

Dendritic Cells

Tools for Mouse and Human Dendritic Cell Research

Table of Contents

Dendritic Cells	2
Human Dendritic Cells	3
Identification of DC Precursor Subsets in Human Peripheral Blood	3
<i>In Vitro</i> Generation of Dendritic Cells	3
Phenotypic Characterization of Dendritic Cells	4
Immunophenotypic Monitoring of MDDCs	4
Custom Antibody Cocktails	4
Antibodies for Analysis of Human Dendritic Cells	5
Functional Analysis	6
T-cell stimulation	6
Cytokine Production	6
Mouse Dendritic Cells	7
Phenotypic Characterization of Dendritic Cells	7
Antibodies for Analysis of Mouse Dendritic Cells	8
Functional Analysis	8
Isolation of Mouse Dendritic Cells	8
References	11-12

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Patents

PE and APC: US 4,520,110; 4,859,582; 5,055,556;

Europe 76,695; Canada 1,179,942 **PerCP:** US 4,876,190 **Cy:** US 5,268,486; 5,486,616; 5,569,587; 5,569,766; 5,627,027 **PE-Cy7:** 4,542,104 **APC-Cy7:** US 5,714,386

Dendritic cells

Dendritic cells (DCs) are highly potent antigen presenting cells (APCs) uniquely able to initiate primary immune responses, including tolerogenic responses.¹⁻⁶ Because of their instrumental role in the immune system and their natural adjuvant properties, there is a great interest in exploiting DCs to develop immunotherapies for cancer, chronic infections and autoimmune disease, as well as for induction of transplant tolerance.⁶⁻⁸ Accordingly, there is increasing research activity, both in the intricacies of basic DC biology, as well as in murine models of disease, and the application of this knowledge to pre-clinical strategies for the manipulation of DCs in human disease.

Changes During Maturation and Differentiation

DC precursors migrate from the bone marrow to practically all lymphoid and non-lymphoid tissues, like skin and lung, where they carry out a sentinel-like function, sensing pathogens. In some cases they may be attracted by a cytokine gradient to an inflammatory site. Upon acquiring a “maturation/danger” signal, immature DCs migrate to secondary lymphoid organs (in response to specific chemokines), maturing along the way (**Figure 1**). DC maturation signals include components of pathogens and cytokines and other molecules associated with inflammation or tissue damage. Maturation of DCs is a terminal differentiation process accompanied by changes in the expression of numerous cell-surface antigens that reflect the changing functional role of the cells.

Immature DCs, such as Langerhans cells in the skin, have high cell-surface expression of receptors that efficiently capture antigen for uptake and processing.^{9,10} In contrast, mature DCs in secondary lymphoid tissues, such as lymph nodes and spleen, have a lower capacity to capture antigens, but are extremely efficient in antigen presentation and stimulating naïve T cells.

During maturation, DCs switch their pattern of chemokine receptor and adhesion molecule expression, allowing them to migrate to the secondary lymphoid tissues.^{2,11} In addition, the maturing DCs increase cell surface expression of peptide-loaded MHC class II as well as adhesion and co-stimulatory molecules, such as CD80 and CD86,

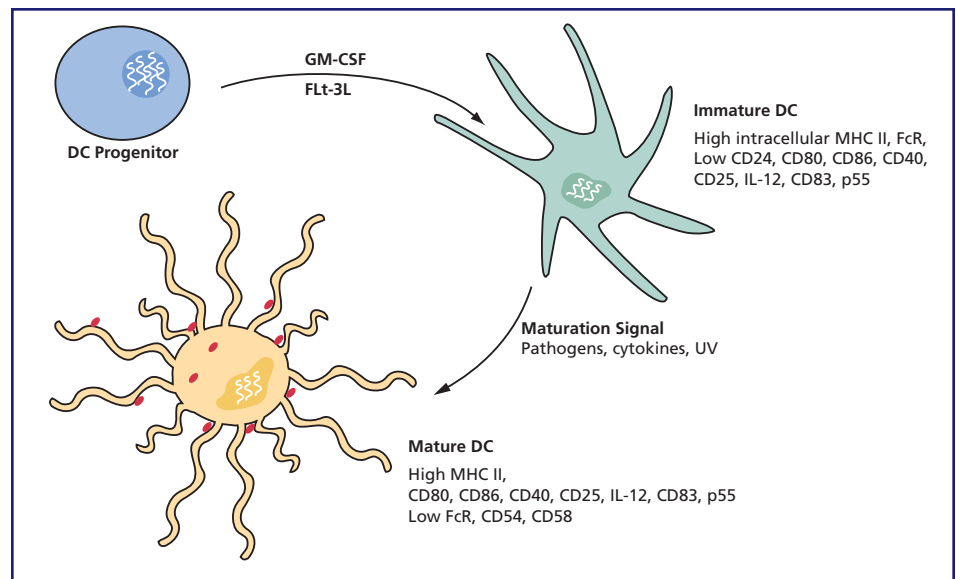


Figure 1. Maturation of dendritic cells. Dendritic cell (DC) progenitors from the bone marrow migrate to lymphoid and non-lymphoid tissues where they respond to maturation signals to fully develop. Maturation of DCs from “immature” to the “mature” stage involves changes in surface expression of antigens involved in antigen presentation and stimulation.

which co-stimulate via the CD28 pathway to allow for a productive response of the T cell.¹² In turn, T cells activate the DCs through the CD40-CD40L interaction to produce cytokines such as IL-12.¹³

Multifunctional Role of DCs in Immunity

Not only are DCs potent initiators of immune responses, they also play an important regulatory role, tuning the immune response⁴ by secreting cytokines that favor the development of Th1 or Th2 effector cells.^{14,15} The issue of exactly which DCs are regulating this, and how, is still an open question. Some studies point to the level of maturity of DCs as being the critical parameter in this¹⁶, others to a role for different lineages.^{4,17} The truth may lie in between these—in a model with a large degree of functional plasticity³.

