DEC-205/CD205+ dendritic cells are abundant in the white pulp of the human spleen, including the border region between the red and white pulp

Maggi Pack,[1] Christine Trumpfheller,[1] Dolca Thomas,[2] Chae Gyu Park,[1] Angela Granelli-Piperno,[1] Christian Münz[1] and Ralph M. Steinman[1]

[1]*The Rockefeller University and* [2]*New York-Presbyterian Hospital/Weill Cornell Medical Center, New York, NY, USA*

doi:10.1111/j.1365-2567.2007.02710.x
Received 24 March 2007; revised 11 July 2007; accepted 1 August 2007.
Correspondence: Dr Maggi Pack, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.
Email: packm@rockefeller.edu
Senior author: Ralph Steinman,
email: steinma@rockefeller.edu

Summary

The distribution of dendritic cells (DCs) and macrophages in the human spleen has received less attention than that of lymphocytes. Here we have addressed this problem with the human DEC-205/CD205 marker ('DEC'), which is an endocytic receptor on DCs that mediates efficient presentation of antigens. DEC was abundant on dendritic profiles in the white pulp but absent from the red pulp, the latter defined with antibodies to two antigens, mannose receptor/CD206 on sinusoidal lining cells, and macrosialin/CD68 on macrophages. Double staining with anti-DEC and anti-CD3 showed the expected concentration of DEC+ cells in the relatively small T-cell areas of the human spleen. DEC+ cells were also found in other regions of the white pulp. In all regions, the DEC+ cells were positive for major histocompatibility complex (MHC) class II and the CD11c integrin but largely immature, with low expression of B7-2/CD86 costimulator and DC-lysosome-associated membrane protein (LAMP)/CD208. When we concentrated on the perifollicular region between the red pulp and the marginal zone, we found macrophages that stained with antibodies to sialoadhesin/CD169 and DC-specific ICAM-3 grabbing non-integrin (SIGN)/CD209, and just inside these cells were DEC+ profiles. The DEC+ DCs were intertwined with cells that stained for the vascular addressin mucosal addressin cell adhesion molecule (MAdCAM). Therefore, anti-DEC-205/CD205 antibodies are useful for identifying DCs in human splenic white pulp and its border region with the red pulp.

Keywords: dendritic cells; DEC-205; human spleen; perifollicular zone; macrophages

Introduction

The initiation of a specific immune response involves the acquisition and presentation of antigen to naïve T lymphocytes by dendritic cells (DCs), particularly in the T-cell areas of secondary lymphoid organs, which are designed to promote DC–T-cell interactions.[1] The capacity of antigen-bearing DCs to select antigen-specific T cells has now been visualized in living lymph nodes of mice.[2]

The spleen is a major lymphoid organ and has been used extensively to study the location of DCs in mice. The DCs are found in large numbers in the marginal zone, particularly in the bridging channels where T cells enter from the marginal zone into the T-cell areas, the periarterial lymphoid sheaths.[3,4] Much less attention has been paid to antigen-presenting cells in the human spleen. Steiniger *et al.* described the presence of sialoadhesin/CD169+ macrophages in the perifollicular zone that surrounds the marginal zone in humans.[5] McIlroy *et al.* have studied DCs with the CD11c marker and have localized CD11c+ DCs in three distinct regions: the T-cell zones, the B-cell zones and the marginal zone. They also concluded that most spleen DCs are immature based on a lack of CD86 and CD83 expression.[6]

Our interest in the human spleen was stimulated by a new approach to analyse and better control the function of DCs *in situ*. The approach uses monoclonal antibodies (mAbs) that selectively target DCs. In the case of mouse DEC-205/CD205 ('DEC'), several antigens have been introduced into the corresponding mAb. Antibody-based delivery improves antigen presentation *in vivo* 100-fold.[7,8] This approach is therefore also being developed as a means to control the immune response during vaccination.[9–11] To extend this approach to humans, the distribution of CD205+ DCs needs to be determined. In

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the human lymph node, CD205+ cells were found to be abundant in the T-cell areas, where the cells coexpressed the CD11c integrin and major histocompatibility complex (MHC) class II, as expected for DCs.[12] Here we extend our studies to CD205+ cells in the human spleen.

The spleen provides two important functions: it is a filtration organ for microbes and senescent red cells in the blood, and it is a secondary lymphoid organ, being the major organ for defence against blood-borne pathogens, in which responses against thymus independent 2 (TI-2) antigens need to be elicited. Anatomically, the spleen is divided into an erythrocyte-rich red pulp and a lymphocyte-rich white pulp that focus on these filtration and immune functions, respectively. The red pulp consists of irregularly shaped blood vessels, the splenic sinuses, which are separated by diffuse strands of macrophage-rich tissue known as the cords of Billroth. White pulp consists mostly of lymphocytes, with T cells dominating in periarterial lymphatic sheaths and B cells in follicles and a peripheral marginal zone.

