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CUTTING EDGE

Cutting Edge: Langerin/CD207 Receptor on Dendritic Cells Mediates Efficient Antigen Presentation on MHC I and II Products In Vivo[1]

Juliana Idoyaga,[2] Cheolho Cheong,[2] Koji Suda, Nao Suda, Jae Y. Kim, Haekyung Lee, Chae Gyu Park,[3,4] and Ralph M. Steinman[3,4]

The targeted delivery of Ags to dendritic cell (DCs) in vivo greatly improves the efficiency of Ag presentation to T cells and allows an analysis of receptor function. To evaluate the function of Langerin/CD207, a receptor expressed by subsets of DCs that frequently coexpress the DEC205/CD205 receptor, we genetically introduced OVA into the C terminus of anti-receptor Ab H chains. Taking advantage of the new L31 mAb to the extracellular domain of mouse Langerin, we find that the hybrid Ab targets appropriate DC subsets in draining lymph nodes and spleen. OVA is then presented efficiently to $CD8^+$ and $CD4^+$ T cells in vivo, which undergo 4–8 cycles of division in 3 days. Peptide MHC I and II complexes persist for days. Dose response studies indicate only modest differences between Langerin and DEC receptors in these functions. Thus, Langerin effectively mediates Ag presentation. The Journal of Immunology,* 2008, 180: 3647–3650.

Dendritic cells (DCs)[5] are specialized APCs able to control T cell immunity in vivo (1). Several DC subpopulations are known (2). These typically express distinct receptors for Ag uptake and capacities for Ag processing (3). Many differentially expressed receptors include molecules with C-type lectin domains (4) such as DEC205/CD205 on $CD8^+$ DCs, dermal DCs, and Langerhans cells (LCs) and 33D1/DCIR2 on $CD8^-$ cells (2, 3, 5, 6).

Langerin/CD207 is a type II transmembrane protein (5). Originally, Langerin was thought to be LC-specific, but it is expressed on other DCs, especially $CD8^+$ DEC^+ DCs in spleen (7) and skin draining lymph nodes (pLN), although at lower levels (6, 8, 9). The expression of Langerin by $CD8^+DEC^+$ DCs is primarily seen in BALB/c mice but is much reduced in C57BL/6 mice (8). Langerin recognizes mannose, *n*-acetylglucosamine, fucose, and sulfated sugars (7, 10, 11). Langerin also binds pathogens such as HIV (12) and *Candida albicans* (13). Langerin is responsible for Birbeck granule formation within LCs (5). Both Birbeck granules and Langerin are involved in endocytosis (5, 14). In human epidermal LCs, Langerin is predominantly found in an endosomal recycling compartment (14). In murine epidermis, Langerin was found to colocalize with MHC II in situ (15). Abs to Langerin can be internalized (5), but it is not known whether this mediates Ag presentation to T cells.

A new way to assess receptor function, including in vivo, is to deliver Ags within anti-receptor mAbs, which selectively bind to the receptor and initiate Ag uptake, processing, and presentation on MHC products. Depending on the maturation state of the targeted DCs, receptor-mediated targeting enhances or inhibits T cell responses (3, 16–18). The role of DC subsets expressing select receptors, as well as the role of the receptors themselves in Ag presentation, might therefore be determined by using specific mAbs as Ag carriers.

In this study, we use a newly developed mAb to the extracellular domain of mouse Langerin to begin to study the consequences of Ag uptake via Langerin in vivo (8). We have cloned the H and L chains of this mAb and engineered the H chain to encode OVA. We will show that OVA targeting to Langerin results in efficient presentation to OVA-specific $CD4^+$ and $CD8^+$ transgenic T cells.

Materials and Methods

Mice

C57BL/6J and BALB/cJ mice were purchased from Charles River or Jackson Laboratories and used at 6–8 wk of age. OVA-specific, TCR transgenic, OT-I (C57BL/6-Tg(TcraTcrb)1100Mjb/J) and OT-II (C57BL/6-Tg(TcraTcrb)425Cbn/J) mice were used as described (17). All animals were maintained under specific pathogen-free conditions and used under institutional guidelines.

