A Monoclonal Antibody to the DEC-205 Endocytosis Receptor on Human Dendritic Cells

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ABSTRACT: DEC-205 is a multilectin receptor for adsorptive endocytosis, expressed in mouse dendritic cells (DC) and some epithelia. DEC-205 is homologous to the macrophage mannose receptor (MMR). A cDNA for murine DEC-205 was used to identify 3 overlapping human DEC-205 clones from a lymphocyte library. The human homologue is a transmembrane protein of 1722 amino acids with 10 externally disposed C-type lectin domains having 77% identity to the mouse counterpart. The NH2 terminal cysteine-rich and fibronectin type II domains were expressed and used to immunize mice. A hybridoma, MG38, which specifically recognized the immunogen was obtained from a DEC-205 knockout mouse. The antibody precipitated a 205 kD protein from metabolically labeled, monocyte-derived DCs. MG38 labeled mature monocyte-derived DCs but showed weak or no labeling of other peripheral blood mononuclear cells. In tissue sections, MG38 identified DEC-205 on thymic cortical epithelium and DCs in the thymic medulla and tonsillar T cell areas. In contrast, an anti-MMR antibody stained DEC-205 negative, macrophages in the thymus cortex, the trabeculae of the thymus and tonsil, as well as efferent lymphatics in the tonsil. Therefore, the MG38 anti-DEC-205 antibody is useful for identifying DCs and reveals clear differences in sites where MMR and DEC-205 are expressed in lymphoid tissues. Human Immunology 61, 729–738 (2000). © American Society for Histocompatibility and Immunogenetics, 2000. Published by Elsevier Science Inc.

KEYWORDS: dendritic cells; antigen receptor; C-type lectin; DEC-205; monoclonal antibody

ABBREVIATIONS

MMR macrophage mannose receptor

DC dendritic cell

INTRODUCTION

Several receptors for adsorptive endocytosis and antigen presentation have now been identified on dendritic cells (DCs). These include Fcγ [1] and Fcɛ [2] receptors and two multilectin receptors, the macrophage mannose receptor (MMR) [3] and DEC-205 [1]. The carbohydrate recognition domains of the MMR show specificity for mannose [3], and the MMR internalizes and presents mannosylated proteins [3, 4]. The ligands for DEC-205 are not yet known, but antibodies to DEC-205 are presented efficiently to Ig-reactive T cells [1]. Both the MMR and DEC-205 contain homologous amino terminal cysteine rich (CR) and fibronectin II domains followed by 8 and 10 contiguous C-type lectin domains respectively. Both molecules contain a single transmembrane domain and a cytosolic region with a coated pit localization sequence for adsorptive uptake.

DEC-205 is expressed by many different sources of mouse DCs [5–7], according to data with a rat-anti-mouse monoclonal antibody, originally termed NLDC-145 [5]. This monoclonal was used to clone DEC-205...
and provide the first information on its function [1]. A monoclonal to mouse MMR has yet to be reported. In contrast, the human MMR is a potent immunogen in mice, and the corresponding monoclonals have revealed abundant expression of the MMR on monocyte-derived DCs [3]. No antibody to human DEC-205 is available, although HuDEC-205 has been cloned independently in our laboratory and by Kato et al. [8].

Here, we set out to prepare monoclonal antibodies to sequences from the predicted protein but only succeeded when we immunized DEC-205 knockout mice. We describe a monoclonal MG38 to huDEC-205. Expression of human DEC-205 is prominent in DCs rather than other cells in human blood. Using monoclonals to MMR and DEC-205, we find that these receptors are expressed in different sites in tissue sections of thymus and tonsil.

MATERIALS AND METHODS

Human DEC-205 cDNA

A cDNA fragment of the 3’ portion of murine DEC-205 was used to screen a human lymphocyte matchmaker cDNA library (EBV-transformed human peripheral blood B lymphocytes) and a human thymus 5’-stretch plus cDNA library in a Ogt10 vector (Clontech Laboratories, Palo Alto, CA, USA). Positive clones were characterized by DNA sequencing on both strands using Sequenase (United State Biochemical, Cleveland, OH, USA), or the dye terminator kit (PE Applied Biosystems, Foster City, CA, USA) and automated sequencing (Applied Biosystems model 371).

