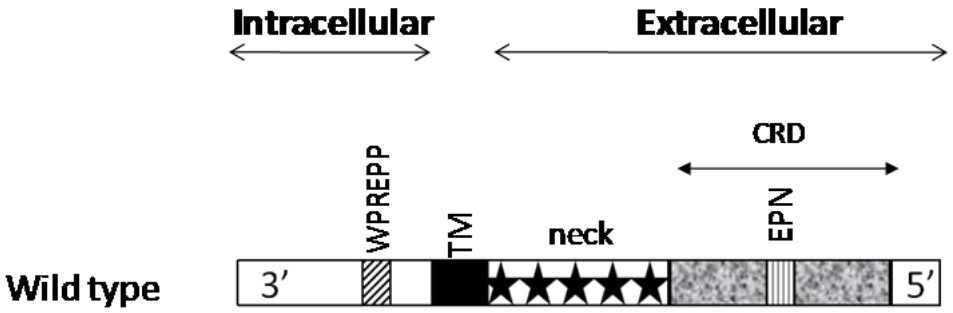
Anti-Langerin/CD207 monoclonal antibodies

Langerin is a protein encoded by the CD207 (Cluster of Differentiation 207) gene. Langerin is a type II transmembrane cell surface receptor produced by Langerhans cells which are immature dendritic cells of the epidermis and mucosa. It is also expressed in several other dendritic cell types including dermal $CD103^+$ DCs and splenic $CD8^+$ DCs.
Langerin is localized in the Birbeck granules, organelles present in the cytoplasm of Langerhans cells and consisting of superimposed and zippered membranes.
Langerin features a single carbohydrate recognition domain (CRD) with mannose-type specificity in its extracellular portion and it has been proposed that mannose binding by this protein leads to internalization of antigen into Birbeck granules and provides access to a nonclassical antigen-processing pathway.
Langerin is unique among the CLRs in that it contains an intracellular domain with a proline-rich motif. Langerin expression has not been reported outside the DC system. (*Valladeau J et al, 2002 J Immunol, 168 : 782-792) (Douillard P et al, 2005 J Invest Dermatol, 125: 983–994).*

Langerin protein domains



Langerhans cells are the first dendritic cells to encounter pathogens entering the body *via* mucosa or skin. Equipped with pattern recognition receptors (PRR), Langerhans cells are able to detect and respond to these pathogens. Langerin is an important PRR which forms a protective barrier against HIV-1 infection.
In addition, antigens targeted to Langerin are presented to T cells to induce an adaptive immune response. Therefore Langerin functions as an innate anti-viral defense mechanism and an antigen receptor involved in adaptive immune responses *(van der Vlist M., Immunol Cell Biol. 2010; 88(4):410-5).*

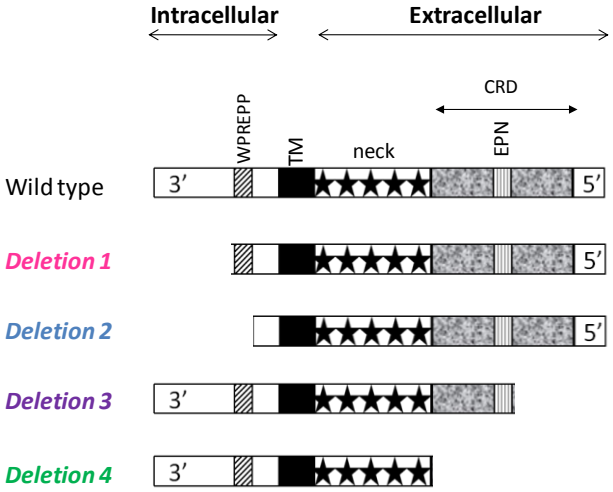
A large panel of anti-Langerin monoclonal antibodies was generated following different immunization programs. Immunizations with either human or murine Langerin were performed, using Langerin-transfected cells, *ex-vivo* isolated Langerhans cells (mouse ear crawl-out) or *in vitro*-derived dendritic cells.
Anti-Langerin monoclonal antibodies were obtained either from mouse or from rat.

Several experiments were performed to characterize the panel of anti-Langerin monoclonal antibodies. Characteristics and applications of representative antibodies are summarized in Table I.