Reagents

Anti-CD16/32, FITC-anti-CD11c, allophycocyanin-anti-CD8, anti-CD4, and PE-Vα2 were from BD Biosciences or eBioscience. Other reagents were

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[5] Abbreviations used in this paper: DC, dendritic cell; CHO, Chinese hamster ovary; LC, Langerhans cell; pLN, skin draining lymph node.

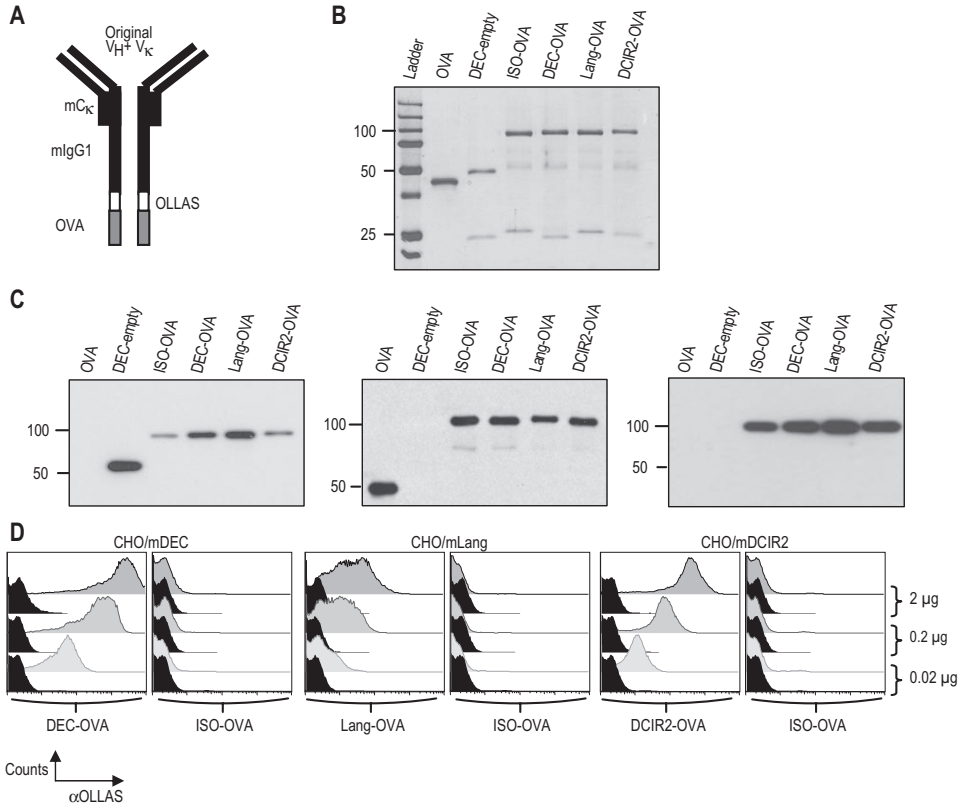

FIGURE 1. Genetic engineering of OVA into the anti-Langerin mAb H chain. *A*, Diagram of engineered mAbs fused to OVA, with OLLAS tag between H chain and OVA. mC_κ, mouse C_κ; mIgG1, mouse IgG1. *B*, Coomassie-stained 10% SDS-PAGE reducing gel comparing chimeric anti-Langerin (Lang), anti-DEC, anti-DCIR2, and control Ig -OLLAS-OVA, with molecular mass in kDa. *C*, Western blots of hybrid mAbs using HRP-conjugated anti-mouse IgG1 (*left*), anti-OVA (*middle*), or anti-OLLAS biotin followed by HRP-conjugated streptavidin (*right*). *D*, Binding of mAbs to CHO/mouse Langerin (mLang), CHO/mouse DEC (mDEC), CHO/mouse DCIR2 (mDCIR2) (gray), and control CHO/Neo cells (black), using graded doses (0.02–2 μg) of recombinant mAbs and anti-OLLAS-Alexa Fluor 647 (αOLLAS). ISO stands for isotype control.