The link that DCs provide between innate and adaptive immunity is also becoming more appreciated. Not only do they mature in response to ‘danger’ signals, thus becoming capable of inducing a productive T cell response, they also trigger a natural response to invading infectious agents by activating macrophages, natural killer (NK) cells, NK-T cells and eosinophils.^{5,18} The discovery that plasmacytoid DCs (P-DCs) are a major source of interferon (IFN)- α and - β , quickly secreting these in response to certain viruses^{19,20,21} has increased the interest in this DC subset.

Dendritic Cell Heterogeneity

Several different types of DCs and DC precursors have been described (e.g., interdigitating reticulum cells in lymphoid organs, blood DCs, Langerhans cells and dermal, or interstitial, DCs) which differ in origin, morphology, localization, maturation state, phenotype, and function.^{2,3,5} Although the cell surface phenotype defining them seems to differ in the species, two generally accepted types of DCs have been described in both mouse and human models that appear to represent different lineages: plasmacytoid DCs (P-DCs) and myeloid DCs (M-DCs).^{2,3,5,17} Provocative differences in certain functionally related measures are being described for P-DCs and M-DCs, including their expression of Toll-like receptors^{3,4}, their use of chemokine receptors²², and their cytokine secretion pattern.²³

Since a combination of factors influence the resulting T cell response, including the DC subset and maturation stage, detailed phenotypic analysis combined with functional studies will be one of the useful approaches in further studying the intricacies of DC biology in physiological as well as pathological conditions.

Identification of DC Precursor Subsets in Human Peripheral Blood

The frequency of DC precursor subsets in human blood has been reported to change in the course of certain diseases, such as HIV infection,^{24, 25} or to be a predictor for acute graft-versus-host disease.²⁶ This measure can thus be potentially informative in understanding the role of DCs in immune regulation in both disease and health.

The primary subsets of DC precursors that have been described in human blood are distinguished by their absence of expression of several lineage markers for monocytes, lymphocytes and NK cells, and the differential expression of CD11c (Integrin α_x) and CD123 (IL-3 $R\alpha$).^{3, 27-31}

Plasmacytoid DCs (P-DCs)

lin^{neg} CD11c^{neg} CD123^{high}

Myeloid DCs (M-DCs)

lin^{neg} CD11c^{high} CD123^{low}

The 3-color and 4-color dendritic value bundles are designed for identification of these two predominant DC precursor subsets in human peripheral blood and enable the determination of the frequency and number of cells in each of these rare DC subsets (Figure 2).

The inclusion of HLA-DR in addition to CD11c, CD123 and lineage cocktail allows the discrimination of CD123⁺ DCs from basophils. Starting with either whole blood or peripheral blood mononuclear cell (PBMC) samples, the 4-color assay requires just one tube per sample (plus one control), while the 3-color assay uses 2 tubes per sample (plus 2 controls). They thus enable quick analysis of these rare subsets with a small sample volume.

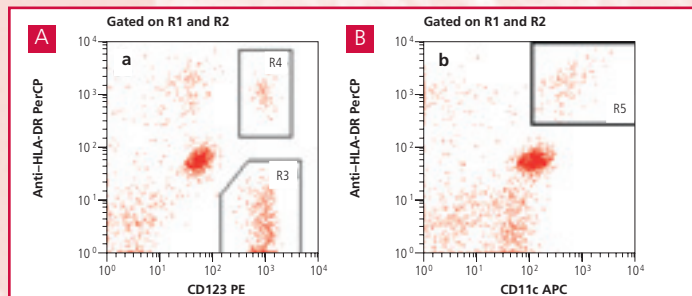


Figure 2. 4-color assay for DC precursor subsets in whole blood. Staining was performed using the 4-color dendritic value bundle following the procedure in the associated Application Note. Data shown was gated on events excluding debris and on lin¹ dim and negative events (not shown). A. Region R3 defines basophils and region R4 CD123⁺ DCs (0.14 % of total). B. Region R5 defines CD11c⁺ DCs (0.21% of total).

DESCRIPTION	CONTENTS (ANTI-)	SIZE	CAT. NO.
3-color dendritic value bundle	Lineage cocktail 1 FITC* (340546), CD123 PE (340545), CD11c PE (333149), HLA-DR PerCP (347402), Mouse IgG1 PE, Mouse IgG2a PE	50 tests	340566
4-color dendritic value bundle	Lineage cocktail 1 FITC* (340546), CD123 PE (340545), CD11c APC (333144), HLA-DR PerCP (347402), Mouse IgG1 PE, Mouse IgG2a APC	50 tests	340565

*The lineage cocktail 1 contains anti-CD3, CD14, CD16, CD19, CD20, CD56

For more information on this assay, please refer to the **Application Note** “Peripheral Blood Dendritic Cells Revealed by Flow Cytometry” available at www.bdbiosciences.com/immunocytometry_systems/application_notes

In Vitro Generation of Dendritic Cells

Because of the very low frequency of DCs in blood and tissues, various protocols for the *ex vivo* preparation of DCs from more readily available cells are widely used, in particular for studies aimed at developing immunotherapies using DCs. This often involves either isolated blood monocytes or CD34⁺ em cells from bone marrow or neonatal cord blood, and their cultivation in the presence of cytokines (e.g., GM-CSF, IL-4 and TNF- α) to induce their differentiation *in vitro*.^{6, 8, 32}

The characterization of the purity and maturation state of such preparations, as well as some *in vitro* measure of functionality, are seen as some of the key factors in achieving standardization between studies.^{6, 8, 32}

Monocyte Isolation

Blood monocytes are the most commonly used precursor cells for generating DCs *in vitro*. For the isolation of human monocytes we offer the BD™ IMag Cell Separation System. Based on a simple yet highly effective direct magnet technology, this easy-to-use system, combined with CD14 magnetic particles, delivers excellent purities and recoveries of monocytes in a few short steps.