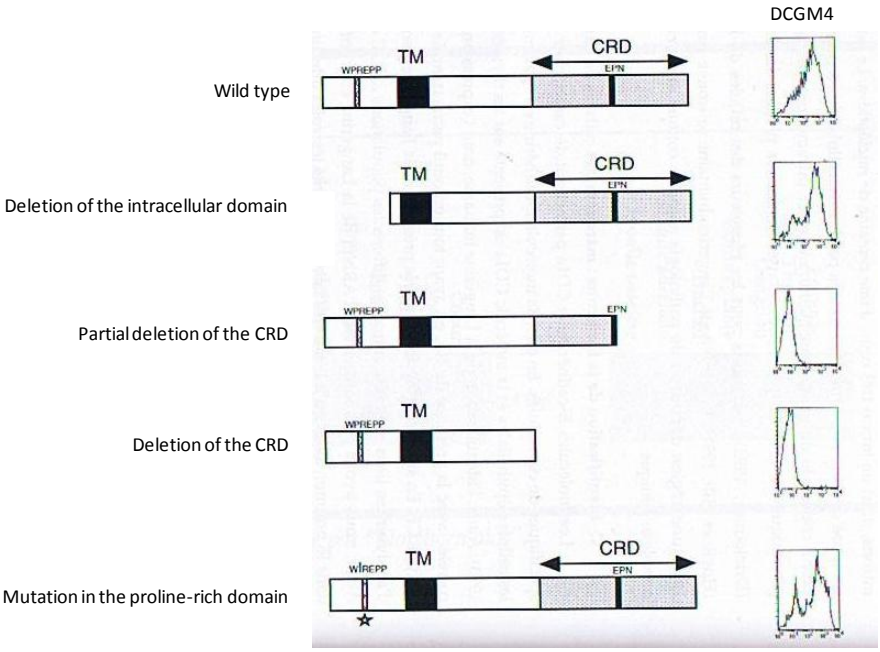
Epitope recognition

A first series of experiments aimed at the identification of the epitope recognized by each antibody along the Langerin molecule. For this purpose, the full length Langerin cDNA was deleted of the different protein domains and transfected in COP5 cells *(Valladeau J, EJI, 1999 ; Pin JJ, personal data).*

Langerin: deletion mutant constructs

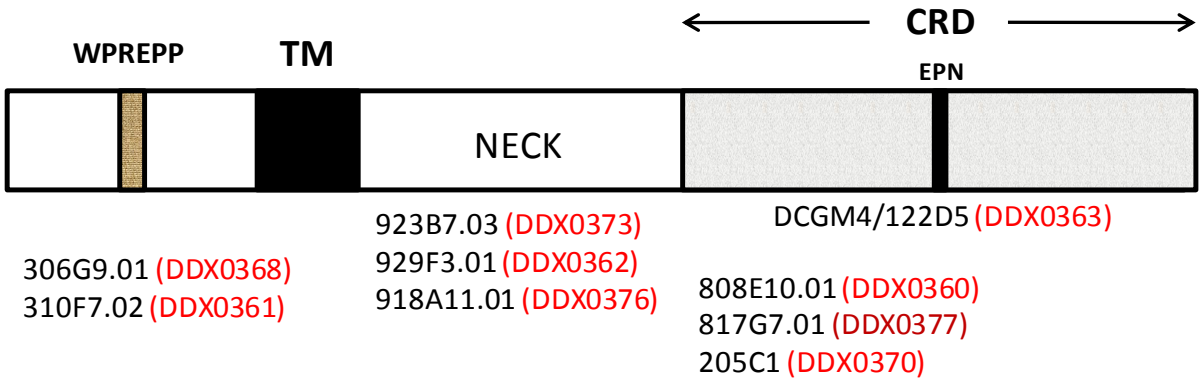


Anti-Langerin monoclonal antibodies were tested in flow cytometry with COP5 cells transfected with the different Langerin constructs, allowing the identification of the epitope recognized by each antibody. An illustration of FACS data obtained with DDX0363 antibody (122D5/DCGM4) is presented below. The overall data are listed in Table I.



Valladeau et al, EJI 1999

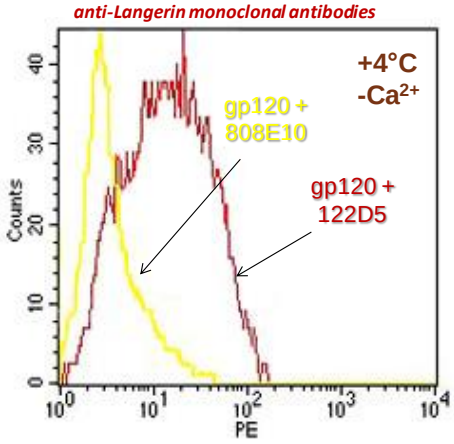
The region recognized by DDX antibodies are represented below



HIV gp120 and Langerin interaction

Mucosa plays a major role in viral spread during HIV infection. Langerin is described to be a cellular receptor for HIV entry by interacting with the HIVgp120 glycoprotein. The panel of monoclonal antibodies anti-Langerin was tested for their ability to block HIVgp120-Langerin interaction. Data are listed in Table I. Furthermore, a specific EMICI (Elisa Monitoring of Innate Conformational Interaction) assay was designed to quantify the circulating anti-gp120 blocking antibodies in the serum of HIV patients *(Canard B, thesis, 2010)*. An illustration of the data obtained with a blocking (808E10) and a non blocking (122D5/DCGM4) clone is presented below:

Inhibition analysis of gp120 binding on Langerin-transfected CHO cells using anti-Langerin monoclonal antibodies



Canard B, Thesis, 2010

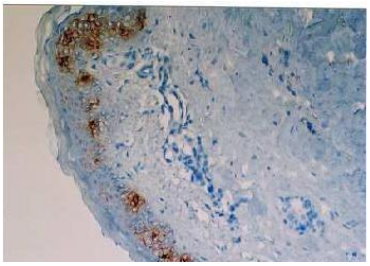
Langerhans cell histiocytosis

Langerhans cell Histiocytosis (LCH) is a rare disease involving clonal proliferation of Langerhans cells, abnormal cells deriving from bone marrow and capable of migrating from