Expression of CR-FnII Domains of hDEC-205 in a Mammalian 293T Cell Expression System
cDNAs were expressed in pEF-BOS [9] modified to carry a 3’ human Fc fragment that was in frame with the insert. DEC-205 leader, CR domain, and FnII domains were amplified from plasmids by PCR using 5’-primers MG31: 5’-CGGGATCCACTAGTCGCGTGCGCCCGAGG - 3’ and 3’-primer MG35: 5’-CTTAAAGCCTGAAAACG GTCCGCGCCGGCGGCCGCATTCTTAT–3’. The 5’-primer contains a SpeI site, while the 3’-primer contains a Not I site and codes for PRR at the junction point of DEC-205 and the Fc domain. The hDEC-205-Fc fusion protein was produced by transiently transfecting 293T cells using calcium phosphate-mediated gene transfer. Medium containing the secreted recombinant protein was harvested after 10 –12 days, and the recombinant protein was recovered by protein A chromatography [10].

Proteolysis and Antigen Purification

The CR-FnII portion of the fusion protein was separated from the Fc tag by papain digestion. Fusion protein bound to Protein A Sepharose was digested with papain (Sigma Chemical Co., St. Louis, MO, USA) at a ratio of enzyme:protein of 1:100 in 1X PBS containing 10 mM EDTA and 10 mM cysteine for 30 min at 37°C (Fig. 1B). The reaction was terminated by adding iodoacetamide to a final concentration of 30 mM. Protein A beads and the bound Fc fragments were removed by centrifugation.

Immunization of DEC-205 Knockout Mice and Hybridoma Production

Purified CR-FnII protein in CFA (prime) or IFA (boost) were injected intraperitonally four times into DEC-205 knockout (DEC-2052/2) mice at 50 μg per inoculation. The DEC-2052/2 mice were produced by deleting the DEC-205 promoter and first coding exon in ES cells as previously performed for Igβ [11]. Initial immunophenotyping showed apparently normal B and T cell development in these mice, as well as T cell dependent immune responses. Sera were harvested from immunized mice and tested for the presence of anti-CR-FnII by western blot. Mice producing antibodies were reboosted with CR-FnII protein in PBS, their spleens were harvested 4 days later, and the splenocytes were fused with SP2/0 cells [10]. Supernatants were screened by ELISA, dot blot, thymus tissue section staining, and FACS analysis.

ELISA

Plates were coated with purified CR-FnII protein at a concentration of 2 μg/ml at 37°C for 2 hrs and blocked
30 min at room temperature with blocking buffer (0.25% BSA in PBS). Fifty µl of supernatant from the growing fusion well was added and incubated at room temperature for 1 h, then washed. HRP-conjugated secondary antibody (1:5,000 dilution in blocking buffer) was added and incubated for 45–60 min at room temperature, then washed. Samples were developed using HRP substrate reagent (Bio-Rad Laboratories, Hercules, CA, USA).

**Dot Blot**

Ten to one hundred nanograms of antigen was loaded directly on the nitrocellulose paper. The nitrocellulose paper was blocked for 30 min at room temperature with 5% dry milk in wash buffer (PBS with 0.05% Tween 20), then incubated with hybridoma supernatants for 60 min at room temperature, and washed with washing buffer. HRP-conjugated-goat anti-mouse antibody (1:10,000 dilution in washing buffer) was added and incubated for 45 min at room temperature, then washed. The blots were developed with ECL detection reagent (NEN Life Science Products, Inc., Boston, MA, USA).

