Editorial

Monocytes and Macrophages in Flow: an ESCCA Initiative on Advanced Analyses of Monocyte Lineage Using Flow Cytometry

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In April 2013, a symposium was organized to highlight different aspects of differentiation and activation of the monocyte-macrophage lineage as analyzed on the flow cytometer. Characterization of this lineage requires knowledge of the maturation process from their progenitors that are present in bone marrow up to the mature monocytic cells in peripheral blood, because each monocytic lineage cell with an aberrant phenotype refers to the corresponding maturation stage. A standardized quantitative analysis will facilitate the monitoring of the pathological processes and the clinical features, such as the outcome of treatment. However, changes in marker expression by variation in intensity, asynchronism, and lineage infidelity must be considered. The dynamics of normal marker expressions in early differentiation stages, e.g. molecules like HLA II, CD64 or CD14, give rise to a hypothesis on their possible role in monocyte ontogeny. Besides their usual role in tissue homeostasis, mature macrophages may also play a similar role in hematopoiesis. This meeting highlighted the large potential of flow cytometric tools available for monitoring of all these aspects in the monocytic and macrophage cell lineage. © 2015 International Clinical Cytometry Society

Key terms: flow cytometry; monocytes; macrophage; myelodysplasia; bactericidy; cell signaling; phosphor-flow

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The European Society for Clinical Cell Analysis in collaboration with the International Federation of Clinical Chemistry organized an international course on flow cytometry in Saint-Etienne (France) in April 2013. Because the monocytic lineage raises a lot of interest due to its multiple functions as a crossing junction between different systems, the course focused on the different differentiation stages of the monocyte-macrophage lineage, their physiological or pathological status, and clinical monitoring in human as well as in animal models.

Furthermore, this meeting aimed at promoting interdisciplinary exchanges of knowledge of the different views from experimental to clinical and from hematological to immunological, between colleagues working on the same cell populations.

Monocytes and macrophages are considered to belong to the same differentiation lineage, but represent sequential stages of a linear development process.

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Monocyte progenitors are initiated in the bone marrow as a common myeloid-monocytic progenitor, the colony-forming unit of granulocytes and monocytes (CFU-GM). Subsequently, these progenitors progressively initiate the myeloid and monocytic lineage. The monocytes circulate in the peripheral blood and eventually migrate into tissues where they accomplish their terminal differentiation into macrophages. Macrophages are cells with extreme plasticity, acquiring different morphological types (alveolar macrophages, Kupffer cells, mesangial macrophages, histiocytes, etc.) according to the microenvironment of the seed and temporal conditions (1). A group of macrophages, named dendritic cells, acquires the capacity of antigen presentation to the cognate immune system. They acquire different phenotypes and such as Langerhans cells in the skin. These cells are strongly adherent to the tissue and thus difficult to analyze by flow cytometry (FCM; (2,3)). They were not addressed in this course.

Lectures focusing on different aspects of monocyte analysis, namely physiology (Ulrich Sack et al. pathology (Frauke Bellos et al and Sergio Matarraz et al. and functional activity (Laura Diaz et al Emilie Coppin et al. are the source of articles in this issue of Cytometry. We are pleased to provide a context and some background for these contributions

PHYSIOLOGY OF THE MONOCYTIC LINEAGE

The monocytic lineage represents a major part of the innate immune system constituting the first line of defense of the body against environmental risks. Macrophages have a high capacity to adhere, migrate, perform phagocytosis, and digest bacteria, immune complexes, debris, and micro-particles. Macrophages are primarily active at body barriers (such as lung, gut, and skin) but also in the reticuloendothelial system in the spleen and liver for the body homeostasis.

Monocytes and macrophages are highly sensitive biosensors for different pathogen associated molecular patterns and damaged tissue residues through their ancestral toll-like receptors (TLRs) and related molecules. Their activation induces a rapid production of cytokines and chemokines in diverse patterns according to the respective risks. Cytokines locally regulate the inflammatory reaction while chemokines attract and prime new effectors. The production is fast and can be massive, ending in septic shock and eventually death of the patient.

