Genetic Evidence of a Functional Monocyte Dichotomy

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Abstract—Human peripheral blood monocytes are found as two distinct populations based upon differential expression of chemokine receptors, adhesion molecules, Fc receptors, and cytokines. cDNA microarray analysis now reveals additional differences between these subsets that suggest dramatically diverse functions. One monocyte subset (CD14++CD16−) appears to be closely paired with neutrophils, and may have as its primary function the removal and recycling of apoptotic neutrophils at sites of inflammation. The other monocyte subset (CD14+CD16+) expresses numerous genes encoding proteins with antimicrobial activity and thus may be more directly involved in peripheral host defense. The production of monocytes capable of efficiently removing dying neutrophils may be necessary to prevent host tissue damage and autoimmune response induction. Therefore, species like humans that produce relatively high levels of circulating neutrophils must also produce relatively high numbers of the recycling monocytes. Conversely, species such as mice and rats that maintain relatively lower levels of circulating neutrophils require fewer recycling monocytes.

KEY WORDS: monocytes; macrophages; differentiation; inflammation; differential display.

INTRODUCTION

For more than 20 years it has been recognized that there are two distinct monocyte subsets in human peripheral blood. The earliest description of these subsets used differential cell surface expression of CD14 and CD16 to define them: the major population in human blood is commonly referred to as CD14++CD16−, while the minor subset is known as CD14+CD16+ [1]. A few years later, it was discovered that the CD14+CD16+ subset produces more inflammatory cytokines than the CD14++CD16− subset, and that the prevalence of these cells in the blood was elevated in several chronic inflammatory diseases [2–4]. For this reason, the CD14+CD16+ subset was labeled "proinflammatory" and the CD14++CD16− subset was termed "conventional."

In 2003, two distinct monocyte subsets were described in the blood of mice using differential cell surface expression of Ly6C/Gr-1, CCR2, CX3CR1, and CD62L [5]. One mouse subset (Ly6C+CCR2+) was found to migrate rapidly into sites of acute inflammation while the other subset (Ly6C-CX3CR1+) did not [6, 7]. For this reason, the former subset was termed "inflammatory" and the latter subset "resident." Unfortunately, this terminology has resulted in a great deal of confusion as it has become clear that the human "proinflammatory" subset is equivalent to the mouse "resident" subset, and the mouse "inflammatory" subset is equivalent to the human "conventional" subset. The nomenclature difficulty has been compounded by the lack of direct correlation of cell surface markers. Mouse monocytes do not express CD14, and no anti-mouse CD16-specific antibodies are available. It is now well established that both human and mouse monocytes

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share a common differential surface expression of CD62L, CCR2, and CX3CR1 [8]. Thus, a human CD62L+ (CD14++CD16−) monocyte is equivalent to a mouse CD62L+ (Ly6C+CCR2+) monocyte and a human CD62L− (CD14+CD16+) monocyte is equivalent to a mouse CD62L− (Ly6C-CX3CR1+) monocyte. Because both monocyte types have "inflammatory" attributes, the ultimate reason for this phenotypic dichotomy remains unclear.

In order to identify additional differences in human monocyte subsets that could provide clues to possible distinctive functions, each subset was isolated by magnetic bead-dependent negative selection from freshly drawn human blood and then subjected to Affymetrix mRNA differential display. Negative selection was chosen as the method of monocyte enrichment or purification rather than positive selection or FACS sorting in order to avoid antibody-induced changes in gene expression.

MATERIALS AND METHODS

Animals

Female C57BL/6 female mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Male Sprague–Dawley rats were obtained from Charles River Laboratories Inc. (Wilmington, MA). All animals were maintained in the animal care facility at Pfizer Global Research & Development (Ann Arbor, MI) and were given food and water *ad libitum*. All protocols for animal use were approved by the Pfizer Institutional Animal Care and Use Committee, which guarantees strict compliance with regulations established by the Animal Welfare Act.