RPMI 1640 and DMEM (Invitrogen Life Technologies/GIBCO), ACK (ammonium chloride potassium) lysing buffer (BioSource International), 30% BSA Solution (Sigma-Aldrich), Alexa Fluor 647 labeling kit (Invitrogen Life Technologies/Molecular Probes), and EZ-Link Biotin (Pierce).

Cloning, engineering and production of anti-Langerin Ab

Cloning of V regions from rat mAbs and engineering of hybrid mAbs with mouse IgG1 H and κ light constant regions was described for mAbs III/10 (control Ig), NLDC145 (anti-DEC205) (16), and 33D1 (anti-DCIR2) (3). Similarly, the V regions of anti-mouse Langerin mAb (8) were cloned from RNAs of the L31 hybridoma with the 5′ RACE system (Invitrogen Life Technologies) using rat IgG2a-specific oligonucleotides (5′-CATCCCAGGGTCA CCATGGA-3′ and 5′-CTGTTGTTTCAGCTGAGGAGACTG-3′) and rat κ-specific oligonucleotides (5′-CAACCTCACAGGTATAGAG-3′ and 5′-G AAGATGGATACAGTTGGTGCAGCATC-3′) following the manufacturer's instructions. An OLLAS epitope sequence was inserted as a linker between the H chain and OVA for the detection of hybrid mAbs by an anti-OLLAS tag mAb (19). Hybrid mAbs were produced (3) and receptor binding was verified using Chinese hamster ovary (CHO) transfected cells by FACS.

Adoptive transfer and T cell proliferation responses

OVA-specific transgenic $CD8^+$ or $CD4^+$ T cells were prepared from lymph node and spleen cell suspensions of OT-I or OT-II mice (17). T cell proliferation was evaluated by injecting individual mice with either 2–3 $\times$ 10^6 OT-I or 3–5 $\times$ 10^6 OT-II cells, labeled at 10^7 cells/ml with 5 μM CFSE (Invitrogen/Molecular Probes) for 10 min at 37°C. Targeting mAbs were injected s.c. 24 h later. CFSE dilution was evaluated 3 days later by FACS after surface staining for CD4 (OT-II) or CD8 (OT-I) and Vα2 (OVA-specific TCR). To address Ag persistence, mAbs were injected 14, 3, or 1 day before T cell transfer.

Evaluation of Ag targeting in vivo

To evaluate mAb targeting to DCs by FACS, animals were injected s.c. with 10 μg of L31-OLLAS-OVA. Spleen and pLN (popliteal, cervical, inguinal, axillary, and brachial) were harvested 24 h later. DCs were enriched by low density on dense BSA (8), and the cells were stained with anti-CD11c-FITC and anti-OLLAS-Alexa Fluor 647. For immunofluorescence, mice were injected s.c. with 25 μg of L31-OLLAS-OVA or control Ig. Twelve hours later, tissue sections were analyzed with a Zeiss LSM 510 system (8).

Statistical analysis

The Student *t* test was applied to reveal significant differences in fold clonal expansion of transgenic T cells, defined as the ratio of the number of cells with more than one division to the number of transgenic T cells in control mice that had not received Ag. Our results are expressed as the mean $\pm$ SE. In the figures, *p* values of $\leq$0.05 are labeled with a single asterisk (*) in contrast to *p* values of $\leq$0.01 (**) or $\leq$0.001 (***).

Results and Discussion

Preparation of anti-Langerin-OLLAS-OVA hybrid mAb

To examine the capacity of the Langerin receptor to process and present proteins through the MHC I and II pathways, we genetically engineered mAbs against Langerin (8), DEC (16), DCIR2 (3), and a nonreactive control Ig (ISO) to express OVA. To track Ab expression and binding, we added a linker tag sequence between the C terminus of the Ab H chain and OVA named OLLAS, recognized by an anti-OLLAS mAb (19) (Fig. 1*A*). In each case, the fusion H chain had a molecular mass of ~110 kDa (Fig. 1*B*) and reacted with anti-mouse Ig, anti-OVA, and anti-OLLAS mAbs by Western blotting (Fig. 1*C*). By FACS, each fusion mAb bound appropriately to CHO transfectants expressing receptor (Fig. 1*D*, gray histograms), but not to untransfected CHO (CHO/Neo; Fig. 1*D*, black histograms), while the control Ig-OVA did not bind to Langerin, DEC, or DCIR2 (Fig. 1*D*). Thus, each mAb to three endocytic receptors can be engineered to express both OVA as an Ag and OLLAS as a sensitive tag.

