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Original Article

mKLRL1 regulates the maturation of dendritic cells and plays important roles in immune tolerance

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Abstract: KLRL1 is a member of C-type lectin-like receptors (CLEC) and preferentially expressed on the surface of immune cells. We have previously illustrated its inhibitory role in Natural killer (NK) cells. Though cloned from dendritic cells (DCs), its role in DCs has not been fully identified. Here, we found that mKLRL1 markedly decreased during DC maturation; mKLRL1-modifed DCs showed enhanced phagocytic capability and reduced ability to induce T cell proliferation, which mimics immature DCs. Further investigation revealed that IL-10 was indispensable for mKLRL1 to suppress DC maturation. And p38 activation was responsible for preferential IL-10 production. Pretreatment with mKLRL1-modified DCs protected mice from subsequently EAE induction, indicating a role in immune tolerance. Taken together, our results have revealed an inhibitory role of KLRL1 in mouse DCs.

Keywords: Dendritic cells, inhibitory receptors, C-type lectin-like receptor, immune regulation

Introduction

Receptors expressed on the surface of immune cells play critical roles in immune response. Especially, immune inhibitory and activating receptors can regulate the functional status of many immunocytes such as DCs, NK cells, $CD4^+$ and $CD8^+$ T cells. Recently, more and more attention have been paid to the roles of immune inhibitory receptors [1-3]. Generally, immune inhibitory receptors express one or more immunoreceptor tyrosine-based inhibition motifs (ITIM), and phosphorylation of the tyrosine in the ITIMs is the central signaling event for the function of these receptors. Upon binding to their ligands, inhibitory receptors can recruit SHP-1/2 and/or SHIP proteins which prevent the activation of cellular signaling cascades by protein dephosphorylation [4]. However, not all inhibitory receptors and their downstream signaling have been identified.

Killer cell lectin-like receptors (KLRs) are characterized by the possession of at least one C-type lectin-like domain derived from six conserved cysteine residues [5, 6]. Most of KLR genes are located within a single genetic locus, the natural killer complex (NKC), located on chromosome 12 in humans and chromosome 6 in mouse [7]. Though these genes are located in NKC, their expression and function are not restricted to NK cells. Growing number of these receptors have now been identified on other immune cells such as macrophages, DCs, CTL, $CD4^+$ and $CD8^+$ T cells [8, 9] or even cancer cells [10].

Previously, we have identified a new KLR inhibitory receptor from human and mouse DCs, designated Killer cell Lectin-like Receptors L1 (KLRL1, GenBank accession number AF247788 and NM_177686 for human and mouse respectively) which has also been reported by three other groups earlier or later [11-14]. Both hKLRL1 and mKLRL1 are type II transmembrane proteins with a typical C-type lectin domain and a putative ITIM motif. We previous indicated that KLRL1 was expressed preferentially in lymphoid tissues and immune cells including DCs, NK cells, macrophages, $CD4^+$ and $CD8^+$ T cells. Additionally, we and others have shown that KLRL1 can recruit both

the protein-tyrosine phosphatases SHP-1 and SHP-2 upon phosphorylation of tyrosine located within the ITIM, suggesting that KLRL1 might be involved in negative regulation of certain signals. In addition, we have illustrated that human KLRL1 is an immune inhibitory receptor which can inhibit the cytotoxicy of NK cells [11].

Dendritic cells play critical roles in the initiation of immune responses and tolerance induction [15-17]. Balanced signaling transmitted via different activating and inhibitory receptors in DCs can regulate the functional status of DCs, thus determining the magnitude and duration of T cells responses. Though cloned from DCs [11], the role of KLRL1 in DC maturation and function have not been fully identified.

In this study, we found that the expression of KLRL1 was high on immature DCs while low on mature DCs, which made us presume an inhibitory role in the maturation of DCs. We thus further investigated the function of KLRL1 in DCs and attempted to identify its role in immune responses. KLRL1 expression reduced the maturation of DCs stimulated by LPS, reduced its potential to prime T cell responses. Mechanically, increased IL-10 production was necessary for the negative regulation of DC function by KLRL1. In vivo pretreatment by KLRL1 modified DCs suppressed subsequent EAE induction, indicating a role of KLRL1 in immune tolerance.

Materials and methods

Mice, cell lines and reagents

C57BL/6J (H-2b), BALB/C (H-2d) mice at 6-8 week of age were purchased from Joint Ventures Sipper BK Experimental Animal (Shanghai, China) and maintained in a pathogen-free environment. OT-2 and IL-10$^{-/-}$ mice were obtained from Jackson Laboratory (Bar Harbor, ME) and bred in specific pathogen-free conditions. All animal experiments were undertaken in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, with the approval of the Scientific Investigation Board of Second Military Medical University (Shanghai, China). Unless stated otherwise, cell lines were obtained from the American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 medium or DMEM (PAA Lab) supplemented with 2 mM glutamine, penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% (vol/vol) heat-inactivated fetal calf serum (FCS; Hyclone, Logan, UT) in a 37°C 5% CO_2 atmosphere. MOG_{35-55} peptides were synthesized and purified by the Chinese Peptide Biotechnology; peptide purity was over 95%. Pertussis toxin from Bordetella pertussis (PTX) was purchased from Sigma.

Culture of BMDCs

BMDCs from C57BL/6J mice were generated as described previously [18, 19]. Briefly, bone marrow progenitors were cultured in 10 ng/ml GM-CSF and 1 ng/ml IL-4 (PeproTech, London, U.K). Non-adherent cells were gently washed out on the second day of culture; the remaining loosely adherent clusters were cultured for additional 3~4 days and immature DCs were positively selected using CD11c magnetic microbeads (Miltenyi Biotec). In some experiments, immature DCs were stimulated with 100 ng/ml LPS.

Real-time quantitative PCR

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. The relative expression level of mKLRL1 mRNA in the culture cells was determined by QRT-PCR, conducted with the SYBR green I master mix kit (Applied Biosystems, Foster city, CA) as instructed. Primers used for mKLRL1 were as follows: 5'-ACAACTTTGGCAACAGA-3' (forward) and 5'-ATTCCAGCACATCCTT-3' (reverse). For mouse β-actin mRNA analysis, the primers were 5'-CTGTCCCTGTATGCCTCTG-3' (forward) and 5'-TGTCAC GCACGATTTCC-3' (reverse). QRT-PCR analyses were performed using Roche Light-Cycler (Indianapolis, IN). The relative expression level of mKLRL1 mRNA was normalized to the level of β-actin expression in each sample using $2^{-\Delta\Delta ct}$ cycle threshold method.

N-glycoside F digestion

pmKLRL1/Flag vectors were generated as described before [11]. NIH3T3 cells transiently transfected with pmKLRL1/Flag vectors were lysed in 10 mM sodium phosphate buffer, pH 6.5, containing 0.1% SDS and 50 mM β-mercaptoethanol. To denature the protein, the cell lysates were heated for 5 minutes at 95°C, and then Nonidet P-40 (final concentration,

1%) and protease inhibitor mixture (Sigma) were added. Aliquots of these preparations were treated with N-glycosidase F (2 mU/ml; Takara) for 20 hours at 37°C. Reactions were stopped by the addition of SDS-PAGE loading buffer, and samples were subjected to Western blot analysis.