Blood Collection

Human blood was obtained from apparently healthy donors in sodium heparin-containing tubes. Blood was collected by cardiac puncture from anaesthetized rats and mice into sodium heparin-containing tubes.

Negative Selection of Human Monocyte Populations

Human PBMC were isolated by density centrifugation over Ficoll–Hypaque (Beckman Coulter Inc., Fullerton, CA) following manufacturer's instructions. The PBMC were washed in 4°C phosphate buffered saline and incubated according to manufacture's instructions with paramagnetic particles conjugated to monoclonal antibodies specific for the following cell surface proteins: CD3, CD19, CD56, CD61, and glycophorin A (Miltenyi Biotec Inc, Auburn, CA). The cells were washed once to remove unbound antibody and then run over a MACS CS separation column (Miltenyi Biotec Inc). The non-adherent cells enriched in monocytes were collected and split into two equal fractions. One fraction was incubated with paramagnetic particles conjugated to a monoclonal antibody specific for CD62L. The other fraction was incubated with paramagnetic particles conjugated to a monoclonal antibody specific for CD16. Each fraction was subjected to a second MACS CS column, and the non-adherent monocytes (CD62L− or CD16−) were collected. The purity of each negatively selected population was determined by flow cytometric analysis. The CD62L− cells were found to be >90% CD14+CD16+CD62L− monocytes. The CD16− cells were found to be >80% CD14++CD16−CD62L+ monocytes. Fewer than 5% contaminating neutrophils were found in each fraction. The majority of non-monocyte contaminating cells in the CD16− fraction appear to be esoinophils (∼5%) as determined by light scatter properties.

Flow Cytometry

For each sample, 0.5 ml of human, rat, or mouse blood was added to polypropylene tubes containing 5 ml of red blood cell lysing buffer (Sigma Chemical Company, St. Louis, MO) for 10 min at 37°C. The cells were then centrifuged, the supernatant was removed and the pellet resuspended in 200 μl FACS buffer (Hanks balanced salt solution with 2% fetal calf serum and 0.02% sodium azide) containing saturating amounts of antibodies directly conjugated to FITC, PE, PE-Cy5 (Cy-chrome™), or biotin and incubated for 30 min at 4°C. The cells were washed twice with FACS buffer and, if necessary, resuspended in 100 μl FACS buffer containing saturating amounts of avidin-conjugated FITC, PE, or PE-Cy5 for 20 min at 4°C. The cells were washed twice more and resuspended in 400 μl FACS buffer for flow cytometric analysis. Stained cells were analyzed on a FACSCalibur (Becton Dickinson, Mountain View, CA) flow cytometer. Fluorescence and forward light scatter signals were collected on 100,000 cells and analyzed using the CellQuest software program (Becton Dickinson). The percentage of cells with a particular fluorescence pattern was determined

by integration of the corresponding populations. Antibodies specific for human CD14, CD16, CD11b, and CD62L, as well as avidin conjugates were purchased from PharMingen (San Diego, CA). Antibodies specific for rat CD11b and CD62L were purchased from PharMingen. Antibodies specific for mouse Ly6C/Ly6G (Gr-1), CD62L, CD11b, and B220 were purchased from PharMingen.

