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Generation of anti-DC-SIGN monoclonal antibodies capable of blocking HIV-1 gp120 binding and reactive on formalin-fixed tissue

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ABSTRACT

DC-SIGN is a C-type lectin of recognized importance in immunology and in the pathogenicity human pathogens. Monoclonal antibodies directed against DC-SIGN have been generated, but their systemic characterization for interfering with binding of the HIV-1 glycoprotein 120 has often been omitted. Moreover, so far, no anti-DC-SIGN monoclonal antibody has been described that recognizes its antigen after formalin fixation and paraffin embedding. In this study, we have generated new anti-DC-SIGN monoclonal antibodies using HeLa cells stably expressing DC-SIGN as immunogen. We have obtained 11 hybridoma clones producing antibodies that recognized DC-SIGN on monocyte-derived dendritic cells and on dermal-type macrophages. Seven monoclonal antibodies displayed a capacity to interfere with DC-SIGN binding to HIV-1 gp120. One recognized DC-SIGN on formalin-fixed dendritic cells and macrophages. Using this antibody we have obtained specific labelling of DC-SIGN and colocalisation with the dermal macrophage marker CD163 on human skin. The described monoclonal anti-human DC-SIGN antibodies will be of use to the scientific community to address fundamental immunology issues, in particular concerning macrophages and dendritic cells, and help elucidate infection events of pathogen targeting DC-SIGN as recognition receptor.

1. Introduction

Among the family of human C-type lectins, DC-SIGN (dendritic cell-specific intracellular adhesion molecules (ICAM)-3 grabbing non-integrin, CD209) is probably the best known. DC-SIGN is a type-II, 404 amino acid transmembrane protein, comprising intracellular signaling signatures and extracellular pattern recognition domains [1]. The intracellular moiety mediates internalization, while the extracellular domain comprises the neck repeats and the C-type carbohydrate-recognition domain (CRD), which binds to carbohydrates rich in mannose. Human DC-SIGN is 77% homologous to L-SIGN on the protein level [2]. The structure of L-SIGN is almost identical to that of DC-SIGN, except for some heterogeneity in the neck region [1].

Human DC-SIGN is expressed by dendritic cells, derived from monocytes (MDDC) or from $CD34^+$ progenitor cells in the presence of GM-CSF and IL-4/IL-13 [3,4]. The cytokines IL-4 or IL-13 are primarily responsible for the induction of DC-SIGN while GM-CSF helps to increase expression levels [3,5]. In addition, DC-SIGN is induced on monocytes by IL-10 and GM-CSF [6]. These cells (MDdM) display phenotypic characteristics of dermal macrophages [6]. L-SIGN is not expressed by MDDCs and it is only found on endothelial cells [7].

The skin and the lymph nodes have been the most closely scrutinized tissues to identify the cells that express DC-SIGN *in vivo*. Although it was initially reported that exclusively DCs express this C-type lectin [8,9], further investigations have shown that dermal macrophages, lymph node subcapsular sinus and lymph node medullary cord macrophages are the main DC-SIGN-expressing cell types *in vivo* [6,10–14]. Only few studies have investigated the expression of DC-SIGN or L-SIGN in diseased human tissues [15].

DC-SIGN is a ligand for ICAM-3, which is expressed by resting T cells [8], and for ICAM-2, expressed by blood and lymphatic endothelium [9]. Best known, however, it the recognition of DC-SIGN and L-SIGN by human pathogens [16]. As endocytic receptors, C-type lectins can then internalize the bound pathogens and participate in infection and/or immune-evasion [16]. For instance, HIV-1 is internalized by MDDCs through the binding of its glycoprotein gp120 with DC-SIGN. Upon contact with T cells, HIV-1, bound to DC-SIGN, is released to productively infect T cells [4,17–19]. L-SIGN can likewise interact with HIV-1 and enhance infection of T-cells in *trans* [2]. In the case of the dengue virus, DC-SIGN interacts with its envelope E glycoprotein and mediates productive infection of

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MDDCs [20,21]. We have recently shown that MDdMs also bind the dengue virus E protein and internalize the virus. However, the MDdMs resist productive infection and sequester the virus in endosomal compartments [6]. It is unknown whether MDdMs would allow dengue virus infection *in trans* alike HIV.

The study of DC-SIGN is therefore of importance not only to better understand the interactions between the antigen presenting cells, DCs/macrophages, and T/endothelial cells, but also to elucidate the pathogenesis of human pathogens. This has triggered an extensive search into molecular tools, such as antibodies, specific for DC-SIGN. A number of mouse monoclonal antibodies (mAbs) have therefore been generated against DC-SIGN. However, only few have been systematically screened for their capacity to efficiently block DC-SIGN interaction with HIV-1 gp120 [4,19]. Moreover, to date, no mAb has been described to recognize DC-SIGN on formalin-fixed, paraffin-embedded cells or tissue. Instead, most studies aimed at identifying DC-SIGN-expressing cells in tissue are conducted with frozen tissue [8–10,12–14,22,23]. In few studies, DC-SIGN was recognized on formalin-fixed sections with polyclonal antibodies [5]. The availability of a mAb reactive with formalin-treated DC-SIGN would facilitate analysis of DC-SIGN expression in human tissue cohorts, that are most commonly available as paraffin blocks. Also, such a mAb would enable the intracellular detection of DC-SIGN which requires cell fixation before permeabilisation.