PRODUCT DESCRIPTION	CLONE	SIZE	CAT. NO.
Human CD14 Magnetic Particles - DM	MoP9	1 x 10 ⁹ cells	557769

Visit www.bdbiosciences.com/bdimag for additional details about the system and its performance.

Phenotypic Characterization of Dendritic Cells

Multicolor flow cytometry allows high-resolution analysis of different cell populations within a heterogeneous sample without physically separating them.

Whether for the analysis of DCs in blood or of isolated or *in vitro*-generated DCs, the expression of numerous markers in addition to the molecules primarily defining the main DC subsets can be of interest. Selected markers may define the migratory potential, the level of maturity, or the potential capacity of an individual cell to respond to particular stimuli, and serve as an adjunct to functional measurements. For example, pathogen recognition receptors, such as Toll-like receptors (TLR), various adhesion molecules, chemokine receptors, and costimulatory molecules have been shown to be either selectively expressed by different DC subsets, or to change in expression during maturation.^{2, 3, 4, 5}

With a wide selection of direct fluorochrome conjugates to many DC-relevant markers (see Table 1), combination of markers for detailed multicolor phenotyping analysis is made easier.

Immunophenotypic Monitoring of MDDCs

The BD™ Multicolor MDDC phenotyping reagent has been designed to identify, enumerate and assess the degree of maturity of *in vitro*-generated monocyte-derived DC (MDDC). This pre-optimized cocktail includes CD209 (DC-SIGN), which is exclusively expressed by dendritic cells that are generated *in vivo*, as well as CD83 (a maturation marker) and CD86, which increases in expression during maturation of DCs. The analysis thus allows enumeration of both immature and mature DCs (Figure 3)³³. In doing so, MDDC preparations can be consistently evaluated for their phenotype across multiple sites.

PRODUCT DESCRIPTION	SIZE	RUO CAT. NO.
BD™ Multicolor MDDC phenotyping reagent CD86 PE / CD209 PerCP-Cy5.5 / CD83 APC	50 tests	334098

Depending on the flow cytometer used for acquisition or analysis, at least one fluorescence channel is available for an additional marker of your choice. For example, the addition of CD14 Alexa Fluor® 488 (Cat. No. 557700) could allow one to monitor monocyte development during the process of MDDC generation.

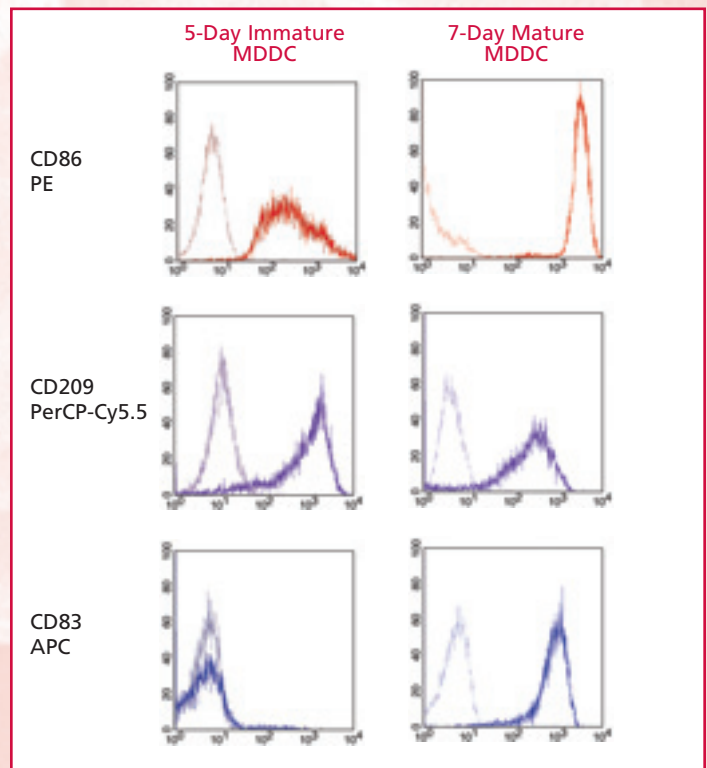


Figure 3. Phenotypic analysis of *in vitro*-generated MDDC. MDDC were prepared according to a published protocol³⁴. 5-day immature or 7-day mature MDDC (matured using GM-CSF, IL-4, IL-1 β , IL-6, TNF- α and PGE-2) were stained with the BD™ Multicolor MDDC phenotyping reagent (Cat. No. 334098, please see data sheet for details of staining) and analyzed on a BD FACSTM brand flow cytometer. The data shown were gated on cells with large scatter characteristics (not shown). Dashed lines show staining with an appropriate isotype control; solid lines show staining for the specificity indicated.

Custom Antibody Cocktails

As continued research identifies additional important markers for immunophenotyping of DCs, or in order to address particular research questions, corresponding multicolor antibody cocktails may be required.

With our Custom Antibody Cocktails we create multi-color reagent combinations (up to 6-color) according to your individual requirements. They are not just mixed. They are designed and pre-tested per your specifications, and are ready to use.

See www.bdbiosciences.com/custom/ for more information, or contact your BD Biosciences representative.