RNA Expression Profiling

Expression profiling was performed using the Affymetrix GeneChip® two-cycle cDNA procedure according to the manufacturer's instructions (Affymetrix Inc., Santa Clara, CA). In the first cycle, 100 ng of total RNA was primed with an oligo dT_{24} primer containing the T7 RNA polymerase recognition sequence and converted into double stranded cDNA using the Superscript III® cDNA synthesis kit (Invitrogen Inc., Carlsbad, CA). Amplified RNA (aRNA) was generated from this template using the Ambion Megascript T7 Kit and purified using the Affymetrix Clean-up module. In the second cycle, single-stranded cDNA was generated using random primers and 600 ng of template aRNA. The resulting product was then primed with oligo dT_{24} T7 primer to produce double-stranded cDNA using the Superscript III® cDNA synthesis kit (Invitrogen Inc., Carlsbad, CA). Biotinylated target cRNA was generated using the Affymetrix GeneChip Expression 3′ Amplification Kit for IVT labeling (Affymetrix Inc., Santa Clara, CA). cRNA target was purified using the Affymetrix Clean-up module and quality and quantity assessed by capillary gel electrophoresis on the Agilent 2100 Bioanalyzer. 15 μg of cRNA was fragmented by metal induced hydrolysis at 94°C, mixed in the standard Affymetrix hybridization cocktail containing hybridization controls, and hybridized to Affymetrix Human 133A 2.0 arrays for 16 h with rotation at 45°C. Arrays were washed and then stained with streptavidin phycoerythrin and anti-streptavidin biotinylated antibody using the Affymetrix Gene Chip Fluidics Station 450. Fluorescent signal intensities were obtained using the Affymetrix Gene Chip Scanner 3000 and quality control parameters were analyzed using Affymetrix Microarray Suite 5.1.

Statistical Data Analysis

Raw data files including signal intensity values and present/absent calls were generated using the Affymetrix GeneChip® Operating Software (GCOS 1.2) software statistical algorithm with default settings. A target intensity of 600 was used for global scaling across individual arrays.

Genes were identified as changing significantly according to the following criteria:

• t test p value ≤0.05
• Fold changes ≥+2.0 and ≤−2.0.
• Mean signal intensity ≥100 for either monocyte subgroup
• At least 50% of the samples in either monocyte subgroup called Present by the Affymetrix algorithm.

Quantitative PCR

TaqMan™ Assay-On-Demand® Gene Expression reagents for detection of differentially expressed mRNAs for validation were obtained from Applied BioSystems, Foster City, CA. cDNA was synthesized from 1 μg of RNA using the High Capacity cDNA Archive Kit (Applied BioSystems Inc.) and Real-time PCR reactions were run using the ABI Prism 7900 HT Sequence Detection System. All samples were run in duplicate in 20 μl reaction volumes using the TaqMan™ Universal PCR Master Mix without AmpErase UNG (Applied BioSystems Inc.). The level of 18S rRNA was determined and used as an internal reference for each sample. The $2^{-\Delta\Delta C}T$ method of Livak and Schmittgen [9] was used to calculate fold change for each transcript measured.

