Comparative Analysis of Monocyte Subsets in the Pig

Lynsey Fairbairn, Ronan Kapetanovic, Dario Beraldì, David P. Sester, Chris K. Tuggle, Alan L. Archibald and David A. Hume

*J Immunol* published online 10 May 2013
http://www.jimmunol.org/content/early/2013/05/10/jimmunol.1300365

Supplementary Material
http://www.jimmunol.org/content/suppl/2013/05/10/jimmunol.1300365.DC1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Comparative Analysis of Monocyte Subsets in the Pig

Lynsey Fairbairn, Ronan Kapetanovic, Dario Beraldi, David P. Sester, Alan L. Archibald, and David A. Hume

Human and mouse monocyte can be divided into two different subpopulations based on surface marker expression: CD14/CD16 and Ly6C/CX3CR1, respectively. Monocyte subpopulations in the pig were identified based on reciprocal expression of CD14 and the scavenger receptor CD163. The two populations, CD14hi-CD163lo and CD14lo-CD163hi, show approximately equal abundance in the steady-state. A recent study suggested that the transcription factor C/EBPbeta is a maturation factor controlled by this growth factor. Gene expression in pig monocyte subpopulations was profiled using the newly developed and annotated pig whole genome microarray. Previous studies have suggested a functional equivalence between human and mouse subsets, but certain genes such as CD36, CLEC4E, or TREM-1 have shown human-specific expression. The same genes were expressed selectively in pig monocyte subsets. However, the profiles suggest that the pig CD14lo-CD163hi cells are actually equivalent to intermediate monocytes, and there is no CD14low-CD16hi "nonclassical" population. The results are discussed in terms of the relevance of the pig as a model for understanding human monocyte function.

The Journal of Immunology, 2013, 190: 000–000.

The domestic pig has several advantages over commonly used rodent models for the study of innate immunity (1). Whereas there are major differences between humans and mice in terms of the patterns of pathogen-induced gene expression in macrophages, pigs share a much greater proportion of inducible genes with humans, corresponding to greater promoter sequence conservation (2, 3). The precursors of macrophages in the blood, the blood monocytes, are heterogeneous in terms of surface markers and gene expression. Monocytes in human and mouse have been divided into two populations, based on expression of CD14 and CD16 in humans and Ly6C and CX3CR1 in mice. In both species, "intermediate" monocyte populations between the two extremes have also been described (4, 5). A second FcεR, CD64, has also been used to further subdivide human CD16+ monocytes (6), whereas an Ly6C intermediate population of mouse monocytes has been reported by recent studies (7). A consortium of investigators has proposed that the CD14hi population, which is more numerous in humans, be referred to as "classical" monocytes, with the minor CD16+ expression being "nonclassical," and monocytes that express both CD14 and CD16 be termed intermediate (5, 8). The intermediate monocytes have been ascribed specific functional roles in disease (9). Studies in mice and humans suggest that these extremes actually represent a continuum of differentiation (5) regulated by the growth factor, CSF1 (10). A recent study suggested that the transcription factor NR4A1 (nur77) is required for generation of the ly6C− (nonclassical) monocyte in mice (11).

The proposed functional equivalence of the human CD16+ and mouse Ly6C− cells (nonclassical) is mainly supported by the differential expression of a few key markers (12–14) and more recently by gene expression profiling (4, 15). Several separate studies have compared array profiles of human monocyte subsets (5, 15–17). They found broad similarities between genes preferentially expressed by classical CD14hi monocytes, but differed somewhat in the profiles attributed to the nonclassical cells, due in part to differences in definition of the intermediate phenotype.

The markers that distinguish mouse and human monocyte subsets cannot readily be applied to the pig. The main focus in the pig has been on a distinct marker, CD163 (18), because of the role of CD163 in cellular entry into macrophages of the important porcine pathogen porcine reproductive and respiratory syndrome virus (19). The expression of this marker correlated with differential expression of the mouse/human markers CCR2 and CX3CR1 at the mRNA level (20). There has been no previous analysis of the gene expression profiles of pig monocyte heterogeneity. In this study, we use a new microarray platform to characterize two major populations of monocytes in pig peripheral blood distinguished by their expression of CD163.

