Dectin-1-activated dendritic cells trigger potent antitumour immunity through the induction of Th9 cells

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Dectin-1 signalling in dendritic cells (DCs) has an important role in triggering protective antifungal Th17 responses. However, whether dectin-1 directs DCs to prime antitumour Th9 cells remains unclear. Here, we show that DCs activated by dectin-1 agonists potently promote naive CD4⁺ T cells to differentiate into Th9 cells. Abrogation of dectin-1 in DCs completely abolishes their Th9-polarizing capability in response to dectin-1 agonist curdlan. Notably, dectin-1 stimulation of DCs upregulates TNFSF15 and OX40L, which are essential for dectin-1-activated DC-induced Th9 cell priming. Mechanistically, dectin-1 activates Syk, Raf1 and NF-κB signalling pathways, resulting in increased p50 and RelB nuclear translocation and TNFSF15 and OX40L expression. Furthermore, immunization of tumour-bearing mice with dectin-1-activated DCs induces potent antitumour response that depends on Th9 cells and IL-9 induced by dectin-1-activated DCs in vivo. Our results identify dectin-1-activated DCs as a powerful inducer of Th9 cells and antitumour immunity and may have important clinical implications.
Naive CD4+ T cells, on antigenic activation, differentiate into various T helper (Th) cell subsets, such as Th1, Th2, Th17 and T regulatory cells (Tregs). Th9 is a recently described Th cell subset characterized by the secretion of interleukin (IL)-9 (refs 3,4). Th9 cells and IL-9 (Th9/IL-9) are pro-inflammatory and appear to function in a broad spectrum of autoimmune diseases and allergic inflammation11. We and others have recently reported that adoptive transfer of Th9 cells induces potent therapeutic immunity against melanoma tumours in mice, better than other Th cells12. We have shown that Th9-derived IL-9 is critical in promoting an efficient host CD8+-CTL-mediated antitumour immune response3,9. Th9-derived IL-9 was also shown to activate mast cells, which may contribute to Th9 cell-induced antitumour activities7. IL-9 has the potential to enhance the survival and proliferation of antitumour effector T cells10. These seminal findings provide an impetus for further investigation of efficient strategies to induce and expand Th9 cells for tumour immunotherapy.

Th9 cells can be generated in vitro by TGF-β and IL-4 in the presence of anti-CD86/CD28 antibodies3,4. However, mechanisms of Th9 cell differentiation under physiological and pathological conditions are poorly understood. Previous investigations showed that IL-1, IL-2, OX40L, TSLP and IL-25 promoted Th9 cell development11-16. However, these factors are not specific for Th9 differentiation because they are also associated with the development of Th1, Th2 and Th17 cells17-21. These investigations suggest that the initiation of Th9 cells depends on some specific profiles of cytokine and costimulatory signals.

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) and play a crucial role in the induction of Th cell subsets2,22,23. Dectin-1, a C-type lectin receptor, is expressed mainly by DCs, macrophages and neutrophils24,25. DCs sense fungal pathogens through dectin-1, which recognizes β-1,3-glucans present on the fungal cell wall, and trigger the host immune response against fungal pathogens26. Dectin-1 triggers Syk and Rafl downstream signalling pathways, which subsequently regulate the activation of canonical and noncanonical NF-kB pathways24. Dectin-1 activation in DCs stimulates the secretion of IL-6, TNF-α and IL-12p40, which polarize naive CD4+ T cells into Th17 and Th1 cells, the key effector cells for antifungal immunity27,28. However, whether dectin-1 activation in DCs favours the induction of antitumour Th9 cells remains unclear.

In this study, we found that dectin-1 activation in DCs potently promotes the induction of Th9 cells. We show that dectin-1 signalling stimulates DCs to overexpress TNFSF15 and OX40L, which are responsible for promoting Th9 cell differentiation primed by dectin-1-activated-DCs in vitro. Syk, Raf1 and NF-kB signalling pathways triggered by dectin-1 are required for dectin-1-induced expression of TNFSF15 and OX40L. Furthermore, immunization of mice bearing melanoma or myeloma tumours with dectin-1-activated DCs induces potent antitumour responses that depend on Th9 cells and IL-9. Our results thus identify dectin-1-activated DCs as a powerful inducer of Th9 cells and antitumour immunity and may have important clinical implications.

Results

Dectin-1-activated DCs enhance Th9 cell priming in vitro. Mouse immature DCs (iDCs) expressed dectin-1 (Supplementary Fig. 1). To address whether dectin-1-activated DCs affected the differentiation of Th9 cells, we matured mouse bone marrow (BM)-derived DCs with TNF-α plus IL-1β (BMDCs) or a selective dectin-1 agonist Curdlan (CurDCs) and stimulated naive CD4+ T cells under Th9-polarizing conditions (without anti-CD28 antibody) with BMDCs or CurDCs. We found that CurDCs efficiently enhanced the development of IL-9-producing Th9 cells (Fig. 1a) and increased IL-9 production at both mRNA and protein levels as compared with BMDCs (Fig. 1b,c). Furthermore, Th9 cells primed by CurDCs expressed significantly higher levels of Th9-related transcription factor Ifng than those primed by BMDCs (Fig. 1d). We also examined the expression of Th1-, Th2- and Th17-related cytokines and transcription factors and found that Th9 cells primed by CurDCs did not express most of the Th1-, Th2- and Th17-related cytokines and transcription factors, such as Ifng, Il4, I15, Il17, Tbx21 and Rorc (Fig. 1c,d), although the Th2-related cytokine Il13 was slightly increased (Fig. 1e). This result demonstrated that CurDCs reinforced Th9 cell differentiation.

To confirm the capability of dectin-1-activated DCs in polarizing naive CD4+ T cells into Th9 cells, we used another dectin-1 agonist Scleroglucan to mature mouse DCs (SciDCs). Similarly, as compared with BMDCs, SciDCs significantly enhanced Th9 cell differentiation (Fig. 1a), leading to higher expression of IL-9, Ifng, Il13 and the Th2-related transcription factor Gata3 (Fig. 1b,d), whereas the expression of other Th-related cytokines and transcription factors remained unchanged (Fig. 1c,d). To examine the role of dectin-1 signalling in activating naturally occurring DCs in Th9 differentiation, mouse spleen CD11c+ cells were isolated, activated by curdlan and cocultured with T cells. Similarly, Curdlan-treated natural DCs drove Th9 differentiation by enhancing Th cell Il9 expression as compared with untreated natural DCs (Supplementary Fig. 2).

