

Research article

## Flow cytometric assessment of the reactivity of a panel of monoclonal antibodies (mAb) against two populations of human dendritic cells (DC)

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### Abstract

**Background:** The identification of antigens on human DC has been a very difficult and elusive task because of the lack of appropriate reagents. Therefore, we evaluated by flow cytometry a panel of mAb that recognize antigens on human DC, aiming to determine the kinetics of DC antigen expression at 7, 14, 21 and 28 days in (i) Dermal DC like cells (Mo-DC) and (ii) Langerhans cell like DC (Mo-LC). In addition we aimed to identify markers for DC subpopulations.

**Results:** It was found at day 7, that mAb BG6, HP-F1, BU10, RFD-1, CMRF-44 recognized <20% of Mo-DC. In contrast, 7H5, ZM3.8, CD1b/c, 55K-2, MMR1.16, MMR190.BB3 and L25 reacted with >50% of Mo-DC. Moreover, 7H5, ZM3.8, CMRF-56, CD1b/c, 55K-2, MMR1.16, MMR190.BB3 and L25 showed increased MFI reactivity against Mo-DC. mAb BG6, BU10 and CMRF-44 recognized <20% Mo-LC while RFD-1 reacted with 21% of Mo-LC. In contrast, HP-F1 showed 87% of Mo-LC positive. Also, 7H5, ZM3.8, RFD-7, MR15-2, CD1b/c, 55K-2, MMR1.16, MMR190.BB3 and L25 reacted with >50% of Mo-LC. The increase in % of positive cells was paralleled by MFI increases.

At day 14, fourteen mAb recognized >50% of the Mo-DC, while five recognized 20-50% of Mo-DC. BG6 reacted with 7% of the Mo-DC. Nineteen mAb recognized >48% of Mo-LC while BG6 had negative reactivity.

At day 21 and 28, all mAb reacted with >20% of Mo-DC and yielded a significant MFI with Mo-DC. Also nineteen mAb yielded significant MFI with Mo-LC while RFD-7 did not.

**Conclusions:** The immunophenotyping assays demonstrated differences between the two DC populations as well as variations in the reactivity of the mAb at diverse time points, suggesting the existence of subpopulations within the Mo-DC and Mo-LC.

### Background

Dendritic cells (DC) are a complex group of mainly bone marrow derived cells that play an important role in the afferent branch of the immune response [1]. However,

DC represents only a minute subpopulation of the peripheral blood mononuclear cells (PBMC), as well as of bulk cellular populations of the lung, intestine, genitourinary tissue, and lymphoid tissue. DC also has been

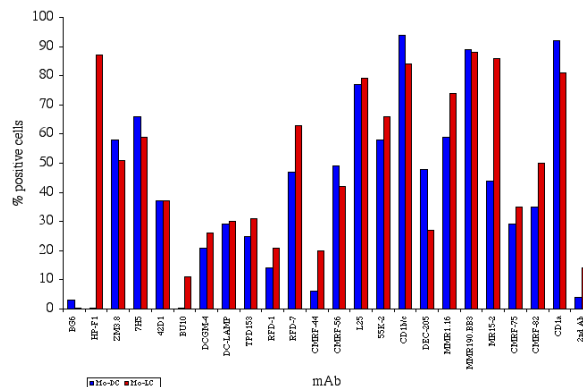
found in the epidermis, dermis and mucous membranes and constituting about 2% of the total cellular population of the human epidermis [2,3]. The Langerhans cells (LC) are a skin derived-DC, that have the capability to travel to the regional lymphoid organs after take up of antigen and undergo there an activation/maturation step. Thereafter, LC interacts and activates T cells. Because of such significant capability to take up soluble antigens, process and present them to responder cells in the lymphoid tissues in the context of the restricted MHC pathway, LC have been considered one of the most important elements in the afferent arm of the immune response [1-4].

Recent, successful efforts to generate DC from PBMC derived monocytes or from CD34 blood precursors by utilizing GM-CSF and IL-4, as well as GM-CSF and/or TNF, has enabled us to obtain PBMC derived DC (Mo-DC) [5-8]. In addition, an approach has been developed to generate LC from isolated monocytes (Mo-LC) [9].

