

Report on the nomenclature for monocytes and dendritic cells in blood 2021 10 up-date
Executive Summary

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Activity of the Committee in 2020 – 2021

The Committee has contributed to a Research Topic on Nomenclature, which was spearheaded by Menno van Zelm and Pablo Engel. Here, LZH has edited two papers and, together with Committee member Marc Dalod and others, has contributed a paper on subsets of the CD1c+ dendritic cells (Heger, Front Immunol, 2020 Sep 30; 11: 559166). Here, we are at a crucial point with respect to nomenclature, because the long known CD14+CD5- subset of the CD1c+ cells, in a single cell sequencing paper, has been called DC3. This is problematic because these cells are characterized by low APC and high cytokine production capacity. In our Front Immunol paper we summarize the current state and we argue that these cells rather might be a member of the monocyte family, than a DC. The paper will be a document for discussion at the next nomenclature meeting.

Also, LZH together with Committee member Ken Shortman and others has written an opinion paper on the nature of pDCs (to be submitted). These cells have been considered an out-sider in the DC family already for long, because it lacks the crucial features of high APC and CCR7-driven migration to lymph nodes. In the paper we point out that these cells, based on their unique feature of high level production of the cytokine type I interferon, might quite well fit with the group of innate lymphocytes. Once published we expect this paper to be a basis for discussions on a revision of the DC nomenclature.

Preparation of a nomenclature up-date workshop

The organization of a workshop, planned for the 2019 IUIS conference in Beijing, has not materialized. The main reason was that the available funding of 12.000 € is short of the required 36.000 €. The format of the next meeting is still unclear and it may be run as a virtual event in 2022. Still, a face-to-face meeting has advantages when it comes to discussion of controversial points. This may be realistic as of 2023.

The later date may be preferred since the Single Cell Sequencing (SCS) approach has generated many clusters, which now need to be consolidated and backed up by biological and medical meaning. This includes definition of cell type versus cell state.

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For the next conference the aim is to arrive at an up-dated nomenclature that

- a) Will include blood monocytes and dendritic cells and their precursor cells
- b) Will work across different species and
- c) Will be applicable to disease including inflammation and malignancy.

The workshop will cover sections on

- a) monocyte precursors
- b) blood monocyte subsets
- c) DC precursors
- d) blood DC subsets
- e) CD1c+ CD14+ cells: DC or monocyte? Appropriateness of DC3
- f) reclassify pDCs
- g) omics to define subsets versus different versions of a subset
- h) single-cell-sequencing: How many subsets are there?
- i) man, mouse and other species
- j) markers in health and disease

For this the nomenclature committee plans on a meeting of 35 leading experts. Participants consist of the current member plus experts covering the new topics. These have been selected from the global community based on their crucial contribution.

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Use of the current IUIS nomenclature

The crucial paper for the new nomenclature is the publication in Blood journal in 2010 (Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJ, Liu YJ, MacPherson G, Randolph GJ, Scherberich J, Schmitz J, Shortman K, Sozzani S, Strobl H, Zembala M, Austyn JM, Lutz MB. Blood. 2010 Oct 21;116(16):e74-8)

As of September 19th 2019 this paper has been referenced more than 1600 times (see www.researchgate.net/publication/45185387).

A search in the Google Scholar webpage in October 2021 gave 1170 papers, which use the term “intermediate monocyte”. This demonstrates that the definition of these previously overlooked cells in the nomenclature proposal has had direct impact on research and now is an established entity. Also, the term “nonclassical monocyte” or “non-classical monocyte” appears 1500 times and “classical monocyte” occurs more than 2500 times. This confirms that the monocyte nomenclature is being widely used even without citing the original paper in Blood.

The nomenclature for DCs in the 2010 proposal names the subsets via defining markers as plasmacytoid CD303+ DCs, myeloid CD1c+ DCs and myeloid CD141+ DCs. While this is being used widely, the terms pDC, DC1 and DC2 are also frequently used. Here, the emerging complexity of subsets of CD1c+ DCs will have to be addressed in a future up-date.

Updating the nomenclature: Monocytes

Impact of single cell sequencing

SCS has been applied to monocytes in many studies since 2017. These studies identified several (up to 14) clusters each (e.g. Kobayashi, 2020, Jiang, JCI insight, 2020, Wen, Annals Transl Med, 2020, Wilk, Nature Medicine, 2020, Schulte-Schreping, Cell, 2020). These studies typically find classical and non-classical monocytes and various numbers of additional clusters. These clusters sometimes appear to represent cell states, like a subset characterized by high cytokine production (Guo, Nature Communications, 2020) or resting and activated versions of classical and non-classical monocytes based on S100A9 expression (Wilk, Nature Medicine, 2020). Often, the different clusters are presented in UMAP plots without any further characterization. Here, cell types and cell states will have to be aligned across different studies and such cells need to be analysed for their functional responses and cell fate.