Two typical patterns have been defined. Macrophages can be classified as (a) proinflammatory macrophages (M1) predominantly producing IL-18, IL-12, IL-26 that promote Th1 and Th17 responses or (b) anti-inflammatory macrophages (M2) more recently named "alternatively activated macrophages" (AAM) that preferentially produce IL-10 and TGFβ with immunoregulatory and tissue-repair activities (4,5). However, it is reasonable that these two patterns are the extreme status of a continuous distribution of intermediate cytokine balances. The variable balance may also occur in the same cells, depending on the context and the timing of the challenge.

Monocytes/macrophages also contribute substantially to the cognitive immune response though presentation of the antigen to T cells and orchestration of the immune reaction. Being able to measure the risks of the challenge, macrophages finely orchestrate the cognitive immune response with respect to the appropriate type, intensity, and length of reaction as well as memory (4,6).

Some mediators that are produced by macrophages express a large (endocrine) acute phase reaction (C-reactive protein, procalcitonin, coagulation, and complement components among other proteins), increasing body temperature, and favoring nervous stress (fever, irritability, anxiety, anorexia). Some cytokines also induce direct vascular changes triggering angiogenesis, permeability, vasodilation, and procoagulation (7,8).

Macrophages play a crucial role in defense against external causes and in inducing and managing inflammation. Furthermore, macrophages can also perform noninflammatory phagocytosis of apoptotic or necrotic cells, inert microparticles, and any debris. In this process, they are clearing damaged tissues due to injury, infection, ischemia, regeneration, or graft rejection and participate in their repair or renewal (9,10). Several tissues possess a very high rate of cell renewal, which is inevitably associated with cell death or aberrancies. This primarily concerns the skin, gut mucosae, lymph nodes, thymus, gonads, and bone marrow (11). Macrophages execute clearance of the debris that is essential to homeostasis in these tissues. In contrast, macrophages also support tissue regeneration by providing growth and angiogenic factors in angiogenesis, osteogenesis, muscle, and nerve tissue regeneration (9). We could question whether, in a clean environment, the continuous maintenance of body homeostasis could even be the predominant activity of macrophages compared with defense against occasional infections.

CHARACTERIZATION OF THE MONOCYTIC LINEAGE

Identification of monocytic lineage cells by FCM is mainly based on the use of lineage-specific monoclonal antibody conjugates (MoAbs; Fig. 1). However, only few MoAbs are really lineage-specific and even fewer are specific to a given maturation stage. Therefore, precise cell characterization requires combinations of several markers (panels; (12,13)).

Mature circulating monocytes are classically characterized by their expression of CD14, usually considered a hallmark, CD13, CD33, CD11b, CD18, dim CD4, and CD64 (Fig. 1). Other markers provide information on their adhesion capacity [e.g., integrin heterodimers, CD54 (ICAM-1), CD49d (VLA-1a)], activation status (e.g., CD68, CD69, and HLA-DR), accessory molecules for T cell activation (e.g., CD70, CD80, CD86), the chemokine receptors CCR1, CCR2, CCR3, CCR4, and CCR5 that regulate the migration of monocytic cells (14–16). Most receptors are expressed at a mean density of ~20,000,

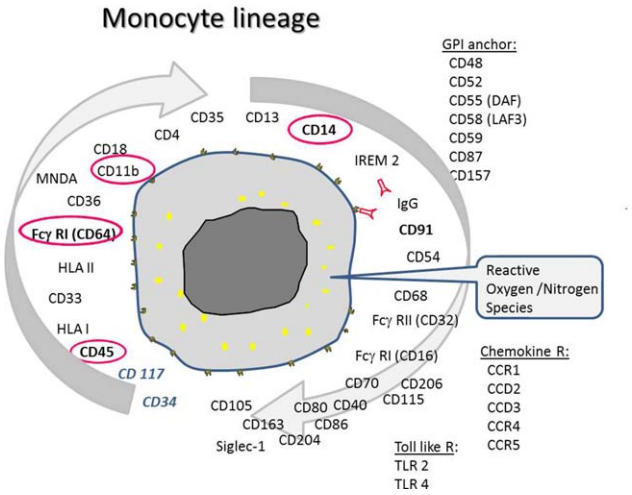


FIG. 1. List of markers of interest for identification of monocyte depending on its maturation and activation stage. Arrows show the maturation progress from left (CD34, CD117 progenitor stage) to right. Marker families are listed including GPI anchor that expression may vary in GPI disorders such as paroxysmal nocturnal hemoglobinuria. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

