Polyisialylation controls dendritic cell trafficking by regulating chemokine recognition


The addition of polyisialic acid to N- and/or O-linked glycans, referred to as polyisialylation, is a rare posttranslational modification that is mainly known to control the developmental plasticity of the nervous system. Here we show that CCR7, the central chemokine receptor controlling immune cell trafficking to secondary lymphatic organs, carries polyisialic acid. This modification is essential for the recognition of the CCR7 ligand CCL21. As a consequence, dendritic cell trafficking is aborted in polyisialyltransferase-deficient mice, manifesting as disturbed lymph node homeostasis and unresponsiveness to inflammatory stimuli. Structure-function analysis of chemokine-receptor interactions reveals that CCL21 adopts an autoinhibited conformation, which is released upon interaction with polyisialic acid. Thus, we describe a glycosylation-mediated immune cell trafficking disorder and its mechanistic basis.

Polyisialylation is a rare posttranslational modification executed by the two enzymes ST8Sia II and ST8Sia IV (7). These polyisialyltransferases generate long α2,8-linked linear homopolymers of sialic acid, which are attached to N- and/or O-linked glycans (2). Polyisialic acid (polysia) is mainly known to control the developmental plasticity of the vertebrate nervous system by modulating cell-cell and cell-matrix adhesions (3). Polyisialic acid (polysia) further promotes cancer growth and metastasis through largely unknown mechanisms (4, 5) and, as such, is pursued as a therapeutic target (6). Recent evidence also suggests various functional implications during immune responses (7–10). We immunologically characterized mice lacking ST8Sia IV (7), the polyisialyltransferase expressed in hematopoietic cells. Under steady-state conditions, mutant animals showed severely reduced cellularity of peripheral lymph node lymphocytes (LNs) (Fig. 1A) and frequently lacked small popliteal LNs (10 LNs missing out of 16 expected). Infiltration of inflammatory stimuli in mutant and control mice failed to trigger LN swelling in the former (Fig. 1B). In contrast,ularity of the spleen did not differ significantly between control and mutant mice (Fig. 1A), which might indicate specific defects in lymphocyte homing to LNs. However, we could not detect polySia on the surface of T and B cells, and we did not observe any cell-autonomous trafficking defects in the lymphocyte compartment (Fig. SL A and B). In contrast, polySia was readily detectable on the surface of dendritic cells (DCs) during steady-state conditions (Fig. 1C, upper panel, and Fig. SIC), and it was additionally elevated upon inflammatory stimulation (Fig. 1C, lower panel). LNs of ST8Sia-deficient mice contained reduced amounts of DC subsets known to migrate from peripheral tissues into the LNs (Fig. 1D). Although

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DCs constitute only ~1% of cells in the LNs, they control LN size by instructing stromal cells to recruit lymphocytes and maintain their homeostasis (12, 13). Hence, a reduced size of the DC compartment might provide a potential explanation for reduced overall LN size. To test whether defective migration from the periphery was responsible for reduced DC numbers in LNs, we performed skin-painting experiments, in which endogenous DCs of the skin are mobilized and subsequently migrate, via the afferent lymphatic vessels, into the draining LN (14). In St8sia4−/− deficient mice, migration of DCs into draining LNs was almost completely abrogated (Fig. 1E).

We next used an in vitro reconstituted system to measure the migratory potential of polySia-deficient DCs. To this end, we generated DCs in vitro from bone marrow precursors. We confirmed that control cells up-regulate polySia upon inflammatory stimulation, whereas St8sia4-deficient cells differentiated normally but completely lacked polySia (fig. S1D). When we co-injected control and St8sia4-deficient DCs into the footpads of wild-type recipient mice, polySia-deficient DCs were completely unable to enter the LNs (Fig. 2A), formally showing that polySia dependency is cell-autonomous. We next incorporated the in vitro-generated DCs into three-dimensional (3D) collagen gels and exposed them to gradients of the chemokines CCL19 and CCL21 (Fig. 2B). By binding to CCR7, these chemokines guide DCs into the draining LN (15). Whereas the migratory response toward gradients of CCL19 was equally efficient for control and knockout cells, polySia-deficient DCs were completely refractory to CCL21 (Fig. 2B). Similarly, signaling, as measured by Akt and extracellular signal-regulated kinase (ERK) phosphorylation, was largely abolished in response to CCL21, whereas the CCL19-triggered signal was comparable to that in control cells (fig. S2). Hence, polySia-deficient DCs were capable of differentiating and migrating regularly but were selectively unresponsive toward CCL21.