RESULTS

Identification of Monocyte subsets

Flow cytometric analysis of human peripheral blood monocyte subsets is most commonly represented by the differential cell surface expression of CD14 and CD16 (Fig. 1A). However, the differential expression of CD62L can also be used to distinguish these same subsets: The CD14++CD16− cells express high levels of CD62L whereas the CD14+CD16+ monocytes lack CD62L expression (Fig. 1B). Thus, anti-CD16 antibodies were used to deplete CD14+CD16+ monocytes and anti-CD62L antibodies were used to deplete CD14++CD16− monocytes by negative selection resulting in untouched monocyte subsets for mRNA analysis. The differential cell surface expression of CD62L can also be used to distinguish equivalent monocyte subsets in mice and rats (Fig. 1C, D).
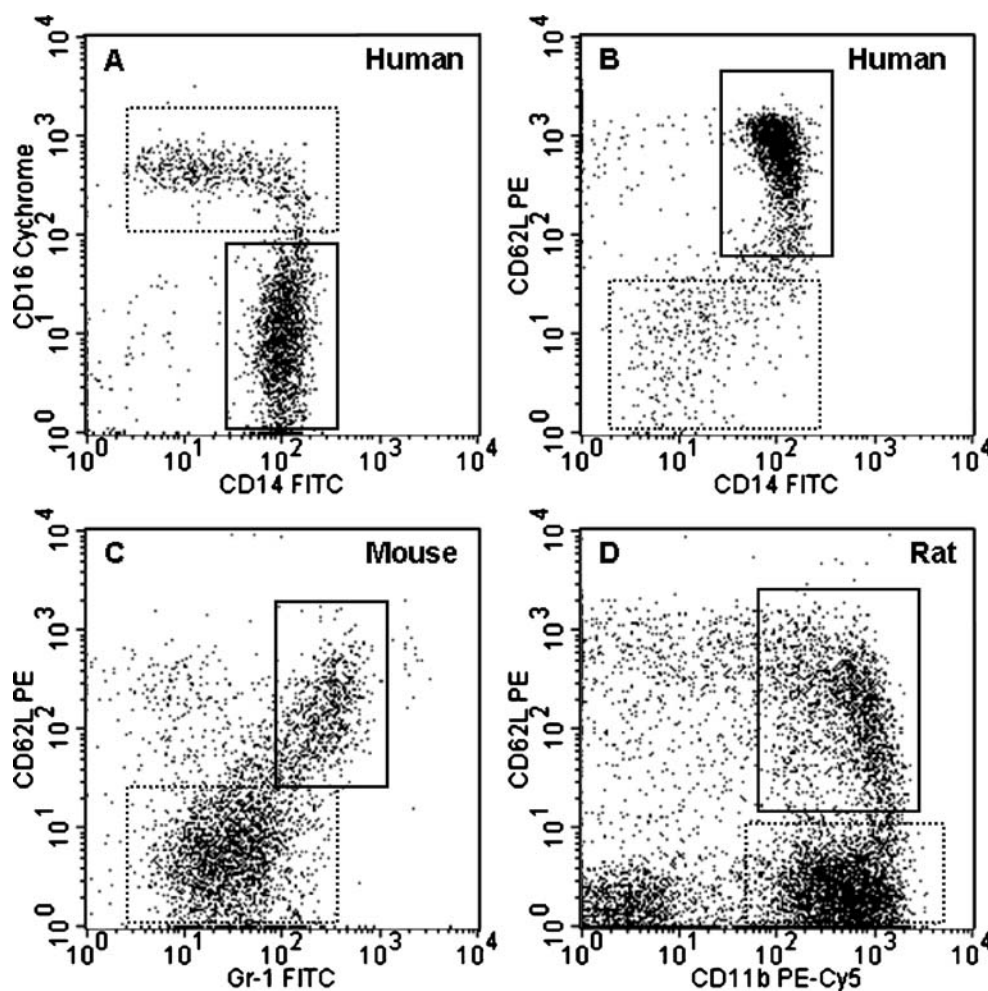
Fig. 1. Flow cytometric analysis of human, mouse, and rat monocytes. Human monocytes are divided into two subsets based on expression of CD14 and CD16 (**A**) or CD14 and CD62L (**B**). CD14+CD16+ monocytes are equivalent to CD14+CD62L− monocytes (dotted boxes), while CD14++CD16− monocytes are equivalent to CD14++CD62L+ monocytes (solid boxes). CD62L expression can also distinguish two equivalent monocyte subsets in mice and rats. Mouse monocytes were first distinguished from other peripheral blood leukocytes by gating on all cells with low orthogonal light scatter followed by gating on cells that express CD11b but not B220 (not shown). The mouse monocytes were then identified as two distinct subsets based on differential expression of Gr-1 and CD62L (**C**). Rat monocytes were first distinguished from other peripheral blood leukocytes by a combination of forward angle light scatter and orthogonal light scatter (data not shown). The rat monocytes were then identified as two distinct subsets based on differential expression of CD11b and CD62L (**D**).

Interestingly, the major monocyte subset in humans (CD62L+) is the minor subset in both mice and rats.