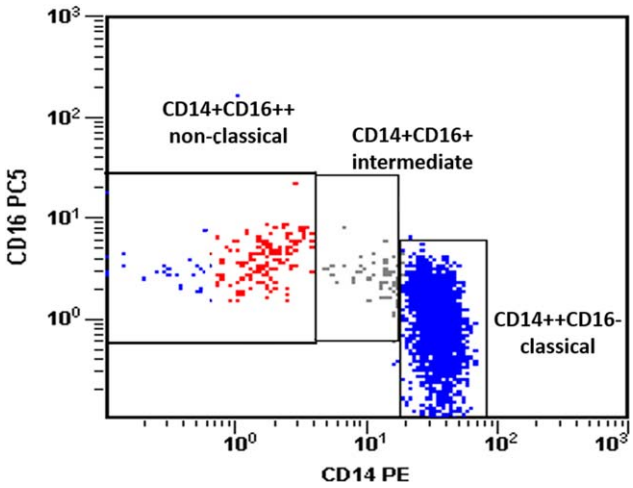


FIG. 2. Intensities of expression of CD14 and CD16 on monocytic cells. Classical monocytes express CD14 strongly and not CD16. However, a small fraction of peripheral monocytes progressively reduce the expression of CD14 and simultaneously acquire the expression of CD16. This rare phenotype has been named anti-inflammatory macrophages (M2) or alternatively activated macrophages (AAM). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

but few are expressed at up to [mt]30,000 molecules per cell ((17); Table 1). Each of these markers corresponds to different monocyte functions.

However, CD14 appears not to be the hallmark for identification of monocytes. Indeed, late maturation of peripheral monocytes (Fig. 2) is accompanied by the expression of Fcγ-RIII (CD16), usually used to identify granulocytes, and the simultaneous decline of its CD14 expression on a small subset of peripheral monocytes (18–22). This is of particular interest as monocyte and granulocyte populations can overlap in forward scatter and side scatter parameters. The CD14-low, CD16-positive monocyte subset corresponds to AMM or M2 macrophages already mentioned (23,24). It has been shown that these M2 cells tend to have lower phagocytosis activity, a higher capacity for cytokine production and antigen presentation that is related to a higher expression of HLA class II (25,26).

Table 1
Absolute Antigen Density on Mature Peripheral Monocytes Expressed as Thousands of Antibody Bound per Cell (x 1000 ABC) Adapted from Bikoue et al. (17)

		Monocytes (*x* 1000 ABC)	Neutrophils (*x* 1000 ABC)
CD11b	Integrin αM	52 ± 18[a]	45 ± 20
CD13	Aminopeptidase N	21 ± 8	11 ± 6
CD14	LPS-Co receptor	114 ± 42	<3
CD16	Fcγ-R III	12 ± 5	408 ± 167
CD18	Integrin β	93 ± 19	49 ± 15
CD35	Complement product	17 ± 6	15 ± 6
CD36	Collagen-R or thrombospondin-R	34 ± 17	11 ± 6
CD44	Hyaluronic-R	201 ± 57	73 ± 23
CD45	Phosphatase	103 ± 44	36 ± 16
CD54	ICAM-I	19 ± 6	12 ± 7

Abbreviations: LPS: lipopolysaccharide; Fcγ: Immunoglobulin gamma receptor; ICAM: intercellular adhesion molecule.
[a]Mean values ± SD.

Because not all monocytes can be identified by CD14 only, recent studies have focused on investigations to find new MoAbs that can characterize all mature monocytes/macrophages. The α2-macroglobulin receptor (CD91) appeared to be reproducibly expressed on monocytes independently of the status of CD14/CD16 phenotypes. Up to 15% of monocytes selected by CD91 did not express CD14 (27–29). Interestingly, CD91 is also known as a common receptor for heat shock proteins gp96, hsp90, and hsp70 (28). CD91 collaborates with collectins like calreticulin (binding mannose residues) and collectin-like C1q that binds cell products such as apoptotic bodies, promoting phagocytosis (27).