**Preparation of Cells**

PBMCs, monocytes, T cells, DCs, and macrophages were prepared from the blood of normal donors as described [12]. After isolation by sedimentation in Ficoll-Hyphaque, PBMC were cultured in RPMI-5% human serum for 1 h. Floating cells were removed, and adherent cells were incubated 6 days in RPMI-5% human serum with GM-CSF (100 U/ml, Leukine, Immunex) and IL-4 (1000 U/ml, Genzyme). Cells were fed on days 2, 4, and 6 with the same medium. Nonattached cells were harvested on day 6 as immature DCs, and to make mature DCs, the cells were cultured 3 more days in the above medium. Cytokines and LPS. Macrophages were also obtained from PBMC by culture in parallel but in the absence of cytokines.

**Immunohistochemistry**

Frozen sections were cut at 10 µ. from surgical specimens of human tonsil and thymus, generously provided by Dr. W.A. Muller (Cornell University Medical College, New York, NY, USA). Sections were cut onto glass slides (Carlson, Peotone, IL, USA), air dried, fixed in acetone and stained as follows. Biotinylated monoclonal antibodies, MG38 anti-DEC-205 and 3.29 anti-MMR [3] (a gift of Dr. A. Lanzavecchia, Basel), were applied at 20 and 5 µg/ml, respectively. Alkaline phosphatase ABC (Vector) was applied for 30 min and the enzyme reaction developed using a BCIP/NBT substrate kit (Vector, Burlingame, CA, USA) according to manufacturer's instructions. Mouse serum (10%) was applied for 15 min, followed by leu 2 anti-CD8 hybridoma culture supernatant overnight and POX-anti mouse Ig (Jackson Immunoresearch, West Grove, PA, USA) for 1 h. Stable DAB (Research Genetics, Huntsville, AL, USA) was used for 6 min to develop the HRP reaction. The slides were mounted in PBS/glycerol. Alternatively, for two color immunofluorescence, thymic sections were labeled with the following sequence: 3.29 MMR at 2 µg/ml followed by FITC-goat anti-human IgG1, then MG38 anti-DEC-205 at 2 µg/ml followed by biotin goat anti-human IgG2b and Cy3 Streptavidin (all secondary antibodies from PharMingen). Stacks of 0.5 µ optical sections were taken by an Olympus epifluorescence microscope equipped with a motorized stage, a cooled CCD camera (Hamamatsu, Japan), and Metamorph software (Universal Imaging). Images were deconvoluted by applying a nearest neighbor algorithm provided by Metamorph.

**Flow Cytometry**

Fresh or permeabilized cells were incubated with hybridoma culture supernatant for 30 min on ice, washed with PBS-1% fetal calf serum, incubated with secondary antibody for 30 min on ice, then washed. Immunoglobulins were used at 1 µg/ml and mAb-containing supernatant was used at 1:1 dilution. The secondary antibody was fluorescein isothiocyanate (FITC)-goat-anti-mouse immunoglobulin (1:200 dilution, Jackson). PE-CD14 (Becton Dickinson, Mansfield, MA, USA), PE-HLA-DR (Becton Dickinson), PE-CD83 (Immunotech) were used for double staining. The antibody to DC-LAMP [13] was provided by Drs. S. Lebecque and S. Saeland of the Laboratory for Immunology Research, Dardilly France. Stained cells were analyzed using FACScan flow cytometer (Becton Dickinson). For permeabilization, cells were fixed in 4% paraformaldehyde (Fisher Scientific Co., Pittsburgh, PA, USA) freshly made in PBS for 30 min on ice, followed by two washes with PBS. Fixed cells were permeabilized with 0.5% saponin (Sigma Chemical Co., St. Louis, MO, USA) in PBS with 1% FCS for 30 min on ice and washed twice using PBS with 1% FCS. To rule out Fc receptor-mediated binding of MG38 to DCs, we verified that the results in the FACS and tissue section studies were not altered by inclusion of 10% human serum in the staining reactions.