To generate a high diversity of DC-SIGN specific antibodies, we have raised mouse anti-DC-SIGN mAbs against HeLa cells expressing DC-SIGN. We obtained 11 mAbs capable of binding DC-SIGN, of which 7 were able to block interaction with HIV-1 gp120 and one mAb could react with DC-SIGN on formalin-fixed, paraffin-embedded cells or tissue.

2. Material and methods

2.1. Generation of anti-DC-SIGN monoclonal antibodies

Balb/c mice (Charles River, Saint Germain sur l'Arbresle, France) were immunised with HeLa cells stably expressing DC-SIGN, provided by Olivier Schwartz, Institut Pasteur, France. Mice were injected i.p. with 2×10^6 HeLa-DC-SIGN cells in combination with complete Freund's adjuvant (CFA, Sigma–Aldrich, L'Isle d'Abeau, France), followed by a boost with HeLa-DC-SIGN cells in incomplete Freund's adjuvant (IFA, Sigma) and another boost without adjuvant. The last boost was realized by injecting 4×10^6 HeLa-DC-SIGN cells in PBS i.v. Six days after the final boost, spleen cells were fused with the murine myeloma SP2/0 (Dendritics, Lyon, France). The hybridomas were selected using standard technology, and supernatants screened for DC-SIGN-specific mAbs by flow cytometry on non-transfected HeLa cells and HeLa-DC-SIGN cells. The isotypes of the generated mAbs were determined using the Isostrip mouse monoclonal antibody isotyping kit (Roche, Meylan, France) or using FITC-conjugated goat anti-IgM mouse and anti-IgG mouse secondary antibodies and flow cytometry. The mAbs are available through Dendritics (www.dendritics.net).

2.2. Screening of the mAb activity against DC-SIGN and L-SIGN by flow cytometry

Given that DC-SIGN and L-SIGN are 73% homologous and are identical in the membrane-proximal repeat region of the ectodomain [1,2], we screened the hybridoma supernatants for activity against cell lines expressing DC-SIGN or L-SIGN. In addition to HeLa-DC-SIGN cells, we used NIH-3T3 stably transfected with DC-SIGN (NIH-3T3/MX-DC-SIGN, from T.D. Martin and V.N. KewalRamani) and L-SIGN (NIH-3T3/MX-L-SIGN, both from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAD, NIH). Cells were amplified in standard complete medium (RPMI comprising glutamine, antibiotics and 10% fetal calf serum (all from Lonza-Invitrogen)) and incubated with the hybridoma supernatant for 30 min on ice. After washing with PBS containing 2% fetal calf serum and 5 mM EDTA, fluorochrome-conjugated goat anti-mouse secondary antibody (Molecular Probes-Invitrogen) was added and incubated for 15 min on ice. After washing, the cells were fixed in PBS-buffered 2% formalin and analysed by flow cytometry on a FACS-Calibur (Becton-Dickinson). The data was processed using the Cellquest Pro software (Becton-Dickinson).

2.3. HIV-1 gp120 binding assay

HeLa and HeLa-DC-SIGN cells were incubated with biotin-conjugated recombinant gp120 HIV-1 IIIB (Immunodiagnostics Inc., Woburn, USA) in PBS, 2 mM $MgCl_2$ and 1 mM $CaCl_2$ for 45 min at 37 °C. After washing with PBS, 2 mM $MgCl_2$ and 1 mM $CaCl_2$ and 1% BSA, the cells were stained with PE-conjugated streptavidin (DAKO, Trappes, France) for 30 min at 4 °C. For the gp120 blocking assay, the cells were pre-incubated with antibodies against DC-SIGN for 15 min at 4 °C before the incubation with biotin-conjugated gp120. The gp120 binding on DC-SIGN was measured by flow cytometry on a FACS-Calibur and data was processed using the CellQuest Pro software.

2.4. Generation of monocyte-derived antigen presenting cells

Monocytes, purified from adult blood by counterflow centrifugal elutriation, were bought from the local blood bank (Etablishement du Sang Français, Alsace, Strasbourg, France). One and a half million monocytes were cultured in 5 mL complete medium (RPMI with ultraglutamine, supplemented with 10 mM Hepes, 100 μg/mL gentamycine and 10% fetal calf serum, all from Lonza-Invitrogen) in the presence of cytokines. For MDDCs, GM-CSF (50 ng/mL) and IL-4 (10 ng/mL) were added, and for MDdMs, GM-CSF (25 ng/mL), IL-10 (25 ng/mL) and M-CSF (10 ng/mL) were used (all cytokines from Immunotools, Friesoythe, Germany). At day 3, the culture was refreshed with 1 mL of complete medium containing cytokines; for MDDCs, GM-CSF (50 ng/mL) and IL-4 (10 ng/mL), and for MDdMs, GM-CSF (12.5 ng/mL) and IL-10 (12.5 ng/mL). At day 5, the cells were harvested and analysed by flow cytometry for expression of CD1a, CD14 and DC-SIGN using anti-CD14 mAb conjugated to PE (Immunotools), anti-DC-SIGN mAb conjugated to Peridinin–chlorophyll–protein complex (PerCP, Becton-Dickinson) and anti-CD1a mAb conjugated to Allophycocyanin (APC, Becton-Dickinson).