Other molecules, such as CD163, a scavenger receptor (30), CD206 (31), the macrophage mannose receptor, and CD115 (the M-CSF receptor) have been associated with type M2 macrophages and could be involved in further differentiation (4,32–34). Another scavenger, CD204, has been associated with poor outcome in lung cancer and possibly a permissive role for tumor growth (35). More markers have been associated with activation (e.g., iglec-1) cytokine or chemokine receptors (15,36).

Interestingly, a number of monocyte markers are GPI-anchored and are defective in paroxysmal nocturnal hemoglobinuria (PNH). Some of these markers are recommended for the detection of PNH clones (e.g., CD14, CD157) in monocytes, though CD14 is normally defective in AAM and dendritic cells without any sign of PNH. Because CD33 and CD91 are not GPI-anchored, they can be used for the monocyte selection within the WBC population in combination with CD45 (37).

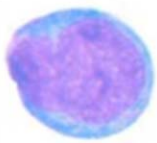
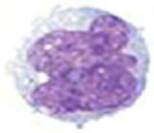
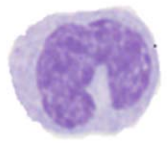
Long Term-Hematopoietic Stem Cell	Common Myeloid Progenitor	Granulocyte Monocyte Progenitor Cell	Monoblast	Promonocyte	Monocyte
	CD34+CD38-CD90+CD45RA	CD34+CD38+CD45RA+CD4-CD15-CD123+	CD34+CD4+CD33+	CD34-CD4+CD33+	CD34-CD4+CD33+
					
CD4		-	-/+	+	+
CD11b		-	-	++	+++
CD13		++	++	+/++	++/+++
CD14		-	-	+/++	+++
CD15		-	-	++	+
CD16		-	-	-	-/+
CD33		+++	+++	+++	+++
CD34		+/++	+	-	-
CD36		-	-	++	+++
CD45		-	+	++	+++
CD64		++	-	++	+++
HLADR		++	++	+++	++/+++

FIG. 3. Phenotypic expression of antigens during the maturation of the monocytic lineage. Many proteins are expressed on monocytic cells depending on the stage of maturation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The early maturation scheme of monocytic lineage cells, which is rather simple, has been described for many years based on morphological criteria, starting with monoblasts followed by pro-monocytes that, in turn, differentiate into mature monocytes ready to leave the bone marrow (Fig. 3). At the earliest stage, monocytic precursors express sialomucin (CD34) and tyrosine-protein kinase c-kit (CD117). In the subsequent stage of the maturation, cells lose their expression of CD34, followed by CD117, while acquiring a bright expression of CD11b (integrin alpha M chain), CD13 (aminopeptidase N), CD33, and CD64 (Fcγ receptor I). At this stage, the monocytic lineage diverges from the myeloid lineage. The monocyte lineage maintains a low side scattering (SSC), acquires the expression of CD14 (endotoxin coreceptor) that increases during maturation from promonocytes to monocytes, and retains a bright expression of HLA-DR, which can be further enhanced by activation and increases their expression of CD45. However, maturating myeloid cells lose HLA-DR and acquire an intermediate to high SSC (Fig. 4). The separation from the myeloid lineage occurs progressively and results in an expression of CD14 of monocytes or an expression of Fcγ-RIII (CD16) and CD15 on myeloid cells. More markers appear progressively on pro-monocytes such as CD4 (an MHC II ligand), CD36 (a collagen and thrombospondin ligand), and CD35 (a complement product receptor) and subsequently these cells are involved in phagocytosis and opsonisation.

In early stages, abnormal phenotypes are difficult to detect on monocytic precursors compared to the cells of the other lineages and other maturation stages. As is shown by Frauke Bellos et al. and Sergio Matarraz et al. (in this issue) a good knowledge is required of the subsequent differentiation stages in comparison to other related lineages, such as the myeloid and erythroid lineage. FCM provides the possibility to analyze a large number of cells and characterize precisely each individual cell with multiple parameters even if it is a small minority among the complex population.

PATHOLOGY

Although cells of the monocytic lineage perform crucial functions for the body, they may also express dysfunctions leading to multiple diseases. The maturation process can fail in different ways leading to cytopenia, maturation disorders [e.g., myelodysplastic syndrome (MDS)], chronic diseases such as chronic myelomonocytic leukemia or even aggressive proliferative diseases, such as acute myelocytic leukemia (AML; (38–40)).