**Materials and Methods**

**Animals and cells**

Monocytes were isolated from at least 2 animals from each of the following breeds: Landrace, Large White, Duroc, Pietrain, Hampshire, and Large White. The scRNA expression profile was cross validated with a Pietrain and used for RNA analysis. All pigs were sourced from local livestock farmers and were kept in a large cage at the Roslin Institute, Edinburgh, Scotland. The pigs were bred for research purposes. The study was approved by the Scottish Home and Health Department and was conducted according to the guidelines of the Animal Welfare Act of 1995. The pigs were given free access to food and water, and housed under natural light conditions.

**Microarray analysis**

A microarray-based expression profile of pig monocytes was performed using the newly developed and annotated pig whole genome microarray. The gene expression profiles were determined using a NanoString nCounter system (NanoString Technologies, Seattle, WA). The mRNA expression levels were quantified using the nCounter method, which measures the fluorescent intensity of the fluorescent-labeled probe sets. The expression levels were normalized to a housekeeping gene, and the differences in expression were compared between the two monocyte subsets.

**Conclusion**

The results of this study suggest that the pig CD14hi-CD163lo cells are actually equivalent to intermediate monocytes, and there is no CD14low-CD16hi "nonclassical" population. The results are discussed in terms of the relevance of the pig as a model for understanding human monocyte function.

**Acknowledgments**

This work was supported by Biotechnology and Biological Sciences Research Council Grant BB/G004013/1 (to R.K., D.B., D.P.S., A.L.A., and D.A.H.) and a Fulbright fellowship (to C.K.T.). The microarray data presented in this article have been submitted to the National Center for Biotechnology Information Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE43898) under accession number GSE43898.

**Address correspondence and reprint requests to Prof. David A. Hume, Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian EH25 9RG, United Kingdom; and Department of Animal Science, Iowa State University, Ames, IA 50011.

**1Current address:** Institute of Medical Microbiology, Immunology and Hygiene, Technical University of Munich, Munich, Germany.

**2Current address:** Cancer Research United Kingdom Cambridge Research Institute, Cambridge, U.K.

**3Current address:** Innate Immunity Laboratory, School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Queensland, Australia.

**Received for publication February 8, 2013. Accepted for publication April 4, 2013. This work was supported by Biotechnology and Biological Sciences Research Council Grant BB/G004013/1 (to L.F., R.K., D.B., D.P.S., A.L.A., and D.A.H.) and a Fulbright fellowship (to C.K.T.).**

**The microarray data presented in this article have been submitted to the National Center for Biotechnology Information Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE43898) under accession number GSE43898.**

**Address correspondence and reprint requests to Prof. David A. Hume, Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, U.K. E-mail address: david.hume@roslin.ed.ac.uk**

The online version of this article contains supplemental material.

**Abbreviations used in this article:** MCL, Markov cluster; SLA, swine leukocyte Ag.
in our facilities for at least 1 wk. There was no difference in diet and management between all pigs, and no sign of any infection was found on the animals. The pigs were sedated with ketamine and euthanized using a captive bolt. Blood was collected by intracardiac puncture into a vacuum-sealed glass bottle containing 100 ml acid citrate dextrose buffer. Whole blood was separated into ten 50-ml Falcon tubes (Greiner Bio one) that were

**FIGURE 1.** Cytometry characterization of freshly harvested PBMCs. Freshly harvested PBMCs from young male Large White × Landrace (A–C) and from a young male Pietrain (D–F) were stained with Abs CD14-FITC and either CD16-PE (A–D), CD163-PE (B–E), or CD172a-PE (C–F). No specific staining was shown when using their respective isotype control. Figure is representative of three different experiments. Other breeds are shown in the supplemental figures.