Genes Differentially Expressed in CD14++CD16− Monocytes

The CD62L+ monocyte subset (CD14++CD16−) expresses relatively high levels of mRNA encoding CCR2, CD62L, and CD11b as expected from the previously-described differential surface expression of these proteins [10] (Table 1). The coordination function of this chemokine receptor, rolling adhesion receptor, and firm adhesion receptor triad allow monocytes to leave the circulation and enter into inflammatory sites several hours after neutrophil entry into the same areas [5–7]. The differential expression of the granulocyte colony stimulating factor (G-CSF) receptor gene *CSF3R* suggests a mechanism whereby the production

Table 1. Genes Differentially Expressed in CD14++CD16− (CD62L+) Monocytes

Gene Name	Gene Symbol	Mean Expression Values		Fold difference	
		CD14++CD16−	CD14+CD16+	Affymetrix	Taqman
Chemotaxis and adhesion molecules					
CCR2	*CCR2*	10,088	412	24.50	7.30
CD62L	*SELL*	13,787	2,271	6.07	3.60
CD11b	*ITGAM*	9,900	2,334	4.24	3.60
Colony stimulating factors					
G-CSF receptor	*CSF3R*	1,226	341	3.59	3.80
Cytokines					
IL-8	*IL8*	7,921	2,909	2.72	ND
Scavenger receptors and recycling enzymes					
CD163	*CD163*	4,046	123	32.94	9.30
CD36	*CD36*	4,220	517	8.17	4.80
CD14	*CD14*	15,113	1,821	8.30	6.30
Macrophage receptor with collagenous structure	*MARCO*	1,057	491	2.15	ND
Macrophage lectin 2 (calcium dependent)	*HML2*	1,417	282	5.03	ND
Complement component C1q receptor	*C1QR*	4,093	1,479	2.77	ND
Acetyl LDL receptor	*SREC*	211	36	5.80	3.50
Acyl coenzyme A:cholesterol acyltransferase	*CES1*	2,172	98	22.15	9.60
Cytochrome P450, subfamily XXVIIA (steroid 27-hydroxylase)	*CYP27A1*	965	123	7.87	5.50
Fatty acid binding protein 5	*FABP5*	1,436	484	2.97	ND

The table shows a selection of genes grouped into functional categories. Mean expression values are reported for three separate analyses from three different blood donors. Fold difference is calculated as the mean of the CD14++CD16− monocyte expression divided by the mean of the CD14+CD16+ expression. Taqman analysis was used to confirm differences from the Affymetrix analysis.
ND = Not determined

of this monocyte subset in the bone marrow is temporally coordinated with the production of granulocytes. The differential expression of the *IL8* gene encoding interleukin-8, a potent neutrophil chemoattractant, further illustrates the connection between CD62L+ monocytes and neutrophils. Thus, the designation of this subset as "inflammatory" appears to be valid.

An additional group of differentially expressed genes provides a clue as to the function of this cell type in the inflammatory process: Several genes found to be differentially expressed in the CD62L+ monocytes encode proteins that are involved in scavenger and recycling functions. *CD163*, the gene with the greatest degree of differential expression in the two monocyte subsets (32-fold difference) encodes a scavenger receptor for hemoglobin [11]. During inflammation, activated neutrophils release superoxide molecules resulting in erythrocyte hemolysis and the release of free hemoglobin that is recovered by monocytes through CD163. The CD62L+ monocytes also express genes encoding scavenger receptors (CD36, C1qR, MARCO) that function in the ingestion of apoptotic cells, including neutrophils [12, 13]. CD14, whose expression defines

this specific monocyte subset, is recognized widely as a member of the LPS-binding TLR4 complex. CD14 also functions independently of TLR4 as a scavenger receptor for removal of apoptotic cells [14]. Another function of CD36 is as a scavenger receptor for free lipoproteins including oxidized low density lipoprotein [15]. Other genes encoding lipid and lipoprotein receptors also are differentially expressed on the CD14++CD16− monocytes, including the fatty acid binding protein 5 (*FABP5*) and the acetyl LDL receptor gene *SREC* [16, 17].