Despite the successful efforts in the generation of DC from blood precursors, the characterization of surface markers on human DC has been a very difficult and elusive task because the lack of appropriate reagents with high specificity for DC identification [4]. However, some molecules whose genes recently have been cloned and sequenced (e.g. CD83, DEC-205) have been found strongly associated with DC [5,6]. In addition, a panel of monoclonal antibodies (e.g. CMRF-44) that recognize molecules on DC has been raised [7]. There is a growing need for cluster and establishment of a common and comprehensive nomenclature for such DC associated molecules, as well as to clarify and define the lineage(s) of DC and the existence of DC subsets. These developments have prompted the set up of diverse approaches that evaluated the reactivity of a group of mAb against populations of DC [10-29]. Therefore and within the scope of this study, we set up a flow cytometry approach and evaluated a panel of 20 mAb against two populations of DC aiming to determine the kinetics of expression of antigens on DC at diverse intervals of time and the likelihood to identify markers for DC subsets [12-29].

**Results and Discussion**

Flow cytometric assessments of monoclonal antibodies (mAb) reactivity against dendritic cells (DC) was undertaken by measurement of the % of reactivity (Figure 1,2,3,4) and mean fluorescent intensity (MFI) of two populations of DC at 7, 14, 21 and 28 days (Table 1). At day 7 (Figure 1), the flow cytometry evaluation showed that, antibodies 70011 (BG6), 70194 (HP-F1 (CD85i)), 70293 (BU10), 70511 (RFD-1) and 70375 (CMRF-44) recognized less than 20 % of Mo-DC. In contrast, 70345 (7H5 (CD85a)), 70358 (ZM3.8 (CD85j)), 70807 (CDIb/c), 70806 (55K-2 fascin), 70776 (MMR1.16 (CD206)),



**Figure 1**  
Percentage of positive cells detected by mAb against Mo-DC and LC-DC after 7 days of culture

70767 (MMR190.BB3 (CD206)) and 70772 (L25) reacted with more than 50% of Mo-DC. Moreover, 70345 (7H5 (CD85a)), 70358 (ZM3.8 (CD85j)), 70376 (CMRF-56), 70807 (CDIb/c), 70806 (55K-2 fascin), 70776 (MMR1.16 (CD206)), 70767 (MMR190.BB3 (CD206)) and 70772 (L25) also showed increase MFI reactivity against Mo-DC (Table 1).

The mAb 70011 (BG6), 70293 (BU10) and 70375 (CMRF-44) recognized less than 20% Mo-LC while 70511 reacted with only 21% of the cells. In contrast, 70194 (HP-F1 (CD85i)) showed that 87% of Mo-LCs were positive. Also, 70345 (7H5 (CD85a)), 70358 (ZM3.8 (CD85j)), 70512 (RFD-7), 70802 (MR15-2 (CD205)), 70807 (CDIb/c), 70806 (55K-2 fascin), 70776 (MMR1.16 (CD206)), 70767 (MMR190.BB3 (CD206)), 70772 (L25) reacted with more than 50% of Mo-LC. The increase in % of positive cells was also paralleled by MFI increases (Table 1).

At day 14 (Figure 2), 14 mAb recognized more than 50% of the Mo-DC, while 70427 (DCGM-4 (CD207)), 70451 (TPD153), 70511 (RFD-1), 70512 (RFD-7) and 70375 (CMRF-44) recognized between 20 and 50% of Mo-DC. 70011 (BG6) reacted with 7% of the Mo-DC. The reactivity of 70011 (BG6) against Mo-LC was indistinguishable from the background while the other nineteen mAb recognized more than 48% of Mo-LC. At day 21 and 28, all the mAb reacted with more than 20% of Mo-DC (Figure 3 and 4). Also, the mAb yielded a significant MFI with Mo-DC (Table 1). In addition, nineteen mAb yielded significant MFI with Mo-LC whilst only 70512 (RFD-7) did not. (Table 1) At day 21 (Figure 3), seventeen mAb reacted with more than 50% of the Mo-DC, only 70346 (42D1