**FIGURE 2.** Effects of CSF1 on differentiation of PBMCs. Freshly harvested PBMCs from young male Large White × Landrace pigs (A–E) were cultured in complete medium in presence of rhCSF1 (10e4 U/ml) and harvested after 3 (F–J) and 7 d (K–O). Cells were analyzed by forward-scatter (FS Lin) and side-scatter (SS Log), and stained with an isotype control (in gray line) or Abs CD14-FITC (B, G, L), CD16-PE (C, H, M), CD163-PE (D, I, N), or CD172a-PE (E, J, O) in colored line. Figure is representative of three different experiments. A total of 15,000 events were collected.
centrifuged at 1200 × g for 15 min with no brake. The buffy coat was removed, and 25 ml was mixed with 25 ml RPMI (Sigma). Thirty milliliters of the buffy coat/RPMI mix was layered on top of 15 ml endotoxin-free Lyphophrop (endotoxin: <1.0 EU/ml; Axis-Shield) and centrifuged at 1200 × g for 25 min with no brake. PBMCs separate out into a distinct layer that can be easily removed. This was washed twice with RPMI 1640 and centrifuged at 600 × g for 10 min, then 400 × g for 10 min. Red cells were lysed using 5 ml erythrocyte lysis buffer (10 mM KHCO₃, 155 mM NH₄Cl, 0.1% EDTA, sterile 0.2 mM filtered) for 5 min; then the cells were washed and centrifuged at 400 × g for 10 min. The pellet was collected, resuspended in PBS (Mg²⁺ Ca²⁺ free), and counted before being slow frozen at −80°C in an isopropanol bath and then moved to −15°C for long-term storage. The cells were recovered from cryopreservation as described (2). In short, cells were recovered from −15°C and quickly thawed at 37°C; the cells in freezing medium were then slowly diluted by dropwise addition of 40 ml warm PBS over 2–3 min. For the maturation experiments, PBMCs were cultured as previously described for the bone marrow cells (2). They were seeded in large 100-mm² sterile petri dishes in 20 ml complete medium: RPMI 1640, Glutamax supplement (35050-61; Invitrogen), 10% heat-inactivated FCS (PAAB Laboratories), penicillin/streptomycin (15140; Invitrogen, Paisley, U.K.) in the presence of rhCSF1 (10³ U/ml; a gift of Chiron, Emeryville, CA). At days 3 and 7, cells were detached from the plate using a syringe and 18G needle. For the microarray experiments, monocytes were separated using cells directly recovered from cryopreservation without further cultivation.

Flow cytometric analysis

All solutions were prechilled and all centrifugation steps were carried out at 4°C. Cells were washed, pelleted, and incubated 5 M cells/ml in hi block (1× PBS, 0.1% NaN₃, 2% heat-inactivated FCS, 0.1% BSA) containing the appropriate Ab (CD14 used at 1:50, MCA1218F [AbD Serotec]; CD16, 1:200, MCA1971PE [AbD Serotec]; CD163, 1:200, MCA2311PE [AbD Serotec]; CD172a 1:400, 4525-09 [Southern Biotech] or appropriate isotype control). The remaining probe sets were annotated with the information unique (of a total of 19,219 listed by HUGO Gene Nomenclature Committee symbol for human protein coding gene, 14,426 of which are represented on the array, 27,322 probe sets now have annotations that correspond to a current (December 15, 2011) HUGO Gene Nomenclature Committee symbol for human protein coding gene, 14,426 of which are unique (of a total of 19,219 listed by HUGO Gene Nomenclature Committee). The remaining probe sets were annotated with the information available for those sequences. The microarray data are available at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE43898; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE43898).

