Biochemical and Biophysical Research Communications 480 (2016) 215–221

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Dendritic cell inhibitory receptor 4 (DCIR4) is preferentially expressed on inflammatory and patrolling monocytes

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ARTICLE INFO

Article history:
Received 23 September 2016
Accepted 13 October 2016
Available online 14 October 2016

Keywords:
Monocytes
Marker
Monoclonal antibody
Differentiation
Lectin

ABSTRACT

Dendritic cell inhibitory receptor 4 (DCIR4, Clec4a1) is a lectin receptor and a member of mouse dendritic cell immunoreceptor family. Due to the lack of antibodies against DCIR4, expression of DCIR4 protein remains unknown. In this study, we established a specific monoclonal antibody against DCIR4 and investigated the expression of DCIR4 among immune cells. We found that DCIR4 was expressed on non-granulocytic subsets of $CD11b^+$ cells in various immune organs including bone marrow, peripheral blood, spleen, skin-associated lymph nodes and mesenteric lymph nodes. $DCIR4^+$ $CD11b^+$ cells were dichotomized into $DCIR4^{High}$ and $DCIR4^{Low}$ cells distinguished by different levels of DCIR4 expression. By screening a panel of cell surface markers for expression, we found that in bone marrow, blood and spleen the $DCIR4^{Low}$ and the $DCIR4^{High}$ cells were Ly-$6C^+$ $CD43^{Low}$ $CD11c^-$ inflammatory$^-$ monocytes and Ly-$6C^-$ $CD43^{HIgh}$ $CD11c^+$ patrolling monocytes, respectively. Using in vitro differentiation system, we also found that differentiation of Ly-$6C^+$ monocytes into dendritic cells greatly diminished expression of DCIR4, while that into macrophages did not significantly affect DCIR4 expression. The establishment of the anti-DCIR4 antibody enables clearer definition of monocytes and provides a novel tool to investigate biology of monocytes and their progenies.

1. Introduction

Dendritic cell inhibitory receptor 4 (DCIR4), with the alternate name Clec4a1, is a lectin receptor and a member of mouse dendritic cell immunoreceptor (DCIR) family [1]. Since its discovery, the expression of mouse DCIR4 is only confirmed at mRNA level in a mouse dendritic cell line [1] and no information is available regarding expression of DCIR4 protein. Because the other members of mouse DCIR family, including DCIR1 [2], DCIR2 [3], DCAR1 [4] and DCAR2 [2], are expressed on distinct populations of myeloid cells, it was expected that DCIR4 might be expressed on cells of myeloid lineage.

Myeloid cells are cells of innate immunity, derived from hematopoietic stem cells and include granulocytes, such as eosinophils and neutrophils, and non-granulocytic cells, including monocytes, macrophages, and dendritic cells. Monocytes are circulating blood leukocytes with phagocytic function that play important roles in inflammatory response [5]. They have a capacity to differentiate into macrophages and dendritic cells (DCs) in vivo and in vitro. Monocytes are separated into two major categories with different functions and different phenotypes. In mouse, the presence and the absence of the expression of Ly-6C define Ly-$6C^+$ inflammatory monocytes and Ly-$6C^-$ patrolling monocytes [6]. Initially, monocytes were thought to exist in circulation and bone marrow, however, spleen is shown to be a reservoir of inflammatory and patrolling monocytes [7]. Recently, inflammatory monocytes were found also in peripheral tissues, such as skin and lung, and in lymph nodes and it was shown that under steady state condition they do not undergo differentiation into macrophages and DCs as they survey peripheral tissues and transport antigens to lymph nodes [8]. Although CD115 (M-CSF receptor) has been used to define monocytes, CD115 is not always detected on all of the monocytes, because the CD115 protein can be cleaved under

Abbreviations: BM, bone marrow; cDCs, conventional dendritic cells; DCIR, dendritic cell immunoreceptor; DCIR4, dendritic cell inhibitory receptor 4; DCs, dendritic cells; IFN-γ, interferon-γ; MLNs, mesenteric lymph nodes; mAb, monoclonal antibody; SLNs, skin-associated lymph nodes.