Next we analysed the effects of dectin-1-activated DCs on other Th cell differentiation. Naive CD4+ T cells were cocultured with BMDCs, CurDCs or dectin-1−/− CurDCs under Th1-, Th2-, Th17- and Treg-polarizing conditions. As compared with BMDCs, CurDCs moderately enhanced Th1 and Th17 differentiation by increasing Ifng, Tbx21, Il17a and Rorc expression, respectively (Supplementary Fig. 3); while dectin-1−/− CurDCs-induced Th1 and Th17 cells expressed less Ifng and Il17a than CurDC-induced Th cells, respectively (Supplementary Fig. 3). Together, these results demonstrated the potency of dectin-1-activated DCs in the induction of Th9 cells.

Th9 induction by curdlan-activated DCs relies on dectin-1. To explore the contribution of dectin-1 to dectin-1-activated DC-induced Th9 cell differentiation, mouse DCs matured with Curdlan plus a dectin-1 blocking antibody (αDectin-1) were used to prime Th9 cells. While Th9 cells primed by αDectin-1-treated BMDCs expressed comparable levels of IL-9, Ifng, Il13 and Gata3 as compared with those primed by BMDCs (Fig. 2a–c), Th9 cells primed by αDectin-1-treated CurDCs expressed significantly lower levels of IL-9, Ifng, Il13 and Gata3 than those primed by CurDCs (Fig. 2a–c). This result indicated that dectin-1 played an important role in directing DCs for Th9 cell induction.

To further confirm the function of dectin-1 in activating DCs for Th9 cell induction, we generated BMDCs and CurDCs from wildtype (WT) and dectin-1−/− CurDCs (Fig. 2d–f). Dectin-1-deficiency did not affect BMDCs in priming Th9 cells, as demonstrated by the similar expression levels of IL-9 mRNA and IL-9 protein by Th9 cells primed by dectin-1−/− CurDCs and WT BMDCs (Fig. 2d,e). Notably, Th9 cells primed by dectin-1−/− CurDCs also expressed much lower levels of Il13 and Gata3 than those primed by WT CurDCs (Fig. 2d–f and Supplementary Fig. 4). Collectively, these data demonstrated the important role of dectin-1 in directing DCs for Th9 cell differentiation.
Dectin-1 stimulates DCs to express TNFSF15 and OX40L. Th cell differentiation relies on specific profiles of cytokines and costimulatory molecules\(^1,2\). To explore the molecular mechanisms by which dectin-1-activated DCs drove Th9 differentiation, we performed gene expression profiling analyses in BMDCs versus CurDCs. Among 26,423 mouse genes, we identified 42 upregulated genes of cytokines, chemokines and costimulatory surface molecules that might be involved in Th9 cell activation, polarization and chemotaxis (Table 1). Besides the cytokines Il12p35 (Il12a), Il12p40 (Il12b) and Tnf which were reportedly upregulated by dectin-1 activation\(^24,27\), our data identified additional new cytokines and costimulatory molecules that were upregulated by Curdlan stimulation, especially the TNF/receptor family members, including Tnfsf15 (Tll1a), Tnfsf4 (Ox40l), Tnfsf8, Tnfsf26 and Tnfsf12a (Table 1). The upregulated expression of TNFSF15 and OX40L by dectin-1-activated DCs compared with BMDCs was confirmed by quantitative real-time PCR (qPCR) (Fig. 3a) and flow cytometry analysis (Fig. 3b). Furthermore, the upregulated expression of TNFSF15 and OX40L was completely abolished in CurDCs and Sc1DCs from dectin-1\(^{-/−}\) mice (Fig. 3c,d). Notably, Curdlan stimulation drove natural DCs to express TNFSF15 and OX40L in vivo (Supplementary Fig. 5). In addition, Curdlan stimulation led to upregulation of DC costimulatory surface proteins CD86, CD40 and CD80 as compared with the treatment with TNF-α/IL-1β (Supplementary Fig. 6), suggesting improved immunogenicity of dectin-1-activated DCs. Together, these results demonstrated that dectin-1 activation stimulated DCs to express a specific profile of cytokines, chemokines and costimulatory molecules, especially the TNF/receptor family members, suggesting the potential mechanisms for dectin-1-activated DCs in the induction of Th9 cells.

**Figure 1 | Dectin-1-activated DCs enhance Th9 cell differentiation in vitro.** Naïve CD4\(^{+}\) T cells from spleens of mice (\(n=3\)–5) were cocultured with DCs matured with TNF-α/IL-1β (BMDC), Curdlan (CurDC) or Scleroglucan (Sc1DC) in the presence of anti-CD3 with (Th9) or without (Th0) addition of Th9-polarizing cytokines TGF-β and IL-4 for 3 days. Culture supernatants and CD4\(^{+}\) T cells separated by the magnetic cell sorting (MACS) were collected for analysis. (a) Cells were stained with anti-CD4 and anti-IL-9 antibodies and subjected to flow cytometry analysis. Numbers in the dot plots represent the percentages of naive CD4\(^{+}\) IL-9\(^{+}\) T cells. Right, summarized results of four independent experiments obtained as at left. (b) ELISA assessed the IL-9 secretion in the cocultures. (c–d) qPCR analysis of Th9-, Th1-, Th2- and Th17-related cytokines (c) and transcription factors (d). Expression was normalized to Gapdh and set at 1 in BMDC-induced Th9 cells. Results shown are the mean ± s.d. of 3–5 independent experiments. *\(P<0.05\); **\(P<0.01\) (Student’s t-test).
ABROGATION OF DECTIN-1 INHIBITS THE CAPABILITY OF DCs TO PRIME TH9 CELLS IN VITRO

Dectin-1 induces TNFSF15 and OX40L via Syk and Raf1.

We first compared the effects of Curdlan versus TNF-α/IL-1β on the activation of Syk and Raf1 signalling pathways in mouse iDCs. Curdlan treatment induced rapid increases of phosphorylated (p)-Syk and p-Raf1 in DCs as compared with the treatment with TNF-α/IL-1β (Fig. 5a), indicating that Curdlan was more potent than TNF-α/IL-1β in activating Syk and Raf1 in DCs. To further confirm the involvement of dectin-1 in Syk and Raf1 activation, iDCs generated from WT or dectin-1−/− mice were used. Dectin-1 deficiency remarkably reduced the expression of p-Syk and p-Raf1 in iDCs treated with Curdlan, whereas it did not affect the phosphorylation of Syk and Raf1 in iDCs treated with TNF-α/IL-1β (Fig. 5b), indicating that Curdlan induced dectin-1-dependent activation of Syk and Raf1 in DCs.