**Table 1: MFI of mAb reactivity against HDC**

|            | Day 7 |       | Day 14 |       | Day 21 |       | Day 28 |       |
|------------|-------|-------|--------|-------|--------|-------|--------|-------|
|            | Mo-DC | Mo-LC | Mo-DC  | Mo-LC | Mo-DC  | Mo-LC | Mo-DC  | Mo-LC |
| BG6        | 72    | 57    | 81     | 149   | 637    | 256   | 644    | 1100  |
| HP-F1      | 48    | 274   | 160    | 199   | 717    | 310   | 615    | 1252  |
| ZM3.8      | 180   | 155   | 332    | 305   | 515    | 226   | 747    | 1025  |
| 7H5        | 176   | 153   | 366    | 402   | 387    | 221   | 382    | 859   |
| 42D1       | 138   | 129   | 175    | 157   | 278    | 150   | 291    | 562   |
| BU10       | 50    | 97    | 171    | 172   | 296    | 161   | 392    | 320   |
| DCGM-4     | 115   | 125   | 177    | 193   | 570    | 288   | 608    | 1317  |
| DC-LAMP    | 148   | 126   | 296    | 205   | 601    | 252   | 567    | 712   |
| TPD153     | 124   | 141   | 189    | 198   | 482    | 510   | 657    | 808   |
| RFD-1      | 102   | 113   | 161    | 175   | 374    | 193   | 376    | 712   |
| RFD-7      | 150   | 167   | 209    | 621   | 165    | 114   | 168    | 158   |
| CMRF-44    | 79    | 111   | 160    | 185   | 348    | 152   | 339    | 115   |
| CMRF-56    | 193   | 200   | 209    | 203   | 977    | 421   | 974    | 2232  |
| L25        | 334   | 353   | 279    | 336   | 866    | 890   | 792    | 1430  |
| 55K-2      | 237   | 228   | 373    | 215   | 1029   | 526   | 1044   | 2020  |
| CD1b/c     | 789   | 515   | 392    | 398   | 408    | 234   | 384    | 654   |
| DEC-205    | 162   | 127   | 227    | 171   | 548    | 281   | 430    | 915   |
| MMR1.16    | 194   | 265   | 319    | 281   | 570    | 272   | 656    | 1147  |
| MMR190.BB3 | 716   | 570   | 702    | 778   | 881    | 447   | 1019   | 1917  |
| MR15-2     | 151   | 278   | 547    | 520   | 721    | 476   | 588    | 817   |
| CMRF-75    | 155   | 209   | 266    | 234   | 432    | 186   | 403    | 507   |
| CMRF-82    | 153   | 182   | 340    | 232   | 760    | 357   | 946    | 1420  |
| CD1a       | 801   | 493   | 423    | 603   | 443    | 232   | 354    | 434   |
| 2nd Ab     | 80    | 106   | 121    | 165   | 97     | 81    | 85     | 105   |

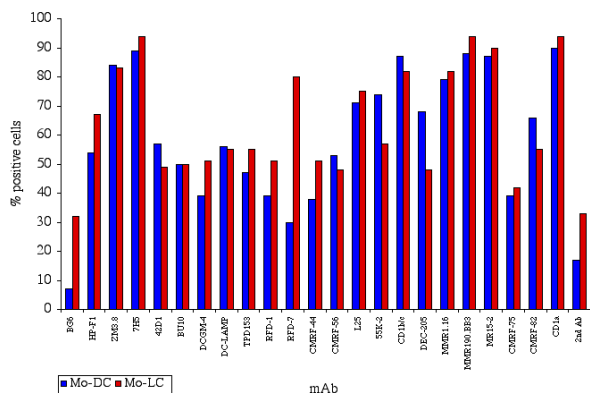
(CD85f)), 70511 (RFD-1) and 70512 (RFD-7) were below 50%. In contrast, only seven mAb reacted with more than 50% Mo-LC but only 70512 (RFD-7) reacted with less than 20% Mo-LC. At day 28 (Figure 4), sixteen mAb reacted with more than 50% Mo-DC. The mAb 70346 (42D1 (CD85f)), 70511 (RFD-1), 70512 (RFD-7) and 70808 (DEC-205 (CD205)) were below 50% while 70375 (CMRF-44) reacted with less than 20% of Mo-LC. Seventeen mAb reacted with more than 50% of Mo-LC.

The simultaneous evaluation on Mo-DC and Mo-LC of FSC (flow cytometric assessment of size) and mAb reactivity at four time points demonstrated the presence of significant variability within the Mo-DC and Mo-LC populations (Figure 5). At day seven, there was several mAb that showed strong reactivity and can be grouped together (Figure 5 panel C mAb-MMR.190.BB3 and panel E mAb-L25). In addition there was another group of mAb with an intermediate level of reactivity that can be grouped together (Figure 5 panel D mAb-7H5 and panel F mAb-ZM-38). However, some mAb showed no reactivity at day seventh (Figure 5 panel A mAb-BG6) compared with the negative control (Figure 5 panel B-Second Ab). In addition, variations in the reactivity of the mAb at di-

verse time points were found. Moreover, at day seven it was found that mAb HP-F1 was negative for Mo-DC and positive for Mo-LC, thus discriminating Mo-DC from Mo-LC. Overall, this set of results demonstrates the existence of antigenic differences between Mo-DC and Mo-LC, even though there was only a single difference in the cytokine mixture utilized. Moreover, within the panel analyzed, it was found that at day 7, there is a mAb that showed reactivity only against Mo-DC with high FSC (mAb-TPD153) or against a fraction of Mo-DC (mAb-DC-LAMP, mAb-55K-2). These results point toward the existence of subsets within the Mo-DC and Mo-LC populations.