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http://dx.doi.org/10.1016/j.bbrc.2016.10.030
0006-291X/

inflammatory condition [9]. Therefore, novel defining markers for mouse monocytes are still needed [10].

In this study, to investigate expression of DCIR4 protein, we established a monoclonal antibody (mAb) against mouse DCIR4. Using the anti-DCIR4 mAb, we demonstrate that the DCIR4-expressing cells are monocytes. In addition, we show that in vitro differentiation of inflammatory monocytes into DCs greatly diminishes expression of DCIR4.

2. Material and methods

2.1. Animals

All animal procedures were approved by the Animal Studies Committee at the University of Tokyo. C57BL/6J mice and LEW/SsN Slc rats were obtained from Nippon SLC (Shizuoka, Japan) and were housed under specific pathogen-free conditions. All the animal experiments were conducted in accordance with the University of Tokyo's rules regarding animal experimentation and the University's animal-experimentation manual.

2.2. cDNA cloning of DCIR4

cDNA of mouse DCIR4 was amplified by PCR from a cDNA library derived from a C57BL/6J mouse spleen using specific primers, (5′-CTGAAGAAAGCAGGTCTCTTCTC-3′) and (5′-GAGTCTGTAGAGAATGCTTCG-3′) for DCIR4 (NCBI accession: BC049354) and cloned into *Sma*I-digested pBlueScriptSK(+) to construct pBlueScript-DCIR4. Sequence of the obtained cDNA was identical to that of the above accession number.

2.3. Cell lines and culture

BWZ.36 cells were provided by N. Shastri (University of California Berkeley, Berkeley, CA). Mouse IL-6-transfected X63 cells and PAI cells were obtained from H. Karasuyama (Tokyo Medical and Dental University, Tokyo, Japan). The above cell lines were cultured in RPMI 1640 medium (Sigma-Aldrich, St Louis, MO) containing 10% heat-inactivated fetal calf serum (FCS, Sigma-Aldrich), 50 μM 2-mercaptoethanol (Sigma-Aldrich), 100 U/mL of penicillin G (Sigma-Aldrich) and 100 μg/mL of streptomycin sulfate (Sigma-Aldrich) (R10 medium). Established hybridomas were cultured in R10 medium supplemented with 1 mM sodium pyruvate (Wako, Osaka, Japan) and 1 × non-essential amino acid (Sigma-Aldrich). The retroviral vector pMXs-IRES-EGFP and the retrovirus packaging cell line Plat-E were provided by T. Kitamura (the University of Tokyo, Tokyo, Japan). Plat-E cells were maintained in DMEM medium (Sigma-Aldrich) containing 10% heat-inactivated FCS, 50 μM 2-mercaptoethanol, 100 U/mL of penicillin G, 100 μg/mL of streptomycin, 10 μg/mL of blasticidin S HCl (Calbiochem) and 1 μg/mL of puromycin (Wako). All the cell lines were cultured at 37 °C under 5% CO_2 and 100% moisture.

2.4. Construction of DCIR4 reporter cells

DCIR4rep-3xFLAG-pMXs-IG, an expression vector for DCIR4 chimeric receptor, which consists of the intracellular domain of the mouse CD3ζ chain (Arg52 through Arg164), the transmembrane region of mouse Ly49A (Ser40 through Ile66), the extracellular region of DCIR4 (Gln77 through Val245) and the 3 × FLAG tag, was constructed on a pMXs-IRES-EGFP vector. Packaging of retro virus and transduction of BWZ.36 cells was performed as described [2]. Establishment of, DCIR3 and DCAR1 reporter cells will be published elsewhere. DCIR1, DCIR2 and DCAR2 reporter cells were established previously [2,11].