Other pathologies are linked to functional deficiencies affecting phagocytosis (41,42) or bactericide activity (43). Dysfunction can be acquired as cell exhaustion after extensive sepsis (44–46). However, inappropriate responses can induce life threatening cytokine storms (septic shock), granulomatosis, or more insidious slowly progressing inflammation that contributes to chronic

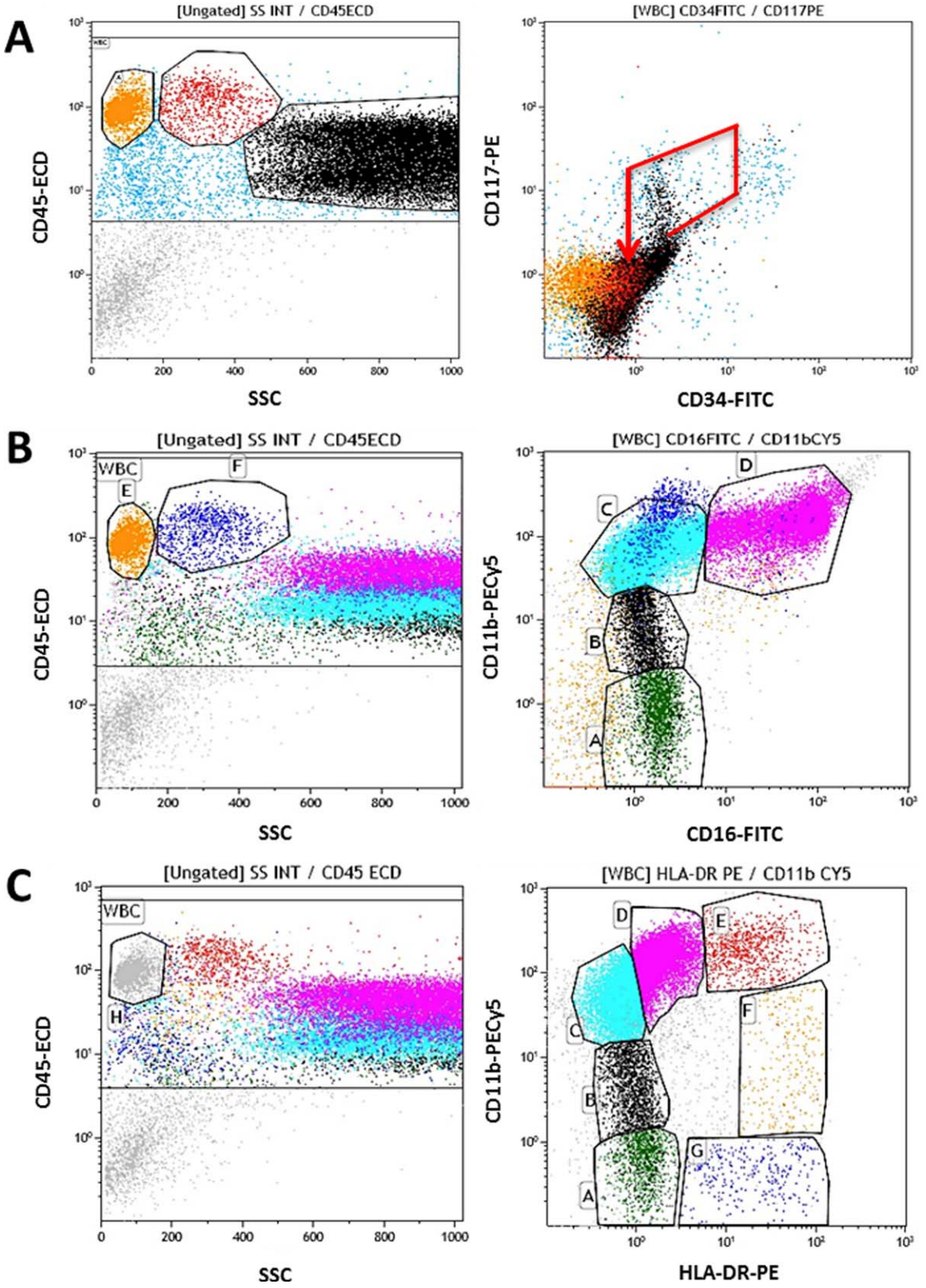


FIG. 4. Phenotypic changes during the maturation of the myelomonocytic progenitors into mature end-stage cells of the monocytic and myeloid lineage. **A**: Myelomonocytic progenitors are located in the $CD45^{dim}/SSC^{dim}$ population (blue). The myelomonocytic progenitors can be selected by plotting CD34 against CD117 (see red arrow). Immature cells differentiate from $CD34^{-}/CD117^{-}$ into $CD34^{+}/CD117^{+}$ (myeloblasts) and into $CD34^{-}/CD117^{+}$ (promyelocytes). **B**: Subsequently, the myeloid and monocytic lineage dichotomy can be visualized by plotting CD11b against CD16 and gated in CD45/SS (plots B). The myeloid cells differentiate from Region A through Regions B and C ($CD11b^{+}/CD16^{-}$) into ultimately Region D ($CD11b^{+}/CD16^{+}$), whereas the monocytic lineage differentiates to higher intensities of CD11b with dim expression of CD16 (dark blue population). **C**: The separation between the monocytic and myeloid lineage is even better defined by plotting HLA-DR against CD11b (Plots C). Monocytic cells sequentially differentiate from progenitors (Region G) to promonocytes (Region F) and subsequently into mature monocytes (Region E), whereas the myeloid cells do not express HLA-DR and differentiate from Regions G, A, B, C into Region D. Remarkably, in the CD45/SS plot, it can be found that myeloid cells during maturation obtain a higher SS and thereafter a higher CD45 expression (see CD45/SS in plots B and C). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

metabolic syndrome (47,48), atherosclerosis (49–51), and other degenerative disorders (52,53). Macrophage contribution has even been shown in neuropsychiatric disorders, such as "mild encephalitis" or Alzheimer's disease (54,55). Furthermore, in association with their feeder and tissue repair activities, paradoxically some tumor-associated macrophages have been shown to support tumor growth as myeloid suppressor cells (9,56).

Monocytes in MDS