Once ingested, the apoptotic cells and lipid fragments must be metabolized, and the CD14++CD16− monocytes express genes that support this function. *CES1* encodes carboxylesterase 1, also known as acyl coenzyme A: cholesterol acyltransferase (ACAT), an enzyme that hydrolizes long chain fatty acids and is required for cellular cholesterol esterification [18]. Sterol 27-hydroxylase (encoded by *CYP27A1*) catalyzes the 27-hydroxylation reaction of cholesterol metabolism in monocytes [19] (Table 1). Thus, the differential expression of multiple genes in the CD14++CD16− monocyte subset suggest that these cells are produced in the bone marrow when neutrophils are produced, follow

Table 2. Genes Differentially Expressed in CD14+CD16+ (CD62L−) Monocytes

Gene Name	Gene Symbol	Mean Expression Values		Fold difference	
		CD14++CD16−	CD14+CD16+	Affymetrix	Taqman
Chemotaxis and adhesion molecules					
CX3CR1	CX3CR1	18,264	38,377	−2.10	−5.30
CD31	PECAM1	3,696	8,476	−2.29	ND
CD11a	ITGAL	4,003	8,644	−2.16	ND
EMR2	EMR2	1,077	4,042	−3.75	−5.00
CD24	CD24	145	3,082	−21.24	ND
Colony stimulating factors					
M-CSF receptor	CSF1R	3,018	9,917	−3.29	−5.70
Cytokines					
Tumor necrosis factor (TNF superfamily, member 2)	TNF	206	731	−3.55	ND
Antibacterial proteins					
Defensin, alpha 1	DEFA1	2,144	38,847	−18.12	−2.40
Defensin, alpha 4, corticostatin	DEFA4	212	5,802	−27.42	−2.20
Complement component 1, q subcomponent, a polypeptide	C1QA	348	2,658	−7.64	−140.10
Complement component 1, q subcomponent, b polypeptide	C1QB	340	2,803	−8.24	ND
Complement component 3	C3	112	1,134	−10.12	−10.80
Lactotransferrin	LTF	550	10,230	−18.60	ND
Lipocalin 2	LCN2	195	4,978	−25.57	ND
Cathepsin L	CTSL	961	4,548	−4.73	−14.40
Cathepsin G	CTSG	66	504	−7.59	ND
Elastase 2, neutrophil	ELA2	27	1,015	−36.94	ND
Surfactant, pulmonary-associated protein D	SFTPD	77	494	−6.42	ND
Antimicrobial LPS-binding protein CAP18	CAMP	234	3,812	−16.30	ND

The table shows a selection of genes grouped into functional categories. Mean expression values are reported for three separate analyses from three different blood donors. Fold difference is calculated as the mean of the CD14++CD16− monocyte expression divided by the mean of the CD14+CD16+ expression. Taqman analysis was used to confirm differences from the Affymetrix analysis.
ND = Not determined

neutrophils into inflammatory sites, and function to ingest and metabolize the dying neutrophils *in situ*.

Genes Differentially Expressed in CD14+CD16+ Monocytes

By contrast, the CD62L− monocytes express none of the scavenging/recycling genes, but instead express genes encoding proteins that are more directly involved in host defense (Table 2). The defensins α1 and α4, and the cathelicidin CAP-18 (encoded by the *CAMP* gene) are peptides or small proteins with direct microbicidal activity [20]. Complement components C1q and C3, and surfactant protein A (SP-A) opsonize microorganisms for efficient ingestion and degradation [21, 22]. Cathepsin G, cathepsin L, and elastase 2 are potent lysosomal proteases that function to kill and digest ingested bacteria [23, 24]. Lactotransferrin and lipocalin 2 kill bacteria by binding to and sequestering iron that is required for bacterial survival [25, 26].