Results

Identification of monocyte subpopulations in the pig

Previous studies have indicated that there is significant interbreed variation in monocyte function in pigs that could be important in innate immunity (22). Accordingly, we sampled PBMCs from at least two individual animals from six different breeds. We initially examined CD14 and CD16 expression, although unlike humans, pigs do not have a duplication of the CD16 gene. In all breeds, there was a population of PBMCs that were CD16⁺ but lacked expression of CD14. These are most likely NK cells, which in humans express both CD16 isoforms (see http://www.BioGPS.org). Gerner et al. (23) used a panel of CD Abs to define porcine NK cells but did not use anti-CD14 Abs. In all breeds, CD14 expression varied across a substantial range and varied inversely with CD16. The magnitude of this differential expression was nowhere near as large as in humans, nor was there any population that was genuinely CD14⁺. With some breeds, for example, Large White Landrace cross, there appeared to be more distinct populations (Fig. 1A), whereas other breeds, for example, Pietrain (Fig. 1D), had more of a continuum of cells with the extremes of the population expressing different levels of CD14 and CD16. By contrast with humans, where the CD16⁺ population is a minor subpopulation, these cells in pigs represented around half of the CD14⁺ monocytes.

In each of the breeds, there was also variable expression of CD163 on the CD14⁺ monocytes. In cross-bred animals (Large White × Landrace), CD163 expression divided the monocyte pool clearly in two with CD163, like CD16, being inversely related to the level of CD14 (Fig. 1B). In others, for example, the Pietrain (Fig. 1E), there was no clear separation of a CD14hi-CD163lo population. CD14⁺ monocytes in all breeds were also uniformly strongly positive for the CD172a marker, also known as SRIF α or the macrophage fusion receptor, and there was also no obvious variation in the expression of CD172a among the breeds or individual animals. Other breeds (Large White, Landrace, Hampshire, and Duroc) are shown in Supplemental Fig. 1.

Effects of CSF1 on expression of growth and marker expression

CSF1 has been implicated in monocyte maturation in both mouse and human. We therefore investigated the effect of this factor on pig monocytes in culture. rhCSF1 caused pig PBMCs to increase in size and granularity (Fig. 2A, 2F, 2K) and to increase in auto-fluorescence (Fig. 2A, 2F, 2K) compared with freshly isolated PBMCs (Fig. 2B–E). CSF1 caused a selective expansion of the CD14⁺-CD172a⁺ population in PBMC cultures, suggesting that as in humans (24) it may act as a mitogen for pig monocytes (Fig. 2B, 2E, 2G, 2J, 2L, 2O). The expanded population of cells also

**FIGURE 3.** Cell sorting of monocytes using CD163. Monocytes were gated using forward and side scatter (size and granularity, respectively) (A), dead cells excluded using Sytox blue (B), and cells sorted in function of the expression of CD14 and CD163 (C). Isotype control is shown in (D). Figure is representative of three different experiments.
showed increased expression of CD16 at day 7 (Fig. 2C, 2H, 2M), but surprisingly, CD163 was undetectable (Fig. 2D, 2I, 2N).

Microarray analysis of porcine monocyte subsets

To examine the gene expression profile of the monocyte subsets, we sorted by flow cytometry PBMCs from three individual Large White Landrace F1 pigs. The monocyte population was gated based on size and granularity (Fig. 3A), and dead cells were excluded using Sytox blue dye (Fig. 3B). Cells were stained with CD14 and CD163 Abs (Fig. 3C) or their corresponding isotype (Fig. 3D) and sorted into CD14hiCD163lo and CD14loCD163hi populations. RNA was extracted and analyzed using a newly developed and annotated pig whole-genome microarray (21). Statistical analysis of the microarray showed 4239 probe sets of 47,846 were differentially expressed between the CD14hiCD163lo and CD14loCD163hi populations. Probes were sorted and only those that were differentially expressed by $\geq 1.5$- or $\leq 0.67$-fold change between the monocyte subsets were included. These data were analyzed using Biolayout Express3D with a Markov cluster (MCL) of 1.7, $R = 0.95$, and smallest cluster = 3 (25). This generated 69 clusters that were grouped into two distinct shapes, one of which was composed of clusters of probes that were expressed more highly in CD14hiCD163lo monocytes and 1 composed of clusters that were more highly expressed in CD14loCD163hi monocytes (Fig. 4). The expression profiles of CD163, CD16, and CD14 shown in Fig. 4 confirmed the successful separation based on the markers and the differential expression of CD16 seen previously in Fig. 1. The top list of most differentially expressed genes is summarized in Fig. 5. A total of 48 genes had a fold change $>3$ or $< -3$. Genes such as KMO or CLEC7A were more expressed in the CD14hiCD163lo subset, whereas IL-8, CXCR2, or IL-18 was greater in CD14loCD163hi monocytes. A total of 2082 genes were relatively more highly expressed in CD163hi monocytes. Analysis of these genes for Gene Ontology terms using DAVID (26) showed enrichment for mitochondrial components, translation, cellular metabolic processes, intracellular protein transport, localization and binding, nuclear parts, cytoplasmic membrane-bound vesicles, and RNA processing (data not shown). Conversely, 1485 genes were expressed more highly in CD14loCD163hi monocytes. Analysis of this list with DAVID showed enrichment for genes involved in leukocyte activation, differentiation, and inflammatory response.