To investigate the role of Syk and Raf1 signalling in directing dectin-1–induced TNFSF15 and OX40L expression in DCs, we explored the specific inhibitor Piceatannol and Raf1–specific inhibitor GW5074 were used during DC maturation. The inhibition of Syk or Raf1 in DCs significantly decreased TNFSF15 and OX40L expression induced by Curdlan (Fig. 5c,d). In addition, by using small interfering RNAs (siRNAs) to specifically silence Syk or Raf1 in iDCs, we found that knockdown of either Syk or Raf1 in DCs reduced TNFSF15 and OX40L expression induced by Curdlan (Fig. 5f,g). To examine the effects of Syk and Raf1 signalling in directing dectin-1–activated DCs for Th cell differentiation, Piceatannol- and GW5074-treated CurDCs were used to prime Th cells. Piceatannol- or GW5074-treated CurDCs were less effective in the induction of Th9, Th1 and Th17 cells than CurDCs, as demonstrated by the decreased expression of Il9, Irf4 and Il17a by Th cells primed by Piceatannol- or GW5074-treated CurDCs compared with control CurDCs (Supplementary Fig. 9). Together, these results demonstrated that dectin-1 induced TNFSF15 and OX40L expression through Syk and Raf1 signalling pathways.
Table 1 | The upregulated genes of cytokines, chemokines and costimulatory surface molecules in CurDCs versus BMDCs identified by gene expression profiling (GEP).

| Rank # | Gene symbol | Gene name | Probe intensity | Fold change |
|--------|-------------|-----------|-----------------|-------------|
| 1      | Edn1        | endothelin 1 | 80,627          | 151.4       |
| 2      | Ccl20       | chemokine (C–C motif) ligand 20 | 3,659 | 118 | 30.9 |
| 3      | Il33        | interleukin 33 | 55,127          | 2,098       |
| 4      | Tnfsf15     | tumor necrosis factor (ligand) superfamily, member 15 | 22,008 | 1,103 | 19.9 |
| 5      | Igf2        | insulin-like growth factor 2 | 60,620 | 3,056 | 19.8 |
| 6      | Ccl4        | chemokine (C–C motif) ligand 4 | 338,983 | 23,896 | 14.2 |
| 8      | Npy         | neuropeptide Y | 11,700          | 848        | 13.2 |
| 9      | Tnf         | tumor necrosis factor | 33,032 | 3,046 | 10.8 |
| 10     | Cd200       | CD200 antigen | 11,305          | 1,457       |
| 11     | Hbegf       | heparin-binding EGF-like growth factor | 48,247 | 6,431 | 7.5 |
| 13     | Ccl3        | chemokine (C–C motif) ligand 3 | 371,668 | 52,647 | 7.1 |
| 14     | Angptl2     | angiopoietin-like 2 | 6,362          | 997        | 6.4 |
| 16     | Inhba       | inhibin beta-A | 134,914 | 21,926 | 6.2 |
| 17     | Penk        | preproenkephalin | 8,547          | 1,590      | 5.4 |
| 18     | Il1a        | interleukin 1 alpha | 209,483 | 46,270 | 4.5 |
| 21     | Csf1        | colony stimulating factor 1 (macrophage) | 115,228 | 28,784 | 4.0 |
| 23     | Nrp1        | neuropilin 1 | 21,066          | 5,783       |
| 24     | Tnfsf8      | tumor necrosis factor (ligand) superfamily, member 8 | 5,670 | 1,561 | 3.6 |
| 25     | Tnfsf26     | tumor necrosis factor receptor superfamily, member 26 | 18,171 | 5,168 | 3.5 |
| 27     | Ccl70       | CD70 antigen | 5,444          | 1,708       |
| 28     | Tnfsf4      | tumor necrosis factor (ligand) superfamily, member 4 | 55,588 | 17,733 | 3.1 |
| 29     | Ccl2        | chemokine (C–C motif) ligand 2 | 11,114 | 3,574 | 3.1 |
| 30     | Nrp1        | neuropilin 1 | 12,982          | 4,189       |
| 32     | Il1f9       | interleukin 1 family, member 9 | 8,432 | 2,888 | 2.9 |
| 33     | Il12b       | interleukin 12b | 5,740          | 2,068       |
| 34     | Igfbp7      | insulin-like growth factor binding protein 7 | 4,935 | 1,799 | 2.7 |
| 35     | Mfge8       | milk fat globule-EGF factor 8 protein | 219,136 | 81,811 | 2.7 |
| 37     | Tnfsf12a    | tumor necrosis factor receptor superfamily, member 12a | 7,312 | 2,880 | 2.5 |
| 38     | Pdgfa       | platelet-derived growth factor, alpha | 12,496 | 4,976 | 2.5 |
| 40     | Il12a       | interleukin 12a | 5,791          | 2,552       |
| 42     | Osm         | oncostatin M | 43,502          | 21,367      |

Figure 3 | Dectin-1 activation in DCs induces TNFSF15 and OX40L expression. DCs generated from mice (n = 3–5) were matured by TNF-α/IL-1β (BMDC), Curdlan (CurDC) or Scleroglucan (Sc1DC) for 48 h. (a) qPCR analysed the mRNA levels of Tnfsf15 and Ox40l in DCs. (b) Flow cytometry analysis of TNFSF15 and OX40L protein surface expression in DCs. Right, summarized results of three independent experiments obtained as the left. MFI, mean fluorescence intensity. BMDCs and CurDCs were generated from WT or dectin-1−/− mice (n = 3). Same as in a and b, the mRNA (c) and protein (d) levels of TNFSF15 and OX40L in DCs were analysed by qPCR and flow cytometry. Results shown are the mean ± s.d. of three independent experiments. *P < 0.05; **P < 0.01 (Student’s t-test).
Dectin-1 induces OX40L and TNFSF15 expression via NF-κB.

To compare the effects of Curdlan versus TNF-α/IL-1β on the activation of NF-κB pathway, mouse iDCs were treated with TNF-α/IL-1β or Curdlan. Curdlan treatment increased the expression of p-IKKα/β and decreased that of IκBα in mouse DCs (Fig. 6a), leading to a remarkable increase of c-Rel, p50 and RelB nuclear translocation and a slight increase of p65 and p52 nuclear translocation as compared with the treatment with TNF-α/IL-1β (Fig. 6b), indicating that Curdlan was more potent in activating NF-κB pathway in DCs than TNF-α/IL-1β. Bortezomib, a proteasome inhibitor, was used to inhibit NF-κB14. We found that mouse DCs matured by Curdlan plus bortezomib expressed less TNFSF15 and OX40L than those matured by Curdlan alone (Fig. 6c,d), indicating that dectin-1 induced TNFSF15 and OX40L expression through NF-κB signalling pathway.