Anti-Langerin targets selectively and systemically to DCs in vivo

To assess targeting of anti-Langerin to DCs in vivo, we injected BALB/c mice s.c. in the footpads with anti-Langerin-OLLAS-OVA or control mAb and detected OLLAS by FACS analysis

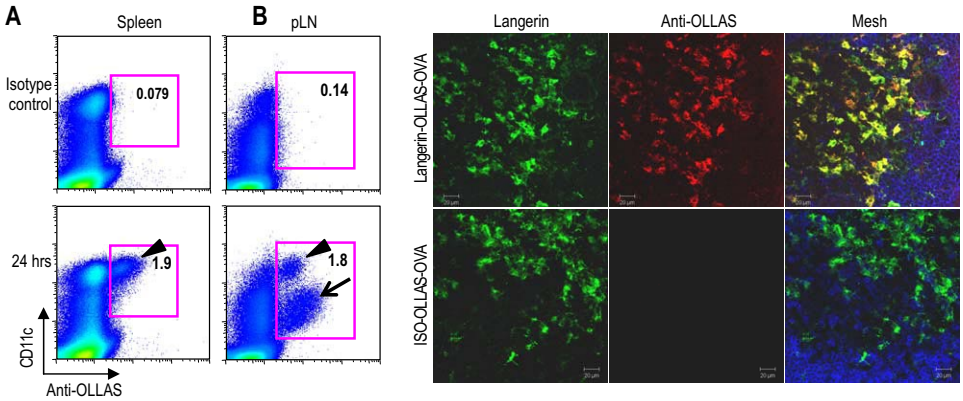

FIGURE 2. Detection of recombinant mAbs after targeting Langerin in vivo. *A*, BALB/c mice were injected s.c. with 10 μg of anti-Langerin or control Ig -OLLAS-OVA. Twenty-four hours later, DCs were enriched and analyzed by FACS using anti-OLLAS-Alexa Fluor 647 mAb. The selectively targeted CD11c^{high} and CD11c^{low} DCs are indicated by an arrowhead and arrow, respectively, where the latter are the tissue-derived Langerin$^+$ cells. *B*, Twelve hours postinoculation with 25 μg of anti-Langerin-OLLAS-OVA to BALB/c mice, lymph node sections were stained with anti-OLLAS biotin and streptavidin-Alexa Fluor 555 (red), anti-Langerin Alexa Fluor 647 (green), and anti-B220-FITC followed by anti-FITC Alexa Fluor 488 (blue). ISO stands for isotype control.

and immunofluorescence. The isotype control mAb (ISO-OLLAS-OVA, where ISO is isotype control) did not bind to DCs (Fig. 2*A*, *top*), whereas anti-Langerin-OLLAS-OVA targeted all subsets of Langerin$^+$ cells, e.g., both CD11c^{high} (~25 ± 5% of Langerin$^+$ cells) and CD11c^{low} (~70 ± 5% of Langerin$^+$ cells) DCs in spleen and in pLN (Fig. 2*A*). By FACS, the DC labeling by anti-OLLAS tag mAb was selective for those CD11c$^+$ cells that also were labeled with anti-Langerin (data not shown). By immunofluorescence of tissue sections, anti-OLLAS also selectively stained cells reactive with anti-Langerin in the T cell area of pLN (Fig. 2*B*). These results indicate that s.c. injection of hybrid anti-Langerin Abs targets Langerin$^+$ DCs selectively and systemically (spleen) in vivo.