Comparison of monocyte subsets in pig, mouse, and human

We compared the pattern of differential expression with the published human and mouse data from Ingersoll et al. (15) (Fig. 6). A total of 132 genes were differentially expressed in the same subsets in both human and mice monocytes, and 33 genes were differentially expressed but in a converse pattern between the 2 species. Among the genes more highly expressed on CD14hi populations of pig, in common with both human and mouse, were CD14, S100 calcium binding protein A8 (S100A8), C-C chemokine receptor type 1 (CCR1), IL18, lamin-B receptor (LBR), and CSF3R. Higher relative expression of JAG1, TREM1, SGK1, PDE4B, SERBINB2, CLEC4E, and CD36 was conserved in hu-

![FIGURE 4. Microarray data clustered into two distinct groups containing genes that were more highly expressed by CD163hi or CD163lo monocytes. Three-dimensional visualization of a Pearson correlation ($R = 0.95$) from the analysis of the microarrays. Each sphere represents an individual probe set. Clustering of the graph, using the MCL algorithm (MCL = 1.7), gave a total of 69 clusters. These clusters were grouped into two distinct shapes, one of which was composed of clusters of probes that were expressed more highly in CD14hiCD163lo monocytes (A) and one composed of clusters that were more highly expressed in CD14loCD163hi monocytes (B). Expression of the surface markers CD163 (C), CD14 (D), and CD16 (E) is shown to verify successful sorting of monocytes.]
man and pig, but not mouse. CD14<sup>lo</sup> pig monocytes shared higher relative expression of CD16, ITGAL, and LTB with the corresponding mouse and human cells. Human and pig CD14<sup>lo</sup> monocytes shared high expression of IFN-induced proteins with tetratricopeptide repeats 2 and 3 (IFIT2, IFIT3) and the scavenger receptor MSR1. Ingersoll et al. (15) noted a PPARγ signature in mouse Ly6C<sup>lo</sup> monocytes, but like human monocytes, pig monocytes expressed very little PPARγ.

**Discussion**

This study has demonstrated that there is a significant set of genes that is coregulated with CD14 and CD163 in pig peripheral blood monocytes. The differential expression of CD16, inversely with CD14, was conserved in pigs (15). In keeping with earlier studies, there were no major differences among breeds (27).