**Cell Lysates and Metabolic Labeling**

Monocytes, T cells, and DCs were harvested, counted, and washed three times with 1× PBS, then resuspended in RIPA buffer (150 mM NaCl, 1% NP40, 0.5% DOC, 0.1% SDS, 50 mM tris-HCl). The following components were added to RIPA buffer before use: 1 mM DTT, 1 mM PMSF and 1× proteinase inhibitor cocktail (1 mg/ml leupeptin, 1 mg/ml antipain, 10 mg/ml benzamidine hydrochloride, 5 mg/ml aprotinin, 10 mg/ml soy bean trypsin Inhibitor, 1 mg/ml pepstatin). Samples
were rotated at 4°C for 30 min, and nuclei removed by spinning in a microfuge for 10 min. Total protein concentrations of the cell lysate was determined using 1× Bradford Assay buffer reagent (Bio-Rad Laboratories, Hercules, CA, USA). For metabolic labeling, cells were washed once with 1X PBS, then resuspended at 1×10^7 cells per 100 mm plate in 10 ml labeling media (DMEM, without methionine, supplemented with 10% dialyzed fetal bovine serum and antibiotics; Gibco-BRL, Grand Island, NY, USA) and incubated at 37°C for 30 min to allow depletion of the intracellular pools of sulfur-containing amino acids. Cells were then labeled by adding 1 mCi of (35S) methionine, for 6 h and lysates were prepared as described.

**Immunoprecipitation (IP)**

Lysates were pre-cleared by incubating with 100 µl of prewashed 50% protein A Sepharose in the cold for 30–60 min. 0.5–1 µg of each test antibody was then mixed with 50 µl of pre-cleared cell lysate and incubated on ice for 1 hr, and 30 µl of prewashed 50% protein A Sepharose was added for and additional hour. Beads were washed 3 times with cold RIPA buffer and analyzed by SDS-PAGE under reducing conditions.

**RESULTS**

**Isolation of Human DEC-205**

We previously reported the isolation of a cDNA clone encoding mouse DEC-205 [1]. By screening both human B cell and thymus libraries with probes derived from the mouse DEC-205 cDNA (see Materials and Methods), we obtained three overlapping cDNAs clones that when combined, encoded the complete human homologue of mouse DEC-205.

The predicted protein encoded by the hDEC-205 cDNAs is a type I transmembrane protein of 1722 amino acids with characteristic features of a group VI C-type lectin. Like mouse DEC-205 the human protein consists of an NH2-terminal signal peptide followed by a cysteine-rich (CR) domain, a fibronectin type II (FnII) domain, ten carbohydrate recognition-like domains (CRDs), and a transmembrane domain, which is followed by a short cytoplasmic tail. Amino acid sequence alignment between the human and mouse cDNAs [1] reveals 77% identity, suggesting functional conservation. Our huDEC-205 sequence is identical to one independently isolated by Kato et al. [8].

Like mouse DEC-205, huDEC-205 is also homologous to other members of the multiple C-type lectin receptors, human macrophage mannose receptor (MMR, 22% identity), a membrane protein implicated in the endocytosis of glycoproteins bearing terminal mannose, fucose, N-acetylgalactosamine, or glucose residues [4, 14]; and human phospholipases A2 (PLA2) receptor (29% identity), which binds to PLA2, that specifically catalyzes the hydrolysis of the 2-ester bond of 3-sn-phosphoglycerides [15, 16].

**A Monoclonal Antibody Reactive with huDEC-205**

Initial attempts to immunize mice with huDEC-205 failed to produce monoclonal antibodies. We reasoned that the failure might be due to the close conservation between human and mouse proteins. To overcome possible cross-tolerance we immunized DEC-knock-out mice. The fusion was initially screened with an ELISA assay against the purified CR-FnII fragment of the immunogen (see Materials and Methods). Reactive monoclonal antibodies were additionally tested by Western blot analysis. Clone MG38 specifically recognized and immunoprecipitated purified CR-FnII but not human Fc (Fig. 2A and B). These results demonstrate that MG38 specifically recognizes an epitope present in the CR-FnII region of human DEC-205. The final step of the screen was to verify MG38 staining of human thymic cortical epithelium, since mouse DEC-205 is abundant on this cell type [5, 6]. MG38 did not react with mouse DCs.