To further determine the role of NF-κB pathway in dectin-1-induced TNFSF15 and OX40L expression, we performed luciferase reporter assays to examine whether these NF-κB molecules could bind directly to Tnfsf15 and Oxl40l promoters and affect their expression. We found that p50-RelB and p52-RelB dimmers could bind to and activate Tnfsf15 promoter (Fig. 6e); while p50, p50-RelB and p52-RelB could bind to and activate Oxl40l promoter (Fig. 6f). Considering that p52 nuclear translocation was only slightly increased by Curdlan treatment, we speculated that p50-RelB was responsible for dectin-1-induced TNFSF15 expression, while p50 and p50-RelB were responsible for dectin-1-induced OX40L expression. Collectively, these results demonstrated that dectin-1-induced TNFSF15 and OX40L expression was dependent on NF-κB signalling pathway.

Dectin-1-activated DCs induce antitumour effects in vivo.

To examine the role of dectin-1-activated DCs in tumour immunotherapy in mice, we generated BMDCs and CurDCs from WT and dectin-1−/− mice, and pulsed them with OVA peptide (323–339). OT-II mice were immunized with OVA peptide-pulsed WT BMDCs, WT CurDCs or dectin-1−/− CurDCs at days 3 and 10 after challenge with B16-OVA melanoma cells. Mice immunized with WT CurDCs displayed greater resistance to melanoma tumour growth than mice immunized with WT BMDCs, while WT BMDC vaccination showed moderate antitumour effects as compared with PBS control (Fig. 7a). Furthermore, idioype (Id)-pulsed CurDCs also induced more potent anti-tumour response than Id-pulsed BMDCs in MPC-11 myeloma Balb/c mouse model (Fig. 7b). These results demonstrated that dectin-1-activated DCs induced potent therapeutic antitumour immunity in vivo. Notably, while blockade of TNFSF15 or OX40L by their specific antibodies partially inhibited the antitumour effects induced by CurDCs (Supplementary Fig. 10), dectin-1−/− Curdc immunization was much less effective than WT CurDCs in inducing anti-melanoma response (Fig. 7a), indicating that CurDCs induced antitumour immunity in a dectin-1-dependent manner.

Notably, a very recent report by HyeMee et al. showed that the dectin-1 agonist Curdlan downregulated OX40L expression on myeloid DCs29, which is exactly the opposite of our finding that dectin-1 upregulated OX40L expression on bone marrow-derived myeloid DCs. The reasons for this discrepancy are unclear. We suggest that the different concentrations of Curdlan used for DC activation in these two studies (5 μg ml−1 in our study versus...
10 μg ml⁻¹ in HyMeE's study) may contribute to the difference of OX40L expression in myeloid DCs (Supplementary Fig. 11).

**Antitumour effects of dectin-1-activated DCs rely on Th9/IL-9.** The observed important role of dectin-1-activated DCs in Th9 cell differentiation in vitro and the antitumour immunity in vivo prompted us to determine whether Th9 cells and IL-9 were involved in mediating the antitumour effects induced by dectin-1-activated DCs in vivo. To determine whether the immunization of dectin-1-activated DCs induces Th9 cells and IL-9 production, OT-II mice were immunized with OVA peptide-pulsed BMDCs or CurDCs. On day 3 after DC immunization, mice were killed, and mouse sera and total leukocytes from spleens and lymph nodes (LN) cells were collected for the assessment of Th9 cell response. While no increase in serum IL-9 was observed in mice immunized with BMDCs as compared with mice treated with PBS (Fig. 7c), mice immunized with CurDCs showed significantly higher levels of serum IL-9 than mice receiving either BMDCs or PBS (Fig. 7c), indicating that dectin-1-activated DC immunization induced IL-9 production. Spleen and LN cells were restimulated with OT-II OVA peptides for 24 h before assay. Interestingly, intracellular staining detected significantly higher percentages of IL-9⁺CD4⁺ (Th9) cells in the spleen and LN cells from mice immunized with CurDCs than mice receiving BMDCs or PBS (Fig. 7d,e), though there was a modest increase of Th9 cells in the spleen and LN cells from mice immunized with BMDCs as compared with PBS control mice (Fig. 7d,e). qPCR and ELISA further confirmed the increase of IL-9 production in mice immunized with CurDCs compared with mice receiving BMDCs or PBS (Fig. 7f and Supplementary Fig. 12). These results demonstrated that dectin-1-activated DCs stimulated the production of Th9 cells and IL-9 in vivo. Dectin-1-activated DCs reportedly promoted the development of Th17 and Th1 cells27. In line with the published observations, we found that dectin-1-activated DC immunization upregulated the expression of Ifng and Il17a by spleen CD4⁺ cells as compared with the treatment with BMDCs or PBS (Fig. 7f and Supplementary Fig. 12).

To further confirm the role of dectin-1 signalling in activating DCs for the induction of Th9 cells and IL-9 production in vivo, we immunized OT-II mice with OVA peptide-pulsed WT CurDCs or dectin-1⁻/⁻ CurDCs. Mouse serum samples, spleen and LN cells were collected for the analysis of Th9 cell response. We found that dectin-1⁻/⁻ CurDC immunization failed to induce Th9 cell development in mice, since the serum IL-9, the number of IL-9⁺CD4⁺ (Th9) cells and the expression of Il9 mRNA were completely abolished in mice immunized with dectin-1⁻/⁻ CurDCs as compared with those immunized with WT CurDCs (Fig. 7c–f).

Th9/IL-9 is known to induce tumour-specific CTL responses8,30. To address whether the induction of Th9/IL-9 by dectin-1-activated DCs in vivo resulted in potent antitumour CTL responses, Balb/c mice were immunized with Id-pulsed BMDCs or CurDCs. CurDC immunization induced higher levels of tumour-specific CTL activity than BMDCs (Fig. 7g). Notably, administration of an IL-9-neutralizing antibody (anti-IL-9) inhibited CTL responses induced by CurDCs (Fig. 7g), indicating that Th9/IL-9 contributes to dectin-1-activated DC-induced CTL responses in vivo.

Finally to define the functional relationship between Th9/IL-9 and dectin-1-activated DC-induced antitumour effects, OT-II mice with established B16-OVA tumours were treated with OVA peptide-pulsed CurDCs in the presence or absence of anti-IL-9.