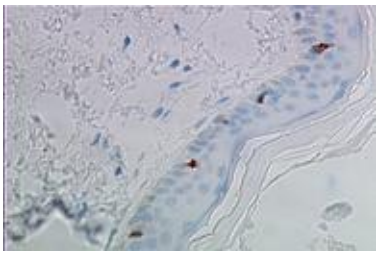
skin to lymph nodes. Clinically, its manifestations range from isolated bone lesions to multisystem disease *(Egeler RM et al, Immunol Rev., 2010, 234(1):213-32).*

Our monoclonal antibodies were tested by immunochemistry on paraffin-embedded tissue section aiming at providing tools for pathological analysis. (All DDX IHC-validated antibodies work on frozen tissue sections). Three clones were demonstrated to work very well in these conditions. An illustration of these data is presented below:

IHC staining of paraffin-embedded tissue sections



DDX0362 (929F3)



DDX0368 (306G9)

DDX0361 (310F7)

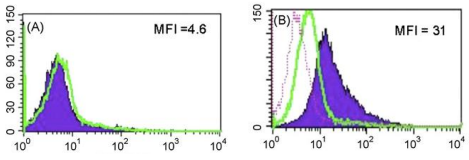
Cross-species reactivity

Several anti-Langerin monoclonal antibodies were tested in other animal species in order to analyze cross-species reactivity. An illustration of staining obtained with swine cells animal is presented below:

Staining of swine Langerin

DDX0361

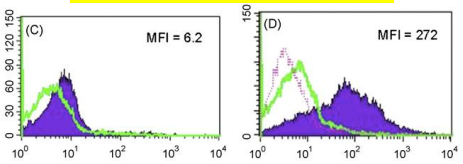
Intracellular FACS staining



Cells derived from overnight culture of porcine skin biopsies were stained permeabilized (B) or not (A) with 310F7.02.

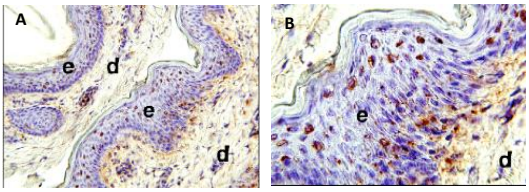
DDX0362

Intracellular FACS staining



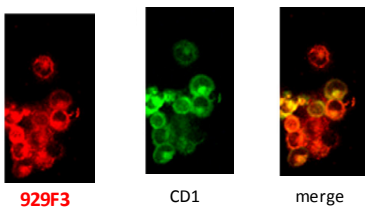
Cells derived from overnight culture of porcine skin biopsies were stained permeabilized (D) or not (C) with 929F3 antibodies and analyzed by flow cytometry.

Immunohistochemical staining



Porcine skin cryosections were stained with 310F7.02 and goat anti-mouse IgG-HRP. A:16X;B:40X;e:epidermis;d:dermis.

Immunofluorescent staining



929F3 CD1 merge

Immunofluorescent staining of porcine Langerhans cells in cytospins of isolated skin DCs with 929F3 (red) and anti-CD1 (green)

Nfon CK et al, Veterinary Immunol and Immunopathol, 2008

Table I: Characteristics of the representative monoclonal antibodies raised against Langerin

Clones Cat#	Host	Cross-species				Isotype	Epitope recognition (Flow cytometry)						Blocking HIVgp120-langerin interaction	Other applications
		H	M	R	S		wild type	deletion 1	deletion 2	deletion 3	deletion 4	Epitope recognized		
122D5/DCGM4 DDX0363	mouse	+				IgG1	+	+	+	-	-	CDR domain (EPN)	-	Antigen-binding (competes with mannan), IHC, IF, flow cytometry, WB, internalization
808 E10 DDX0360	mouse	+				IgG1	+	+	+	+	+/-	CDR domain	+	Flow cytometry, IF, IHC
817 G7 DDX0377	mouse	+				IgG1	+	+	+	+	+/-	CDR domain	+	Flow cytometry
306 G9 DDX0368	mouse	+	+	+	+	IgG1	+	+	-	+	+	Proline-rich domain	-	Flow cytometry, IF, IHC
310 F7 DDX0361	mouse	+	+		+	IgG1	+	-	-	+	+	Intracellular domain	-	Flow cytometry, IF, IHC
205C1 DDX0370	mouse		+			IgM	+							Flow cytometry, IF
923 B7 DDX0373	rat	+	+			IgG2a	+	+	+	+	+	Neck domain	-	Flow cytometry, IF, IHC
918A11 DDX0376	rat	+	+	+		IgG1	+	+	+	+	+	Neck domain	-	Flow cytometry, IF, IHC
929 F3 DDX0362	rat	+	+	+	+	IgG2a	+	+	+	+	+	Neck domain	-	Flow cytometry, IF, IHC

Several other clones available on request