Of note, the studies on the reactivity of two set of mAb (mannose receptor and immunoglobulin-like transcript molecules) raised very interesting considerations about the role of these antigens in the functionality of DC.

This study showed that DC expressed significant amounts of two lectin-type receptors: the mannose receptor (MMR) detected by the mAbs MR 15-2 (CD206), MMR1.16 (CD206), and MMR190.BB3 (CD206?), and the antigen DEC-205 detected by the mAbs DEC-205



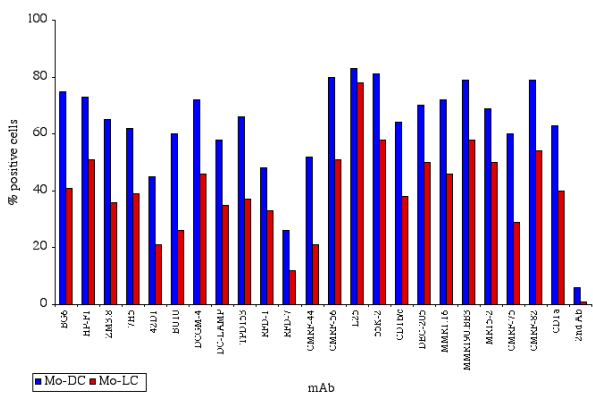
**Figure 2**  
Percentage of positive cells detected by mAb against Mo-DC and LC-DC after 14 days of culture

(CD205) and MMRI-4 (CD205). Both molecules belong to a family of transmembrane C-type lectins. Both molecules have (i) a cysteine-rich domain, (ii) a fibronectin type II domain, (iii) multiple C-type carbohydrate recognition-like domains, (iv) a transmembrane domain and (v) a short cytoplasmic tail [6,12,24,25,27]. Both molecules the MMR [24,25,27] and DEC-205 [6,12], have been implicated in the uptake of carbohydrate-conjugated antigens by DC. MMR has been found on the cell surface of macrophages and its carbohydrate recognition domains mediate the endocytosis of (i) glycoconjugates containing mannose, (ii) fucose, (iii) acetylglucosamine, (iv) glucose residues, (v) microorganisms expressing

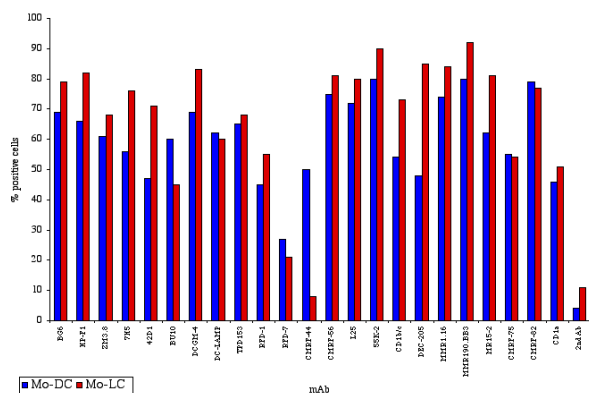
mannose or acetylglucosamine on the surface. Notably, all these terminal sugars are neither common membrane components of mammalian cells nor common components of serum proteins. Therefore, it is feasible to consider that this two lectin-type receptors: the mannose receptor (MMR) and DEC-205 may be involved in the discrimination between self and non-self antigens as well and could contribute to enhance the capability of dendritic cells to generate primary T cell responses against infectious agents and soluble antigens that carry these types of carbohydrates.

In addition, this study provides the first comparison of the distribution of the MMR and DEC-205 on two human blood derived DC, Mo-LC and Mo-DC.