**Figure 5 | Dectin-1 induces TNFSF15 and OX40L expression through Syk and Raf1.** (a) iDCs generated from mice (n = 3–5) were stimulated with TNF-α/IL-1β (T/I) or Curdlan (Cur) for 0.5 or 1 h. Cell lysates were prepared and subjected to western blot analysis using indicated antibodies. (b) iDCs were prepared from WT and dectin-1⁻/⁻ mice (n = 3) and stimulated with TNF-α/IL-1β or Curdlan for 0.5 h. Cell lysates were subjected to western blot analysis using indicated antibodies. iDCs were matured by TNF-α/IL-1β (BMDCs) or Curdlan (CurDCs) in the presence of piceatannol (Pic), GW5074 (GW) or DMSO as control for 48 h. (c) qPCR assessed Tnfsf15 and Ox40l expression in DCs. (d) Flow cytometry of TNFSF15 and OX40L expression in DCs. Right, summarized results of three independent experiments obtained as at left. MFI, mean fluorescence intensity. (e) Mouse serum samples, spleen and LN cells were collected for the analysis of Th9 cell response. While no increase of serum IL-9 was observed in mice immunized with BMDCs as compared with mice treated with PBS (Fig. 7c), mice immunized with CurDCs showed significantly higher levels of serum IL-9 than mice receiving either BMDCs or PBS (Fig. 7c), indicating that dectin-1-activated DC immunization induced IL-9 production. Spleen and LN cells were restimulated with OT-II OVA peptides for 24 h before assay. Interestingly, intracellular staining detected significantly higher percentages of IL-9⁺CD4⁺ (Th9) cells in the spleen and LN cells from mice immunized with CurDCs than mice receiving BMDCs or PBS (Fig. 7d,e), though there was a modest increase of Th9 cells in the spleen and LN cells from mice immunized with BMDCs as compared with PBS control mice (Fig. 7d,e). qPCR and ELISA further confirmed the increase of IL-9 production in mice immunized with CurDCs compared with mice receiving BMDCs or PBS (Fig. 7f and Supplementary Fig. 12). These results demonstrated that dectin-1-activated DCs stimulated the production of Th9 cells and IL-9 in vivo. Dectin-1-activated DCs reportedly promoted the development of Th17 and Th1 cells27. In line with the published observations, we found that dectin-1-activated DC immunization upregulated the expression of Ifng and Il17a by spleen CD4⁺ cells as compared with the treatment with BMDCs or PBS (Fig. 7f and Supplementary Fig. 12).

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Finally to define the functional relationship between Th9/IL-9 and dectin-1-activated DC-induced antitumour effects, OT-II mice with established B16-OVA tumours were treated with OVA peptide-pulsed CurDCs in the presence or absence of anti-IL-9.
We found that blockade of IL-9 with anti-IL-9 abolished the antitumour effects induced by dectin-1-activated DCs (Fig. 7h), demonstrating the important role of Th9/IL-9 in mediating the antitumour effects induced by dectin-1-activated DCs in vivo. Collectively, these data demonstrated that dectin-1-activated DCs mediated their antitumour effects via their ability to induce Th9 cells and IL-9 production in vivo.

Dectin-1-NF-κB signalling induced DC production of a specific profile of cytokines, chemokines and costimulatory surface molecules, including TNFSF15 and OX40L, but little Th1, Th2 and Th17 cytokines. MyD88-mediated signals also activate NF-κB pathway. However, we found that lipopolysaccharide (LPS), a potent stimulator of MyD88/NF-κB molecules. Luciferase reporter assay showed NF-κB-dependent activation of Tnfsf15 (e) and Ox40l (f) promoter in 293T cells. Data are representative of at least three (a, b, c, d, e, f) independent experiments. *P<0.05; **P<0.01 (Student’s t-test).

**Discussion**

Th9 cells have been shown to mediate potent antitumour effects in vivo7,8; therefore, investigation of more efficient strategies to induce and expand Th9 cells in vivo may have high clinical significance in tumour immunotherapy. Dectin-1 signalling has been shown to induce Th17 and Th1 cell responses in vivo, which are essential for protecting a mammalian host from fungal infection27,28,32. However, whether dectin-1-activated DCs promote the development of antitumour Th9 cells remains unknown. In this study, we found that DCs activated by dectin-1 agonists potently promoted Th9 cell differentiation, and such Th9 cells expressed high levels of IL-9- and Th9-related transcription factor IRF4, but not Th1-, Th2- and Th17-related cytokines and transcription factors. This result was confirmed by using a dectin-1 blocking antibody or dectin-1−/− DCs as they almost
completely abrogated the capability of dectin-1 agonist-activated DCs in promoting Th9 cell induction. Importantly, in the functional tests, we found that dectin-1-activated DCs induced potent antitumour immunity against established tumours. Mechanistic studies revealed that immunization with dectin-1-activated DCs efficiently promoted the production of Th9 cells and IL-9, which were required for the antitumour effects induced by dectin-1-activated DCs. Thus, our data established the role for dectin-1 in activating DCs for the induction of potent Th9 cell responses against cancers in vivo.

Previous studies showed that dectin-1 activation in DCs induces the expression of TNF-α, IL-6, IL-23 and IL-12, which are related to Th17 and Th1 cell differentiation. In this study, we identified other cytokines and costimulatory molecules, especially the TNF/receptor family members TNFSF15, OX40L, TNFSF8, TNFRSF26 and TNFRSF12a, that were upregulated by dectin-1 signalling and important for Th9 differentiation. Functional tests showed that dectin-1-induced overexpression of TNFSF15 and OX40L is responsible for the enhanced Th9 cell response induced by dectin-1-activated DCs. This result is