Western blot assay

The cells were lysed in cell lysis buffer (Cell Signaling Technology) supplemented with protease inhibitor mixture (Calbiochem, San Diego, CA) for 20 min on ice. The protein concentration was determined by BCA assay (Pierce, Rockford, IL). Equal amounts of extracts were loaded on each lane of 10% SDS-PAGE, electrophoresed, and transferred to nitrocellulose membrane (Schleicher & Schuell BioScience). Membrane was blocked in 1 × TBST with 5% nonfat milk for 2 h and hybridized with primary antibodies at 1/1000 overnight at 4°C. After three washes with 1 × TBST for a total of 30 min, the membrane was incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. SuperSignal West Femto Maximum Sensitivity substrate (Pierce) was used for the chemiluminescent detection. Anti-mouse MICL/CLEC12A (KLRL1) antibody was purchased from R&D. Antibodies specific to ERK1/2, p38, JNK, TLR4, IκBα, IKKα/β, phosphor-ERK1/2, phospho-p38, phospho-JNK, β-actin, phospho-IκBα, phospho-IKKα/β, and their respective horseradish peroxidase-coupled secondary antibodies were purchased from Cell Signaling Technology.

mKLRL1 recombinant adenovirus construction and genetic modification of DCs

Coding region of full-length of mKLRL1 (primes were: 5'-GCGGTACCATGTCTGAAGAAATTGT-3' and 5'-CCGAAGCTTCTACCTGCTATCCTCTG-3') was cloned into the frame of pShuttle-CMV 1.0 vector (Stratagene), with pShuttle-CMV 1.0 vector as negative control, then linearized using Pme I (New England BioLabs) before co-transformation of BJ5183 bacteria with pAdeasy-1. The recombinant plasmids were digested with Pac I (New England BioLabs) before transfection of the HEK293 packaging cell line, incubated for 10 days when detached cells were observed in the growth medium, and then were harvested and subjected to three rounds of freeze/thaw to prepare the primary viral stocks. The recombinant adenovirus were purified by Vivapure AdenoPACK 100RT (Sartorius) and amplification of a virus stock is achieved by infection of HEK293 cultures with a low passage virus stock. Immature DCs were transfected with mKLRL1 recombinant adenovirus (Ad-mKLRL1) or recombinant control adenovirus (Ad-ctrl) at a MOI of 1:100 for 6 hours in serum-free RPMI1640, then washed twice, and cultured in fresh DC medium for functional detection.

Adenovirus-mediated RNA interference

Three pairs of small interfering RNA (siRNA) oligonucleotides specific for mKLRL1, were synthesized and transfected into RAW264.7 cells using LipofectAMINE 2000 (Invitrogen). Silencing efficiency was confirmed by RT-PCR and the one which has the highest silencing efficiency (mKLRL1-siRNA: ACCAGAGCACAAAUGUAAAtt) was used to construct recombinant interfering adenovirus. Recombinant mKLRL1 siRNA adenoviruses (Ad-Si-mKLRL1) or nonsilensing control adenoviruses (Ad-Si-ctrl) were generated using the pSilencer adeno 1.0-CMV System (Ambion, Austin, TX) as instructed. For infection, cells were exposed to adenovirus for 6 hours, then were washed with PBS and incubated in fresh medium.

Analysis of phenotypes, cytokines production and phagocytic capability

For cell surface marker analysis, cells were incubated for 15 min at 4°C with PE-conjugated mAbs to CD40, FITC-conjugated mAbs to CD80, CD86 (BD Pharmingen) in labeling solution, and then assayed by flow cytometry conducted on FACSCalibur (BD Biosciences), and the data were analyzed with CellQuest Version 3.3 software (BD Biosciences). IL-10, TNF-α, IL-6, IL-12p70, and IL-1β levels in the supernatants were measured with ELISA Kits (R&D Systems, Minneapolis, MN) according to the manufacturer's protocols. The phagocytic capability of DCs was assessed by uptake of FITC-conjugated OVA (Invitrogen) at a final concentration of 100 μg/ml in RPMI1640 containing 10% FCS at 37°C for 4 h, then washed with cold PBS twice and assayed by flow cytometry. Data were analyzed by CellQuest Version 3.3 software. Cells incubated with OVA-FITC at 4°C were used as a negative control.

Allogeneic mixed lymphocyte reaction (MLR) and antigen presentation assay

Immature DCs from C57BL/6J mice were transfected with Ad-mKLRL1, Ad-ctrl or not for 6 h, then washed with cold PBS to remove redundant adenovirus in the medium and cultured for 24 hours, and stimulated with LPS for 24 hours. DCs (2×10^4/well) were then co-cultured with $CD4^+$ T cells purified from BALB/C mice splenocytes (2×10^5/well) for 5 days. Cells were harvested and double-stained with CD4-FITC and 7-AAD, then resuspended in 200 μl PBS. The number of $CD4^+$ $7\text{-}AAD^-$ cells was calculated by flow cytometry. To detect the ability of antigen presentation, splenic $CD4^+$ T cells from OT-2 mice were positively selected by MACS for use as antigen-specific responders, then co-cultured with DCs in the presence of $OVA_{323-339}$ peptide at a ratio of 1:10 (DC/T) in round-bottom 96-well plates (1×10^5 T cells/200 μl/well) for 5 days. The total number of live $CD4^+$ T cells in each well was measured by flow cytometry.

Assay for effect of mKLRL1-modified DCs on EAE

Immature DCs cultured for 5 days were seeded at a density of 2×10^6/well and were transfected with Ad-mKLRL1, Ad-ctrl for 6 hours, then washed gently to remove the redundant adenovirus in the medium and cultured for 24 hours, then stimulated with 100 ng/ml LPS for 24 hours. After loading MOG_{35-55} peptides, the cells were intraperitoneally injected C57BL/6J mice (2×10^6 cells per 0.5 ml/mice). After 24 hours, mice were challenged to EAE induction and the disease development was observed.

Induction and evaluation of EAE

Acute EAE was induced in C57BL/6J mice at 6-8 weeks of age by subcutaneous immunization with 300 μg of the MOG_{35-55} peptide in complete Fruend's adjuvant containing heat-killed Bacillus Calmette-Guerin vaccine (BCG, 10 mg/ml). Pertussis toxin (PTX, 400 ng/mouse; Sigma) in PBS was administered intraperitoneally on the day of immunization and 48 hours later. Mice were weighed and examined daily for disease symptoms; they were assigned scores for disease severity with the following EAE scoring scale: 0, no clinical signs; 1, limp tail; 2, paraparesis (weakness, incomplete paralysis of one or two hindlimbs); 3, paraplegia (complete paralysis of two hindlimbs); 4, paraplegia with forelimb weakness or paralysis; and 5, moribund state or death.

Statistical analysis

Data were compared using the Student *t* test. A P value <0.05 was considered statistically significant.

Results

mKLRL1 protein is glycosylated and down-regulated during DC maturation

To detect the expression of mKLRL1 protein, pmKLRL1/Flag recombinant vectors were transiently transfected into NIH3T3 cells and examined with anti-mouse MICL/CLEC12A (mKLRL1) antibody. As shown in **Figure 1A**, two approximately 42 kDa and 38 kDa protein bands were observed. Both of the apparent molecular masses were considerably larger than that predicted from the amino acid sequence, indicating that mKLRL1 protein was likely to be modified post translation. In the previous study, we found that hKLRL1 is a glycoprotein which contains 6 putative N-glycosylation sites. According to its sequence analysis, mKLRL1 also contains 4 putative *N*-glycosylation sites within the stalk and C-type lectin domain (data not shown). Therefore, the cell lysates of NIH3T3 cells above were then treated with peptide *N*-glycosidase F. In accord with our presumption, the apparent molecular mass of the protein was reduced to about 32 kDa, consistent with the calculated molecular mass of mKLRL1 protein and showed only one prominent band (**Figure 1A**).