MDS are clonal hematopoietic stem cell disorders with morphological, physiological, and phenotypic aberrancies. MDS are heterogeneous, biologically complex, and clinically variable. In MDS, one or more of the three cell lineages, myeloid, monocytic, and the erythroid lineage, can be variably affected ending in a high cell death rate, cytopenia, and high risk of differentiation into leukemic clones. MDS clones can simultaneously express antigens that are usually expressed on normal cells but at different stages of maturation (e.g., late expression of CD34, CD117) or at another intensity level or they can express antigens that are usually restricted to other lineages. Frauke Bellos and colleagues (in this issue) described a convenient method of diagnosis of MDS by determining (a) a different expression of antigens in comparison with those on normal bone marrow cells (e.g., CD11b, CD13, CD16, CD45, HLA-DR, and CD14), (b) an asynchronous expression of maturation markers with abnormal persistence of very primitive markers (CD34 and/or CD117), and (c) possible lineage infidelities with unexpected expressions (e.g., CD2, CD5, CD7, CD19, and CD56). Additionally, they described the modulation of the myeloid nuclear differentiation antigen that is usually highly expressed in the monocytic lineage.

Sergio Matarraz and colleagues (in this issue) described aberrant phenotypes in AML. AML cells are arrested in their maturation process at an early stage of development (i.e., monoblastic or promonocytic stages) frequently with persistence of expression of CD34 and CD117. The high expression of CD11b, CD33, CD64, and HLA-DR identify the monocytic lineage on which aberrant expressions of various markers could be determined.

Monocytes in Inflammatory States

Ulrich Sack et al. (in this issue) discussed the strong expression of CD64, the Fcγ-RI, on the monocytic and myeloid lineage. CD64 is involved in phagocytosis of immunoglobulin-bound antigens, but it is also capable of transmitting signals that activate cells and interfere with their function. CD64 expression is easily upregulated in impending situations such as infections or tissue damage (42). The modulation of CD64 density occurs within a few hours and is correlated with the production of proinflammatory cytokines, among them type I interferon (57). Monitoring of the CD64 density was shown to be clinically relevant in monitoring sepsis (58,59).