Figure 7 | Role of Th9/IL-9 in dectin-1-activated DC-induced antitumour effects in vivo. (a) OT-II mice were injected subcutaneously with 1 × 10⁵ B16-OVA cells. On day 3 after tumour challenge, mice (n = 10 per group) were given two weekly subcutaneously immunizations with 1 × 10⁶ OVA peptide-pulsed BMDCs or CurDCs generated from WT or dectin-1−/− mice. Mice received PBS served as controls. Shown are the tumour growth curves. (b) Balb/c mice were injected subcutaneously with 1 × 10⁶ MPC-11 myeloma cells. On day 6 after tumour challenge, mice (n = 10 per group) were given two weekly subcutaneously immunizations with 1 × 10⁶ KLH-Id-pulsed BMDCs or CurDCs. Mice received PBS served as controls. Shown are the tumour growth curves. (c) OT-II mice (n = 4–5 per group) were injected subcutaneously with 1 × 10⁵ B16-OVA cells. On day 3 after tumour challenge, mice were immunized with OVA-peptide-pulsed BMDCs, CurDCs, dectin1−/− BMDCs or dectin-1−/− CurDCs. PBS served as control. On day 3 after the immunization, serum samples and total leukocytes from LNs and spleen cells were collected and restimulated with 5 μg ml⁻¹ OVA223-233 peptides for 24 h. (d) ELISA analysis of serum IL-9. (e) Flow cytometry analysis of IL-9-producing CD4⁺ T cells. Numbers in the dot plots represent the percentages of CD4⁺ IL-9⁺ T cells. (f) Summarized results of three independent experiments obtained in (d). (g) qPCR analysis of the indicated genes. (h) Balb/c mice (n = 3 per group) were immunized twice (1 week apart) with 1 × 10⁵ Id-KLH-pulsed BMDCs or CurDCs. PBS served as a control. Mice were given control IgG or an IL-9 neutralizing antibody (xIL9) every 3 days beginning 1 day after the first immunization. Results shown are MIP-1α-specific cytotoxicity of spleen T cells from the mice. * shows P < 0.05, comparing CurDC with BMDC, BMDC + xIL9 or CurDC + xIL9. (h) OT-II mice were injected subcutaneously with 1 × 10⁵ B16-OVA cells. On day 3 after tumour challenge, mice (n = 10 per group) received two weekly immunizations with 1 × 10⁵ OVA peptide-pulsed BMDCs, LpsDCs or CurDCs. Mice received PBS served as controls. Mice were given control IgG or xIL9 every 3 days beginning 1 day after the first immunization. Shown are the tumour growth curves. * showing P < 0.05, comparing CurDC with each of the five groups as indicated. In a–g, data are presented as mean ± s.d. of two combined in vivo experiments (a,b) or at least three (c–g) independent in vitro experiments. In h, data from 10 mice per group are used. *P < 0.05; **P < 0.01 (Student’s t-test).
consistent with previous observations that ligation of OX40 inhibits the production of regulatory T cells and Th17 cells and diverts CD4+ T cells to Th9 cells.11 Interestingly, a recent study reported that TNFSF15 potently promotes Th9 differentiation and the pathogenicity of IL-9-dependent allergic diseases.33 Thus, we identify TNF family members TNFSF15 and OX40L as key mediators for promoting Th9 differentiation primed by dectin-1-activated DCs. OX40L/OX40 were suggested to be unique in the TNF/receptor family in promoting Th9 cell induction.33 In contrast, we and others found that TNFSF15 is also a powerful inducer of Th9 cells. Thus, other TNF family members may also be involved in the development of antitumour Th9 cells. Further studies will be necessary to investigate the function of other TNF/receptor family members expressed by dectin-1-activated DCs in the induction of Th9 cells and antitumour immunity.

Engagement of dectin-1 by β-glucan was shown to activate the transcription factor NF-κB through Syk- and Rafl-dependent signalling pathways, leading to the production of IL-6, IL-1β, and IL-23.34 However, the dectin-1 downstream signalling pathways responsible for TNFSF15 and OX40L expression were not defined. In this study, we found that dectin-1 agonists potently activated Syk, Rafl and NF-κB signalling pathways in DCs, more powerful than regular DC maturation reagents TNF-α and IL-1β. Thus, we speculated that Syk, Rafl and NF-κB signalling pathways triggered by dectin-1 might be responsible for TNFSF15 and OX40L expression. Indeed, we found that blocking Syk, Rafl and NF-κB signalling by their specific inhibitors or siRNAs inhibited the expression of TNFSF15 and OX40L in dectin-1-activated DCs. Furthermore, we found that p52-RelB and p50-RelB dimmers directly bound to and activated Tnf/sf15 promoter, while p52-RelB, p50-RelB and p50 directly bound to and activated OX40 promoter, providing direct evidence for NF-κB signalling in promoting TNFSF15 and OX40L expression. Thus, we identify Syk, Rafl and NF-κB signalling pathways as key downstream pathways responsible for TNFSF15 and OX40L expression induced by dectin-1 in DCs. Nevertheless, we cannot exclude the possibility that some other factors that regulate TNFSF15 and OX40L expression might be induced through dectin-1-triggered Syk, Rafl and NF-κB signalling pathways.

In this study, we found that dectin-1-activated DCs induced potent antitumour effects in vivo. However, cellular mechanisms underlying the antitumour effects were not determined. Dectin-1 agonists have been shown to induce Th17 and Th1 cell responses in vivo. In this study, we found that dectin-1-activated DCs induced potent Th9 cell responses in vivo. Th1 cells are a traditional antitumour Th subset,36 while Th17 cells may also play a role in antitumour immunity.37 Th9 cells were found to induce potent antitumour immunity in mouse models, better than Th1 and Th17 cells.37,8 Based on these observations, we speculated that the antitumour effects of dectin-1-activated DCs in vivo mainly relied on Th9 cells and IL-9 induced by dectin-1-activated DCs. Indeed, we found that blockade of IL-9 with anti-IL-9 antibody abolished the antitumour effects induced by dectin-1-activated DCs. Thus, our data demonstrated that Th9 cells are the main effector cells in mediating the antitumour effects induced by dectin-1-activated DCs.

IL-9 expressed by dectin-1-activated DC-induced Th9 cells could have the potential to improve Th17/Th9 cell survival and proliferation in vivo and in vitro. In addition, curdum may improve DC survival as compared with LPS, which exhibits detrimental effects on DC survival.38 As our results showed that the improved efficacy of CurDCs to control the established tumour is dependent on IL-9, we believe that even if CurDC improved the T-cell activation, proliferation or survival or DC survival, Th9 induction by CurDCs played the most important role for the observed antitumour responses.

Neutralization of IL-9 was shown to promote melanoma tumour growth in C57BL/6 mice. However, in this study, we observed no significant difference in B16-OVA tumour growth between mice received IL-9 neutralization antibodies and control IgG in either PBS-, BMDMC- or LpsDC-treated groups. Reasons for this discrepancy could be that (i) we used OT-II mice in this study which largely lack CD8+ T cells, the important antitumour effector cells and (ii) the beginning of administration of anti-IL-9 antibodies was on day 4 after tumour challenge in this study versus day 0 in previous studies.

In summary, our study demonstrates dectin-1-activated DCs as a powerful inducer of antitumour Th9 cells in vitro and in vivo and identifies TNFSF15 and OX40L as key factors in mediating Th9 cell differentiation primed by dectin-1-activated DCs. Syk, Rafl and NF-κB signalling pathways triggered by dectin-1 were required for dectin-1-induced OX40L and TNFSF15 expression. Finally, dectin-1-activated DCs induce potent therapeutic antitumour effects in mouse models, and the antitumour effects depended on induced Th9/IL-9. Our findings may have important clinical implications.