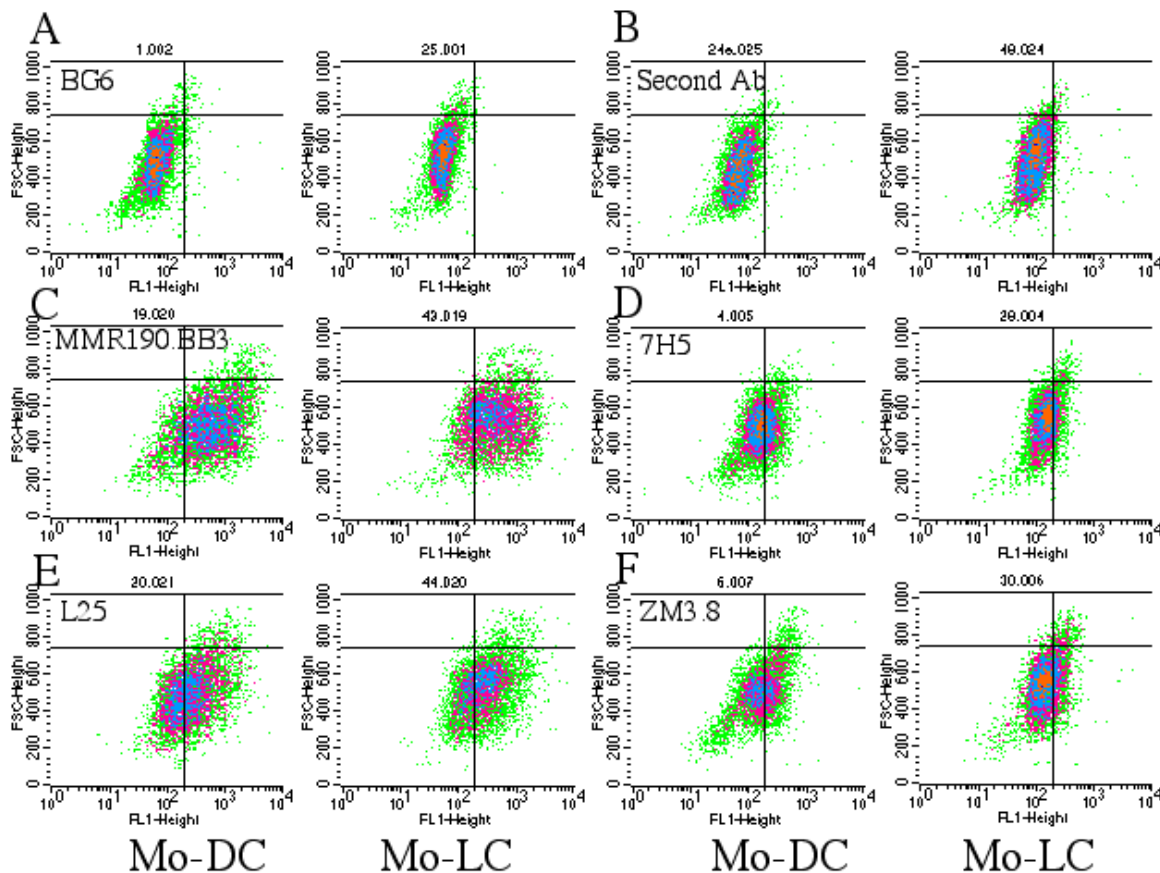
A panel of mAb that recognize several immunoglobulin-like transcript molecules (ILT) was also evaluated in this study. The mAb HP-F1 (CD85i) identified ILT2 [13], ZM3.8 (CD85j) recognized ILT3 [17], 42D1 (CD85f) identified ILT4 [16] and 7H5 (CD85a) recognized ILT5 [15]. One significant feature of the ILTs molecules is their capability to bind MHC class I molecules [13]. Furthermore, the molecules ILT2, ILT3, ILT4, and ILT5 have been considered to play a role as inhibitory receptors because they carry the immunoreceptor tyrosine-based inhibitory motif (ITIM) in the intracytoplasmic tail. Because of (i) the binding capability of ILT2-5 to MHC class I molecules [13,17], (ii) the presence of inhibitory motifs within the cytoplasmic tail of ILT2-5 [13,15-17] and (iii) the identification of ILT2-5, within diverse populations of dendritic cells [10,17], is likely that the ILT molecules identified on DC may not only perform a key role as the receptor for the "missing self [30] but also



**Figure 3**  
Percentage of positive cells detected by mAb against Mo-DC and LC-DC after 21 days of culture



**Figure 4**  
Percentage of positive cells detected by mAb against Mo-DC and LC-DC after 28 days of culture



**Figure 5**

Density plots of Mo-DC and LC-DC reactivity to five mAb after 7 days of culture. Density plot representation of FSC and mAb reactivity at day seventh has been utilized to demonstrate the presence of significant variability within the Mo-DC and Mo-LC populations. Panel A displayed mAb-BG6 in comparison with the negative control that is shown in panel B-Second Ab. Panel C showed mAb-MMR190.BB3. Panel D displayed mAb-7H5. Panel E showed mAb-L25. Panel F displayed mAb-ZM38.

could contribute to control the initial steps of activation of the immune response; Thus, this would tune up the fine balance between the activation signals provided by the simultaneous cross-linking of MHC class I or II and co-stimulatory molecules during the interaction between DC with T cells and the inhibitory signals mediated through the whole variety of ILTs molecules carrying ITIM motifs. Further functional studies of the role of ILTs on DC will provide insight toward the understanding of these mechanisms and its role in the regulation of the DC function.