2.5. Preparation of formalin-fixed, paraffin-embedded monocyte-derived antigen presenting cells

Five hundred thousand monocytes, MDDCs or MDdMs were fixed in phosphate-buffered 4% formalin for 1 h at room temperature. They were then centrifuged and washed twice in Tris-buffered saline. A drop of melted 1% agarose was added to the tube and the cell-pellet was gently incorporated into the agarose by tapping the tube. After agarose solidification on ice, the pellet was removed from the eppendorf tube and placed into a larger recipient. The cell-agarose pellet was dehydrated under shaking at room temperature in successive steps of 70% ethanol (30 min), 95% ethanol (30 min), 100% ethanol (2× 1 h), 100% butanol (2× 1 h). Melted paraffin was then added and the butanol was allowed to evaporate at 62 °C for 24 h. Finally, the cell-agarose pellets were included in paraffin blocks.

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Table 1
Summary of the anti-human DC-SIGN monoclonal antibodies, their reactivity by flow cytometry and by immunofluorescence on formalin-fixed and paraffin-embedded tissue and their capacity to block DC-SIGN–HIV-1 gp120 binding.

mAbs	Flow cytometry					Immunofluorescence (paraffin)			Functionality
	NIH-3T3 DC-SIGN	NIH-3T3 L-SIGN	MDDC	MDdM	Monocytes	MDDC	MDdM	Monocytes	HIV blockage
120C11	+	–	+	+	–	–	–	–	++
111H2	+	+	+	+	–	+	+	–	+/–
120E12	+	–	+	+	–	–	–	–	+
108H8*	+	–	+	+	–	–	–	–	+
112G6	+	+	+	+	–	–	–	–	+
111E3	+	+	+	+	–	–	–	–	–
103F12	+	–	+	+	–	–	–	–	–
104D6	+	–	+	+	–	–	–	–	+
105G10*	+	–	+	+	–	–	–	–	+
106A4	+	–	+	+	–	–	–	–	+
120E12 * IgM	+	–	+	+	–	–	–	–	–

2.6. Preparation of formalin-fixed, paraffin-embedded skin sections

Fresh skin was obtained from patients undergoing abdominoplasties according to institutional guidelines and after approval of the bioethical review board. Skin pieces were fixed in 4% formalin for 24 h at 4 °C, pinned down onto paraffin plates. They were then rinsed in Tris-buffered saline and dehydrated under shaking at room temperature in 70% ethanol (2 h), 95% ethanol (2 h), 100% ethanol (2× 2 h), 100% butanol (2× 2 h). Melted paraffin was added and the butanol was allowed to evaporate at 62 °C for 24 h. The skin was included in paraffin blocks.

2.7. Immunolabelling of fixed cell and skin-sections

Formalin-fixed and paraffin-embedded sections were cut onto SuperFrost-Plus slides (O. Kindler, Freiburg, Germany), and allowed to dry at 58 °C overnight. The sections were deparaffinated by incubations in toluene for 10 min, ethanol for 10 min and in water for 5 min. Antigens were retrieved by heating for 30 min in 10 mM EDTA pH 8. After allowing the sections to cool to room temperature, the labelling was done in Tris-buffered saline containing primary mAbs, diluted in 1% goat serum, and incubated for 2 h at room temperature. After washing, fluorochrome-conjugated secondary Abs were added for 1 h at room temperature. Nuclear counter-coloration was done with DAPI (Molecular Probes-Invitrogen). Sections were mounted in liquid mounting medium (DAKO Fluomount) and visualized on an Axiscope 2 microscope (Zeiss) equipped with a digital camera (Nikon, DXM 1200C). The images were processed with the Photoshop software.

3. Results

3.1. Identification of anti-DC-SIGN mAbs by flow cytometry

To generate human DC-SIGN-specific mAbs, we immunised Balb/c mice with HeLa cells stably expressing human DC-SIGN. After 3 boosts, splenic B cells were fused with a murine myeloma and hybridoma clones selected. Specific binding of hybridoma supernatants to HeLa-DC-SIGN was individually tested by flow cytometry. A second selection round was performed on NIH-3T3 fibroblasts, which stably expressed DC-SIGN or L-SIGN. L-SIGN was added to this screen as both molecules share strong homology and are identical in the membrane-proximal repeat region of the ectodomain [1,2]. Table 1 lists all the mAbs found to react specifically with NIH-3T3-DC-SIGN cells or to cross-react with NIH-3T3-L-SIGN cells. We found that 8 of 11 hybridoma supernatants reactive to DC-SIGN were specific for DC-SIGN and that the remaining 3 recognized DC-SIGN and L-SIGN. To determine the immunoglobulin class, the FACS analysis was repeated with anti-IgM and IgG-specific fluorochrome-conjugated secondary Abs. We found that 2 of the 11 mAbs were of the IgM isotype (Table 1). Fig. 1A shows a representative FACS-profile for the mAbs 120C11, 111H2 and 103F12, as well as for the DC-SIGN/L-SIGN reactive commercial mAbs used as control.