Methods

Mice and cell lines. C57BL/6, Balb/c, OX40−/− (B6.129S4-Tnfrsf4tm1Nik/J) and OT-II (C57BL/6-Tg(Thacre)125Shui/J) mice were purchased from the Jackson Laboratory. Dectin-1−/− mice were provided by G. Brown (University of Aberdeen, Aberdeen, Scotland), and the lack of dectin-1 expression in mouse DCs was confirmed by flow cytometry and qPCR (Supplementary Fig. 1). Mice were bred and maintained in pathogen-free facilities at the First Hospital Animal Center of Jilin University. Six-to-eight-week-old mice were used for experiments. All animal experimental procedures were reviewed and approved by the Animal Ethical Committee of First Hospital of Jilin University.

B16 and B16-OVA melanoma cell lines (ATCC) were cultured in Iscove’s modified Dulbecco’s media (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS, HyClone) and 100 U ml−1 penicillin (Invitrogen) and 100 mg ml−1 streptomycin (Invitrogen). MPMC-11 myeloma cells were purchased in RPMI 1640 supplemented with FBS (10%), l-glutamine (2 mM) and penicillin/streptomycin.

Reagents. Recombinant mouse GM-CSF, TNF-α, IL-1β, IL-4, IL-9 and human TGF-β were purchased from Peprotech. TNFSF15 was purchased from R&D Systems. MHC class II restricted OT-II OVA peptide (OVAA32–339, ISQAVHAAHAEINEAGR) was purchased from Genscript. Functional anti-mouse CD3ε and CD28 antibodies (mAbs) were purchased from ebioscience. Dectin-1-blocking mAb was purchased from Invivogen. TNFSF15 neutralization mAb and OX40L-blocking mAb were purchased from R&D Systems. Curdlan and Scleroglucan were purchased from Sigma-Aldrich and Invivogen, respectively. Piceatannol (a Syk inhibitor) and GW5074 (a Raf1 kinase inhibitor) were purchased from Calbiochem. Bortezomib, a NF-κB inhibitor, was purchased from Selleckchem.

Dendritic cell generation. BMDMs were generated as described previously.39 In brief, BM cells (4 × 107 ml−1) were cultured in RPMI 1640 complete medium supplemented with GM-CSF (20 ng ml−1) and IL-4 (10 ng ml−1). At day 4, culture medium was removed and replaced with fresh GM-CSF and IL-4. BMDCs were collected as immature DCs (iDCs) and matured in fresh medium containing TNF-α (10 ng ml−1) and IL-1β (10 ng ml−1) (BMDMs). In some cultures, iDCs were matured with Curdlan (3 μg ml−1) or Scleroglucan (10 μg ml−1). Cells were matured for 48 h, and at day 9, semi-adherent cells were collected as immature DCs (iDCs) for further experiments or gene expression analysis by flow cytometry and qPCR.

In blocking experiments, iDCs were matured with TNF-α/IL-1β or Curdlan in the presence of a blocking anti-dectin-1 antibody (5 μg ml−1) or control IgG (5 μg ml−1) for 2 days. mDCs were collected for in vitro Th9 cell differentiation. In some experiments, iDCs were generated from WT and dectin-1−/− C57BL/6 mice and matured with TNF-α/IL-1β. Curdlan or Scleroglucan for 2 days. mDCs were collected for further experiments or gene expression analysis by flow cytometry and qPCR.

In some experiments, Piceatannol (40 μM), GW5074 (1 μM) or bortezomib (10 nM) was added to the medium during DC maturation, and cultures with added DMSO (0.1%) Sigma were served as control. Cells were matured for 48 h; and mDCs were collected and analysed by flow cytometry and qPCR. Cytokines in culture supernatants were assessed by ELISA.
Supernatants were analysed by qPCR and ELISA. In some experiments, blocking anti-OX40L mAb (5 μg per well) was added to culture medium. In some experiments, a blocking anti-OX40L mAb (5 μg per well) was added to culture medium.

**In vitro Th9 cell differentiation.** Naive CD4+ T cells (CD4 + CD25 - CD62L+) were obtained from mouse spleen cells by fluorescence-activated cell sorter (FACS) as previously described.10 Naive CD4+ T cells (1 × 10^6 per well) were cultured with BMDCs or dendritic-1-activated DCs (1 × 10^5 per well) in the presence of plate-bound anti-CD3 (2 μg ml⁻¹) plus TGF-β (3 ng ml⁻¹) and IL-4 (10 ng ml⁻¹). Th0 cells were generated without addition of TGF-β and IL-4 in the culture medium. In some experiments, a blocking anti-OX40L mAb (5 μg ml⁻¹) or a neutralization anti-TNFSF15 mAb (5 μg ml⁻¹) were added. After 3 days of culture, cells were collected and analysed for gene expression at the mRNA or protein levels by flow cytometry, qPCR and ELISA.

To examine the Th9-polarizing function of TNFSF15, CD4+ naive T cells (1 × 10^6 per well) were cultured under Th0 or Th9 polarizing conditions in the presence of anti-CD3 and anti-CD28 (2 μg ml⁻¹) and matured with TNF-α and IL-12 (10 ng ml⁻¹) plus TGF-β (5 μg ml⁻¹) or a neutralization anti-TNFSF15 mAb (5 μg ml⁻¹) were added. After 3 days of culture, cells were collected and analysed for gene expression at the mRNA or protein levels by flow cytometry, qPCR and ELISA.

**In vitro differentiation of Th1/2/17/Treg cells.** Sorted naive CD4+ T cells (1 × 10^6 per well) were cultured with DCs (1 × 10^5 per well) in the presence of plate-bound anti-CD3 (1 μg ml⁻¹) plus soluble anti-CD28 (2 μg ml⁻¹) and Th polarizing cytokines: IL-12 (4 ng ml⁻¹) for Th1, IL-4 (10 ng ml⁻¹) for Th2, IL-6 (20 ng ml⁻¹) plus TGF-β (5 μg ml⁻¹) for Th17 and TGF-β (5 μg ml⁻¹) for Treg. IL-12 and IL-6 were purchased from eBioscience. After 3 days of culture, cells were collected and analysed for gene expression by qPCR.

**qPCR and western blot analyses.** qPCR was performed as previously described.10 Total RNA was extracted from cells by using an RNeasy Mini kit (Qiagen) according to the manufacturer’s instructions. The mRNA levels of Dectin1, Il1b, Ifng, Il4, Il5, Il13, Il17, Tnf, Tfrsf15, Ox40l, Sp1, Ifna, Tbx21, Gat3 and Rorc by Th cells or DCs were analysed. Expression was normalized to the expression of the house-keeping gene Gapdh. Primer sets used for these analyses are listed in Supplementary Table 1.