As we previously reported, mKLRL1 is expressed in mouse bone marrow-derived DCs, NK cells, $CD4^+$ T cells, $CD8^+$ T cells and macrophages [11]. DCs play unique roles in the immune responses. Therefore, the expression of mKLRL1 during DC maturation was further monitored by real-time quantitative PCR assay. Our results revealed that the expression of mKLRL1 markedly decreased during maturation of BMDCs in vitro (**Figure 1B**). Moreover, upon LPS stimulation, the expression of mKLRL1 was significantly down-regulated. CpG stimulation could also down-regulate the expression level of mKLRL1 on DCs, though with smaller extent than LPS stimulation (**Figure 1C**). These results indicated that mKLRL1 pro-

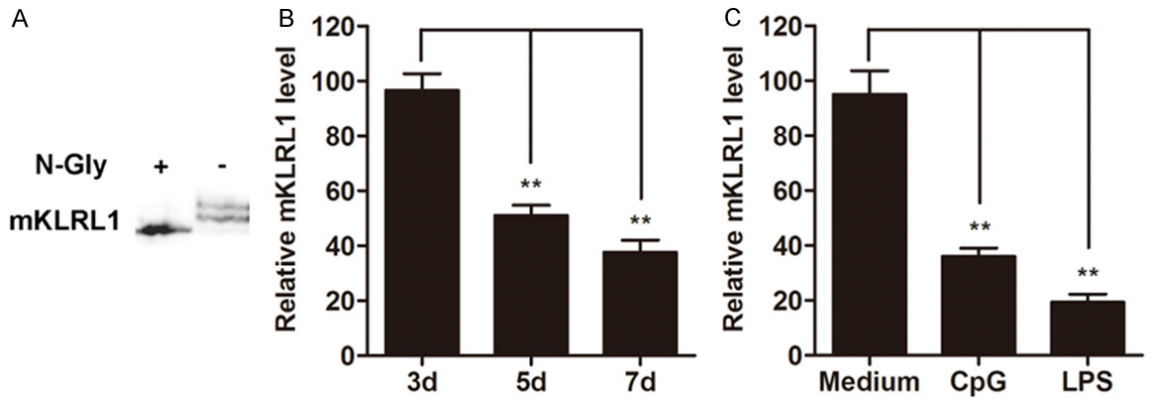

Figure 1. mKLRL1 is glycosylated and down-regulated during DC maturation. A. Immunoblot annlysis of lysates treated with N-glycosidase F (N-Gly) or not from NIH3T3 cells transfected with Flag-tagged mKLRL1. B. Quantiative PCR analysis of mKLRL1 mRNA from BMDCs at different days during culture. C. Quantitative PCR analysis of mKLRL1 mRNA from BMDCs stimulated with LPS or CpG for 24 h. Data shown are means ± SD. Similar results were obtained in at least three independent experiments. $^{**}P<0.01$.

tein might be differently glycosylated and the expression of mKLRL1 was closely related to the maturation and activation of DCs.

mKLRL1 negatively regulates the function of DCs upon LPS stimulation

DCs play critical roles in the initiation of immune response and induction of tolerance. Our above results demonstrated that the expression of mKLRL1 greatly decreased during maturation of DCs especially after LPS stimulation, suggesting that mKLRL1 may play inhibitory role during DC maturation. To further investigate the role of mKLRL1, a recombinant adenovirus expressing mKLRL1 (Ad-mKLRL1) was constructed. Immature DCs were then transfected with Ad-mKLRL1 or Ad-ctrl, as shown in **Figure 2A**, the expression level of mKLRL1 in Ad-mKLRL1 transfected DCs was significantly increased.

Since the expression level of mKLRL1 was greatly decreased during DC maturation, we wondered whether mKLRL1 could regulate the expression of co-stimulatory molecules. In contrast to Ad-ctrl transfected DCs, the expression of CD80 and CD86 were decreased on mKLRL1-modified DCs upon LPS stimulation; however, CD40 expression was not significantly changed (**Figure 2B**). We then tested the phagocytic capability of KLRL1-modificed DCs by flow cytometry. As shown in **Figure 2C**, Ad-mKLRL1 transfected DCs have much stronger phagocytic capability than control DCs. In contrast, the proliferation of allogeneic T cells stimulated with mKLRL1-modified DCs was significantly decreased in an MLR assay, as compared with Ad-ctrl transfected DCs (**Figure 2D**). To further investigate the role of mKLRL1 in antigen-specific immune response, OT-2 cells were used as responders and mKLRL1-modified DC as stimulators. After 5 days stimulation in the presence of $OVA_{323-329}$ peptides, mKLRL1-modified DCs showed impaired ability to promote Ag-specific response, as determined by the decreased proliferation of T cells (**Figure 2E**). All of these results indicated that mKLRL1-modified DCs might resist LPS-induced maturation.

We further examined the impact of mKLRL1 over-expression on cytokines production. mKLRL1-modified DCs secreted high level of IL-10 and low level of TNF-α upon LPS stimulation (**Figure 2F**). However, we did not detect any significant changes in the secretion of IL-6, IL-1β or IL-12 as a result of mKLRL1 over-expression (data not shown). We also examined the production of NO and no marked change was found between mKLRL1-modified DC and Ad-ctrl transfected DCs (data not shown). Taken together, all of these results indicated that, mKLRL1-modified DCs exhibited some distinguishing features which belong to immature DCs, suggesting that mKLRL1 may negatively regulate the function of DCs and probably play a regulatory role during DC maturation.

Preferential IL-10 production is important for mKLRL1 to negatively regulate the function of DCs

Our above data have shown KLRL1 promotes IL-10 production from DCs. IL-10 is an important regulatory cytokine which is involved in