Here we report that most DEC+ cells in the human spleen are CD11c+ and that, in addition to the white pulp proper, DEC+ DCs are found in a border region between the red and white pulp, in the location occupied by mucosal addressin cell adhesion molecule (MAdCAM)+ cells, but are distinct from phagocytes expressing CD169/sialoadhesin, DC-specific ICAM-3 grabbing non-integrin (SIGN)/CD209 and mannose receptor/CD206. Most of the DEC+ cells in all regions of the human spleen were found to be immature, consistent with previous observations using CD11c to mark splenic DCs.[6]

Materials and methods

Human tissue

Spleens were obtained from brain-dead organ transplant donors through the Regional Organ Procurement Organization/Organ Donor Network at the Islet Cell Transplant Program at Weill Cornell Medical College. Ethical guidelines regulating the use of human tissues were followed and written informed consent was obtained from the appropriate next of kin. No information about the donors was made available to us except that the splenic tissue was regarded as normal.

Immunofluorescence

Spleen tissue was frozen in OCT Compound (Sakura Finetechnical Co, Tokyo, Japan) and stored at −80°. Frozen sections (6 to 8 μm) prepared using a Microm cryostat (Microm Laborgeräte, Walldorf, Germany) were air-dried and stored at −20°. Upon thawing, sections were air-dried, fixed for 10 min in acetone, rehydrated in phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA) and stained for 30–60 min with primary antibody (Table 1). After washing in PBS, sections were stained with Alexa Fluor 488- or Alexa Fluor 568-conjugated anti-mouse subclass-specific secondary antibodies (Molecular Probes, Eugene, OR) diluted in PBS/1% BSA to 2 μg/ml. Most slides were viewed on a Molecular Devices Olympus AX70 deconvolution microscope (Olympus America Inc, Lake Success, NJ) running METAMORPH Meta Imaging

Table 1. Antibodies used for immunofluorescence

Antibody	Specificity	Clone	Isotype	Final [ ]	Source
CD3	T cells	SK7	IgG1	2 μg/ml	BD
CD11c	α_x integrin	KB90	IgG1	2 μg/ml	Dako
		SHCL-3	IgG2b	2·5 μg/ml	BD
CD14	Monocytes	MϕP9	IgG2b	5 μg/ml	BD
CD19	B cells	HIB19	IgG1	2 μg/ml	BD
CD20	B cells	L27	IgG1	2 μg/ml	BD
CD23	FDC:FcεRII	M-L233	IgG1	1 μg/ml	BD
CD68	Lysosomes/macrosialin	MO814	IgG1	4 μg/ml	Dako
CD74	Invariant chain	LN2	IgG1	10 μg/ml	GeneTex
HLA-DR	Class II	L243	IgG2a	0·5 μg/ml	BD
CD86	B7-2	2331:FUN-1	IgG1	2·5 μg/ml	Pharmingen
CD169	Sialoadhesin	HSn 7D2	IgG1	1·3 μg/ml	Novus Biological
CD205	DEC-205	MG38-2	IgG2b	1 μg/ml	Our lab
		Clone #77	IgG1	1 μg/ml	Our lab
CD206	MMR	19.2	IgG1	1·5 μg/ml	A. Lanzavecchia
CD208	DC-LAMP	104.G4	IgG1	4 μg/ml	Immunotech
CD209	DC-SIGN	Several	IgG1, IgG2a	1 μg/ml	Our lab
Anti-IgM	B cell surface immunoglobulin	SA-DA4	IgG1	5 μg/ml	eBioscience
Anti-MAdCAM-1	Mucosal addressin cell adhesion molecule	31468	IgG1	1 μg/ml	Serotec

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series software (Universal Imaging Corporation, West Chester, PA). Slides were also examined and photographed in the RU Bio-Imaging Resource Center on an upright laser scanning Zeiss Axioplan confocal microscope (Carl Zeiss Microimaging, Jena, Germany) running LSM 510 software (Carl Zeiss Microimaging).

Cloning and production of anti-human DEC-205 monoclonal antibody

Because the original MG38-2 hybridoma to human receptor DEC-205 was found to produce both κ and λ light chains, we cloned from total hybridoma RNA the single heavy chain and the DEC-binding λ light chain.[13] The variable (V) region was produced with the 5′-RACE PCR kit (Invitrogen, Carlsbad, CA) using primers for the 3′-end of mouse immunoglobulin G2b (IgG2b) and immunoglobulin λ. To obtain full-length heavy and light chain immunoglobulin cDNA, the V regions were cloned in frame with a signal peptide and the respective mouse immunoglobulin heavy and light constant domains. Monoclonal antibody (mAb) was produced by transient transfection (calcium phosphate) in 293 T cells in serum-free Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with Nutridoma SP (Roche, Nutley, NJ), purified on protein G columns (GE Healthcare Biosciences, Uppsala, Sweden), and characterized by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and western blot. The integrity of the mAb was further characterized on immature DCs by fluorescence-activated cell sorting (FACS) using a phycoerythrin (PE)-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA).