3.2. Application of the novel mAbs for monitoring monocyte differentiation

DC-SIGN is expressed by immature dendritic cells (MDDC), derived from monocytes in the presence of GM-CSF and IL-4 [3]. In addition, dermal-type macrophages (MDdM), obtained by culturing monocytes in M-CSF, GM-CSF and IL-10 also express DC-SIGN [6]. We tested the newly generated mAbs for recognition of DC-SIGN expressed by MDDCs and MDdMs. All 11 DC-SIGN-reactive mAbs recognized MDDCs and MDdMs with similar sensitivity (Table 1). Fig. 1B shows the flow cytometry profiles of 120C11, 111H2 and 103F12, as well as for the two commercial DC-SIGN/L-SIGN reactive mAbs. The L-SIGN-specific mAb did not recognize the cells, showing that neither MDDCs nor MDdMs carried L-SIGN.

3.3. Determination of HIV gp120-blocking anti-DC-SIGN mAbs

Among the different pathogens for which DC-SIGN has affinity [16], DC-SIGN can bind HIV-1 by interacting with its envelope glycoprotein (gp) 120 [17,19,24,25]. Therefore, we screened the mAbs for blocking the DC-SIGN–gp120 interaction. Recombinant gp120-coupled to biotin and revealed by PE-streptavidin efficiently bound HeLa-DC-SIGN cells, as monitored by flow cytometry (Fig. 2A). Using this assay, we measured the ability of the mAbs to prevent DC-SIGN from binding to gp120. Among the 11 mAbs, 8 mAbs displayed an ability to interfere with the DC-SIGN–gp120 interaction (Table 1). Fig. 2B shows three mAbs with complete, intermediate and no blocking ability. A titration curve with the efficient blocking mAb 120C11 shows that its gp120-DC-SIGN inhibition is almost complete at 30 μg/mL (Fig. 2C).

3.4. Identification of a mAb recognizing formalin-fixed and paraffin-embedded DC-SIGN

In view of the fact, that, so far, no mouse mAb has been described to recognize DC-SIGN on formalin-fixed and paraffin-embedded cells or tissue, we screened the mAbs for this capacity on MDDCs and MDdMs. As negative control, we included monocytes, which do not express DC-SIGN or L-SIGN [9]. After deparaffination and hydration, the cell-sections were incubated with the hybridoma

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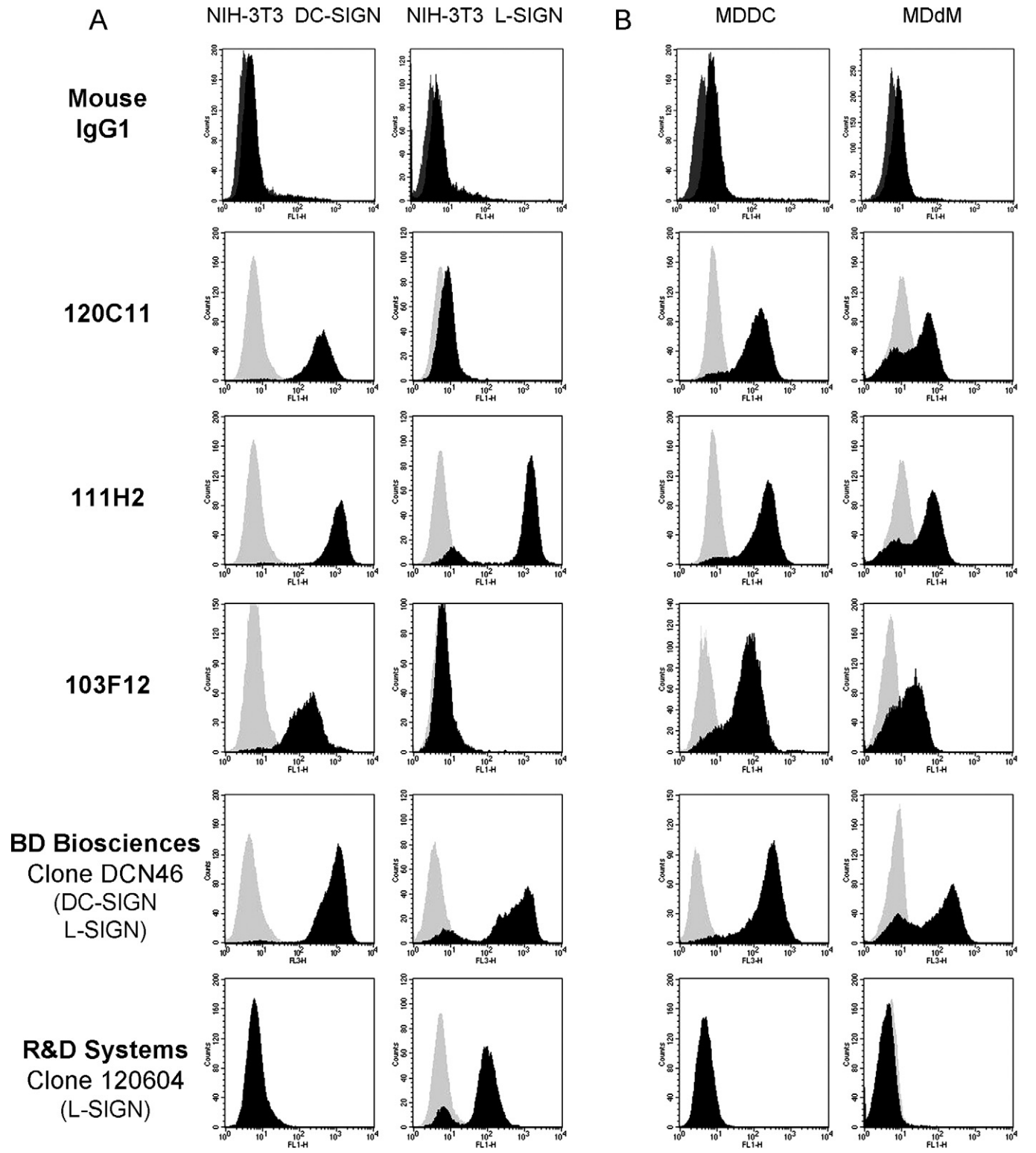