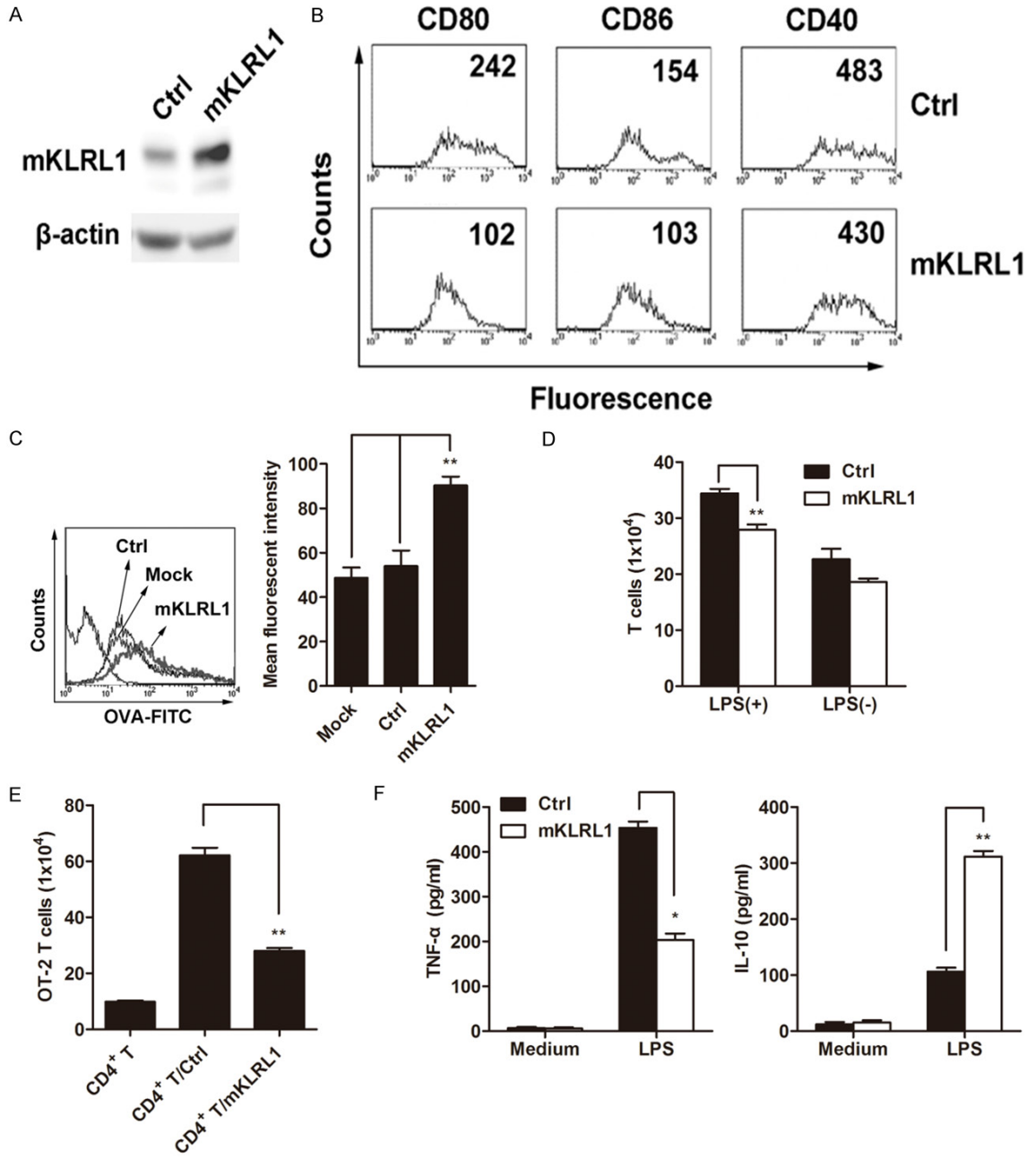

Figure 2. mKLRL1 negatively regulates the maturation of DCs. A. Immunoblot analysis of mKLRL1 in BMDCs transfected with Ad-mKLRL1 or Ad-ctrl. B. Flow cytometry analysis of surface markers (CD80, CD86, CD40) on Ad-mKLRL1 or Ad-ctrl modified BMDCs stimulated with LPS for 24 h. Numbers in histograms indicate the geometric mean fluorescence in each group. C. Ad-mKLRL1 or Ad-ctrl modified BMDCs were cultured with FITC-conjugated OVA for 4 h and phagocytic capability were assessed by flow cytomertry. D. Ad-mKLRL1 or Ad-ctrl modified BMDCs were co-cultured with allogenic $CD4^+$ T cells for 5 days and the total numbers of $CD4^+$ T cells were measured. E. Ad-mKLRL1 or Ad-ctrl modified BMDCs were co-cultured with $CD4^+$ T cells from OT-2 mice in the presence of $OVA_{323-329}$ peptide for 5 days and the total numbers of $CD4^+$ T cells were measured. F. ELSIA analysis of IL-10 and TNF-α in Ad-mKLRL1 or Ad-ctrl modified BMDCs treated with medium alone or stimulated with LPS. Data shown are means ± SD, and represent one of at least three independent experiments with similar results. **P*<0.05, ***P*<0.01.

immune suppression. It is also reported that autocrine IL-10 serves as a potent mechanism in limiting the maturation of monocyte-derived DCs and their capacity to initiate Th1 responses. Therefore, we presumed that mKLRL1 might regulate the function of DCs by autocrine

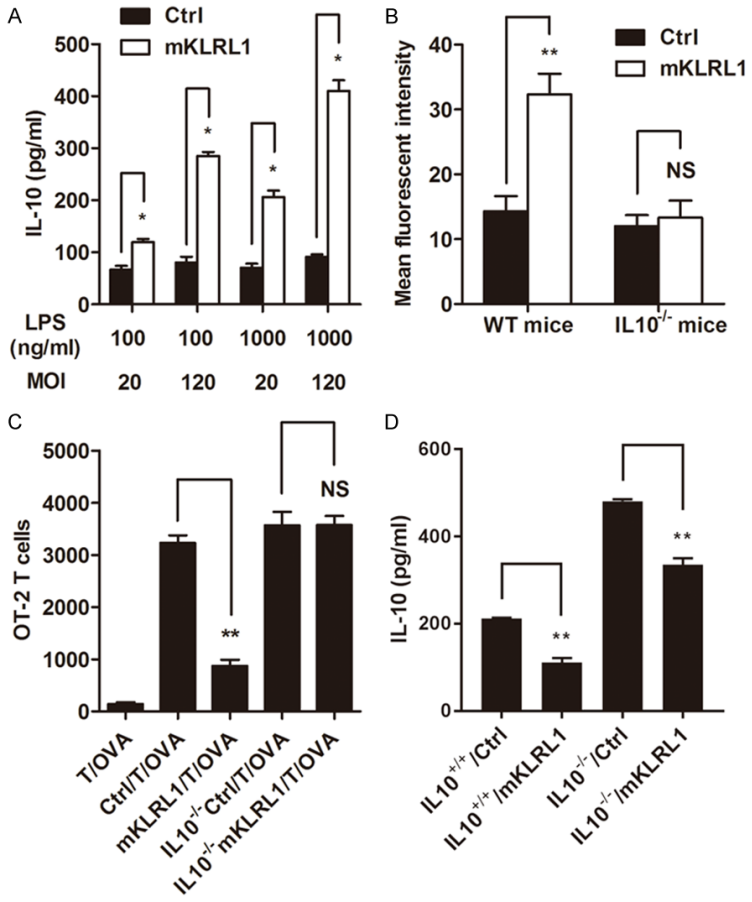

Figure 3. IL-10 is necessary for mKLRL1 to negatively regulate DC function. A. ELISA analysis of IL-10 in Ad-mKLRL1 or Ad-ctrl modified (MOI=20 or 120) BMDCs stimulated with 100 ng/ml or 1 μg/ml LPS. B. Ad-mKLRL1 or Ad-ctrl modified BMDCs from wild-type (WT) mice or IL-10$^{-/-}$ mice were cultured with FITC-conjugated OVA for 4 h and phagocytic capability were assessed by flow cytometry. C. Ad-mKLRL1 or Ad-ctrl modified BMDCs from WT mice or IL10$^{-/-}$ mice were co-cultured with CD4^{+} T cells from OT-2 mice presence of $OVA_{323-329}$ peptide for 5 days and the total numbers of CD4^{+} T cells were measured. D. ELISA analysis of IL-10 in Ad-mKLRL1 or Ad-ctrl modified BMDCs from WT mice or IL10$^{-/-}$ mice. Data shown were the geometric mean fluorescence of each test sample. Data shown are means ± SD, and representative of at least three independent experiments with similar results. $^{**}P<0.01$, NS indicates not significant.

IL-10. As shown in **Figure 3A**, compared with Ad-ctrl transfected DCs, mKLRL1-modified DCs produced high level of IL-10 dose-dependently, and also had positive correlation with multiplicity of infection (MOI) of the recombinant adenovirus infection.

To further investigate the significance of IL-10 for the inhibitory role of mKLRL1 on DCs, we then compared the biological functions of mKLRL1-modified DCs from wild-type and IL-10 deficient C57BL/6J mice. As shown in **Figure 3B**, mKLRL1-modified DCs from wide-type mice showed much stronger phagocytic capability than the corresponding Ad-ctrl group, whereas mKLRL1-modified DCs from IL-10 deficient mice had a comparable phagocytic capability with Ad-ctrl transfected DCs, indicating an important role of IL-10 in regulating the phagocytic capability of DCs by mKLRL1.

We also examined the role of IL-10 in the T cell priming ability of DCs regulated by mKLRL1. As shown in **Figure 3C**, compared with Ad-ctrl transfected DCs, mKLRL1-modified DCs from wide-type mice had impaired ability to induce OVA-specific CD4^{+} T cells proliferation, whereas there was no significant difference between DCs from IL-10 deficient mice.

We further detected the TNF expression (**Figure 3D**) in Ad-mKLRL1 or Ad-ctrl modified IL10$^{-/-}$ BMDCs compared with WT BMDCs. We found that IL-10 knockout lead to conspicuous upregulation of TNF expression in BMDCs stimulated with LPS. Ad-mKLRL1 modification still can inhibit TNF expression in IL10$^{-/-}$ BMDCs but the suppressive effect is weaker than that in WT cells. To sum up, all of these results demonstrated that IL-10 might be a vital effector molecule for mKLRL1 to display its negative regulatory role on DCs.

Increased p38 MAPK activation is necessary for the preferential expression of IL-10 in mKLRL1-modified DCs

To further investigate the signal pathways involved in the production of IL-10 by mKLRL1-modified DCs, we then examined the activation of some signal pathways. First, the increased secretion of IL-10 by mKLRL1-modified DCs was LPS-stimulation dependent (**Figure 4A**).