Other antibodies

Our panel of mAbs is described in Table 1. The results with cloned MG38-2 were confirmed with several other newly produced anti-DEC-205 mAbs. These were produced by one of us (CGP) following immunization of DEC knockout mice with the extracellular domain of human DEC-205, and will be described in a future publication.

Results

DEC/CD205 staining is in the white pulp

When we carried out immunofluorescence staining of frozen sections of human spleen with a mAb to CD205, we noted numerous dendritic profiles in large areas of the spleen (Fig. 1). We proceeded to use a panel of

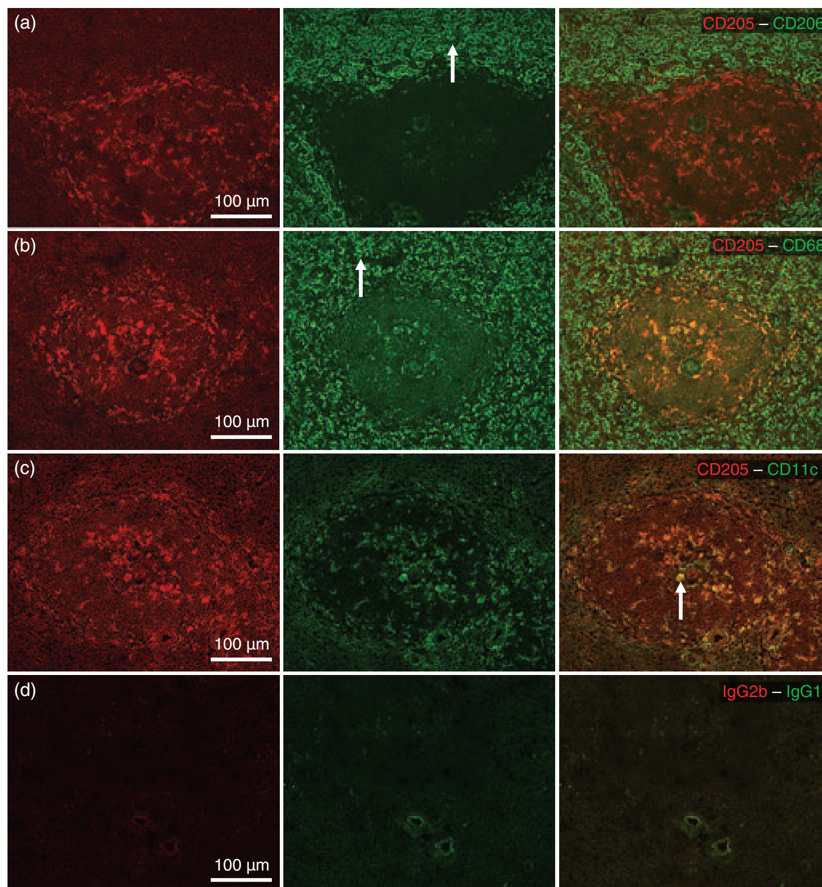

Figure 1. Cells expressing DEC-205/CD205 at high levels are restricted to the white pulp of the human spleen. Double staining of spleen sections was carried out with anti-DEC-205 and antibodies (0·5 µg/ml) to macrophage mannose receptor (MMR)/CD206 (a), CD68 (b), CD11c (c), or non-reactive isotype controls (d). Bar, 100 µm. The distribution and phenotype of dendritic cells in these sections are representative of all 13 spleens examined. (a) Arrow points to $CD206^+$ endothelial cells in the red pulp; (b) arrow points to $CD68^+$ macrophages throughout the red pulp; (c) arrow points to a typical cell where CD11c and DEC are colocalized.

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mAbs to more precisely define the regions of the spleen containing DEC$^+$ cells as well as the phenotype of these cells.

An antibody to the macrophage mannose receptor (MMR/CD205)[14] has been shown previously to mark endothelial cells lining venous sinuses in the red pulp.[15] Similarly, we observed string-like labelling for CD206 on the sinus-lining endothelial cells throughout the red pulp (arrow, Fig. 1a). Staining with anti-DEC-205 was confined to the white pulp and was not seen in the CD206-rich red pulp areas (Fig. 1a).

The red pulp was also defined with an antibody to CD68/microsialin, a lysosomal-associated glycoprotein that is abundant in macrophages.[16] Anti-CD68 stained macrophages throughout the red pulp (arrow, Fig. 1b). Previous research by Martinez-Pomares *et al.* showed that the CD68$^+$ macrophages are distinct from the CD206$^+$ sinus-lining endothelium, a result that is confirmed here. Double staining for CD68 and CD205 confirmed a lack of DEC labelling of red pulp macrophages but showed clear colabelling in the white pulp, as observed previously in mice.[17] Therefore, DEC$^+$ cells are restricted to the white pulp regions of the human spleen, and are not found in the red pulp with its CD206$^+$ sinusoids and CD68$^+$ macrophages.