2.5. Establishment of mAb against DCIR4

Immunization of rats with the DCIR4 reporter cells and preparation of hybridomas were performed as described previously [2]. The supernatants from the hybridomas were assayed for the capacity to bind DCIR4 reporter cells by reporter cell assays or flow cytometry as described [2]. We successfully established an anti-DCIR4 mAb, MH7E7 (rat IgG1, κ-light chain).

2.6. Antibodies and flow cytometry assays

FITC-conjugated mAbs against mouse CD3ε (145-2C11), CD11c (N418), CD45RA/B220 (RA3-6B2), CD80 (16-10A1), CD86 (GL-1), CD103 (2E7), F4/80 (BM8), IA-IE (M5/114.15.2), Ly-6C (HK1.4) and Ly-6G (1A8) and PE-conjugated mAbs against mouse CD11b (M1/70), CD11c (N418), CD115 (AF598) and CD117 (2B8) were obtained from BioLegend (San Diego, CA). PE-conjugated mAb against Siglec-F (ES22-10D8) and FITC-conjugated mAbs against CD335 (29A1.4.9) and CD43 (L11) were from Milteny-Biotec (Bergisch Gladbach, Germany). PE-conjugated mAb against CD49b (DX5) was from BD-Bioscience (San Jose, CA). mAbs against mouse Fcγ receptors (2.4G2), CD8α (53–6.72), CD11b (M1/70) and NK1.1 (PK136) were purified from supernatants of hybridomas obtained from ATCC (Manassas, VA) with affinity chromatography using Hi-Trap Protein G HP columns (GE Healthcare Japan Corporation, Tokyo, Japan), the latter three of which were then labeled with FITC (Sigma-Aldrich) or DyLight 633 NHS-ester (Thermo-Fischer Scientific). Anti-DCIR4 mAb (MH7E7) established in this study was also purified from the culture supernatants of hybridoma and then biotinylated using EZ-Link Sulfo-NHS-LC-Biotin (Thermo-Fischer Scientific) or labeled with DyLight 650 NHS Ester (Thermo-Fischer Scientific).

For staining of the reporter cells, the cells were stained with hybridoma supernatants or the purified mAb and then stained with PE-conjugated goat $F(ab')_2$ anti-mouse IgG (H+L) (Beckman Coulter, Fullerton, CA).

For staining of mouse immune cells, PBS containing 1 mM EDTA, 0.1% BSA (Wako) and 0.1% NaN_3 was used. The single cell suspensions prepared from various immune organs were incubated with anti-mouse Fcγ receptors mAb (2.4G2) before the addition of mAbs. Biotinylated anti-DCIR4 (MH7E7) mAb was used in combination with PE-conjugated streptavidin (BioLegend). Propidium iodide (Sigma-Aldrich) was added at the final concentration of 1 μg/ml immediately before flow cytometry acquisition to exclude dead cells from analysis. The data were acquired with a FACSCalibur system (BD Biosciences) and were analyzed with FlowJo software (TreeStar, San Carlos, CA). Background staining was estimated using fluorochrome-conjugated or biotinylated isotype control mAbs: rat IgG2a (anti-human AICL, 3G72) or rat IgG1 control mAbs (anti-human KLRF1, H206), both of which were prepared in our laboratory [12], or rat IgG1 (RTK2071), rat IgG2a (RTK2758), rat IgG2b (RTK4530), rat IgG2c (RTK4174) or Armenian hamster IgG (HTK888) control mAbs from BioLegend or mouse IgG2a (eBM2a) mAb from eBioscience (San Diego, CA).

2.7. Preparation of single cell suspensions of various immune organs

8-16 weeks-old female C57BL6/J mice were used. Single cell suspensions from spleens, mesenteric lymph nodes (MLNs) and skin-associated lymph nodes (SLNs) that include axillary, inguinal and popliteal LNs were prepared by passing through cell strainers. Bone marrow (BM) was flushed from femurs and tibiae with PBS and passed through nylon mesh. Peripheral blood was collected in a syringe with 20 μL of 0.5 M EDTA (pH 8.0) by cardiac puncture under anesthesia. Spleen, BM and peripheral blood cells were