Human Dendritic Cells

Table1. Antibodies for Analysis of Human Dendritic Cells

Marker	Expression/Relevance*	Fluorochromes										FITC
		APC-Cy7	PE-Cy7	Alexa Fluor® 700	PerCP-Cy5.5	PerCP	PE-Cy5	Alexa Fluor® 647	APC	PE	Alexa Fluor® 488	
Lineage cocktail**	DCs are negative											
CD1a	▲ mat											
CD1b												
CD1d												
CD11c	M											
CD14	monocytes											
CD28	T cell											
CD33	M											
CD36	P > M											
CD40	▲ mat (co-stimulation)											
CD45RA	P > M											
CD54	▲ mat (adhesion)											
CD62L	P > M (homing)											
CD80	▲ mat (co-stimulation)											
CD83	mature											
CD86	▲ mat (co-stimulation)											
CD123	P											
CD150 (SLAMF6)	upon activ'n by costim.											
CD152 (CTLA-4)	on T cell											
CD154 (CD40L)	on T cell											
CD205 (DEC205)	on most (Ag uptake)	unconjugated										
CD206 (MMR)	Ag uptake											
CD209 (DC-SIGN)	DC-specific (migration & T cell adhesion)											
B7-H2	▼ mat (co-stimulation)											
CCR1	▼ mat											
CCR5	▼ mat											
CCR6	▼ mat											
CCR7	▲ mat P & M (and LC)											
CXCR3	▼ mat											
CXCR4	▼ mat											
CMRF-56	early activ'n Ag											
CMRF-58	mid-mat P; few M											
FDC	follicular DC											
HLA-DR	▲ mat (Ag presentation)											
TLR-1	M	unconjugated										
TLR-3	M (intracel)	unconjugated										
TLR-4	M	unconjugated										
TLR-5	M	unconjugated										
Intracellular Markers												
BrdU	proliferation detection											
COX-1, COX-2												
IFN-γ	activ'n dependent											
IL-1β	activ'n dependent											
IL-6	activ'n dependent											
IL-8	activ'n dependent											
IL-10	activ'n dependent											
IL-12p40/p70	activ'n dependent											
IL-12p70	activ'n dependent											
MIP-1α	activ'n dependent											
MIP-1β	activ'n dependent											
TNF-α	activ'n dependent											

* please note that most of these markers are also expressed on cells other than dendritic cells; in an attempt to summarize the salient feature of each marker, we may have not accounted for some tissue-specific differences in expression that have been reported in the literature. Please refer to the Technical Data Sheet for more information.

** Lineage cocktail 1 contains anti-CD3, CD14, CD16, CD19, CD20, CD56. Alexa refers to Alexa Fluor® dyes.

Details about each antibody can be found on our web at www.bdbiosciences.com

For more information about the fluorochromes and typical instrument configurations, please go to www.bdbiosciences.com/colors or www.bdbiosciences.com/spectra

Expression Key: P plasmacytoid DC mat mature
M myeloid (conventional) DC imm immature

Functional Analysis

T-Cell Stimulation

The most widely used *in vitro* measure of dendritic cell function is DCs capacity to stimulate T cells to proliferate in a mixed lymphocyte reaction (MLR). Proliferation is conventionally measured by incorporation of tritiated [³H]thymidine into DNA. Alternatively, one can measure the incorporation of the thymidine analog bromodeoxyuridine (BrdU) using antibodies to BrdU and flow cytometry.³⁵⁻³⁷

A key extension of this method, which is employed in the BrdU flow reagents presented here, is its compatibility with antibodies to additional markers, such as cytokines or cell surface molecules^{38, 39}. This allows determination of the frequency and nature of individual cells that have undergone proliferation, enabling a more complete analysis of functional activities at the single-cell level. This methodology has been used to assess the lymphoproliferative response induced by DCs.⁴⁰

The data shown here (Figure 4) illustrates the use of flow cytometry for the combined measurement of BrdU incorporation and cytokine production by T-cells after allogenic MLR with MDDCs. A similar approach can be used to measure antigen-specific T-cell stimulation by dendritic cells.⁴¹

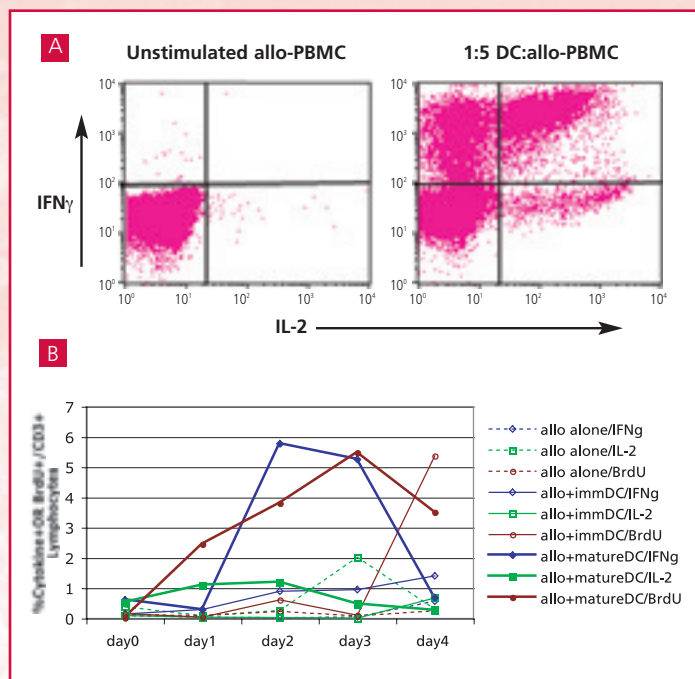


Figure 4. Allo-stimulation by Immature and Mature MDDC. Mature human MDDC (cultured in GM-CSF, IL-4, IL-1 β , IL-6, TNF- α and PGE-2) were prepared following a published protocol⁴². Immature or mature MDDC were mixed with allogeneic PBMC at ratios of 1:5 and cultured for 24h, 48h, 72h or 96h. BrdU and brefeldin A were added to the cultures 6 h before harvesting at each time point. Cells were treated with FACS Lysing solution for 10 min, washed, treated with FACS Permeabilizing solution 2 for 10 min, washed twice and stained with BD FastImmune™ BrdU FITC with DNase (Cat. No. 340649), and anti IL-2 PE + CD3 PerCP and IFN- γ APC for 60 minutes. A. To illustrate the analysis, IFN- γ and IL-2 staining of CD3-positive cells at one time-point (48h) and a DC:PBMC ratio of 1:5 is shown. B. Kinetics of production of IL-2, IFN- γ , or of BrdU incorporation, as % positive among the CD3-positive lymphocytes. More detail on the protocol can be found in the Application Note "Simultaneous detection of proliferation and cytokine expression in peripheral blood mononuclear cells" on our web: www.bdbiosciences.com/fastimmune