To verify that the DEC$^+$ cells were DCs, we double labelled for the integrin CD11c, which is expressed at high levels on many types of DCs in human blood and lymph node[12,18] as well as DCs generated from monocytes in culture.[19] The CD11c$^+$ cells were primarily found in the white pulp, just like DEC-205, and the two markers colocalized on the same profiles (arrow, Fig. 1c) with only rare exceptions, i.e. almost all of the DEC$^+$ cells were CD11c$^+$ and vice versa. Isotype control mAbs were negative (Fig. 1d). We conclude that, in the human spleen, DEC-205 is expressed predominantly on CD11c$^+$ DCs in the white pulp nodules.

DEC$^+$ cells are found in the T-cell areas of the human spleen

We looked at higher power at the T-cell areas, a standard site in which DCs are localized in peripheral lymphoid tissues.[3] As defined by staining with anti-CD3, the T-cell-rich regions of human spleen comprised a relatively small proportion of the white pulp in all 13 specimens that we examined (Fig. 2a). These CD3-rich areas were near a vessel(s) and contained a high density of stellate DEC$^+$ cells (Figs 2a and b; arrows Fig. 2b). The T-cell areas of the human spleen were not devoid of B cells, as indicated by staining with anti-CD20, but the T-cell area DCs were CD20 negative (arrow, Fig. 2c). It was also apparent that DEC$^+$ cells were numerous in non-T-cell areas. The localization of DEC$^+$ CD11c$^+$ cells in B-cell areas of the human spleens is surprising, as this has not been observed in previous work in mice, and this will be the subject of future research.

Double staining with anti-CD68 confirmed that many CD68$^+$ cells were in the T-cell areas double labelled for DEC, although the two markers were in different parts of the cell (arrow, Fig. 2d). Colabelling with anti-CD11c indicated that essentially all of the DEC$^+$ cells were CD11c$^+$ (arrow, Fig. 2e). However, an occasional CD11c$^+$ cell was DEC$^-$ and also a rare DEC$^+$ cell was CD11c$^-$ (not shown). Therefore, DEC$^+$ DCs are abundant in the T-cell areas of human spleen.

DEC$^+$ cells in the T-cell areas do not express markers for DC maturation

To obtain some initial information on the functional properties of the DCs in the T-cell areas, we first verified that the DEC$^+$ cells could be double labelled with anti-HLA-DR (Fig. 3a) and anti-CD74/invariant chain (Fig. 3b) mAbs. We next examined markers for DC maturation. DC-lysosomal-associated membrane protein (LAMP) (CD208) is a lysosomal antigen that is induced upon maturation of monocyte-derived DCs in culture.[20] Serial sections (not shown) revealed that, at low power (arrows, Fig. 3b), the expression of DC-LAMP was restricted to the T-cell areas, while at higher magnification (arrow, Fig. 3c) only a subpopulation of DEC$^+$ cells expressed DC-LAMP.

The expression of the costimulatory molecule CD86 is increased upon DC maturation.[21] At low magnification (not shown), CD86 was expressed in both T-cell and B-cell areas but the frequency of positive cells was low. Double staining showed that most CD86$^+$ cells were DEC$^+$. In the T-cell areas, the DEC$^+$ cells were typically CD86 low or negative (arrow, Fig. 3d) but occasional fields showed higher numbers of DEC$^+$ CD86$^+$ cells (Fig. 3e). Taken together, these observations indicate that most DEC$^+$ DCs in the T-cell area are in an immature functional state, as was concluded by McIlroy for CD11c$^+$ cells.[6]

DEC$^+$ cells at the periphery of splenic white pulp are distinct from CD169$^+$ and CD209$^+$ cells in the perifollicular region

In addition to the DEC$^+$ DCs in the T-cell and B-cell areas of human spleen, DEC$^+$ profiles were found in the border region between the red and white pulp. In contrast to the mouse spleen, the human spleen possesses an additional region, known as the perifollicular zone, surrounding the marginal zone.[22,23] Steiniger *et al.* used an antibody to sialoadhesin (CD169) to define macrophages in the perifollicular zone.[5] We included two other antibodies (specific for MMR/CD206 and DC-SIGN/CD209) as well as CD169 to define this region, and the expression of these antigens by DEC$^+$ cells.