Targeting proteins to DCs and LCs in vivo via anti-Langerin

C57BL/6 mice were injected with graded doses of each OLLAS-OVA fusion mAb 1 day after inoculating CFSE-labeled OT-I or OT-II T cells, the latter to monitor Ag presentation in vivo. After 3 days, pLN and spleen were evaluated for T cell proliferation by CFSE dilution and the total number of expanded CFSE-low cells. Anti-DEC, anti-Langerin, and anti-DCIR2- OLLAS-OVA induced responses by MHC I-restricted CD8$^+$ OT-I cells, as well as MHC II-restricted OT-II cells, in both spleen and pLN. Virtually all T cells entered cell cycle and underwent 4–8 cell divisions after a single dose of 3 μg of anti-Langerin-OLLAS-OVA (Fig. 3*A*).

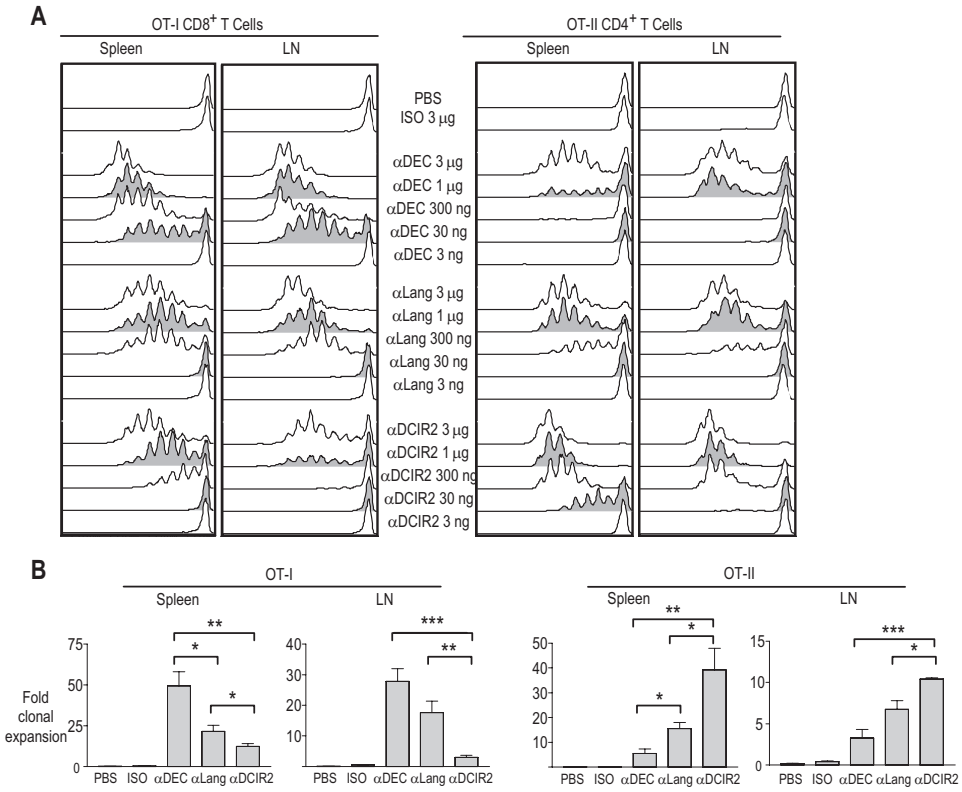

FIGURE 3. Anti-Langerin-OVA induces strong in vivo proliferation of CD8$^+$ and CD4$^+$ transgenic T cells. *A*, C57BL/6 mice were injected i.v. with 2–5 × 10^6 CFSE-labeled OT-I or OT-II T cells and, 24 h later, with graded doses of the indicated OLLAS-OVA fusion mAb s.c. Three days after the hybrid mAb injection, pLN and spleen were harvested and expansion of CD8$^+$ or CD4$^+$Vα2 cells was evaluated by FACS for CFSE dilution. Panels are typical of 2–4 experiments. *B*, Bar graphs to quantify T cells undergoing more than one division (fold clonal expansion; see *Materials and Methods*) on day 3 after the inoculation of 3 μg of anti-Langerin, anti-DEC, or anti-DCIR2 -OLLAS-OVA. Means and SE values are shown for six experiments. The Greek letter alpha (α) stands for "anti," and ISO stands for isotype control.