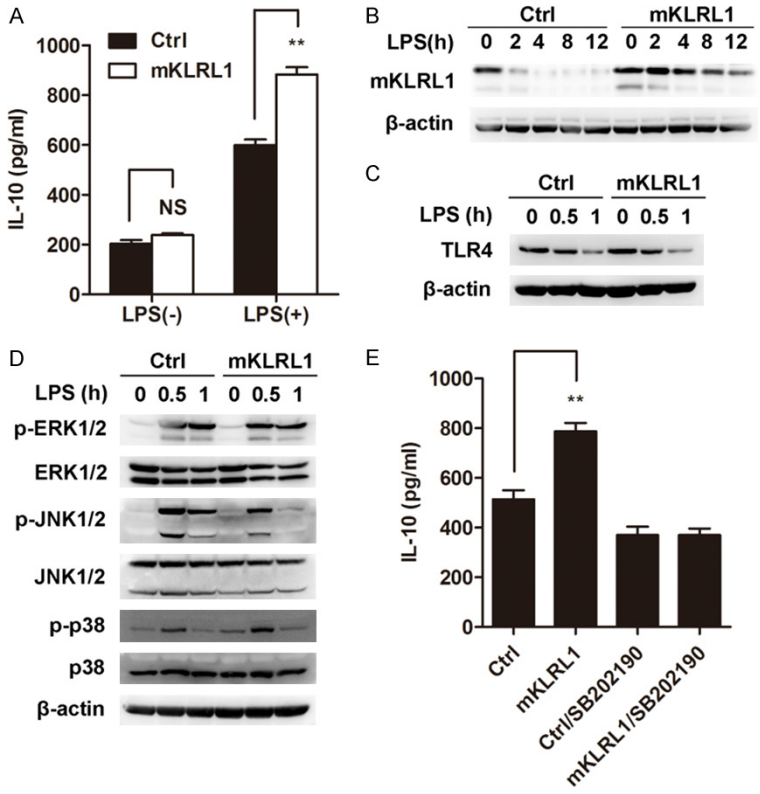

Figure 4. Increased p38 activation is necessary for mKLRL1 induced IL-10 expression. A. ELISA analysis of IL-10 in Ad-mKLRL1 or Ad-ctrl modified BMDCs stimulated with LPS or not. B. Immunoblot analysis of KLRL1 expression in Ad-mKLRL1 or Ad-ctrl modified DCs post LPS stimulation. C, D. Immunoblot analysis of TLR4 (C) p38 (p-p38), JNK1/2 (p-JNK1/2) and ERK1/2 (p-ERK1/2) (D) in Ad-mKLRL1 or Ad-ctrl modified BMDCs stimulated with LPS at different time points. E. ELISA analysis of IL-10 in Ad-mKLRL1 or Ad-ctrl modified BMDCs stimulated with LPS in the presence of p38 inhibitor SB202190 or not. Data shown are means ± SD, and represent one of at least three independent experiments with similar results. $^{**}P<0.01$, NS indicates not significant.

mKLRL1 is constitutively expressed in DCs and LPS stimulation can dramatically down-regulate mKLRL1 expression (**Figure 4B**). We then examined the expression and phosphorylation of some inter-mediators of TLR4 signaling in LPS-stimulated DCs, in order to find the pathways responsible for preferential IL-10 production. We found that TLR4 was comparably expressed in mKLRL1-modified DCs and Ad-ctrl transfected DCs both before and after LPS stimulation (**Figure 4C**), excluding KLRL1 regulates DC function through TLR4 expression.

MAPKs, including ERK, JNK, and p38, can be activated by LPS stimulation and play different roles in IL-10 production in DCs. We wondered whether ERK, JNK, and p38 are differentially regulated in mKLRL1-modified DCs. As shown in **Figure 4D**, upon LPS stimulation, ERK1/2 was comparably activated in both mKLRL1-modifed DCs and Ad-ctrl transfected DCs; however, compared with Ad-ctrl transfected DCs, mKLRL1-modified DCs expressed a lower level of activated JNK1/2 but a higher level of activated p38 upon LPS stimulation, suggesting that these differently regulated pathways might be involved in the regulatory role of mKLRL1 in DCs.

To further examine whether the activated p38 pathway was responsible for the preferential IL-10 production in mKLRL1-modifed DCs, cells were treated with SB202190, an inhibitor for p38 pathway. We found that SB202190 pretreatment inhibited IL-10 production in mKLRL1-modified DCs (**Figure 4E**), indicating that increased p38 activation is likely necessary for mKLRL1-modifed DCs to produce more IL-10 upon LPS stimulation.

mKLRL1 negatively regulates LPS-induced NF-κB activation

NF-κB pathway plays an important role in inflammatory reaction. We then tested the activation of NF-κB pathway in mKLRL1-modified DCs. Our results showed that, compared with Ad-ctrl transfected DCs, the phosphorylation of I-κB in mKLRL1-modified DCs was suppressed. Similar results were obtained in the detection of IKKα/β kinase activation (**Figure 5A**). As IL-10 was necessary for KLRL1 mediated inhibition of DC phagocytosis, T cell activation and TNF production. So we further explored the impact of IL-10 in NF-κB pathway. As shown in **Figure 5B** mKLRL1 modification still diminished the phosphorylation of I-κB and IKKα/β but the effect was not as remarkable as in WT cells. So we thought KLRL1 mediated inhibition of NF-κB pathway was partially dependent on IL-10.

To confirm the negative role of mKLRL1 on NF-κB activation, we constructed mKLRL1 interfering RNA-expressing recombinant adeno-

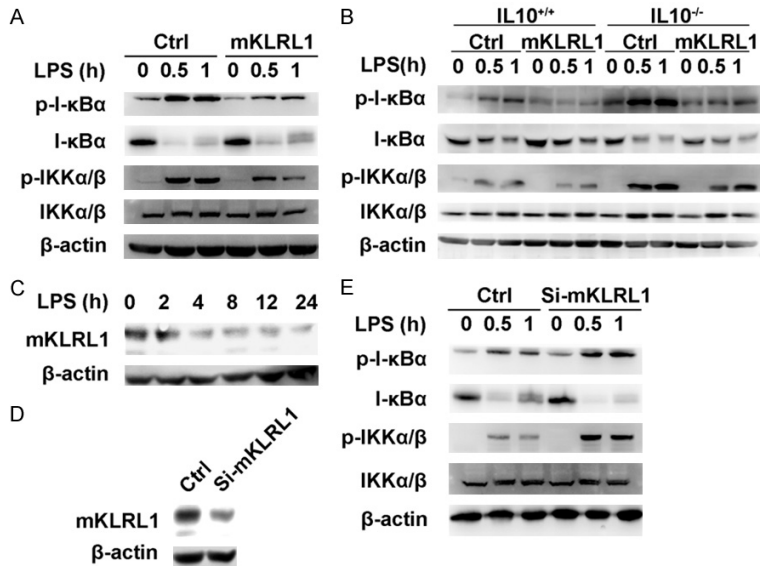

Figure 5. mKLRL1 negatively regulates LPS-induced NF-κB activation. A. Immunoblot analysis of I-κBα (p-I-κBα) and IKKα/β (p-IKKα/β) in Ad-mKLRL1 or Ad-ctrl modified BMDCs stimulated with LPS at different time points. B. Immunoblot analysis of I-κBα (p-I-κBα) and IKKα/β (p-IKKα/β) in Ad-mKLRL1 or Ad-ctrl modified BMDCs from WT or IL10$^{-/-}$ mice stimulated with LPS at different time points. C. Immunoblot analysis of mKLRL1 in Raw264.7 cells stimulated with LPS at different time points. D. Immunoblot analysis of mKLRL1 in Raw264.7 cells transfected with Ad-Si-mKLRL1 or Ad-Si-ctrl. E. Immunoblot analysis of I-κBα (p-I-κBα) and IKKα/β (p-IKKα/β) in Ad-Si-mKLRL1 or Ad-Si-ctrl modified Raw264.7 cells stimulated with LPS at different time points. Data shown represent one of at least three independent experiments with similar results.

virus (Ad-Si-mKLRL1). Since mKLRL1 expression was strongly decreased during DC maturation while adenovirus transfection could stimulate DC maturation; therefore, we use RAW264.7 cells to detect the activation of NF-κB pathway after mKLRL1 silencing. We found that LPS stimulation could also decrease mKLRL1 expression in RAW264.7 cells (**Figure 5C**). mKLRL1 expression in Ad-Si-mKLRL1 transfected RAW264.7 cells was decreased significantly as compared with that in Ad-Si-ctrl transfected cells (**Figure 5D**). We then transfected RAW264.7 cells with Ad-Si-mKLRL1 or Ad-Si-ctrl, and found that the phosphorylations of I-κB and IKKα/β kinase were increased in mKLRL1-silenced RAW264.7 cells after stimulated with LPS (**Figure 5E**). Taken together, these results indicated a negatively regulatory role of mKLRL1 on activation of NF-κB signal pathway and mKLRL1-modified DCs might restrict LPS-induced maturation by suppressing NF-κB activation.

mKLRL1 in the induction of antigen-specific immune tolerance

The maturation state of DCs plays a role in determining the immune response or tolerance. Our study on mKLRL1 showed that mKLRL1 expression was greatly decreased during DC maturation, and mKLRL1-modified DCs had stronger phagocytic capability but impaired ability to induce T cell proliferation, which mostly mimics the characteristic of immature DCs. It has been shown that immature DCs could initiate T cell tolerance directly. We thus presumed that mKLRL1 expression on DCs might have much significance in the direction of immune response, and mKLRL1 might play a role in the induction of immune tolerance.