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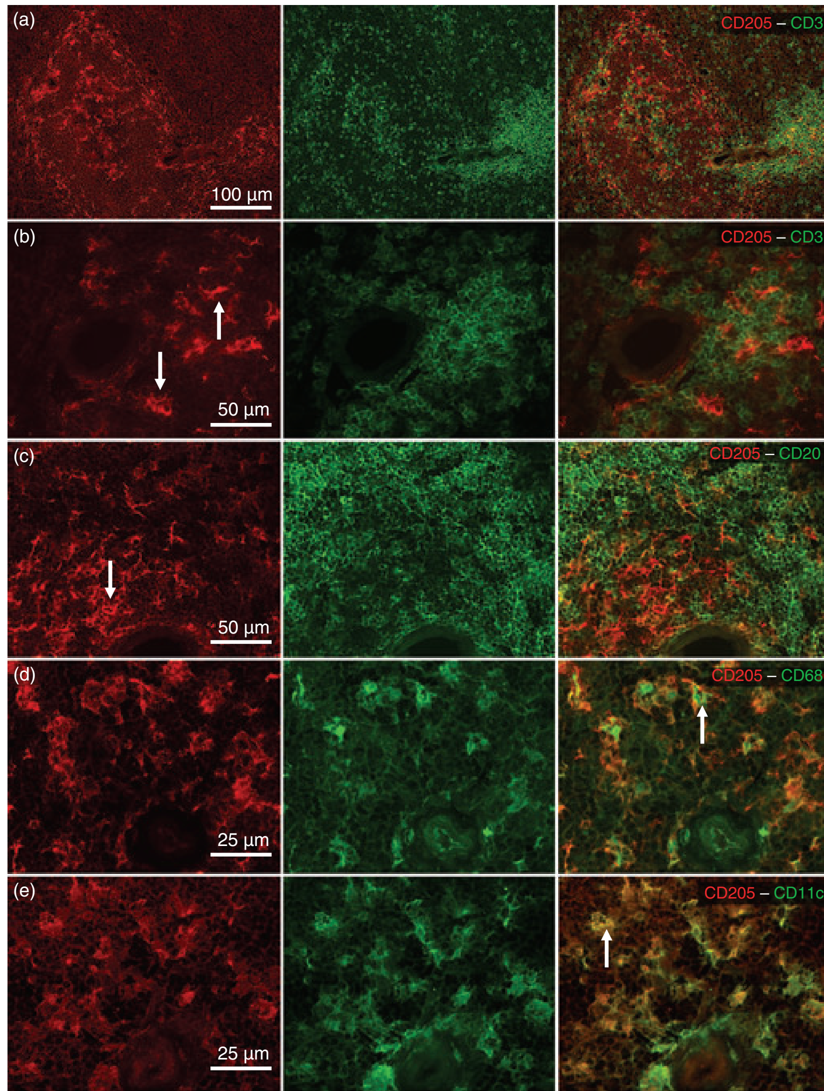

Figure 2. Markers of DEC$^+$ cells in the T-cell areas of the human spleen. Double staining of spleen sections was carried out with anti-DEC-205 and antibodies to CD3 (a, b), CD20 (c), CD68 (d) and CD11c (e). Bar, 25 (a, b, d, e) or 100 (c) µm. (b) Arrow points to stellate DEC$^+$ cells; (c) arrow points to a representative DEC$^+$ cell that is CD20 negative; (d) arrow points to a cell where DEC and CD68 appear in different parts of the cell; (e) arrow points to a representative cell where DEC and CD11c colocalize.

Expression of CD206, as it stains the endothelial cells lining the venous sinuses, was used to define the red pulp. At low and high power (arrows, Figs 4a and b), DEC$^+$ cells were found interior to the region defined by CD206 staining. CD169 stains cells, presumably macrophages, forming sheaths around the capillaries that surround the white pulp. When we double stained for CD169 and DEC (Fig. 4c), a ring of DEC$^+$ cells was seen interior to the cells stained with anti-CD169 (arrow, Fig. 4c). This places DEC$^+$ cells just inside the ring of cells defined by CD169 and therefore interior to the CD169$^+$ region of the perifollicular region described by Steiniger *et al.*

Using antibodies to DC-SIGN/CD209,[12] we found that this marker was not expressed on either the macrophages of the red pulp or the DCs of the white pulp. However, a relatively low frequency of cells, which were strongly positive for DC-SIGN, was noted at the border of the red and white pulp. Some CD169$^+$ cells stained for CD209 while other profiles were singly positive for each marker. Double staining with anti-CD206 and anti-DC-SIGN showed that CD209 defined a broken ring of cells just inside the region stained for CD206 (arrow, Fig. 4d). The CD209$^+$ cells were quite large and irregularly shaped. When we double stained for DEC and CD209, most DEC$^+$ cells were distinct from and interior to the CD209$^+$ cells (arrow, Fig. 4e), although occasional cells were found where colocalization was seen. Therefore, DC-SIGN$^+$ cells are in the perifollicular zone along with the CD169$^+$ cells.

In the marginal zone, i.e. the B-cell area around primary and secondary follicles, Steiniger *et al.* found that, unlike in the mouse, CD169$^+$ cells were absent. They were further able to distinguish the marginal zone from the perifollicular zone by B-cell staining, as they described IgM$^+$ CD20$^+$ cells at the outer region of the marginal zone becoming more scattered into the perifollicular zone.[5] We therefore used IgM to define and delimit the outer marginal zone from the perifollicular area. When we double stained for