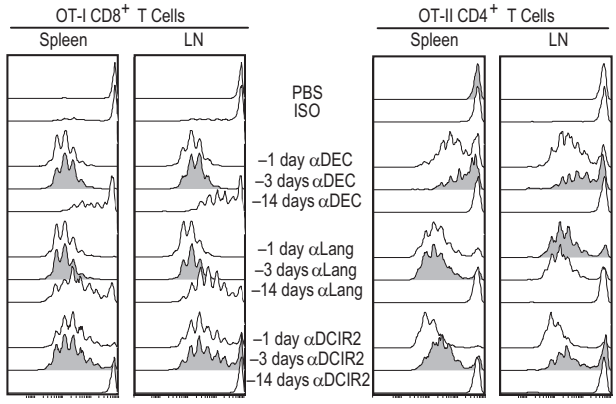

FIGURE 4. Prolonged MHC I and II presentation after anti-Langerin targeting. C57BL/6 mice were inoculated with the indicated OLLAS-OVA fusion mAb. One, 3, or 14 days later 2–5 × 10^6 CFSE-labeled OT-I or OT-II cells were transferred. Proliferation in pLN and spleen was followed by FACS 3 days later. One of two similar experiments is shown. The Greek letter alpha (α) stands for "anti," and ISO stands for isotype control.

Similar results were obtained when we inoculated fusion mAbs lacking the OLLAS tag, indicating that the OLLAS sequence did not affect Ag presentation in vivo (data not shown). We also reproduced prior work (3) showing that 33D1 anti-DCIR2, which selectively targets $CD8^-$ DCs, was more efficient in inducing $CD4^+$ T cell expansion while anti-DEC was more efficient for $CD8^+$ T cells (Fig. 3*B*). Anti-Langerin-OVA was intermediate in its behavior, slightly less effective than anti-DEC for triggering OT-I expansion but more effective for OT-II T cells (Fig. 3*B*). The observations with OT-II cells in C57BL/6 mice were confirmed on DO11.10 T cells in BALB/c mice (data not shown). Taken together, Langerin delivers OVA Ag for efficient presentation on MHC I and II products.

Long-lasting presentation of OVA delivered via anti-Langerin

To evaluate the persistence of presented Ag, we injected the different OVA fusion mAbs and 1, 3, or 14 days later we injected CFSE-labeled, OVA-specific OT-I or OT-II cells. Presentation to $CD8^+$ T cells after one dose of anti-Langerin-OLLAS-OVA was long-lasting (Fig. 4), being vigorous in pLN and spleen 14 days after mAb injection. In contrast, as shown previously (3) presentation by $CD8^-$ DCs via anti-DCIR2 persisted for 3 days (Fig. 4). Functional peptide MHC complexes persisted to a slightly greater extent at day 14 when OVA was targeted via Langerin rather than DEC (Fig. 4).

Prior observations had shown that Langerin acts as an endocytic receptor in vitro with cultured human LCs. In this study we show that Langerin mediates Ag presentation in vivo by targeting the receptor selectively within the new L31 mAb to the Langerin extracellular domain (8). Langerin delivers Ag to both $CD4^+$ and $CD8^+$ T cells, and the presentation is prolonged for several days. When anti-Langerin-OVA is compared with anti-DEC-OVA where DEC and Langerin expression is to a large extent confined to the same subsets of DCs, e.g., $CD8^+$ DCs and LCs, anti-Langerin is slightly less efficient than anti-DEC for presentation on MHC I and more efficient for MHC II, and Langerin-based presentation also seems to persist slightly longer. In contrast, when anti-Langerin-OVA is compared with anti-DCIR2-OVA, presentation of anti-Langerin is less efficient for MHC II but more efficient to MHC I. Although the OT-I and OT-II transgenic T cells have been valuable in this study for carrying out quantitative comparisons of the efficacy of different endocytic receptors, the functional consequences of Ag presentation are best conducted with other more clinically relevant Ags and the infrequent T cells in the polyclonal repertoire.

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Disclosures

The authors have no financial conflict of interest.

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