Fig. 1. Uncovering anti-DC-SIGN-specific mAbs by flow cytometry. (A) NIH-3T3 mouse fibroblasts stably transfected with DC-SIGN or L-SIGN were incubated with mouse isotype control (IgG1), upper panels, and with hybridoma supernatants. Binding to the cells was revealed by a fluorochrome-labelled anti-mouse secondary Ab. Shown are three anti-DC-SIGN hybridoma mAbs, of which one (111H2) also reacts with L-SIGN. Specific labelling is in black, control labelling in grey was secondary Ab only. As control, two commercially available mAbs were included, of which one (clone DCN46) recognizes DC-SIGN and L-SIGN and one (clone 120604) is specific for L-SIGN. (B) As for panel A, only that monocyte-derived dendritic cells (MDDCs) and monocyte-derived dermal macrophages (MDdMs) were used instead of the transfected murine cell line.

supernatants, washed and bound mAb revealed with Alexa 595-conjugated anti-mouse Ig secondary Ab. As listed in Table 1 and shown in Fig. 3A, one mAb was identified (111H2), which clearly stained MDDCs and MDdMs but not monocytes. The MDdMs expressed CD163 and could therefore be double-labelled with anti-CD163 mAb (clone 10D6). As the 111H2 clone is an IgG2b and the anti-CD163 mAb is an IgG1, double labelling was performed with isotype-specific secondary Ab. CD163 is a scavenger receptor for