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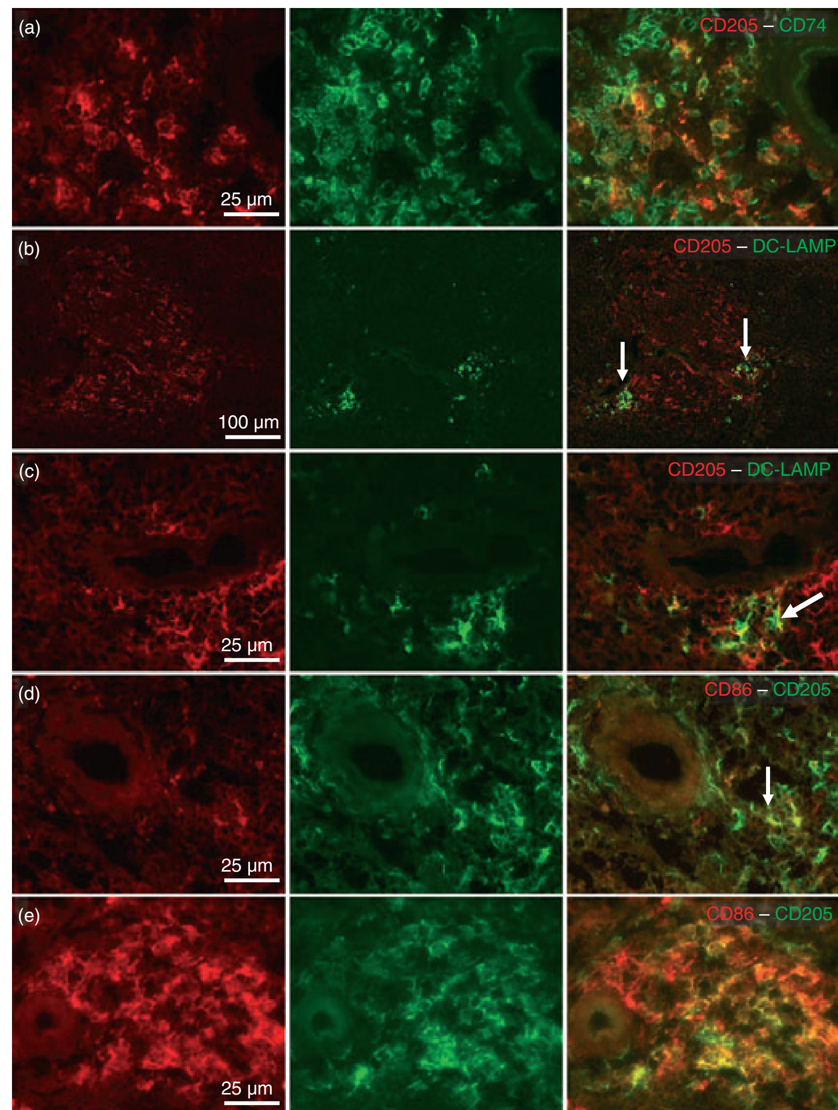

Figure 3. Dendritic cells (DCs) in T-cell areas of the human spleen do not express high levels of costimulatory molecules. Double staining of spleen sections was carried out with anti-DEC and antibodies to CD74 (a) DC-lysosomal-associated membrane protein (LAMP) (b, c) and CD86 (d, e). (b) Arrows indicate that expression of DC-LAMP is restricted to T cell areas; (c) arrow indicates that only a subpopulation of DEC+ cells express DC-LAMP; (d) arrow points to DEC+ cells with low expression of CD86.

DEC and IgM, we observed that most of the DEC staining in the border region was outside the region defined by IgM (Fig. 4f), i.e. outside the marginal zone.

DEC+ DCs in the perifollicular region colocalize with the MAdCAM-1 addressin

Steiniger *et al.* have also described MAdCAM-1-positive cells in the perifollicular and marginal zone regions.[24] When we double stained for DEC and MAdCAM-1, the latter stained rings of elongated branched cells around the white pulp nodules (arrows, Fig. 5a). DEC+ cells were found to be closely associated with MAdCAM-1+ cells (dotted box, Fig. 5a, and arrows, Figs 5b and c), sometimes as double rings in this area. Double staining with DC-SIGN and MAdCAM-1 placed the DC-SIGN+ cells (arrows, Figs 5d and e) outside the region defined by MAdCAM-1. We conclude that DEC-205/CD205-positive cells are found in the border region between the red and white pulp outside the marginal zone area defined by IgM and directly within the rings of cells that express MAdCAM-1 (Fig. 5f).

Discussion

Whereas the literature offers a large body of work describing the vascular and cellular circulation and the microstructure of the mouse spleen, species differences are well documented,[4,25] and there exist important gaps in the delineation of potential antigen-capturing and -presenting cells in the human spleen. Similarly, only a few studies have attempted to define the phenotype and distribution of human spleen DCs *in situ* and none has attempted to identify DCs with the DEC-205 marker. Studying isolated cells in suspension, Kato *et al.* described expression of DEC-205 on DCs and monocytes from blood, as well as low-level expression by other cell types including natural killer (NK) cells, T cells and plasmacytoid blood DCs,[26] confirming