To verify this hypothesis, we then observed the protective effect of mKLRL1-modified DCs on EAE induction. Immature DCs were transfected with Ad-mKLRL1 or Ad-ctrl, and stimulated with LPS for 24 hours, then pulsed with MOG_{35-55} peptides and injected into wide-type C57BL/6 mice i.p. One day after injection, we induced EAE in these mice and observed the disease development. As shown in **Figure 6A**, a markedly delayed onset and a generally lower grade disease were observed in mice pretreated with mKLRL1-modified DCs after immunized with MOG_{35-55} peptide. In addition, mice pretreated with Ad-ctrl transfected DCs showed a decrease in mean body weight after EAE induction, whereas mice pretreated with mKLRL1-modified DCs showed little change (**Figure 6B**).

To further detect the proliferation of antigen-specific T cell in vivo, we then obtained the spleens and lymph nodes from each groups 14 days post EAE induction. As shown in **Figure 6C**, spleens from mice pretreated with Ad-ctrl transfected DCs obviously swelled compared with those from mice pretreated with mKLRL1-

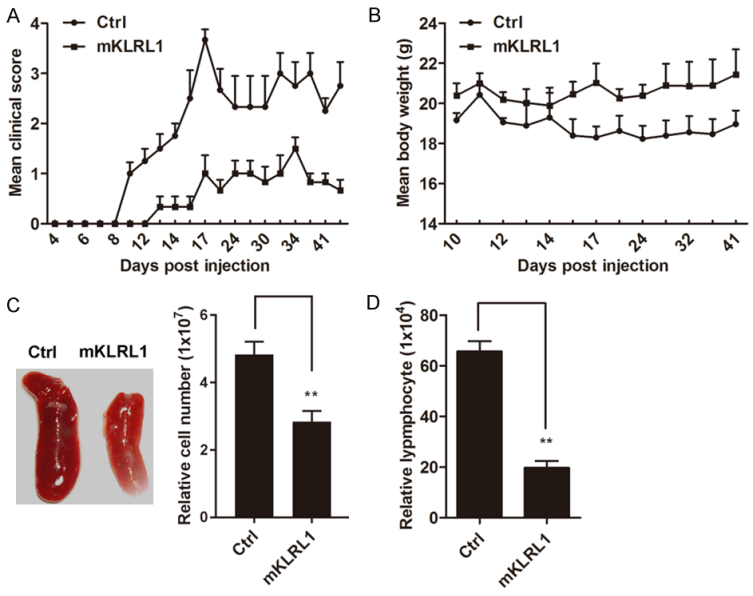

Figure 6. Mice pretreated with mKLRL1-modified DCs are protected from EAE. A. EAE scores of mice injected with Ad-mKLRL1 or Ad-ctrl modified BMDCs (n=6). B. Mean body weight of mice after EAE induction in (A). C. Spleens and total number of splenic cells in mice 14 days after EAE induction (n=3 mice per group). D. Lymphocytes from lymph nodes in mice after 14 days EAE induction were stimulated with MOG_{33-55} and their proliferation was evaluated by cell counting (n=3 per group). Data shown are means ± SD, and represent one of at least three independent experiments with similar results. $^{**}P<0.01$.

modified DCs. Lymphocytes from lymph nodes of each group of mice were obtained and stimulated with MOG_{35-55} peptide, and their proliferation was then evaluated. We found that, MOG_{35-55} stimulation could greatly increase the proliferation of lymphocytes from mice pretreated with Ad-ctrl transfected DCs more than those from mice pretreated with mKLRL1-modified DCs in vitro (**Figure 6D**). Taken together, these results indicated that mice pretreated with mKLRL1-modified DCs could be protected from subsequently EAE induction, and the inhibitory receptor mKLRL1 might play a role in the induction of antigen-specific immune tolerance through regulating the maturation of DCs.

Discussion

In recent years, a growing number of killer cell lectin-like receptors (KLRs) have been identified, both of human and mouse origin [5]. In contrast to their NK counterparts that primarily control cellular activation through recognition of MHC class I and related molecules, these receptors appear to have a far more diverse range of functions and ligands [20]. Some of KLRs possess activating or inhibitory signaling motifs that trigger downstream signaling events, suggesting the role for these receptors as positive or negative regulators.

Generally, KLRs family include Ly49 (KLRA), NKR-P1 (KLRB), CD94/NKG2 (KLRD/KLRC), KLRE1, KLRF-1, MAFA-1 (KLRG1), KLRH1 and NKG2D (KLRK). The Ly49 family of lectin-like receptors were the first class I-binding receptors to be characterized in mouse NK cells, and contain both stimulatory and inhibitory receptors. The NKR-P1 (KLRB) family also consists of both activating and inhibitory members [21]. The CD94/NKG2A heterodimer constitutes an inhibitory receptor that has been proposed to be involved in the recognition of a wide variety of HLA-A, -B, and -C allotypes [22]. However, the association of CD94 with other NKG2 proteins lacking ITIMs (i.e., NKG2C) may form receptors with activating function [23]. KLRE1 does not contain an ITIM in the cytoplasmic tail in its predicted proteins, but may form a functional heterodimer with a yet unidentified ITIM-bearing partner to generate an inhibitory receptor complex and inhibit immune function [24]. KLRF-1, also called NKp80, contains two non-typical tyrosine based motifs in the cytoplasmic tail and may recognize an unidentified ligand on T cells. Stimulation of NK cells with anti-KLRF-1 antibodies induces cytolytic activity and Ca^{2+} mobilization [25]. KLRG1 has an ITIM in its cytoplasmic domain, and mAb-mediated cross-linking of KLRG1 inhibits NK cell function [26, 27]. The function of KLRH1 is unknown; however, it also contains an ITIM which indicates an inhibitory function [28]. For many members of KLR family, the natural ligands are unknown and their biological functions have rarely been investigated in detail.

In our previous study, we have identified a new inhibitory receptor from human and mouse DCs [11], designated KLRL1 which is identical to MICL, DCAL-2 and Clec12A reported by three

other groups earlier or later [12-14]. KLRL1 is a type II transmembrane protein with an immunoreceptor tyrosine-based inhibitory motif (ITIM) and a C-type lectin-like domain. The KLRL1 gene is located in the central region of the NK gene complex (NKC) in both human and mice. Our previous studies indicated that both hKLRL1 and mKLRL1 are preferentially expressed in lymphoid tissues and immune cells and we also found that, as a novel NK cells receptor, hKLRL1 could inhibit natural killer cell cytotoxicity.

Although NKC-encoding receptors are mostly expressed on NK cells and mainly involved in their functional regulation, these receptors are found widely distributed on other immune cells which indicate a more important role for them [20]. DCs play a vital regulatory role in immune response, and accumulating evidences have demonstrated that integration of inhibitory and activating signals in DCs can instruct DC differentiation, maturation, and activation [29]. The balance between divergent receptors establishes a threshold of DC activation and allows for homeostasis between induction of tolerance or immunity [30]. Interference with unilateral signaling always would result in an altered balance of signals on DCs, which in turn affects the maturation and function of the DC themselves [31-33]. As an inhibitory receptor, the expression of mKLRL1 markedly decreased during DC maturation. Interestingly, others have shown that MICL (identical to KLRL1) is down-regulated on activated leukocytes following recruitment to a focus of acute inflammation in vivo [14]. This results and ours are both consistent with the hypothesis that activation can be facilitated by down-regulating or removal of inhibitory molecules.