Cytokine Production

Another important measure of the functional potential of DCs is their ability to produce certain cytokines upon stimulation. Through their secretion of particular cytokines (and chemokines) at particular times, DCs contribute to regulating their own migration, the recruitment of other immune cells, and the polarization of the T-cell response.^{2, 3, 42}

The detection of cytokine-producing cells by intracellular staining and analysis by flow cytometry allows the cytokine-producing phenotype of individual cells within mixed populations to be analyzed.⁴³ This is made possible because cytokine staining can be combined with the analysis of cell surface markers used for subset definition. Although numerous studies have utilized the advantages of this technology to study T-cell responses, it is just starting to be applied to study dendritic cells.^{44, 45, 46, 47}

In addition, using the same staining procedure, other functional measures can be analyzed, such as BrdU incorporation (as described above, for T-cells), and expression of cyclooxygenase-1 and -2 (COX-1 and COX-2).^{33, 48} COX-2 is a pivotal regulator of prostaglandin biosynthesis that is upregulated during the course of inflammation.

Intracellular Cytokine Staining Products

PRODUCT GROUP	SPECIFICITIES*
BD FastImmune™ Cytokine System	Human IL-1 α , IL-1 β , IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-13, IFN- γ , TNF- α
More info: www.bdbiosciences.com/fastimmune	
BD Pharmingen™ Intracellular Cytokine Staining Reagents (using BD Cytofix/Cytoperm)	Human IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p40/p70, IL-12p70, IL-13, IL-16, GM-CSF, GRO- α , IFN- γ , IP-10, MCP-1, MCP-3, MIP-1 α , MIP-1 β , RANTES, TNF- α , TNF- β
	Mouse IL-2, IL-3, IL-4, IL-5, IL-6, IL-12p40/p70, IL-17, IFN- γ , MCP-1, MDC (ABCD-1), TNF- α
More info: www.bdbiosciences.com/immune-function	
Follow link to Intracellular Staining	

*As direct fluorochrome conjugates. For currently available conjugates, please refer to the e-catalog on our web

BrdU Flow Products

PRODUCT DESCRIPTION	SIZE	RUO CAT. NO.
BD FastImmune™ Anti-BrdU FITC (with DNase)	50 tests	340649
BD Pharmingen™ BrdU Flow Kit (FITC) Contents: FITC-conjugated Anti-BrdU Antibody, BD Cytofix/Cytoperm™ Buffer, BD Perm/Wash™ Buffer, BD Cytoperm™ Plus Buffer, 7-AAD, BrdU, DNase	50 tests	559619
BD Pharmingen™ BrdU Flow Kit (APC) Contents: as for 559619, but with APC-conjugated Anti-BrdU	50 tests	552598

Details about these products and the recommended protocols can be found under the catalog numbers at www.bdbiosciences.com

In the mouse, the majority of DCs express CD11c. Two main subsets have been defined, primarily based on their differential expression of CD11b and CD45R/B220, which appear to functionally correspond to myeloid (or conventional) and plasmacytoid precursor DCs. These subsets can be further subdivided based on the differential expression of CD4, CD8a and CD205 (DEC205), with CD8a⁺ subsets being found in both lineages.^{3, 5, 49} The definitive relationships of the various subsets described in different studies and from different tissues, as well as the potential functional implications, remain to be established.

Table 2: Phenotype of Murine Dendritic Cells^{3, 5, 49}

	Conventional DC	Plasmacytoid pre-DC
Surface Phenotype		
CD11c	high	intermediate
CD11b	+	-
CD45R/B220	-	+
Functional measures		
IFN- α *	-	+

*secreted upon appropriate activation

Phenotypic Characterization of Mouse Dendritic Cells

For the phenotypic analysis of DCs from mouse tissues or of isolated and cultured DCs various antibodies may be used. In addition to the cell surface markers defining the abovementioned subsets, a number of molecules that change in expression during maturation, such as costimulatory molecules, adhesion molecules, and chemokine receptors, clearly have potential functional implications, and may help define functional subsets of DCs.

To mention just one example, a recently discovered member of the B7 family, B7-DC, whose expression is inducible on DCs *in vitro*, appears to participate in bi-directional communication. Ligation of B7-DC on DCs stimulate those DCs in addition to regulating the responses of receptor-bearing T cells⁵⁰. Phenotypic analysis of splenic DCs for their expression of B7-DC is shown in Figure 5.

