on migratory behaviour of skin DC.