Western blot assay was performed as previously described.10 Anti-mouse phosphorylated (p)-Syk, Syk, pRail, Raf1, p-IERK3/2, 4E-binding protein-2, p50, p55, c-Rel, RelB, p52, β-actin and HDAC1 antibodies were purchased from Cell Signaling Technology (CST). RIPA Buffer (cat #: 9806) was purchased from Thermo Scientific. Images have been cropped for presentation. Full-size images are present in Supplementary Figs 14 and 15.

**Gene-expression profiling.** Immature DCs were generated from Balb/c mice and matured with TNF-α/IL-1β or Curdlan for 48 h. mDCs were collected and stored in Trizol reagent (Invitrogen) at −80°C. All samples were sent to OneArray (http://www.onearray.com.cn/, Beijing, China) for transcription profiling via genome-wide microarrays, and the subsequent data analysis was also performed by OneArray.

**RNA interference.** RNA interference was performed as previously described.10 In brief, day 6 iDCs were transfected with 50 nM siRNA with transfection reagent DharmaFECT (Dharmacon) according to the manufacturer’s protocol. Silencing was confirmed at the mRNA levels by qPCR. On day 8, iDCs were matured with TNF-α/IL-1β or Curdlan for 48 h. On day 10, mDCs were collected and analysed by qPCR and flow cytometry staining. siRNAs used are listed in Supplementary Table 2.

** Luciferase reporter assay.** The luciferase reporter vector pGL4.10, a control vector pGL4.74 and expression vectors for NF-κB molecules p50, p65, c-Rel, p52 and RelB were purchased from Addgene. A 2500-bp mouse Oxs15 promoter and a 2500-bp mouse Oxs15 promoter were separately inserted into pGL4.10 (mOs1040-pGAL4.10 and mTnsfs15-pGAL4.10). HEK293T cells were transiently transfected with mOs1040-pGAL4.10 (0.25 μg per well), mTnsfs15-pGAL4.10 (0.25 μg per well) or pGL4.74 (0.05 μg per well) and expression vectors (0.5 μg per well) for NF-κB molecules by Lipofectamine 2000 (Invitrogen). Promoter activity was measured with Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s instructions. Values are normalized to internal control and expressed as the mean ± s.d. of relative luciferase units.

**In vivo functional tests for dectin-1-activated DCs.** iDCs generated from WT or dectin-1−/− C57BL/6 mice were matured with TNF-α (10 ng ml⁻¹) plus IL-1β (10 ng ml⁻¹) or Curdlan (5 μg ml⁻¹) for 48 h, and pulsed with OT-II OVA peptide (OVA257–264, 10 μg ml⁻¹) for additional 6 h. Treated DCs were collected for mouse immunization. In the therapeutic model, 1 × 10^6 B16-OVA cells were injected subcutaneously into OT-II mice. On day 3 after tumour challenge, mice were randomly divided into groups and immunized subcutaneously with 1 × 10^6 treated DCs. Mice were immunized twice (1 week apart). Mice injected with PBS served as controls. In some experiments, mice were given control IgG or neutralization anti-IL-9 antibody (Zll-9, 100 μg per mouse) every 3 days beginning 1 day after the first DC immunization. Tumour development was monitored over time. Tumour volume was calculated by the formula: 3.14 × (mean diameter)^2/6. Mice were killed when the tumour diameter reached to the range between 1.5 and 2 cm.

**OT-II mice** were challenged with B16-OVA tumour cells and immunized with OVA-peptide-pulsed DCs as described above. On day 3 after DC immunizations, serum samples were collected and total leukocytes from tumour-derived LN and spleens were restimulated with 5 μg ml⁻¹ OVA252–264 peptide and 339 peptide for 24 h. Cytokine expression was analysed by qPCR, ELISA and intracellular staining.

**In vivo therapeutic potency of dCSs in priming therapeutic immunity against naive tumour-specific antigens, a myeloma tumour model.** MPC-11 was used. MPC-11 cells secrete an Id protein (IgG2b) which is a naive tumour-specific antigen. To enhance its immunogenicity, Id protein was conjugated to keyhole limpet hemocyanin (KLH, EMD Biosciences) as described previously.10 iDCs generated from Balb/c mice were pulsed with Id-KLH (50 μg ml⁻¹) for 6 h, and matured with TNF-α/IL-1β or Curdlan for additional 48 h. MPC-11 cells (1 × 10^6) were injected subcutaneously into Balb/c mice. On day 6 after tumour challenge, mice were grouped and immunized subcutaneously with 1 × 10^6 Id-KLH-pulsed DCs. Mice were immunized twice (1 week apart) and tumour development was monitored over time. On day 3 after the second immunization, spleen cells were collected and re-stimulated with Id-KLH-pulsed BMDCs or CurDCs with addition of control IgG or OVA-9 for 48 h.

**Cytotoxicity assay.** The cytotoxicity assay was performed as previously described.10 In brief, Id protein secreted by MPC-11 cells was used as tumour antigen. Balb/c mice (n = 3 per group) were immunized twice (1 week apart) with 1 × 10^6 Id-KLH-pulsed BMDCs or CurDCs. Mice treated with PBS served as control. Mice were given control IgG or an IL-9 neutralizing antibody (Zll-9,100 μg per mouse) every 3 days beginning 1 day after the first DC immunization. On day 2 after the second immunization, spleen cells from the mice were re-stimulated with Id-KLH-pulsed BMDCs for 48 h and used as effector cells. MPC-11 cells labelled with 5 μM CFSE were used as target cells, whereas MOPC-315 labelled with 0.5 μM CFSE were used as non-target cells.

**Statistical analysis.** The Student’s t-test was used to compare various experimental groups. A P value of <0.05 was considered significant.

**Data availability.** The microarray data of BMDCs and CurDCs are stored in the GEO repository and is accessible under the accession number GSE81111. All data are available within the article (as figure source data or Supplementary Information Files) and/or from the authors on request.

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Author contributions
S.W. and Y.Z. initiated the study, designed the experiments and wrote the paper; S.W., Y.Z., X.C., J.C., Y.W., Y.J., X.Z., G.T. and W.Z. performed the experiments and amplified it. S.W. and Q.Y. initiated the study, designed the experiments and wrote the paper; S.W., Y.Z., X.C., J.C., Y.W., Y.J., X.Z., G.T. and W.Z. performed the experiments and statistical analyses; L.Y. and X.M. read and edited the manuscript; L.Y., S.G., H.Y. and H.X. provided critical suggestions to this study.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

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