LPS-stimulated mKLRL1-modified DCs secreted high level of IL-10 and low level of TNF-α. IL-10 is one of important immunomodulatory cytokines and generally considered to limit immune and inflammatory responses [34]. IL-10 has also been identified as a major factor that can strongly inhibit DC maturation induced by different stimuli [35]. Some previous studies have suggested that autocrine IL-10 is important in maintaining DCs in an immature state and serves as a potent mechanism for limiting the maturation of monocyte-derived DCs and their capacity to initiate Th1 responses [36, 37]. Our results indicated that inhibitory receptor mKLRL1 might negatively regulate the maturation and function of DCs by increasing IL-10 production through promoting activation of p38. In accordance, several recent reports also revealed the activation of MAPK p38 are responsible for the enhanced production of IL-10 [35, 38-40].

It is shown that the direction of the immune response toward immunity or tolerance mainly depends on the stage of maturation and functional properties of DCs [41]. DCs with immature characteristics are tolerogenic to T cells, while mature DCs are immunogenic [42]. In our experiments, mKLRL1-modified DCs upon LPS stimulation showed many characteristics similar with those of immature DCs, including higher endocytic capability, lower expression levels of costimulatory molecules and less efficient T cell priming. With EAE model, we further observed that, mice pretreated with mKLRL1-modified DCs could be protected from subsequently EAE induction to a certain extent which was exhibited by markedly delayed onset and the improved nervous system function, suggesting that mKLRL1 probably plays a role in the induction and maintenance of antigen-specific immune tolerance.

Actually, Chen et al [13] had described the suppressive role of mKLRL1 (DCAL-2) in human DC maturation and cytokine production. In the study they utilized anti-DCAL-2 mAb to crosslink DCAL-2 on iDCs. Antibody mediated crosslink can definitely activate downstream signal pathways, and may exert certain biological function in these cells. However, this may be artificial signals and too strong than which really happens in vivo, as the paucity knowledge of its physiological ligands. In addition, besides act as receptors, mKLRL1 may also be activated by other outside-in signals and mediate inside-out signals as integrins, or act as docking site for other signal molecules. In this study, we made use of adenovirus infection to modify the expression of mKLRL1 to study its role in DC maturation. Overexpression of mKLRL1 could facilitate the interaction between mKLRL1 and its ligands, and accumulate and prolong the outside-in/inside-out signals by KLRL1, as it is quickly downregulated after LPS stimulation. Ligation of DCAL-2 indeed changed some phenotypes of DC with or with-

out TLR-ligands stimulation. By overexpression of mKLRL1, we observed some accordant phenomenon such as reduced DC maturation and enhanced IL-10 production as DCAL2 cross-linking in human DCs. However, anti-DCAL-2 stimulation induced modest MAPK pathway activation and cytokines production, while overexpression of mKLRL1 reduced activation of Jnk signals but unregulated p38 activation after LPS stimulation. We don't have enough data to conclude if ligation of mKLRL1 was required for its suppressive function. Antibody mediated ligation or overexpression of mKLRL1 may both cause complicated cross-talk with other signal pathways and it deserve further research.

Recently, great attentions have been paid to the receptors on DCs especially those inhibitory receptors which have been reported to play a regulatory role in determining DC maturation. It has been demonstrated that the antigen-presenting function of monocytes and dendritic cells is modulated by the expression of ILT-3 and ILT-4 [43], and ILT-4 could also alter maturation of dendritic cells [44]. Inhibitory receptors ILT3 and ILT4 belong to immunoglobulin superfamily which are structurally and functionally related to killer cell-inhibitory receptors. Another inhibitory receptor on DCs, FcγRIIb was highly expressed in immature DCs, and markedly down-regulated in mature DCs [45, 46], which mostly resembles our KLRL1. Interestingly, some studies have also indicated that FcγRIIb is important in the maintenance of tolerance [45, 46]. Therefore, these results and ours indicated that some inhibitory receptors can regulate DC maturation and activation. Changes of the expression levels of these receptors might have impact on DC function and probably results in an altered balance between tolerance and immunity.

In conclusion, we examined the regulatory role of mKLRL1 in DCs and in the immune response. We found mKLRL1 negatively regulated the function of DCs upon LPS stimulation and mKLRL1 might also play a role in the induction of immune tolerance. Recently, KLRL1 has also been used to mediate antigen uptake and cross-presentation on DCs to elicit efficient anti-tumor responses [47]. Future studies are still required to identify its naturally recognized ligands and elucidate more physiologic functions of this receptor.

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Disclosure of conflict of interest

None.

Abbreviations

KLRL1, killer cell lectin-like receptor L1; mKLRL1, mouse killer cell lectin-like receptor L1; DCs, dendritic cells; BMDC, bone marrow-derived dendritic cells; QRT-PCR, quantitative real-time PCR; MOI, multiplicity of infection; OVA, Ovalbumin; EAE, experimental autoimmune encephalomyelitis.

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References

[1] Ravetch JV and Lanier LL. Immune inhibitory receptors. Science 2000; 290: 84-9.
[2] Fife BT, Pauken KE, Eagar TN, Obu T, Wu J, Tang Q, Azuma M, Krummel MF and Bluestone JA. Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. Nat Immunol 2009; 10: 1185-92.
[3] Yawata M, Yawata N, Draghi M, Partheniou F, Little AM and Parham P. MHC class I-specific inhibitory receptors and their ligands structure diverse human NK-cell repertoires toward a balance of missing self-response. Blood 2008; 112: 2369-80.
[4] Attanasio J and Wherry EJ. Costimulatory and coinhibitory receptor pathways in infectious disease. Immunity 2016; 44: 1052-68.
[5] Redelinghuys P and Brown GD. Inhibitory C-type lectin receptors in myeloid cells. Immunol Lett 2011; 136: 1-12.
[6] Dambuza IM and Brown GD. C-type lectins in immunity: recent developments. Curr Opin Immunol 2015; 32: 21-7.
[7] Renedo M, Arce I, Montgomery K, Roda-Navarro P, Lee E, Kucherlapati R and Fernandez-Ruiz E. A sequence-ready physical map of the re-