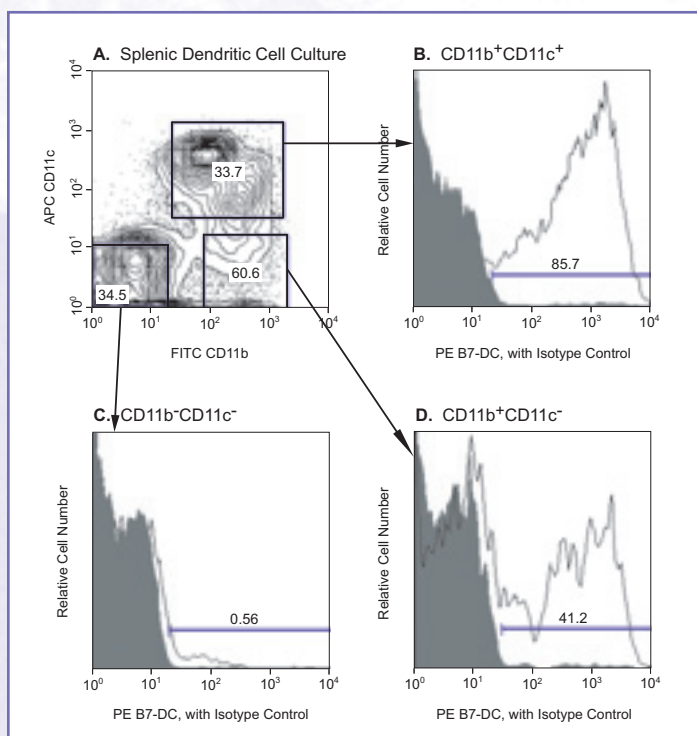


Figure 5. Splenic dendritic cells (DCs) express B7-DC. Splenic DCs were generated from BALB/c mice. Adherent cells were supplemented with 1000 U/ml of recombinant mouse GM-CSF (Cat. no. 554586). The DCs were harvested after overnight incubation at 37°C and stained with either PE-conjugated anti-mouse B7-DC mAb TY25 (open histograms) or PE-conjugated Rat IgG2a, κ -isotype control mAb R35-95 (Cat. no. 554689, filled histograms) in the presence of Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. no. 553141/553142). DCs were identified by staining with FITC-conjugated anti-mouse CD11b mAb M1/70 (Cat. no. 553310/557396) and APC-conjugated anti-mouse CD11c mAb HL3 (Cat. no. 550261). Dead cells were removed from analysis by propidium iodide gating. Panel A displays the gating of three leukocyte populations: CD11b⁺CD11c⁺ DCs, CD11b⁺CD11c⁻ DCs, and CD11b⁻CD11c⁻ non-DCs. The data, which are representative of three separate experiments, show that B7-DC expression was highest (~86%) on the CD11b⁺CD11c⁺ population (Panel B), intermediate (~41%) on the CD11b⁺CD11c⁻ population (Panel D), and very low (<1%) on the CD11b⁻CD11c⁻ population (Panel C). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system, and the data were analyzed using FlowJo (TreeStar, Inc., San Carlos, CA).

Table 3. Antibodies for Analysis of Mouse Dendritic Cells

Marker	Expression Relevance*	Fluorochromes											
		APC-Cy7	PE-Cy7	Alexa Fluor® 700	PerCP-Cy5.5	PerCP	PE-Cy5	Alexa Fluor® 647	APC	PE	Alexa Fluor® 488	FITC	Pacific® Blue
CD1d	P > M (Ag present'n)												
CD4	fine subsetting												
CD8a	fine subsetting												
CD11b	on M; dim/- on P												
CD11c	on all (differentially)												
CD23	Ag Uptake												
CD40	▲ mat M (co-stimuton)												
CD45R/B220	P								PE-Texas Red®				
CD45RA	P												
CD54	Adhesion/Homing												
CD62L	P > M; Adhes/Homing												
CD80	on most; co-stimulation												
CD86	M > P; ▲ mat; co-stimul'n												
CD152 (CTL-4)	T cell												
CD154 (CD40L)	T cell												
B7-H1	co-stimulation/inhibition												
B7-DC (PD-L2)	inducible; co-stim/inhib'n												
CCR5	▼ mat												
CCR6	on subsets												
CXCR4	preferentially P												
CIRE (mDC-SIGN)	subsets of P and M	biotin											
DC Marker (33D1)	on most ▲ mat												
FIRE (F4/80-like)	subsets (M); ▼ on activ'n												
MHC class II	mat (Ag presentation)	numerous haplotype reactivities are available - please refer to our catalog or web											
TLR4-MD-2	LPS signaling												
Intracellular Markers													
BrdU	proliferation detection												
IFN-γ	activ'n dependent												
IL-6	activ'n dependent												
IL-10	activ'n dependent												
IL-12 p40/p70	activ'n dependent												
TNF	activ'n dependent												

*please note that most of these markers are also expressed on cells other than dendritic cells; in an attempt to summarize the salient feature of each marker, we may have not accounted for some tissue-specific differences in expression that have been reported in the literature

Most antibodies listed are also available as biotin conjugates, which can be combined with our wide selection of fluorochrome-conjugated streptavidin. Alexa refers to Alexa Fluor® dyes.

For more information about the fluorochromes and typical instrument configurations, please go to www.bdbiosciences.com/colors or www.bdbiosciences.com/spectra

Expression Key: P plasmacytoid DC
M myeloid (conventional) DC
> higher expression than
mat mature
imm immature

Functional Analysis

For functional analysis, the same types of assays described for the human system are also available for mouse, such as measurement of intracellular or secreted cytokine, a flow-based proliferation assay, and several other measures of immune function and antigen-specific responses. For more information, go to www.bdbiosciences.com/immune_function/.

In addition, for many of the molecules involved in DC-T cell interaction, we also offer function-modulating (blocking or stimulatory) antibodies in a No Azide/Low Endotoxin (NA/LE) preparation for cellular assays.



Isolation of Mouse Dendritic Cells

For the enrichment of DCs from mouse tissues we offer the BD™ IMag Mouse Dendritic Cell Enrichment Set. Based on a simple yet highly effective direct magnet technology, this easy-to-use system delivers enriched, untouched dendritic cells in a few short steps.