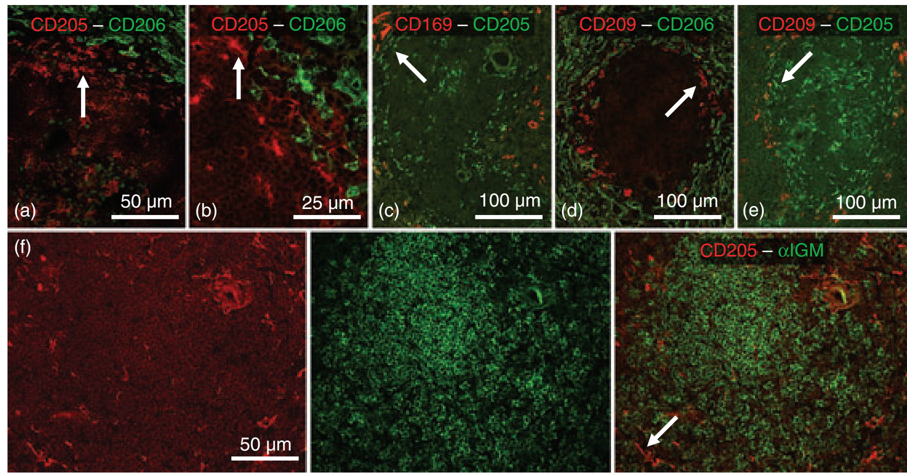

Figure 4. DEC$^+$ cells are found at the border region between the red and white pulp. (a, b, c, e, f) Double staining of spleen sections was carried out with anti-DEC and antibodies to CD206 (a, b), CD169 (c), CD209 (e), and immunoglobulin M (IgM) (f). (d) Double staining of spleen sections was carried out with anti-DC-specific ICAM-3 grabbing non-integrin (SIGN)/CD209 and macrophage mannose receptor (MMR)/CD206. Bar, 25 (b), 50 (a, f) or 100 (c, d, e) µm. (a, b) Arrows indicate DEC$^+$ cells occupying a position interior to the region defined by CD206 staining; (c) arrow points to a ring of DEC$^+$ cells interior to the cells stained with anti-CD169; (d) arrow points to a broken ring of CD209$^+$ cells that lies just inside the region stained for CD206; (e) arrow points to a DEC$^+$ cell distinct from and interior to the CD209 cells; (f) arrow points to a DEC$^+$ cell outside the region defined by IgM.

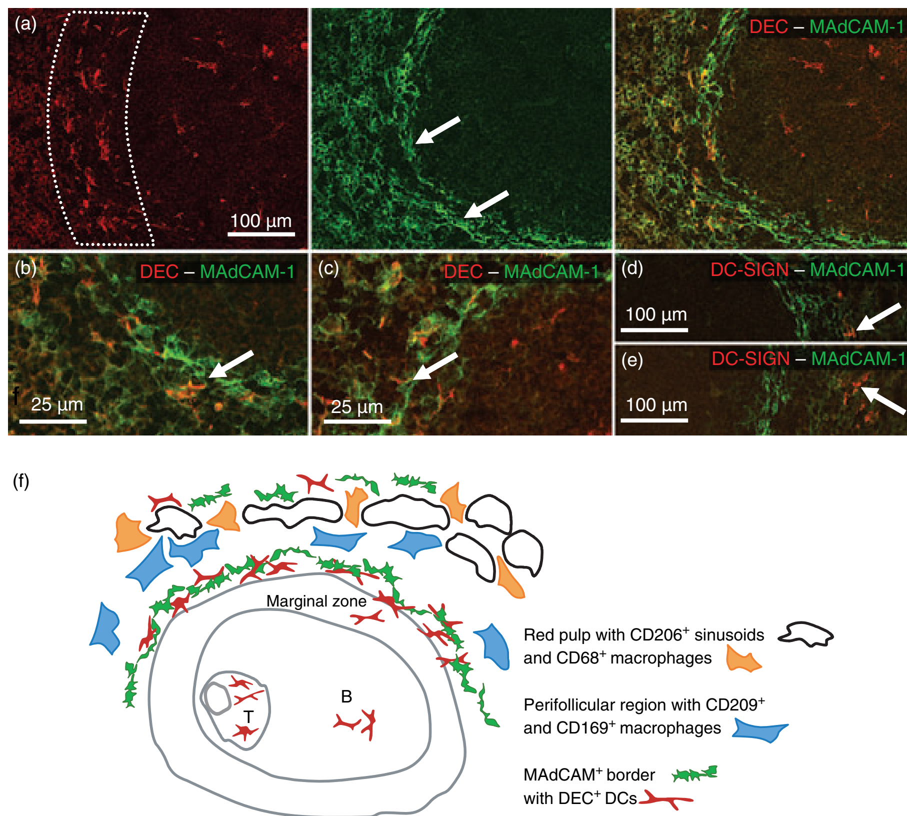

Figure 5. DEC$^+$ cells are closely associated with mucosal addressin cell adhesion molecule (MAdCAM)$^+$ cells in the perifollicular region. Double staining of spleen sections was carried out with anti-DEC-205 and antibodies to MAdCAM-1 (a–c) and DC-specific ICAM-3 grabbing non-integrin (SIGN) (d, e). Bar, 25 (b, c) or 100 (a, d, e) µm. F is a diagram describing the border region of human splenic white and red pulp, as defined with the monoclonal antibodies used in this paper. (a) Arrows point to MAdCAM-1 staining of rings of elongated branched cells around the white pulp nodules; (b, c) arrows point to DEC$^+$ cells in close association with MAdCAM-1$^+$ cells; (d, e) arrows point to DC-SIGN$^+$ cells outside the region defined by MAdCAM-1.