gion containing the human natural killer gene complex on chromosome 12p12.3-p13.2. Genomics 2000; 65: 129-36.
[8] Gunturi A, Berg RE and Forman J. Preferential survival of CD8 T and NK cells expressing high levels of CD94. J Immunol 2003; 170: 1737-45.
[9] Meyers JH, Ryu A, Monney L, Nguyen K, Greenfield EA, Freeman GJ and Kuchroo VK. Cutting edge: CD94/NKG2 is expressed on Th1 but not Th2 cells and costimulates Th1 effector functions. J Immunol 2002; 169: 5382-6.
[10] Benitez AC, Dai Z, Mann HH, Reeves RS, Margineantu DH, Gooley TA, Groh V and Spies T. Expression, signaling proficiency, and stimulatory function of the NKG2D lymphocyte receptor in human cancer cells. Proc Natl Acad Sci U S A 2011; 108: 4081-6.
[11] Han Y, Zhang M, Li N, Chen T, Zhang Y, Wan T and Cao X. KLRL1, a novel killer cell lectinlike receptor, inhibits natural killer cell cytotoxicity. Blood 2004; 104: 2858-66.
[12] Marshall AS, Willment JA, Lin HH, Williams DL, Gordon S and Brown GD. Identification and characterization of a novel human myeloid inhibitory C-type lectin-like receptor (MICL) that is predominantly expressed on granulocytes and monocytes. J Biol Chem 2004; 279: 14792-802.
[13] Chen CH, Floyd H, Olson NE, Magaletti D, Li C, Draves K and Clark EA. Dendritic-cell-associated C-type lectin 2 (DCAL-2) alters dendritic-cell maturation and cytokine production. Blood 2006; 107: 1459-67.
[14] Marshall AS, Willment JA, Pyz E, Dennehy KM, Reid DM, Dri P, Gordon S, Wong SY and Brown GD. Human MICL (CLEC12A) is differentially glycosylated and is down-regulated following cellular activation. Eur J Immunol 2006; 36: 2159-69.
[15] Hasegawa H and Matsumoto T. Mechanisms of tolerance induction by dendritic cells in vivo. Front Immunol 2018; 9: 350.
[16] Qian C and Cao X. Dendritic cells in the regulation of immunity and inflammation. Semin Immunol 2018; 35: 3-11.
[17] Veglia F and Gabrilovich DI. Dendritic cells in cancer: the role revisited. Curr Opin Immunol 2017; 45: 43-51.
[18] Xu S, Han Y, Xu X, Bao Y, Zhang M and Cao X. IL-17A-producing gammadelta T cells promote CTL responses against Listeria monocytogenes infection by enhancing dendritic cell cross-presentation. J Immunol 2010; 185: 5879-87.
[19] He W, Ma J, Chen Y, Jiang X, Wang Y, Shi T, Zhang Q, Yang Y, Jiang X, Yin S, Zheng A, Lu J and Zheng Y. Ephedrine hydrochloride protects mice from staphylococcus aureus-induced peritonitis. Am J Transl Res 2018; 10: 670-683.
[20] Martinet L and Smyth MJ. Balancing natural killer cell activation through paired receptors. Nat Rev Immunol 2015; 15: 243-54.
[21] Ljutic B, Carlyle JR, Filipp D, Nakagawa R, Julius M and Zuniga-Pflucker JC. Functional requirements for signaling through the stimulatory and inhibitory mouse NKR-P1 (CD161) NK cell receptors. J Immunol 2005; 174: 4789-96.
[22] Gunturi A, Berg RE, Crossley E, Murray S and Forman J. The role of TCR stimulation and TGF-beta in controlling the expression of CD94/NKG2A receptors on CD8 T cells. Eur J Immunol 2005; 35: 766-75.
[23] Guma M, Busch LK, Salazar-Fontana LI, Bellosillo B, Morte C, Garcia P and Lopez-Botet M. The CD94/NKG2C killer lectin-like receptor constitutes an alternative activation pathway for a subset of CD8+ T cells. Eur J Immunol 2005; 35: 2071-80.
[24] Saether PC, Westgaard IH, Hoelsbrekken SE, Benjamin J, Lanier LL, Fossum S and Dissen E. KLRE/I1 and KLRE/I2: a novel pair of heterodimeric receptors that inversely regulate NK cell cytotoxicity. J Immunol 2008; 181: 3177-82.
[25] Dennehy KM, Klimosch SN and Steinle A. Cutting edge: NKp80 uses an atypical hemi-ITAM to trigger NK cytotoxicity. J Immunol 2011; 186: 657-61.
[26] Grundemann C, Schwartzkopff S, Koschella M, Schweier O, Peters C, Voehringer D and Pircher H. The NK receptor KLRG1 is dispensable for virus-induced NK and CD8+ T-cell differentiation and function in vivo. Eur J Immunol 2010; 40: 1303-14.
[27] Huntington ND, Tabarias H, Fairfax K, Brady J, Hayakawa Y, Degli-Esposti MA, Smyth MJ, Tarlinton DM and Nutt SL. NK cell maturation and peripheral homeostasis is associated with KLRG1 up-regulation. J Immunol 2007; 178: 4764-70.
[28] Naper C, Hayashi S, Lovik G, Kveberg L, Niemi EC, Rolstad B, Dissen E, Ryan JC and Vaage JT. Characterization of a novel killer cell lectin-like receptor (KLRH1) expressed by alloreactive rat NK cells. J Immunol 2002; 168: 5147-54.
[29] Schlitzer A, McGovern N and Ginhoux F. Dendritic cells and monocyte-derived cells: two complementary and integrated functional systems. Semin Cell Dev Biol 2015; 41: 9-22.
[30] Boruchov AM, Heller G, Veri MC, Bonvini E, Ravetch JV and Young JW. Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. J Clin Invest 2005; 115: 2914-23.
[31] Adema GJ. Dendritic cells from bench to bedside and back. Immunol Lett 2009; 122: 128-30.

[32] Joffre O, Nolte MA, Sporri R and Reis e Sousa C. Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. Immunol Rev 2009; 227: 234-47.
[33] van Vliet SJ, den Dunnen J, Gringhuis SI, Geijtenbeek TB and van Kooyk Y. Innate signaling and regulation of Dendritic cell immunity. Curr Opin Immunol 2007; 19: 435-40.
[34] Ip WKE, Hoshi N, Shouval DS, Snapper S and Medzhitov R. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. Science 2017; 356: 513-519.
[35] Mittal SK and Roche PA. Suppression of antigen presentation by IL-10. Curr Opin Immunol 2015; 34: 22-7.
[36] Kajino K, Nakamura I, Bamba H, Sawai T and Ogasawara K. Involvement of IL-10 in exhaustion of myeloid dendritic cells and rescue by CD40 stimulation. Immunology 2007; 120: 28-37.
[37] Kawakami Y, Inagaki N, Salek-Ardakani S, Kitaura J, Tanaka H, Nagao K, Kawakami Y, Xiao W, Nagai H, Croft M and Kawakami T. Regulation of dendritic cell maturation and function by Bruton's tyrosine kinase via IL-10 and Stat3. Proc Natl Acad Sci U S A 2006; 103: 153-8.
[38] Wang B, Rao YH, Inoue M, Hao R, Lai CH, Chen D, McDonald SL, Choi MC, Wang Q, Shinohara ML and Yao TP. Microtubule acetylation amplifies p38 kinase signalling and anti-inflammatory IL-10 production. Nat Commun 2014; 5: 3479.
[39] Imbrechts M, De Samblancx K, Fierens K, Brisse E, Vandenhaute J, Mitera T, Libert C, Smets I, Goris A, Wouters C and Matthys P. IFN-gamma stimulates CpG-induced IL-10 production in B cells via p38 and JNK signalling pathways. Eur J Immunol 2018; 48: 1506-1521.
[40] Li H, Hu D, Fan H, Zhang Y, LeSage GD, Caudle Y, Stuart C, Liu Z and Yin D. Beta-Arrestin 2 negatively regulates toll-like receptor 4 (TLR4)-triggered inflammatory signaling via targeting p38 MAPK and interleukin 10. J Biol Chem 2014; 289: 23075-85.
[41] Tan JK and O'Neill HC. Maturation requirements for dendritic cells in T cell stimulation leading to tolerance versus immunity. J Leukoc Biol 2005; 78: 319-24.
[42] Hubert P, Jacobs N, Caberg JH, Boniver J and Delvenne P. The cross-talk between dendritic and regulatory T cells: good or evil? J Leukoc Biol 2007; 82: 781-94.
[43] Vlad G, Chang CC, Colovai AI, Berloco P, Cortesini R and Suciu-Foca N. Immunoglobulin-like transcript 3: a crucial regulator of dendritic cell function. Hum Immunol 2009; 70: 340-4.
[44] Liang S, Ristich V, Arase H, Dausset J, Carosella ED and Horuzsko A. Modulation of dendritic cell differentiation by HLA-G and ILT4 requires the IL-6–STAT3 signaling pathway. Proc Natl Acad Sci U S A 2008; 105: 8357-62.
[45] van Montfoort N, 't Hoen PA, Mangsbo SM, Camps MG, Boross P, Melief CJ, Ossendorp F and Verbeek JS. Fcgamma receptor IIb strongly regulates Fcgamma receptor-facilitated T cell activation by dendritic cells. J Immunol 2012; 189: 92-101.
[46] Desai DD, Harbers SO, Flores M, Colonna L, Downie MP, Bergtold A, Jung S and Clynes R. Fc gamma receptor IIB on dendritic cells enforces peripheral tolerance by inhibiting effector T cell responses. J Immunol 2007; 178: 6217-26.
[47] Hutten TJ, Thordardottir S, Fredrix H, Janssen L, Woestenenk R, Tel J, Joosten B, Cambi A, Heemskerk MH, Franssen GM, Boerman OC, Bakker LB, Jansen JH, Schaap N, Dolstra H and Hobo W. CLEC12A-mediated antigen uptake and cross-presentation by human dendritic cell subsets efficiently boost tumor-reactive T cell responses. J Immunol 2016; 197: 2715-25.