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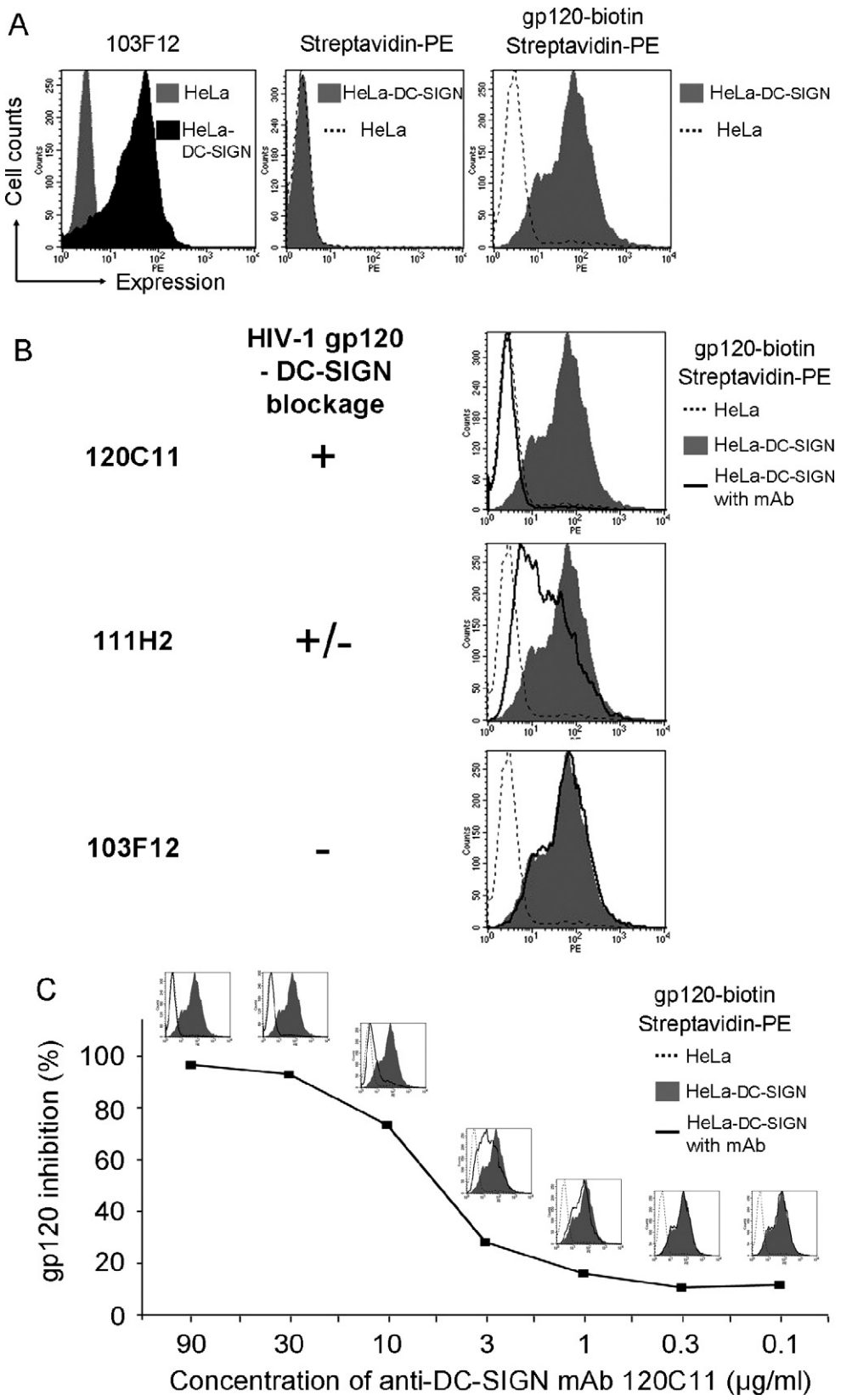

Fig. 2. Identification of DC-SIGN-specific mAbs capable of blocking DC-SIGN–HIV-1 gp120 interaction. (A) Left: grey filled histogram represents binding of anti-DC-SIGN mAb 103F12 followed by PE-secondary Ab to HeLa cells and black filled histogram to HeLa-DC-SIGN cells. Middle: HeLa-DC-SIGN cells (grey filled histogram) of HeLa cells (open stippled histogram) were incubated with PE-streptavidin only. Right: HeLa-DC-SIGN cells (grey filled histogram) of HeLa cells (open stippled histogram) were incubated with biotin-gp120 followed by PE-streptavidin. (B) For the gp120 blocking assay, HeLa or HeLa-DC-SIGN cells were incubated with biotin-gp120 followed by PE-streptavidin with or without the mAbs. (C) Gp120 binding to HeLa-DC-SIGN cells in the presence of different concentration of the blocking mAb 120C11. Above each point is shown the flow-cytometry analysis used to calculate the gp120 binding blockage. The % inhibition is the labelling of HeLa-DC-SIGN cells by gp120 in the presence of the mAb minus 100.