DESCRIPTION	SIZE	CAT. NO.
BD IMag Mouse Dendritic Cell Enrichment Set - DM Contents: CD2, CD3e, CD45R/B220, CD49b, CD147, Ly-6G and LY-6C, TER-119 Erythroid cells biotinylated antibody cocktail and BD IMag™ SAv particles	1 x 10 ⁹ cells	557955

For additional details about this product, and its performance, please refer to the Technical Data Sheet, or visit www.bdbiosciences.com/bdimag

References

1. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature* 392:245-252.
2. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu Y-J, et al. 2000. Immunobiology of dendritic cells. *Annu. Rev. Immunol.* 18:767-811.
3. Shortman, K., Liu, Y.J. 2002. Mouse and human dendritic cell subtypes. *Nat. Rev. Immunol.* 2: 151-161.
4. Palucka, K. and Banchereau, J. 2002. How dendritic cells and microbes interact to elicit or subvert protective immune responses. *Curr. Opin. Immunol.* 14: 420-431.
5. Lipscomb, M.F. and Masten, B.J. 2002. Dendritic cells: Immune regulators in health and disease. *Physiol. Rev.* 82:97-130.
6. O'Neill, D., Adams, S. and Bhardwaj, N. 2004. Manipulating dendritic cell biology for the active immunotherapy of cancer. *Blood* 104: 2235-2246
7. Woltman, A.M. and van Kooten, C. 2003. Functional modulation of dendritic cells to suppress adaptive immune responses. *J Leuk. Biol* 73:428-441.
8. Figdor, C.G., De Vries, I.J.M., Lesterhuis, W.J. and Melief, C.J.M. 2004. Dendritic cell immunotherapy: mapping the way. *Nature Medicine* 10: 475-480.
9. Jiang, W., W. J. Swiggard, C. Heufler, M. Peng, A. Mirza, R. M. Steinman, and M. C. Nussenzweig. 1995. The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature* 375:151-155.
10. Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia. 1995. 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.* 182:389-400.
11. Sallusto, F., P. Schaerli, P. Loetscher, C. Schaniel, D. Lenig, CR Mackay, S. Qin, and A. Lanzavecchia. 1998. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur J Immunol.* 28(9): 2760-9.
12. Cella, M., F. Sallusto, and A. Lanzavechia. 1997. Origin, maturation and antigen presenting function of dendritic cells. *Curr. Opin. Immunol.* 9:10-16.
13. Koch, F., U. Stanzl, P. Jennewein, K. Janke, C. Heufler, E. Kampgen, N. Romani, and G. Schuler. 1996. High level of IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. *J. Exp. Med.* 184:741-746.
14. Moser, M., K. M. Murphy. 2000. Dendritic cell regulation of TH1-TH2 development. *Nat. Immunol.* 1:199.
15. Langenkamp, A., M. Messi, A. Lanzavecchia, F. Sallusto. 2000. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nat. Immunol.* 1:311.
16. Lutz, M.B. and Schuler, G. 2002. Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? *Trends Immunol.* 23: 445-449.
17. Grabbe, S., Kämpgen, E. and Schuler, G. 2000. Dendritic cells: multi-lineal and multi-functional. *Immunology Today* 21: 431-433.
18. Reis e Sousa, C. 2004. Activation of dendritic cells: translating innate into adaptive immunity. *Curr. Opin. Immunol.* 16: 21-15.
19. Yonezawa, A, Morita, R, Takaori-Kondo, A, Kadowaki N, Kitawaki, T, Hori, T and Uchiyama, T. 2003. Natural Alpha Interferon-Producing Cells Respond to Human Immunodeficiency Virus Type 1 with Alpha Interferon Production and Maturation into Dendritic Cells. *J. Virol.* 77: 3777-3784.
20. Dalod M, Salazar-Mather, TP, Malmgaard, L, Lewis, C, Asselin-Paturel, C, Brière, F, Trinchieri, G and Biron, CA 2002. Interferon α/β and Interleukin 12 Responses to Viral Infections: Pathways Regulating Dendritic Cell Cytokine Expression *In Vivo*. *J. Ex. Med.* 195: 517-528.
21. Diebold SS, Montoya M, Unger H, Alexopoulou L, Roy P, Haswell LE, Al-Shamkhani A, Flavell R, Borrow P, Reis e Sousa C. 2003. Viral infection switches non-plasmacytoid dendritic cells into high interferon producers. *Nature*. 424:324-8
22. Penna, G., Sozzani, S. and Adorini, L. 2001. Cutting edge: Selective use of chemokine receptors by plasmacytoid dendritic cells. *J. Immunol.* 167: 1862-1866.
23. Penna, G., Vulcano, M., Roncari, A., Facchetti, F., Sozzani, S. and Adorini, L. 2002. Cutting edge: Differential chemokine production by myeloid and plasmacytoid dendritic cells. *J. Immunol.* 169: 6673-6676.
24. Barron MA, Blyveis N, Palmer BE, MaWhinney S, Wilson CC., 2003. Influence of plasma viremia on defects in number and immunophenotype of blood dendritic cell subsets in human immunodeficiency virus 1-infected individuals. *J. Infect. Dis.* 187 : 26-37.
25. Chehimi J, Campbell DE, Azzoni L, Bacheller D, Papasavvas E, Jerandi G, Mounzer K, Kostman J, Trinchieri G, Montaner LJ. 2002. Persistent decreases in blood plasmacytoid dendritic cell number and function despite effective highly active antiretroviral therapy and increased blood myeloid dendritic cells in HIV-infected individuals. *J Immunol.* 168(9):4796-801.
26. Reddy, V., Iturraspe, J.A., Tzolas, A.C., Meier-Kriesche, H.-U., Schold, J. and Wingard, J.R. 2004. Low dendritic cell count after allogeneic hematopoietic stem cell transplantation predicts relapse, death, and acute graft-versus-host disease. *Blood* 103: 4330-4335.
27. Olweus J, BitMansour A, Warnke R, et al. 1997. Dendritic cell ontogeny: a human dendritic cell lineage of myeloid origin. *Proc Natl Acad Sci USA.* 94:12551-12556.
28. Hart DNJ, McKenzie JL. 1988. Isolation and characterization of human tonsil dendritic cells. *J Exp Med.* 168:157-170.
29. Grouard G, Rissoan M, Filgueira L, Durand I, Banchereau J, Liu J. 1997. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin-3 (IL-3) and CD40 ligand. *J Exp Med.*;185:1101-1111.
30. O'Doherty U, Peng M, Gezelter S, et al. 1994. Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. *Immunology*;82:487-493.
31. Thomas R, Lipsky PE. 1994. Human peripheral blood dendritic cell subsets. Isolation and characterization of precursor and mature antigen-presenting cells. *J Immunol.* 153:4016-4027.
32. Nestle, F. O., Banchereau, J. and Hart, D. 2001. Dendritic cells: On the move from bench to bedside. *Nat. Medicine* 7: 761-765.