**huDEC-205 Is Expressed by LPS Activated Monocyte-derived Dendritic Cells**

To determine whether monocyte derived DCs expressed huDEC-205, we performed immunoprecipitation studies. Metabolically labeled monocyte-derived DCs yielded a single band migrating at a molecular weight that was greater than the 205 kDa seen with murine DEC-205 [7] (Fig. 3). A protein with the same molecular weight was also precipitated by rabbit polyclonal antibodies that were raised against mouse DEC-205 (unpublished results and [6, 7]). We presume that the higher apparent molecular weight of human DEC-205 reflects differences due to the primary sequence or posttranslational modifications.

**FACS Analysis of DEC-205 Expression**

We used FACS analysis to additionally examine expression of huDEC-205 during DC development and maturation (Fig. 4). Immature DCs, mature DCs and macrophages were prepared from monocytes. Surface-bound as well as total DEC-205 was visualized in live and fixed permeabilized cells, respectively. Other antibodies included a non-reactive IgG2b (termed Y-Ae; isotype-matched to MG38), CD21 (B cell marker), DC-LAMP (DC marker), and CD83 (DC marker). Double labeling was used to identify the DCs as CD14^− HLA-DR^−^, and CD83^+^.

Only a small fraction of the immature DC preparation expressed high DEC-205 (Fig. 4A; <5% are DEC-205^+^ HLA-DR^+^, as indicated by the arrow). However, the
number of DEC-205\textsuperscript{+} cells was dramatically increased (\textgtr 85\%) in the mature DC population (Fig. 4A). Staining was increased considerably by permeabilizing the cells with saponin (Fig. 4A). Increased DEC-205 expression was correlated with higher levels of CD83 and DC-LAMP, both antigens being characteristic of mature DCs (Fig. 4B). In contrast, little or no staining was observed in other types of blood leukocytes present in PBMC (not shown). However, EBV transformed B-LCL did stain with MG38, which is not surprising given the fact that our human DEC-205 cDNA was obtained from a B-LCL library.

Differences in the Expression of MMR and DEC-205 in Sections of Lymphoid Tissue

Because monoclonals to human MMR had been prepared previously [3], we were in a position to use monoclonals to compare the \textit{in vivo} expression of MMR with DEC-205. We studied sections of human tonsil and thymus. In tonsil, presumptive DCs in the T cell areas stained for DEC-205 (Fig. 5A, 5B). In contrast, the MMR was found on scattered presumptive macrophages in the septae; when found near the T cell area, the MMR was clearly present on vascular profiles, presumably efferent lymphatics (arrow, Fig. 5D). The latter originate in the T cell regions to carry tonsillar lymphocytes to the cervical and other lymph nodes in the neck (Fig. 5C, 5D). Neither DEC-205 nor MMR were found on blood vessels.

In thymus, DEC-205 stained the cortical epithelium (red stain in Fig. 6 left) and large dendritic profiles in the thymic medulla (red stain in Fig. 6 right). The latter medullary profiles double labeled for the DC-restricted marker DC-LAMP (not shown). In contrast, the MMR was found on scattered cells, presumably macrophages, in the thymic cortex and septae (green stain in Fig. 6 left) and on infrequent profiles in the medulla (green stain in Fig. 6 right). Two color immunofluorescence of thin 1 micron optical sections, examined by deconvolution microscopy, clearly showed that DEC-205 and MMR were expressed at different sites in the thymus. Therefore, these two homologous multilectins are expressed in dif-
FIGURE 4 (A) DEC-205 is induced in mature DCs. Flow cytometry on live (surface) or saponin-permeabilized monocyte-derived macrophages, immature DCs, and mature DCs. Double staining of cells with MG38, detected by FITC-goat-anti-mouse Ig, and PE-antibodies: PE-CD14, PE-HLA-DR, and PE-CD83. A small subset of mature DEC-205\(^+\) HLA-DR\(^+\) cells in the immature DC preparation is arrowed.
B

Surface

Monoclonal Antibody to Human DEC-205 Receptor

FIGURE 4 (B)  DEC-205 is induced in mature DCs. Flow cytometry on live (surface) or saponin-permeabilized monocyte-derived macrophages, immature DCs, and mature DCs. Histograms of DEC-205, CD83, DC-LAMP, and CD21 expression. Mature DCs, but not immature DCs or macrophages, expressed high levels of DEC-205, CD83 and DC-LAMP.
ferent sites in lymphoid tissues, and only DEC-205 is abundant on DCs.