### Conclusions

The immunophenotyping assays described in this report enabled us to determine in human DC: (i) the existence

of differences between Mo-DC and Mo-LC populations; (ii) the existence of subsets within the Mo-DC and Mo-LC populations; (iii) the kinetics of antigens expression at diverse intervals of time on DC; and (iv) specific markers for subpopulations of DC.

### Material and methods

#### *Monoclonal antibodies (mAb)*

A panel of mAb with potential reactivity against DC was evaluated by setting up a flow cytometry approach. The panel of antibodies was kindly provided by Dr. D. Hart. Antibodies are listed in Table 21 {CD1a, CD1b/c, BG6, HP-F1 (CD85i), BU10, RFD-1, CMRF-44, 7H5 (CD85a), ZM3.8 (CD85j), 55K-2 (fascin), MMR1.16, MMR190.BB3 (CD206), L25, CMRF-56, RFD-7, MR15-2

**Table 2: List of monoclonal antibodies**

| Sample | Panel number       | Monoclonal antibody (CD*) | Reference |
|--------|--------------------|---------------------------|-----------|
| 1      | 70011              | BG6                       | 12        |
| 2      | 70194              | HP-FI(CD85i)ILT2          | 13        |
| 3      | 70293              | BU10                      | 14        |
| 4      | 70345              | 7H5 (CD85a) ILT5          | 15        |
| 5      | 70346              | 42D1 (CD85f)ILT4          | 16        |
| 6      | 70358              | ZM3.8 (CD85j) ILT3        | 17        |
| 7      | 70427              | DCGM-4 (CD207) Langerin   | 18        |
| 8      | 70435              | DC-LAMP (CD208)           | 19        |
| 9      | 70451              | TPD153                    | 20        |
| 10     | 70511              | RFD-1                     | 21        |
| 11     | 70512              | RFD-7                     | 22        |
| 12     | 70375              | CMRF-44                   | 7         |
| 13     | 70376              | CMRF-56                   | 23        |
| 14     | 70802              | MR15-2 (CD206)            | 24        |
| 15     | 70808              | DEC-205 (CD205)           | 6,12,25   |
| 16     | 70807              | CD1b/c                    | 31        |
| 17     | 70806              | 55K-2 fascin              | 26        |
| 18     | 70776              | MMR1.16 (CD206)           | 27        |
| 19     | 70767              | MMR190.BB3 (CD206?)       | 27        |
| 20     | 70772              | L25                       | 28        |
| 21     | Positive Control 1 | CMRF-75                   | 12        |
| 22     | Positive Control 2 | CMRF-82                   | 12        |
| 23     | Positive Control 3 | CD1a                      | 31        |
| 24     | Second Ab          | 2nd Ab                    | 31        |
| 25     | 70875              | AZN-D1 (CD209)            | 29        |
| 26     | 70876              | AZN-D2 (CD209)            | 29        |
| 27     | 70874              | MMRI-4 (CD205)            | 12,25     |

(CD\*): Hart DNJ., Clark GJ. MacDonald K., Kato M., Vuckovic S., Lopez A., Wykes M., Munster D.. 7<sup>th</sup> Leucocyte Differentiation antigen workshop, DC section summary. D. Mason, Editor, Leukocyte Typing VII, Oxford University Press. Oxford. In Press 2001 [12]

(CD205), DCGM-4 (CD207), TPD153, 42D1 (CD85f), DEC-205 (CD205), MMRI-4 (CD205), DC-LAMP (CD208), AZN-D1 (CD209), AZN-D2 (CD209), CMRF-75, CMRF-82, CD11c, CD80, CD86 and HLA-DR} [12–29,31]. The panel of antibodies that was utilized in the Dendritic cell section of the 7<sup>th</sup> workshop for HLDA is listed in table 2[12–29].

#### Cell culture

Adherent PBMC were obtained from a normal healthy donor and cultured in 25-cm<sup>2</sup> tissue culture flasks, with complete medium for DC culture {Complete Medium: RPMI 1640 with 25 mM Hepes buffer with L-glutamine (Gifco BRL, Basel Switzerland), supplemented with 10% heat-inactivated FBS, and 1 ml of Antibiotic/Antimycotic (Gifco BRL)} and supplemented with rhGM-CSF (250 ng/ml) +rhIL-4 (100 ng/ml) in order to generate Dermal DC like (Mo-DC). Adherent PBMC were also cultured in 25-cm<sup>2</sup> tissue culture flasks, with rhGM-CSF (250 ng/ml) +rhIL-4 (100 ng/ml) and rhTGF- $\beta$ 1 (10 ng/ml) in order to generate DC like Langerhans cell (Mo-LC) [31]. Afterwards, the DC (Mo-DC and Mo-LC) cultures were

incubated at 37°C, 5% CO<sub>2</sub> for 7, 14, 21 and 28 days. Every week, half volume of the medium was removed and replaced with fresh medium without adding additional cytokines.