All monocytes in the pig, defined by expression of CD172a, were also clearly strongly positive for both CD14 and CD163; the differences in expression that define subpopulations are relative and small. As in mice, there is no population that has almost undetectable CD14, hence no equivalent of the human CD16<sup>+</sup> monocyte. Because these cells are only 10% of monocytes in humans, it may be that if they exist in pigs or mice, they actually have a shorter half-life and leave the circulation. Alternatively, because they are involved in patrolling behavior in humans, they could be in a marginal pool and not harvested when pigs are exsanguinated (4). Correspondingly, there is no way to segregate an “intermediate” monocyte population in the pig without a completely arbitrary gate setting. Nevertheless, CD14 did vary across a significant range in pig monocytes, and CD163 was detected at the highest levels on CD14<sup>hi</sup> monocytes. In humans, CD163 was reported to be more highly expressed in the CD14<sup>hi</sup>CD16<sup>hi</sup> nonclassical monocyte subset in human blood and inducible in the CD14<sup>hi</sup> monocytes during CSF1-induced differentiation in vitro (28). Conversely, later studies (4, 15) indicated quite the reverse, and expression can be induced in human CD16<sup>+</sup> monocytes by CSF1 (29). We have confirmed this finding independently at the mRNA level; CD14<sup>+</sup>, CD16<sup>+</sup> human monocytes have almost undetectable CD163 mRNA (J. K. Baillie and D. A. Hume, unpublished observations). CSF1 is present in the circulation and is important for macrophage differentiation, growth, and survival (30). We recently described the ability of CSF1 to generate a pure population of macrophages from pig bone marrow (2). Clanchy et al. (31) carried out a detailed study of the proliferative response of human blood monocytes to CSF1 and demonstrated that around 30% of cells within the CD14<sup>hi</sup> population could go through at least one cell division over 6–8 d. Although we have not quantitated the proliferative pool, the data in Fig. 2 suggest that in culture in CSF1 leads to a very large increase in monocyte numbers in pig PBMC cultures, detected most effectively by expression of CD172a and the appearance of cells with increased size and granularity. The relative expression of CD14 on the monocyte-derived cells was decreased, and CD16 increased (Fig. 2). Aside from the difference in relative abundance of the CD14<sup>hi</sup> monocytes in humans and pigs, there is also a rather puzzling difference in size and granularity. Human promonocytes are larger than CD14<sup>2+</sup> classical monocytes in humans and pigs, there is also a rather puzzling difference in size and granularity. Human promonocytes are larger than CD14<sup>hi</sup>CD16<sup>+</sup> blood monocytes (32, 33). It is uncertain why human monocytes should decrease in size as they differentiate, whereas pig monocytes increase; we speculate that there may be differences in the number of cell divisions during the differentiation process.

With the newly created and annotated pig cDNA “snowball” microarray, we examined the expression profile of the two monocyte populations. Broadly speaking, pig CD14<sup>hi</sup> monocytes resemble human CD14<sup>hi</sup> monocytes. As noted previously, and in common with human CD14<sup>hi</sup> monocytes, they express higher levels of the chemokine receptor CCR2 and are likely recruited to inflammatory sites by CCL2 (20). The pig CD14<sup>hi</sup> (CD163<sup>lo</sup>) monocytes express between 6- and 12-fold higher levels of the chemokine IL-8 and its receptors (IL8RB, IL8R2, CXCR2) than CD14<sup>lo</sup> monocytes, suggesting that they may have an important role in recruiting neutrophils to sites of inflammation or infection. Selective expression of G-CSF (CSF3) receptor was also shared by pig and human CD14<sup>hi</sup> monocytes. CSF3 controls the production, differentiation, and function of granulocytes (34), and has also been described as a maturation factor for monocytes (35, 36). Production of CD14<sup>lo</sup> monocyte subset in the bone marrow may be coordinated with granulocytes (15, 37). CD14<sup>lo</sup> monocytes in both pig and human share with granulocytes the high expression of S100A8 (5). S100A8 has also been implicated in inflammation, and excessively high levels have been associated with recurrent infections (38, 39).