DISCUSSION

The availability of monoclonal antibodies to human MMR and DEC-205 now makes it possible to look more closely at the tissue distribution of these homologous, multilectin receptors for adsorptive endocytosis. A single monoclonal has been available to mouse DEC-205 but only polyclonals and antisense RNA probes for the mouse MMR. For human, several monoclonals to the MMR have been produced but none to human DEC-205. We have been able to secure the MG38 monoclonal to human DEC-205 by immunizing DEC-205 knockout mice with the NH2-terminal region of the recently cloned human DEC-205. The MG38 antibody reacts with the immunogen produced from DEC-205 cDNA, it precipitates a 205 kD polypeptide from metabolically labeled mature DCs, and it stains the thymic cortical epithelium much like the rat anti-mouse DEC-205 antibody. On isolated human blood cells, the expression of DEC-205 is similar to what is known for mouse DEC-205 [5, 7, 17]. The extent of analysis in the human system is still limited, but DEC-205 is expressed on DCs derived from blood monocytes, while PBMCs stain weakly or not at all with MG38. The presence of weak FACS signals in other leukocytes is in keeping with the fact that our cDNA was derived from a human lymphocyte library.

In tissue sections of tonsil and thymus, DEC-205 and MMR are expressed on different cells. As in the mouse, MG38 stains large dendritic profiles in the T cell areas of tonsil and the medulla of the thymus, as well as the thymic epithelium. In contrast, the MMR is found on scattered macrophages in the trabeculae or septae of both organs, and on efferent lymphatic endothelium in the tonsil. Recently [18], it has been reported that the mouse MMR is often expressed on lymphatic vessels in mouse lymphoid tissues, but not in the T cell areas, a known site for the localization of DCs. Two other studies have shown that a polyclonal antibody to the mouse MMR also stains sinusoidal lining cells in lymph node and liver, but does not stain DCs in the T cell areas [18, 19]. In sum, even though MMR and DEC-205 are homologous in structure and expressed on monocyte-derived

FIGURE 5 Expression of human DEC-205 and MMR in select regions of human tonsil. At low power (A,C), a T cell area (left) is next to a vessel rich (*) nonparenchymal region (right); at high power, a T cell area is shown (B,D). The sections were labeled in brown for CD8 to identify T cells and in blue for DEC-205 (MG 38; panels A,B) or MMR (mAb 3.29; panels C,D). The MMR, when found in the T cell area, is on lymphatic vessels (D, arrow), while in nonparenchymal regions, MMR is found on scattered cells, probably macrophages (C). DEC-205 is found on DCs in the T cell area (B).
DCs, the tissue distribution of these two endocytosis receptors is quite different.

Because both the MMR and DEC-205 are found on DCs produced in vitro from human monocytes with GM-CSF and IL-4, it is possible that both are involved in the presentation of foreign antigens. Recently, it has been shown that blood derived mouse monocytes can capture particles in a peripheral extravascular site and then enter the lymph and T cell areas of the draining lymph node to become DCs [20]. Monoclonals are not yet available to verify the expression of mouse MMR and DEC-205 on monocyte-derived DCs in vivo. However, in the steady state, the MMR and DEC-205 are expressed in different sites in lymphoid tissues and may have distinct functions. We speculate that a biological role of the MMR in sinusoidal endothelium is to clear self proteins that have been altered to expose mannosyl residues. In contrast, DEC-205 on thymic epithelium and DCs in the thymic medulla and T cell areas may function to capture self proteins for thymic and peripheral selection events.

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