The Mo-DC and Mo-LC populations were harvested and evaluated at 7, 14, 21 and 28 days

#### Flow cytometry analysis

Cells (10<sup>5</sup> cells/100  $\mu$ l) were incubated with 10  $\mu$ l of mAb on ice for 30'. Cells were washed, twice with wash buffer (Becton Dickinson, San Jose Ca), and stained with 10  $\mu$ l (1:100 antibody dilution) anti-mouse Ig FITC-labeled on ice for 30', washed once and fixed with Cellfix™ (Becton Dickinson, San Jose Ca). Thereafter, cells were acquired (10<sup>4</sup> cells per sample) and analyzed on a FACSCalibur (Becton Dickinson, San Jose Ca). Cells were acquired and gated by FSC and SSC. The gated cells were analyzed by (i) histograms displaying the fluorescent reactivity collected in fluorescence 1 (green channel); (ii) Density plots displaying the fluorescent reactivity collected in fluorescence 1 (green channel) against the FSC [31]. Flow

cytometry measurement on non-stained or stained only with the second antibody (secondary antibody) were performed and used as control populations. Secondary antibody only stained cells at day 7 served to determine the markers and the quadrant borders. At least 99% of these cells were located in the lower left quadrant (negative). Three independent experiments were performed. Data were analyzed with CellQuest (Becton Dickinson, San Jose Ca). Statistical analysis (mean, standard deviation and graphics) was performed with Microsoft Excel.