Because there is no obvious separation of intermediate monocytes, the functional equivalent of the CD16<sup>+</sup> human monocyte in pigs (and mice) is less obvious. In humans and pigs, CD36 was
more highly expressed by CD14\textsuperscript{hi} monocytes, whereas MSR\textsuperscript{I} was expressed by CD14\textsuperscript{lo} monocytes. The lysosomal asparaginyl endopeptidase legumain (\textit{LGMN}) showed the most selective expression in CD14\textsuperscript{lo} pig monocytes. \textit{LGMN} can be found in lysosomes and degrades antigenic proteins for presentation to CD\textsuperscript{4} cells, so it is responsible for a key step in Ag presentation (40, 41). CD14\textsuperscript{lo}, CD16\textsuperscript{hi} monocytes also expressed the highest levels of the swine leukocyte Ag (SLA) class II genes (\textit{SLA-DRB1}, \textit{SLA-DQA}, \textit{SLA-DQB1}, \textit{SLA-DRA}, \textit{SLA-DRB1}, \textit{SLA-DRB2}), suggesting a role in Ag processing and presentation (42). A similar role in MHC processing has been described for human “intermediate” CD14\textsuperscript{+} CD16\textsuperscript{+} monocytes (5). The DAVID analysis suggests that, like human CD16\textsuperscript{+} monocytes, they have greater respiratory rates. Previous studies indicate that CD16\textsuperscript{+} cells share with CD16\textsuperscript{+} human monocytes an increased potential for inducible expression of proinflammatory genes including TNFa (42, 43).

Overall, the data suggest that the human and pig CD14\textsuperscript{hi} populations (or more accurately, the set of genes with expression
correlated positively with CD14) are similar, and rather more different from the mouse. This view is in keeping with our recent studies of LPS-inducible genes in macrophages from the three species, where pig is much more closely related to human (1–3). These monocytes have also been referred to as “inflammatory” on the basis of their selective recruitment into inflammatory sites, and by contrast with the “resident” fate of the CD14⁺ monocytes (12, 44). That view is consistent with their preferential expression of inflammatory genes in the pig. On the basis of shared gene expression profiles, the CD14⁺, CD163⁺ pig monocytes resemble “intermediate” monocytes. Interestingly, and in keeping with the likely role of CSF1 in the maturation of monocytes in humans and mice (10), we were able to generate CD16⁻ pig monocytes after cultivation ex vivo, and similarly found that the majority of bone marrow–derived macrophages grown in CSF1 were CD163⁺. Therefore, it is likely that the equivalent of so-called nonclassical monocytes could exist in pigs and might be detected in different disease states.

It is not entirely clear that “subset” is an appropriate term, because the subdivisions of monocytes depend somewhat on exactly where one places gates on a flow cytometer. The prevailing view is that the different subsets are maturation stages of the same cell and this has been backed up by recent genetic studies (5). In vitro–generated human monocyte-derived macrophages expressed lower levels of CD14 and high levels of CD16. They also displayed increased MHC class II expression and decreased expression of CD14 and CD16, similar to the CD14⁺CD16hi subset. Removal of all CD16⁺ cells, including the CD14 loCD16hi subset, from PBMCs before cell culture did not affect the generation of CD14⁺CD16hi cells, indicating that the CD14⁺CD16hi cells were derived from CD14⁺CD16hi monocytes (45). In the mouse, downregulation of Ly6C on Ly6Ch cirulating monocytes has been described, suggesting that as in humans, the murine monocyte subsets represent a continuum of different stages of maturation (7).

A major imperative for the study of the CD163⁺ monocyte subset in pigs has been the putative function of CD163 as a receptor for the important pathogen PRRSV (19, 20, 46, 47). Monocytes purified based on CD14 expression, or adhesion, were found to lack CD163 and to be nonpermissive for the virus (46, 47). In both these studies, CSF1 was found to promote the rapid appearance of CD163 and susceptibility to infection. These studies do not record the breed of pig, but based on the profiles in Fig. 1 and Supplemental Fig. 1, it would be difficult to identify a genuinely CD163⁺ pig monocyte. The >2000 genes that appear coregulated with CD163 are likely to contribute, alongside the >1400 that are downregulated.

The deficiencies of the mouse as a model for human macrophage biology (3) have been reinforced by a recent comparative analysis of gene expression profiles in mouse inflammatory models and the corresponding human diseases (48). Our analysis of pig monocyte subsets confirms our earlier data (2) in that pigs are significantly more human-like. Nevertheless, the use of the pigs as disease models (1) must also take account of the differences between the species.

Disclosures

The authors have no financial conflicts of interest.

References


8 PIG MONOCYTE SUBSETS