FUNCTIONAL ACTIVITY OF MONOCYTES/MACROPHAGES

Macrophages have an exceptional capacity to phagocytize foreign materials. This phagocytosis makes use of several membrane receptors (e.g., CD64, CD35, CD91, CD163, and CD204) to capture and internalize microparticles. The digestion of phagocytized products is enhanced by their oxidative burst activity and production of reactive oxygen species (ROS) by NADPH-oxidase that can be defective in some genetic or acquired circumstances. Production of ROS reduces substrates such as dichloro-dihydro-fluorescein-diacetate (DCHF) or dihydro-rhodamine 123 that become fluorescent and can be quantified by FCM (60).

Monocytes are associated with more vital metabolic activities that can also be explored by FCM. Among them, the peroxisomes provide β-oxidation with very long chain fatty acids (VLCFA) and degradation of hydrogen peroxide (H_2O_2) using catalases (61,62). ATP-binding cassettes and acyl-CoA oxidase 1 are used. These activities are related to oxidative stress and directly influenced by nutrition and ageing (63). This is proven by the fact that peroxisome dysfunction is involved in metabolic syndromes and in several inflammatory and/or degenerative diseases. The expression of peroxisomal ABC transporters, Abcd1, Abcd2, and Abcd3, as well as of peroxisomal enzymes Acox1 and catalase can be measured by FCM using specific MoAbs.

Laura Diaz et al. (this issue) presented that, besides ROS, monocytes also produce reactive nitrogen species (RNS) derived from nitrogen oxide. ROS and NOS metabolisms can join at some point, producing peroxynitrite (ONOO) from NO and $O2^{-}$ that causes protein nitrosylation with simultaneous decline of ROS function. Peroxynitrite interacts with lipids, DNA, and proteins via direct oxidative reactions or via indirect, radical-mediated mechanisms. These reactions trigger cellular responses ranging from subtle modulations of cell signaling to overwhelming oxidative injury that can eventually commit cells to necrosis or apoptosis (64). ROS and NOS can also be studied by FCM, giving rise to the so-called "flow-cytoenzymology" concept (65). NO production is measured using 4-Amino-5-Methyl amino-2',7'-difluorofluorescein diacetate (DAF-FM DA), while DHR 123 is activated by various ROS, including ONOO and superoxide, but not by NO. MitoSOX is readily oxidized by superoxide only. Most of these activities are located in mitochondria. A simplified method is described for simultaneously analyzing the kinetics of these enzymatic activities in peripheral monocytes and named "real-time" FCM concept.

Each receptor ligation induces stepwise signaling events through the nuclear gene regulators. The signaling can be measured by the detection of phosphorylation of several cytoplasmic key proteins. This phosphorylation is a very early event after receptor ligation and can be altered in several diseases. Spontaneous autonomous activation is responsible for some hematologic disorders. The phosphorylated epitopes are small and

Table 2
Main Markers Available for Phenotypic and Functional Analysis of Monocyte Macrophages by FCM

	Markers	Relevance
CMP	CD34, CD117, CD38	Progenitors
Monoblast	$CD33^{++}$, $CD64^{+}$, $CD34^{+}$ (early), $CD117^{+}$ (early), CD45, HLA DR, CD36	Early myelopoiesis; Myelodysplasia
Promonocyte	**$CD14^{++}$**, $CD13^{++}$, $CD15^{-}$, $CD16^{-}$, $CD11b^{+}$, $CD11c^{+}$, CD35	
Monocyte M1	$CD45^{++}$, CD14, CD36, CD4, CD62L, CD64, CD91, CD163, CD204, CD206, CD115	
Monocyte M2	CD1a, **$CD16^{+}$ $CD14^{-}$** CD163	AAM
Activation	CD69, CD38, CD206, and CD115 (M2)	M1/M2
GPI Anchor	CD14, CD157, CD24, CD16, CD55, CD59, CD48, CD52, CD87, CD109	PNH Clone
Chemokine receptors	CCR1, CCR2, CCR3, CCR4, and CCR5	Activation status
Oxidative burst	DCHF or dihydro-rhodamine 123	Metabolic syndrome inflammatory and/or degenerative diseases
Reactive Nitrogen Species	4-Amino-5-Methyl amino-2',7'-difluorofluorescein diacetate (DAF-FM DA)	Inflammatory and/or degenerative diseases
Phospho-flow (most frequently analyzed)	pAKT, pERK (TLR4) pSTAT5 (GM-CSF)	Metabolic syndrome inflammatory and/or degenerative diseases