En route from the periphery into the LN, CCL21 mediates two key steps: (i) directed interstitial migration toward the dermal lymphatic vessel and (ii) migration from the LN’s subcapsular sinus into the deep T cell area. To exclude any confounding effects of CCL19, we probed DC migration in CCl19-deficient hosts. To bypass the skin and directly measure migration within the LN, we injected DCs into the afferent lymphatic vessel (fig. S3A) (16). Within the LN,
polySia-deficient DCs behaved like control cells and entered the deep T cell parenchyma, whereas Ccr7-deficient DCs were unable to leave the subcapsular sinus, as previously shown (Fig. 2C) (16). Next, we selectively probed migration within the skin and co-incubated skin explants with polySia-deficient and control DCs. We found that only control cells entered the dermal lymphatic vessels, whereas polySia-deficient DCs did not even infiltrate the dermal interstitium (Fig. 2D), therefore precisely phenocopying Ccr7-deficient DCs (17). Similarly, in cultured ear explants of St8sia4-deficient mice, DCs remained in the interstitium and failed to enter the lumen of lymphatic vessels, in contrast to DCs in control tissue (Fig. S3B). Hence, DCs require polySia to sense dermal CCL21, whereas sensing of LN CCL21 is independent of polySia. Tissue context-specific presentation of CCL21 might explain why T cells, which do not express polySia, are able to home into LNs of Ccl19-deficient mice (18), although they only responded to CCL19 and not to CCL21, when exposed to soluble chemokine (fig. S3C). This prompted us to study the molecular mechanism underlying polySia-dependent CCL21 sensing.

Although the chemokine domain of CCL21 is structurally similar to that of CCL19 (19, 20), CCL21 carries a positively charged C-terminal extension, which mediates binding to glycans, particularly heparan sulfate residues (21). It has been suggested that, via these residues, CCL21 might also interact with negatively charged polySia (9). Consequently, cell surface polySia might act as a co-receptor and increase the local availability of CCL21 for CCR7 (22). Of the seven proteins that were described as polySia carriers (2), only neuropilin-2 was expressed on mature DCs. To further investigate CCR7 as a direct target of polysialylation, we used a human embryonic kidney (HEK) 293 cell system. When green fluorescent protein (GFP) was immunoprecipitated from lysates of cells coexpressing a CCR7-GFP fusion protein and St8Sia IV, polySia

Fig. 2. PolySia affects CCL21 sensing in peripheral tissues. (A) Footpad injection of TAMRA (tetramethylrhodamine azide)– and CFSE (carboxyfluorescein diacetate succinimidyl ester)–labeled St8sia4+/+ and control bone marrow DCs into wild-type recipient mice. Mice were euthanized 48 hours after injection and analyzed by flow cytometry (left) and immunohistochemistry (right). Cryosections of popliteal (pop) LNs were stained against laminin. Scale bar, 100 μm. (B) The left panels show single cell tracks of St8sia4+/+ and control bone marrow DCs migrating within 3D collagen matrices toward CCL19 and CCL21 gradients (indicated by gray wedges; 0.33 μM per gel). Average velocities (v) ± SD and directionality (d) are indicated for each genotype and condition. On the right is an automated analysis of y-directed velocities of DC migration in 3D collagen gels. The curves show average speeds in the y direction over time ± SD from eight independent experiments with cells isolated from at least three different mice. The inset illustrates the experimental setup. (C) Intra-lymphatic injection of TAMRA-labeled St8sia4+/+ control, or Ccr7−/− bone marrow DCs into Ccl19−/− recipient mice. Ten hours after injection, popliteal LNs were stained against laminin to visualize LN architecture (parenchyma and cortical sinuses). The graph on the right shows average migratory distances ± SD of TAMRA+ DCs from the LN edge to the parenchyma (at least five mice per group). Scale bar, 250 μm. (D) On the left are z-stack projections of wild-type ear sheets incubated with St8sia4+/+ and control bone marrow DCs and stained against LYVE1. Quantification of cells inside lymphatic vessels is shown in the graph on the right. Bars indicate average values ± SD of five different fields per view of three independent experiments. Scale bar, 100 μm. For all bar graphs, differences between groups were examined by two-tailed Student’s t tests [(A) and (D), unpaired; (C), paired].
could be detected in the precipitate, whereas it was absent in precipitates of both single transfectants but not in double transfectants (Fig. 3B). Further biochemical and mutational analysis in HEK293 cells indicated that polySia was attached to both the N- and O-linked glycans of CCR7, because inhibition of either N- or O-glycosylation did not fully abrogate polysialylation of CCR7 (fig. S4, D and E). To substantiate that CCR7 is polysialylated in DCs, we used flow cytometry to analyze cell-surface levels of polySia in Ccr7-deficient and control DCs and found reduced polySia on Ccr7-deficient cells (Fig. 3C). This further suggests that, apart from the chemokine domain conformation of full-length CCL21, residues belonging to the C-terminus (fig. S5C). Together, these data suggest that CCL21’s C terminus structurally alters its chemokine domain, probably by transient binding, and that this structural alteration is abrogated by polySia binding to CCL21’s C-terminal extension.