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### References

- Steinman RM: **The dendritic cell system and its role in immunogenicity.** *Annu Rev Immunol.* 1991, **9**:271-296
- Katz SI, Cooper KD, Iijima M, Tsuchida T: **The role of Langerhans cells in antigen presentation.** *J Invest Dermatol.* 1985, **85**:96s-98s
- Cella M, Sallusto F, Lanzavecchia A: **Origin, maturation and antigen presenting function of dendritic cells.** *Curr Opin Immunol* 1997, **9**:10-16
- Banchereau J, Steinman RM: **Dendritic cells and the control of immunity.** *Nature* 1998, **392**:245-252
- Bender A, Sapp M, Schuler G, Steinman RM, Bhardwaj N: **Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood.** *J Immunol Methods.* 1996, **196**:121-135
- Jiang W, Swiggard WJ, Heufler C, Peng M, Mirza A, Steinman RM, Nussenzweig MC: **The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing.** *Nature.* 1995, **375**:151-155
- Hock BD, Starling GC, Daniel PB, Hart DN: **Characterization of CMRF-44, a novel monoclonal antibody to an activation antigen expressed by the allostimulatory cells within peripheral blood, including dendritic cells.** *Immunology.* 1994, **83**:573-581
- Sallusto F, Lanzavecchia A: **Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha.** *J Exp Med.* 1994, **179**:1109-1118
- Geissmann F, Prost C, Monnet JP, Dy M, Brousse N, Hermine O: **Transforming growth factor beta1, in the presence of granulocyte/macrophage colony-stimulating factor and interleukin 4, induces differentiation of human peripheral blood monocytes into dendritic Langerhans cells.** *J Exp Med.* 1998, **187**:961-966
- Nunez R, Filgueira L: **Flow cytometry assessment of monoclonal antibody (mAb) reactivities against Dendritic cells (DC) In, D. Mason, Editor, Leukocyte Typing VII, Oxford University Press. Oxford.** 2001
- Nunez R, Filgueira L, Nunez C: **Flow cytometric assessment on human dendritic cell lines (HDCL) of monoclonal antibody (mAb) reactivities and determination of surface antigen expression by cytokine modulation. In: Mason D., Editor, Leukocyte Typing VII. Oxford: Oxford University Press.** 2001
- Hart DNJ, dark GJ, MacDonald K, Kato M, Vuckovic S, Lopez A, Wykes M, Munster D: **7th Leukocyte Differentiation antigen workshop, DC section summary. D. Mason, Editor, Leukocyte Typing VII, Oxford University Press. Oxford.** 2001
- Colonna M, Navarro F, Bellon T, Llano M, Garcia P, Samaridis J, Angman L, Cella M, Lopez-Botet M: **A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells.** *J Exp Med.* 1997, **186**:1809-1818
- Bosseloir A, Heinen E, Defrance T, Bouzazha F, Antoine N, Simar LJ: **Moabs MASS16 and 5B5, two fibroblast markers, recognize human follicular dendritic cells.** *Immunol Lett.* 1994, **42**:49-54
- Lane RD, Hegazy MG, Reimann EM: **Subcellular localization of glycogen synthase with monoclonal antibodies.** *Biochem Int.* 1989, **18**:961-970
- Colonna M, Samaridis J, Angman L: **Molecular characterization of two novel C-type lectin-like receptors, one of which is selectively expressed in human dendritic cells.** *Eur J Immunol.* 2000, **30**:697-704
- Cella M, Dohring C, Samaridis J, Dessing M, Brockhaus M, Lanzavecchia A, Colonna M: **A novel inhibitory receptor (ILT3) expressed on monocytes, macrophages, and dendritic cells involved in antigen processing.** *J Exp Med.* 1997, **185**:1743-1751
- Valladeau J, Duvert-Frances V, Pin JJ, Dezutter-Dambuyant C, Vincent C, Massacrier C, Vincent J, Yoneda K, Banchereau J, Caux C, Davoust J, Saeland S: **The monoclonal antibody DCGM4 recognizes Langerin, a protein specific of Langerhans cells, and is rapidly internalized from the cell surface.** *Eur J Immunol.* 1999, **29**:2695-2704
- de Saint-Vis B, Vincent J, Vandenabeele S, Vanbervliet B, Pin JJ, Ait-Yahia S, Patel S, Mattei MG, Banchereau J, Zurawski S, Davoust J, Caux C, Lebecque S: **A novel lysosome-associated membrane glycoprotein, DC-LAMP, induced upon DC maturation, is transiently expressed in MHC class II compartment.** *Immunity.* 1998, **9**:325-336
- Pulford K, Jones M, Banham AH, Haralambieva E, Mason DY: **Lymphocyte-specific protein 1: a specific marker of human leucocytes.** *Immunology.* 1999, **96**:262-271
- Mishra BB, Poulter LW, Janossy G, James DG: **The distribution of lymphoid and macrophage like cell subsets of sarcoid and Kveim granulomata: possible mechanism of negative PPD reaction in sarcoidosis.** *Clin Exp Immunol.* 1983, **54**:705-715
- Roholl PJ, Kleyne J, Prins ME, Hooijkaas H, Vroom TM, Van Unnik JA: **Immunologic marker analysis of normal and malignant histiocytes. A comparative study of monoclonal antibodies for diagnostic purposes.** *Am J Clin Pathol.* 1988, **89**:187-194
- Hock BD, Feamley DB, Boyce A, McLellan AD, Sorg RV, Summers KL, Hart DN: **Human dendritic cells express a 95 kDa activation/differentiation antigen defined by CMRF-56.** *Tissue Antigens.* 1999, **53**:320-334
- Barrett-Bergshoeff M, Noorman F, Bos R, Rijken DC: **Monoclonal antibodies against the human mannose receptor that inhibit the binding of tissue-type plasminogen activator.** *Thromb Haemost* 1997, **77**:718-724
- Kato M, Neil TK, Feamley DB, McLellan AD, Vuckovic S, Hart DN: **Expression of multilectin receptors and comparative FITC-dextran uptake by human dendritic cells.** *Int Immunol* 2000, **12**:1511-1519
- Hu W, McCrea PD, Deavers M, Kavanagh JJ, Kudelka AP, Verschraegen CF: **Increased expression of fascin, motility associated protein, in cell cultures derived from ovarian cancer and in borderline and carcinomatous ovarian tumors.** *Clin Exp Metastasis.* 2000, **18**:83-188
- Sallusto F, Cella M, Danieli C, Lanzavecchia A: **Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products.** *J Exp Med* 1995, **182**:389-400
- Clayberger C, Krensky AM, McIntyre BW, Koller TD, Parham P, Brodsky F, Linn DJ, Evans EL: **Identification and characterization of two novel lymphocyte function-associated antigens, L24 and L25.** *J Immunol.* 1987, **138**:1510-1514
- Geijtenbeek TB, Torensma R, van Vliet SJ, van Duijnhoven GC, Adema GJ, van Kooyk Y, Figdor CG: **Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses.** *Cell.* 2000, **100**:575-585
- Yokoyama WM: **What goes up must come down: the emerging spectrum of inhibitory receptors.** *J Exp Med.* 1997, **186**:1803-1808
- Nunez R: **Assessment of surface markers and functionality of Dendritic cells. Unit 9.17 in Robinson J.P., Darzynkiewicz Z., Dean P., Orfao A., Rabinovitch P., Tanke H., Wheelless L. eds. Current protocols in Cytometry. New York. John Wiley & Sons, Inc. 2001, Supplement 17:9.17.1-9.17.15**