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results from mice.[27] In sections, however, the DEC-205-rich cells that we describe here are primarily if not exclusively DCs because they double label for CD11c, and carry other myeloid markers such as CD68 rather than the B-cell markers CD19 and CD20.

With the caveat that sensitivity limitations of immunohistochemistry may preclude detection of low levels of antigen expression, we used antibody to CD205, also known as DEC-205, to study frozen sections of spleens from 13 organ donors by immunocytochemistry. DEC-205 is an antigen uptake and processing receptor that belongs to a family of type I transmembrane multilectin receptors that includes the MMR and the phospholipase A2 R (PLA2R).[28] Antibodies to DEC-205 target vaccine proteins selectively and efficiently to mouse DCs *in vivo*. This preclinical vaccine research used the mouse antibody to DEC-205, originally termed NLDC145,[29,30] which stains interdigitating cells, Langerhans cells and thymic epithelia and has been used to localize mouse DCs in tissues and to phenotype mouse DC subsets. However, we are now readying anti-human DEC mAbs for evaluation in vaccines in humans.

McIlroy *et al*. localized DCs in human spleen using antibodies to CD11c.[6] These authors observed strongly CD11c+ DCs in three distinct regions: at the periphery of the white pulp, in the T-cell zones and in the B-cell zones. The careful study of Steiniger *et al*. defined the perifollicular zone as a region occupied by strongly sialoadhesin+ macrophages.[5] Our results using antibodies to DEC-205 are consistent with these previous reports.

We also found DEC+ zones in three regions: the T-cell areas, the B-cell areas, and the inner aspect of the perifollicular zone. Although the T-cell areas of the human spleen are relatively small, numerous DEC+ cells were present in the T-cell zones and they were positive for CD11c, DR and CD74. The interesting feature of DCs in the T-cell areas is that only small groups of cells express markers of maturation such as DC-LAMP/CD208 and the costimulator CD86. McIlroy *et al*. also found that CD11c+ DCs were distributed evenly throughout the T-cell zones and that only a minority of cells, present only in some spleens, were mature, expressing CD86 and CD83.[6] They proposed that some spleen DCs in a subset of organ donors had been activated *in vivo*, perhaps in the process of antigen presentation and specific immune response induction. We would like to suggest that, as in mice, the function of most DEC+ DCs in the human spleen is to capture harmless self and environmental antigens for purposes of tolerance[7] and that the few mature DCs have captured antigens for purposes of immunity.

DCs in the B-cell areas were also immature and will be the subject of a future report, given the fact that, in mice, DCs are not typically found in B-cell areas, in contrast to all 13 human spleens that we studied here.

The finding of DEC+ cells in the border region between the red and white pulp led us to define their location within the microarchitecture of the spleen (Fig. 5f). In mice, DEC+ cells are found in the T-cell areas but not in the border region of red and white pulp, at least with current staining methods. The situation is complicated in the human spleen by the presence of an additional compartment unique to the human spleen, the perifollicular zone, which was initially described by van Krieken and te Velde.[22,23] Steiniger *et al*. defined the perifollicular zone between the red pulp and the marginal zone as a region occupied by strongly sialoadhesin-positive macrophages.[5] They further distinguished the perifollicular zone from the marginal zone, which they defined as a region occupied by IgM+ IgD− cells. Steiniger also found that MAdCAM-1, a vascular addressin expressed in gut lamina propria and high endothelial venules of the lymph node and Peyer's patch, is expressed on cells in this region.

We have found that, around the white pulp nodule, the scattered DCs were largely distinct from CD209+ and CD169+ macrophages. We have located the position of the DEC+ cells as inside the red pulp region (defined by staining of the sinusoids for MMR/CD206), inside the perifollicular region (defined by staining with CD169 and CD209 antibodies) and outside the marginal zone (stained with anti-IgM). We have pinpointed the location of the DEC+ cells to the region occupied by MAdCAM+ cells. The finding of DEC+ cells in the border region between the red and white pulp could be significant because this region, as Steiniger *et al*. have suggested, probably represents a compartment where antigen and recirculating lymphocytes enter the organ. DCs in this site are therefore positioned to capture antigens.

Acknowledgements

We gratefully acknowledge the expertise and help of Alison North, Shivaprasad Bhuvanendran and Mathieu Marchand in the Rockefeller University Bio-imaging Resource Center, and Judy Adams for preparation of the manuscript. Grant support was provided by a grant to RMS from NIAID (AI40045).

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