33. Ghanekar, S.A., Bhatia, S., Ruttenberg, J.J., Maecker, H.T., Maino, V.T. and Waters, C.A. 2004. Phenotypic and functional characterization of immature and mature monocyte-derived dendritic cells activated with inflammatory stimuli. Abstract Number W13.129, 4th Annual conference of FOCIS, July 2004. Available at http://info.bdbiosciences.de/SGFOCIS_2004_MDDC.pdf
34. Marovich, M.A., Mascola, J.R., Eller, M.A., et al. 2002. Preparation of clinical-grade recombinant canarypox-human immunodeficiency virus vaccine-loaded human dendritic cells. *J. Infect Dis* 185: 1242-1252.
35. Miltenburger, H. G., G. Sachse and M. Schliermann. 1987. S-phase cell detection with a monoclonal antibody. *Dev. Biol. Stand.* 66: 91-99.
36. Gratzner, H. G. and R. C. Leif. 1981. An immunofluorescence method for monitoring DNA synthesis by flow cytometry. *Cytometry*. 1:385-393.
37. Toba, K., E. F. Winton and R. A. Bray. 1992. Improved staining method for the simultaneous flow cytofluorometric analysis of DNA content, S-phase fraction, and surface phenotype using single laser instrumentation. *Cytometry*. 13:60-67.
38. Mehta BA, Maino VC. 1997. Simultaneous detection of DNA synthesis and cytokine production in staphylococcal enterotoxin B activated CD4+ T lymphocytes by flow cytometry. *J Immunol Methods*. 208:49-59.
39. Waldrop S, Pitcher C, Peterson D, Maino V, Picker L. 1997 Determination of antigen-specific memory/effector CD4+ T cell frequencies by flow cytometry. *J Clin Invest*. 99:1739-1750.
40. Hsieh, S.-M., Pan, S.-C., Hung, C.-C., Tsai, H.-C., Chen, M.-Y., Lee, C.-N. and Chang, S.-C. 2001. Kinetics of antigen-induced phenotypic and functional maturation of human monocyte-derived dendritic cells. *J. Immunol.* 167: 6286-6291.
41. Maecker, HT, Ghanekar, SA, Suni, MA, He, X-S, Picker, LJ and Maino, VC. 2001. Factors Affecting the Efficiency of CD8+ T Cell Cross-Priming with Exogenous Antigens. *J. Immunol.* 166: 7268-7275. See publication for an explanation of the conditions that produced these results.
42. Sallusto, F, Palermo, B, Lenig, D, Miettinen, M, Matikainen, S, Julkunen, I, Forster, R, Burgstahler, R, Lipp, M, Lanzavecchia, A. 1999. Distinct patterns and kinetics of chemokine production regulate dendritic cell function. *Eur. J. Immunol.* 29: 1617-25.
43. Carter, LL and Swain, SL. 1997. Single cell analysis of cytokine production. *Curr Opin Immunol.* 9(2): 177-82.
44. Ghanekar, SA, Zheng L, Logar A, Navratil J, Borowski L, Gupta P, and Rinaldo C. 1996. *J. Immunol.* 157: 4028-4036.
45. Willmann K, Dunne JF. 2000. A flow cytometric immune function assay for human peripheral blood dendritic cells. *J. Leukoc. Biol.* 67:536-44.
46. Bueno C, Almeida J, Alguero MC, Sanchez ML, Vaquero JM, Laso FJ, San Miguel JF, Escibano L, and Orfao A 2001. Flow Cytometric analysis of cytokine production by normal human peripheral blood dendritic cells and monocytes: comparative analysis of different stimuli, secretion-blocking agents and incubation periods. *Cytometry* 46: 33-40
47. Krug A, Uppaluri R, Facchetti F, Dorner BG, Sheehan KCF, Schreiber RD, Cella M and Colonna M. 2002. Cutting Edge: IFN-Producing Cells Respond to CXCR3 Ligands in the presence of CXCL12 and Secrete Inflammatory Chemokines upon Activation. *J. Immunol.* 169:6079-6083
48. Ruitenberg, JJ and Waters, CA 2003. A rapid flow cytometric method for the detection of intracellular cyclooxygenases in human whole blood monocytes and a COX-2 inducible human cell line. *J. Immunol. Methods* 274: 93-104.
49. O'Keeffe, M., Hochrein, H., Vremec, D., Caminschi, I., Miller, J.L., Anders, E.M., Wu, L., Lahoud, M.H., Henri, S., Scott, B., Hertzog, P., Tatarczuch, L. and Shortman, K. 2002. Mouse plasmacytoid cells: Long-lived cells, heterogeneous in surface phenotype and function, that differentiate into CD8+ dendritic cells only after microbial stimulus. *J. Exp. Med.* 196: 1207-1319.
50. Nguyen, L.T., Radhakrishnan, S., Ciric, B., Tamada, K., Shin, T., Pardoll, D.M., Chen, L., Rodriguez, M. and Pease, L.R. 2002. Cross-linking the B7 family molecule B7-DC directly activates immune functions of dendritic cells. *J. Exp. Med.* 196: 1393-1398. See publication for an explanation of the conditions that produced these results.

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