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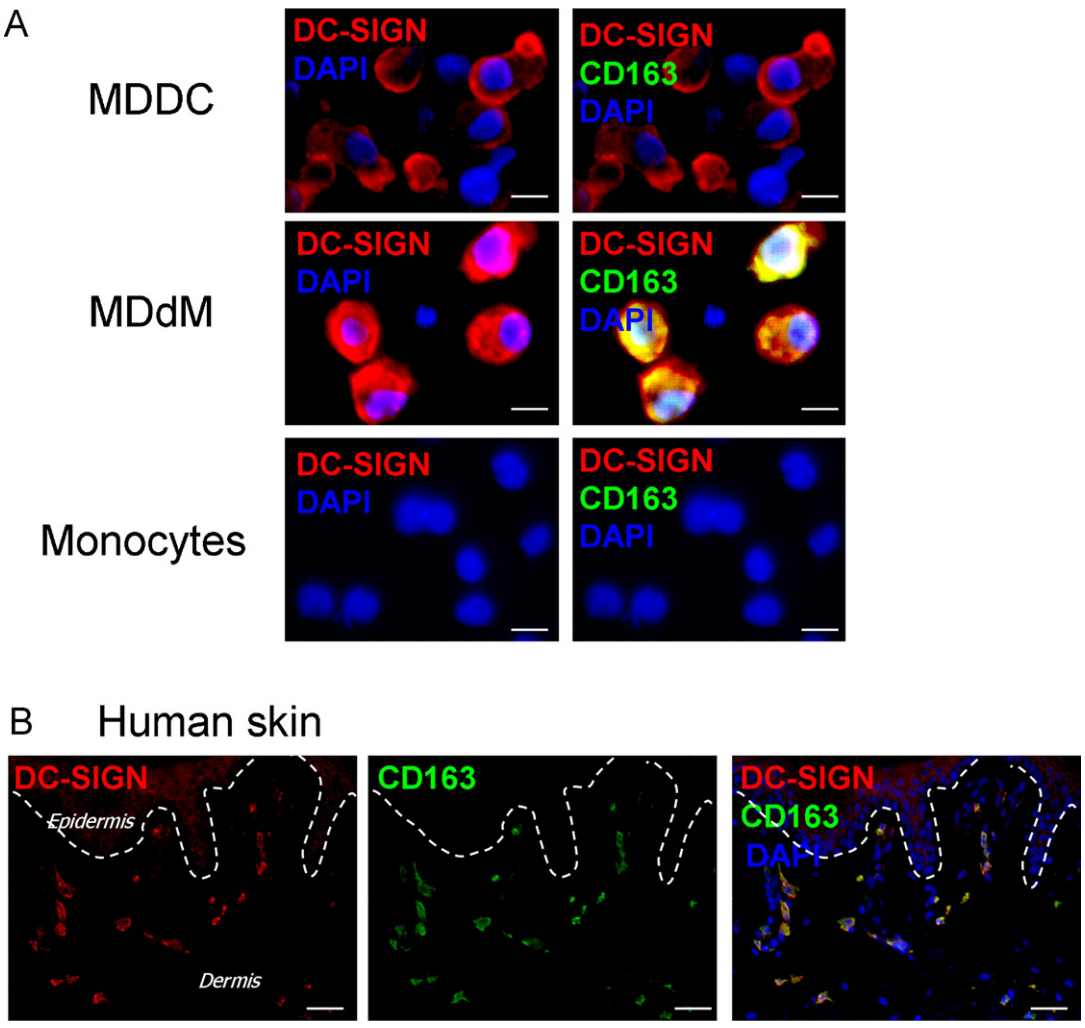

Fig. 3. Identification of mAbs capable of recognizing formalin-fixed DC-SIGN on isolated cells and on tissue. (A) Monocyte-derived dendritic cells (MDDCs), monocyte-derived dermal macrophages (MDdMs) and monocytes were formalin-fixed and embedded in paraffin. Cell sections were prepared and incubated with the clone 111H2 (IgG2b). The mAb was revealed by Alexa-597-secondary Ab specific for mouse IgG2b. In addition, the cells were incubated with an anti-CD163 mAb (IgG1), which was detected by an Alexa-488-anti-mouse IgG1 secondary Ab. Only MDdMs co-express DC-SIGN and CD163. Cell nuclei were colored with DAPI. Scale bars represent 20 μm. (B) Formalin-fixed and paraffin-embedded human skin sections were prepared and incubated with the anti-DC-SIGN mAb clone 111H2, and/or the anti-CD163 mAb. Their specific antigen-recognition was revealed with isotype-specific secondary Ab, as for panel A. Where indicated, cell nuclei were colored with DAPI. Scale bars represent 100 μm.

hemoglobin/haptoglobin complexes and one of the most reliable markers to distinguish resident tissue macrophages from dermal DCs [12,26].

It has been shown by different laboratories that DC-SIGN is predominantly expressed by the dermal macrophages using Factor XIIIa [12], CD14 [10] or the macrophage mannose receptor [14] as marker to distinguish the macrophages from the dermal DCs. As dermal macrophages express CD163 [12,26], we performed a double labelling with the anti-DC-SIGN and the anti-CD163 mAbs on fixed and paraffin-embedded human skin sections. As shown in Fig. 3B, the anti-DC-SIGN and the anti-CD163 mAbs clearly identified the same cells in the dermis. Therefore, the 111H2 clone recognized formalin-fixed DC-SIGN on isolated cells and on human tissue and further confirms the exclusive expression of DC-SIGN by dermal macrophages.

4. Discussion

In this paper, we have described the generation and the characterization of anti-human DC-SIGN mAbs. Using an immunisation protocol with HeLa cells expressing DC-SIGN, we have obtained 11 mAbs that recognized DC-SIGN on transfected cell lines and on cells which naturally express this C-type lectin, immature MDDCs and MDdMs cultured from monocytes. Of the 11 hybridomas, 8 mAbs were capable of interfering with DC-SIGN–HIV-1 gp120 interaction, and we have described the first mAb that recognizes formalin-fixed DC-SIGN. Using this mAb we have performed double labelling of dermal macrophages with an anti-CD163 mAb, which is reactive only on formalin-fixed tissue.

Previously, mAbs against DC-SIGN were generated using MDDCs as immunogen [3,8] or a combination of DNA-vaccination and purified recombinant protein [13]. Although the first protocol of raising mAbs against MDDCs resulted in hybridomas recognizing DC-SIGN, their number was low; three clones were isolated by Geijtenbeek et al. [8] and one by Relloso et al. [3]. Other investigators did not succeed using this technique and instead turned to the combination of DNA-vaccination and purified recombinant protein, which yielded 12 clones that all recognized DC-SIGN on MDDCs [13]. Therefore, in terms of the number of positive hybridoma clones obtained, our protocol of immunisation with a cell-line transfected with the DC-SIGN was comparable to that using DNA-vaccination/purified recombinant protein. An advantage of our

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protocol is that no protein purification is necessary, which can be technically challenging and can introduce structure alterations. Moreover, post-translational modification may differ depending on the cell source of the recombinant protein. In a similar manner to our protocol, 6 anti-DC-SIGN/L-SIGN antibody producing hybridoma clones were generated by R&D Systems using NIH-3T3 cells transfected with DC-SIGN or L-SIGN [4].