To functionally challenge the autoinhibition model, we took advantage of the fact that polySia-insensitive chemokine CCL19 naturally lacks a C-terminal extension and produced a recombinant chemokine with CCL21’s C terminus transplanted to CCL19 (Fig. 4, D right panel). Chemotaxis assays revealed that control DCs responded to the chimeric chemokine with kinetics that were more similar to those observed for CCL21 than for CCL19 (Fig. 4, D left panel). The maximal directed response of wild-type DCs toward the chimera was slightly diminished in comparison with the response toward CCL19. Most relevantly, transplantation of CCL21’s C terminus to CCL19 conferred polySia sensitivity, because the response of polySia-deficient DCs to the chimeric chemokine was largely abrogated. These data corroborate a model in which polySia binding to CCL21 transiently interacts with the chemokine domain of CCL21, thereby providing a putative structural correlate of functional autoinhibition. When polySia was titrated to full-length CCL21, residues belonging to the putative autoinhibition signature shifted to positions similar to those observed in the spectra of truncated CCL21 (Fig. 4C and fig. S5B). Hence, in the presence of polySia, the chemokine domain of full-length CCL21 adopts a similar conformation as that of C-terminally truncated CCL21. PolySia binding to truncated CCL21 was considerably weaker than to full-length CCL21, confirming that interactions mainly take place via the C terminus (fig. S5C).
our findings may be therapeutically relevant. In the spread of metastatic tumors, suggesting that ficking, the CCR7 axis is also centrally involved between two alternative ligands. Apart from being 190

coupled receptor allows discrimination similar C-terminal extensions. The finding that polySia dependency is restricted to the skin sug-
gests that, depending on the molecular context, polySia dependency is restricted to the skin sug-
CCL21 can either be present in the autoinhibited
increasing concentrations of polySia DP9 (grays to cyan; DP, degree of poly-
merization). On the right, residues within CCL21-FL with significant polySia-
CCL21 with significant polySia-
CCL21-FL titrated with increasing concentrations of polySia DP9 (grays to cyan; DP, degree of polymer-
CCL21 with significant polySia-
duced chemical shift perturbations are colored cyan. (D) Migration of mature bone marrow DCs toward the chimeric chemokine (left) and schematic rep-
resentations of chemokines used for in vitro migration assays (right).

Fig. 4. Identification of an autoinhibitory interaction site within CCL21
(A) Migration of mature bone marrow DCs within 3D collagen gels toward truncated CCL21 (CCL21trunc). Shown are average speeds in the y direction over time ± SD from eight independent experiments with cells generated from at least three different mice. (B) The left panel shows overlays of a portion of the 15N-1H HSQC (heteronuclear single-quantum coherence) spectra of full-length CCL21 (CCL21-FL) residues 1 to 111 (black) and CCL21trunc residues 1 to 79 (purple) (ppm, parts per million). Red arrows indicate chemical shifts of the respective residue. On the right, the first 79 amino acids of the CCL21-FL structure are depicted with residues (purple) that showed significant changes in chemical shift perturbations upon truncation. The red circle indicates a putative autoinhibitory site. (C) The left panel shows overlays of a portion of the 15N-1H HSQC spectra of CCL21-FL 1 to 111 (black) and CCL21-FL titrated with increasing concentrations of polySia DP9 (grays to cyan; DP, degree of polymerization). On the right, residues within CCL21-FL with significant polySia-

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A chemokine's sugary release

As immune cells survey the body for pathogens, they circulate through the blood and migrate through the lymphatic system. The latter route allows for tissues and lymph nodes—the central hubs of the immune system—to communicate. Kiermaier *et al.* reveal the importance of the monosaccharide sialic acid in keeping immune cells in motion. Multiple sialic acids decorate the surface CCR7 on immune cells. CCR7 recognizes proteins called chemokines, which direct where cells move in the body. Sialic acids on CCR7 release one such chemokine present on lymph node endothelial cells from an inhibited state, allowing immune cells to enter lymph nodes.

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