The expression of so many antimicrobial genes suggests that the CD62L− (CD14+CD16+) monocytes may function as sentries of the innate immune response, perhaps located at barrier sites throughout the body. These cells express the *TNF* gene, and produce higher levels of TNF-α than their CD62L+ (CD14++CD16−) counterparts [2]. TNF-α is a multifunctional cytokine that initiates many of the early responses in inflammation [27]. The CD14+CD16+ monocytes may function at the earliest stage of the innate immune response by directly responding to invading microorganisms and releasing a multifunctional cytokine that initiates the acute phase response, directing the production of additional antibacterial proteins and G-CSF [28]. The G-CSF would promote the production of more potent microbicidal neutrophils and the CD14++CD16− monocytes that remove them once their job is finished.

The differential expression of the gene encoding the M-CSF receptor (*CSF1R*) by the CD14+CD16+ monocytes also provides some insight into the function

of this subset. Saleh et al. [29] reported that human volunteers injected with recombinant M-CSF demonstrated a dramatic increase in the proportion of CD14+CD16+ monocytes in their blood, suggesting that this growth factor functions selectively on this one monocyte subset. Osteopetrotic mice (*op/op*) have a genetic defect that makes them M-CSF deficient, resulting in an interesting pattern of monocyte and macrophage production [30]. These mice have severe reductions in circulating monocytes, and almost complete absence of osteoclasts, resident peritoneal macrophages, splenic marginal zone metallophils, and lymph node subcapsular sinus macrophages. However, other macrophages are produced at nearly normal or reduced levels including microglia, Kupffer cells, Langerhans cells, splenic red pulp macrophages and lung alveolar macrophages. These observations may provide insight into the ultimate fate of the M-CSF-responsive CD62L− monocytes as they differentiate into tissue macrophages.

Correlation of Neutrophil and Monocyte Levels

Humans are somewhat unique among the mammals in that neutrophils account for a relatively high percentage (50–70%) of their peripheral blood leukocytes [31]. By contrast, neutrophils represent only 10–20% of the leukocytes in the blood of mice and rats [32] (Fig. 2A). If, as the genetic data suggest, CD14++CD16− monocytes function primarily to remove apoptotic neutrophils, then it would be expected that more of these monocytes would be produced in species that produce more neutrophils. As shown in Fig. 2B, in human blood CD62L+ (CD14++CD16−) monocytes represent the major subset (82%) whereas in rats (39%) and mice (30%), CD62L+ monocytes are the minor subset. Thus, the relative production of neutrophils correlates quite well with the relative proportion of CD62L+ monocytes in the circulation.

DISCUSSION

This analysis of differential gene expression in human monocyte subsets suggests that these subsets have distinctly different functions in the innate immune response. The pattern of expression of genes encoding adhesion molecules, chemokine receptors, differentiation factors, and defensive proteins implies that the CD14+CD16+ monocyte subset, or their macrophage
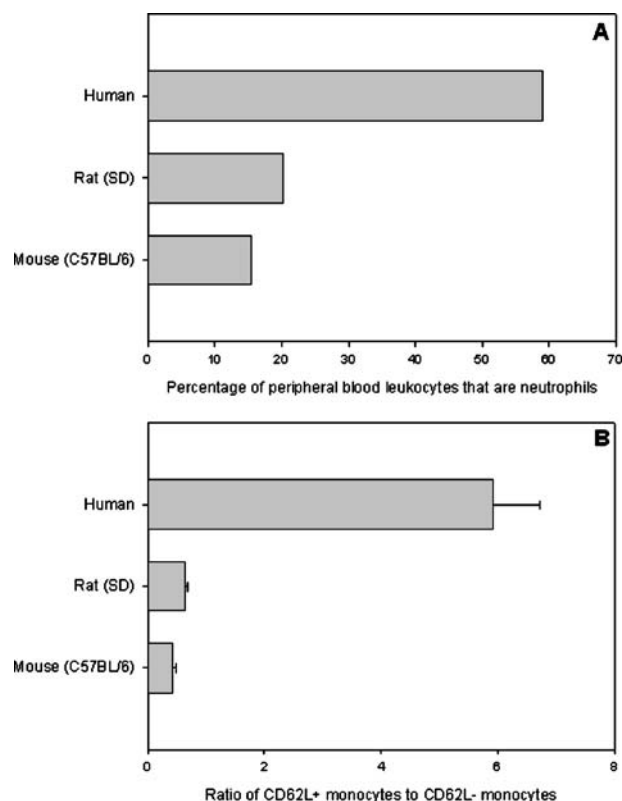