difficult to detect in the overcrowded region directly under the cell membrane. Proper cell permeabilization is critical for a good access to signaling complex without epitope degradation for immunolabeling. Most of the published analyses are performed using western blot. FCM methods have been previously developed for labeling phosphorylated proteins, but were tedious with poor sensitivity until now (66,67). Emilie Coppin and colleagues (in this issue) presented a new and elegant method that strongly improves FCM analysis of TLR4 (Ras/ERK) or GM-CSF (Jak/Stat5) phosphorylation at the single cell level, termed "phospho-flow" analysis. The new technique is simplified, bringing real benefits in time and higher sensitivity. Monitoring the intracellular phosphorylation of receptors adds one extra level of information on monocyte/macrophage reaction that could be induced by any ligand such as cytokines or inflammatory products such as bacterial extracts or PAMS. Furthermore, constitutive aberrant signaling has been recently associated with some leukemias (68,69) and new therapeutic drugs, such as tyrosine kinase inhibitors, have shown their strong therapeutic efficacy on theses specific types of leukemia. This "phospho-flow" analysis now allows a cost and time effective screening of patients for personalized therapy.

CONCLUDING REMARKS

The combination of multiple perspectives on the monocyte-macrophage system raises a number of questions on its physiological and potentially pathological aspects. The Darwin paradigm explains the origin of species diversity, through hazardous changes and selection on utility. We could postulate that membrane expression of functional receptors could have appeared by chance, but would be conserved (only) if useful or, even more, if it yields an advantage to the lineage. If we apply this paradigm to cell phenotypes, we could ask what advantage each of these markers brings at certain stages of maturation of the lineage. As an example, what role CD11b, CD13, and CD64 could play in cell ontogenesis, lineage orientation, growth, or maturation? Integrin expression could explain the direct effect of the matrix environment and mesenchymal cells that play a crucial role in bone marrow physiology (70), but what is the role of MHC class II molecules other than an improbable antigen presenting function in this stage of cell development and at this anatomical site? Furthermore, immunoglobulins can also interact with monocytes (and myelocytes) through the different Fcγ-receptors. Interestingly, the Fcγ-RII, CD32 (71) seems not to be involved in this early stage. If natural, well-known receptors (like immunoglobulins and T-cell receptors) are not expected in bone marrow physiology, what would then be their ligand? Similarly, CD14, a co-receptor of TLR whose best-known function is being the LPS co-receptor, appears early in the cell maturation of monocytes where LPS is supposed to be absent. Is there any other role for CD14? However, an ontogenic role of CD14 could also explain myelopoiesis disorders during sepsis that are frequently associated with cytopenia and immune-paralysis?

As discussed, a fraction of mature, peripheral monocytes acquire the expression of CD16 (Fcγ-RIII) that comes with an increased capacity for antigen presentation. Could this improve the antigen presentation capacity by selecting the antigen capture, as B cells do, using their membrane immunoglobulins? Intriguingly, this M2 cell function increases with loss of phenotype specificity of CD14, whereas expression of CD16 is more frequently associated with neutrophils and NK cells.

As expected, crossing views on one lineage using the available expertise and tools (Table 2) has indeed raised

several hypothesis of interest that could help in the fields of hematology or immunology.

In conclusion, the monocyte is an interesting cell that deserves more attention. Good choices in the combination of specific markers present in the available panels together with a good gating strategy leads to precise characterization of hematological disorders in reference to the normal maturation process. Similarly, on peripheral monocytes, multiple parameters are informative on disease characterization and monitoring, providing some standardization, in bacterial infection and acute or chronic inflammatory disease. Good examples are modulation of expression of CD64 or HLA-DR on monocytes during sepsis. The role of receptors expressed during monocyte ontogeny remains a source of questions. Finally, the possible role that macrophages play in hematopoiesis regulation and bone marrow regeneration is a promising area that should be explored.

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