As expected from the high amino-acid conservation between DC-SIGN and L-SIGN, we obtained mAbs that cross-reacted with L-SIGN. Likewise, of the 3 mAbs raised against MDDCs by Geijtenbeek and coworkers, 2 reacted with L-SIGN [2]. Of the 12 clones obtained by Granelli-Piperno and coworkers, 11 mAbs cross-reacted with L-SIGN. We found that 3 clones cross-reacted with L-SIGN, they other 8 were specific for DC-SIGN. Whether this higher yield of DC-SIGN-specific mAbs is the result of using transfected cell lines as immunogen is unclear. One possibility is that post-translational modifications on DC-SIGN are introduced by HeLa cells, which distinguish conserved protein regions from L-SIGN. As expected, no L-SIGN specific mAbs were isolated. All our mAbs recognized MDDCs, which carry only DC-SIGN at their cell surface but not L-SIGN [2]. By using an L-SIGN-specific commercial mAb, we confirmed this finding and extended it to MDdMs. This shows that irrespective of whether monocytes are activated by IL-4 or IL-10, they express DC-SIGN but not L-SIGN.

Of the 11 mAbs, 7 showed a capacity to interfere with HIV-1 gp-120 binding. All of them were DC-SIGN-specific except one, which recognized also L-SIGN. The clone AZN-D1 isolated by Geijtenbeek et al. [8] and a number of mAbs obtained after immunisation with NIH-3T3–DC-SIGN cells by Wu et al. [4] also showed a capacity to block binding of DC-SIGN to gp120. Moreover, of these 7 DC-SIGN/L-SIGN mAbs [4], 6 could reduce HIV infection of T cells *in trans* by THP-1-DC-SIGN cells [4]. Whether the described anti-DC-SIGN mAbs here will find application in anti-HIV therapy is currently unclear, as previous studies using HIV-1–DC-SIGN blocking mAbs have uncovered that they only partially protected transmission of HIV-1 to T cells *in trans* when more relevant cells than transfected cell lines were used (such as MDDCs) [4,18]. Also, *Rhesus* macaque DCs efficiently transmit primate lentiviruses in the absence of DC-SIGN expression [27]. It is likely that receptors other than DC-SIGN also play a role in HIV-1 transmission [24], such as the macrophage mannose receptor, another C-type lectin [28]. Despite this, DC-SIGN remains an important drug target in an effort to prevent early infection and transmission events of HIV-1 [29]. Interestingly, in a similar manner to HIV-1, the dengue virus also binds both to DC-SIGN and the macrophage mannose receptor [20,21,30]. However, in a study of human polymorphism of the DC-SIGN-encoding gene, a clear correlation was established between DC-SIGN levels dictated by promoter mutations and dengue fever [31]. So far, anti-DC-SIGN mAbs have not yet been taken into clinical trials to prevent the development of dengue fever. Before such applications, the mapping of DC-SIGN–glycoprotein E interactions and tests of prevention of MDDC infection will be preliminary requirements. The described antibodies would certainly be useful in addressing these issues.

Of the 11 mAb, only one was reactive to DC-SIGN on formalin-fixed cells or tissue. The scarcity of such a mAb may provide an explanation to why, so far, no mAb reactive to formalin-fixed DC-SIGN has been found. Still, surprisingly, none of the clones isolated by other laboratories [3,4,8,13] (23 in total) were tested for recognition of formalin-fixed DC-SIGN. To our knowledge, the described clone here represents the first mAb reactive to formalin-fixed DC-SIGN. Being of the IgG2b isotype, it allowed us to combine it with an anti-CD163 mAb (IgG1), which is frequently used by anatomo-pathologistes but only labels formalin-fixed cells. Using this antibody combination, we showed that the $CD163^+$ dermal macrophages express DC-SIGN. This mAb will facilitate the study of this C-type lectin in cohorts of diseased tissues, which are commonly conserved as paraffin blocks. Moreover, the mAb will facilitate the study of DC-SIGN expression by flow cytometry on cells, which are fixed for intracellular staining, or because they had been infected with a human pathogen and formalin-treatment is performed for biosecurity reasons.

In summary, in view of the importance of DC-SIGN in fundamental immunology and in research applied to elucidate the infection events of human pathogens, we have generated novel anti-DC-SIGN mAbs. They could be of therapeutic value against HIV-1 infections or against other DC-SIGN recognizing human pathogens such as the dengue virus. We have presented the first mAb reactive to DC-SIGN on formalin-fixed and paraffin-embedded tissue.

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