Fig. 2. Higher levels of circulating neutrophils are associated with higher levels of CD62L+ monocytes. Neutrophils account for 59% of human peripheral blood leukocytes, but only 20.1% of Sprague–Dawley rats, and 10.5% of C57BL/6 mice (**A**). The CD62L+ (CD14++CD16−) monocyte subset accounts for 81.9% of total human monocytes, but only 39.1% of total rat monocytes and 29.6% of total mouse monocytes (**B**).

progeny, serve as sentries in the peripheral tissues that are capable of directly killing invading microorganisms. By contrast, the pattern of expression of genes encoding multiple proteins with scavenging or recycling function suggests that the CD14++CD16− monocytes are responsible for the removal of dying cells or cellular debris, but are ill equipped to fight pathogens. By expressing genes encoding adhesion molecules that are also expressed on neutrophils, the CD14++CD16− monocytes are situated to migrate to the same tissue sites as neutrophils, suggesting that it is neutrophil recycling that is a major task of this subset.

The differential expression of the G-CSF receptor by the CD14++CD16− monocytes suggests the possibility that the induction of this subset in the bone marrow could be developmentally correlated with the G-CSF-dependent production of neutrophils. This mechanism would ensure that whenever neutrophils

are produced, the cells required to dispose of those neutrophils are also produced. Two species that produce relatively few neutrophils (mice and rats) have relatively few recycling monocytes, whereas in humans, high levels of circulating neutrophils are associated with high levels of recycling monocytes. As reagents become available to distinguish monocyte subsets in more animal species, it will be informative to learn whether the correlation between the level of circulating neutrophils and the levels of CD62L+ monocytes holds true.

The results of this study are consistent with a cost–benefit perspective of monocyte development [33]. Phagocytic cells of the myeloid lineage are capable of producing a battery of potent bactericidal agents, including reactive oxygen species, nitric oxide, neutral proteases, and acid hydrolases that not only kill the invading pathogen, but also can inflict severe collateral damage on nearby host tissues. In order to balance the defensive benefits of these agents with their potential toxic costs, nature appears to have developed two different compromise solutions. The first solution was to produce phagocytes with a limited arsenal of antimicrobial agents which allows them to kill most (but not all) invading pathogens. The advantage of carrying fewer toxic substances within these phagocytes is that they can live for a relatively long period of time without damaging themselves or the tissues in which they reside. CD14+CD16+ monocytes and their tissue macrophage progeny fit this description.

The second compromise solution was to produce maximally lethal phagocytes (neutrophils), but limit their exposure within healthy tissues. Thus, neutrophils have a short life span, are produced in limited quantities in healthy individuals, and are restricted primarily to the circulatory system. Upon infection, neutrophils can be produced rapidly in the bone marrow and migrate specifically to the site of infection, with minimal dissemination into healthy tissues. A second phagocyte is also required to dispose of the neutrophils and their battery of toxic agents once the invading pathogen has been neutralized. CD14++CD16− monocytes and their macrophage progeny fit this description. Species that have a high level of circulating neutrophils and a high frequency of CD14++CD16− monocytes (like humans) may require a more potent defense system due to higher levels of exposure to pathogens [34]. Alternatively, such species may be able to offset the toxic and metabolic liabilities associated with this defense system better than other species that are more reliant on CD14+CD16+ monocytes.

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