Non-classical monocytes

6-sulfo LacNAc+ (slan+) cells

For more than a decade slan+ cells have been addressed as a distinct type of dendritic cells and they were dubbed slanDCs (Schäkel, Immunity, 2006). The monocyte nature of slan+ blood cells has been pointed out by the Ziegler-Heitbrock team already early on (Siedlar, Immunobiology, 2000). Still the name slanDC was maintained well into 2015. Transcriptomic work by the Ziegler-Heitbrock team and the van de Loosdrecht team has then unequivocally demonstrated assignment of slan+ cells to the monocytes (Hofer et al, BLOOD, 2015, Leeuwen-Kerkhoff, J Leuk Biol 2017) and this was supported by comparative functional studies (Calzetti, JACI, 2018). In the light of this evidence the team that had proposed the slanDC nomenclature accepted that slan identifies a monocyte subset (Olaru, JCIinsight, 2018). Also, the same team consolidated this view in a review paper (Ahmad, Frontiers in Immunology, 2019). A review by the Ziegler-Heitbrock team has given an overview on the slan+ cells and has pointed out the open questions (Hofer et al, Frontiers in Immunology, 2019). This includes the question whether slan can be used to define the non-classical monocytes. The importance of slan as a marker has been highlighted in a clinical study on CMML (Tarfi, Haematologica, 2019, Haematologica, 2020), which demonstrated that a decrease of the slan+ non-classical monocytes below 1.7% contributes to diagnosis of the disease also when there is concurrent inflammation.

More recently a series of papers have supported the usefulness of the slan marker in clinical studies of monocyte subsets in man: In COVID-19 a depletion of slan⁺ monocytes was demonstrated (Farias, 2021, Zingaropoli, 2021, Trombetta, *Frontiers Immunology*, 2021). Also, slan⁺ monocytes are decreased in multiple myeloma, B cell lymphoma and after IFN-therapy of HCV infection (Lamarthee, *Oncoimmunology*, 2018, Gallou, 2021, Vita, 2020).

The inclusion of slan as a marker for non-classical monocytes will be proposed at the next Nomenclature Committee Meeting.

The term “patrolling” monocytes for the non-classical monocytes appears to be fading away. This may be due to the demonstration (by the team that coined this term) that the patrolling (=crawling) behavior is equally evident for the classical monocytes in man (Collison, Geissmann, *J Immunol* 2015). Also, in the mouse it is the classical monocytes that patrol the liver vasculature (Dal-Secco, *JEM*, 2015).

Regarding non-classical monocytes Patel et al (*J Exp Med*, 2017) have shown using deuterium labelling that the life span of these cells with about 7 days is much longer compared to classical monocytes with about 1 day. Also they have confirmed expression of slan on the non-classicals. The kinetic data in man are in contrast to the mouse where similar studies have shown a half-life of 2 days for the non-classical monocytes (Yona, *Immunity*, 38, 79–91, 2013). While we propose a unified nomenclature for man and mouse these data again remind us of the fundamental differences we encounter between these two species for monocyte subsets.

Intermediate monocytes

For the intermediate monocytes a series of publications has appeared since its delineation in the original nomenclature document (Ziegler-Heitbrock, *Blood*, 2010). This included transcriptome studies which show unique signatures (Wong, *Blood*, 2011, Zawada, *Blood*, 2011). While it appears that these cells are, in fact, in transition, their definition as a separate cell population also for clinical purposes is warranted: Several clinical reports have shown the increase the CD14⁺⁺CD16⁺ intermediate monocytes in inflammatory conditions.

The dissection of intermediate and non-classical monocytes in flow cytometry is not straightforward. An improvement may come with the use of an additional marker like slan or CCR2 as suggested by Wong et al (*Immunological Research*, 2012). CCR2 has been tested in a series of studies on cardiovascular disease (Shantsila, *Thromb Haemost.* 2013; 110: 340-8., *J Thromb Haemost.* 2011;9:1056-66). However, no relevant advantages of this approach have been demonstrated and molecular studies that document a superior separation when using CCR2 are lacking.

On the other hand a clear separation of these cells from the non-classical monocytes was demonstrated when using the marker slan (Hofer, *Blood*, 2015). This definition was supported by both molecular data and clinical studies showing an increase of slan⁻ CD14⁺ CD16⁺ monocytes in sarcoidosis. These data await independent confirmation before a recommendation on the definition of intermediate monocytes as slan⁻ CD16⁺ monocytes can be made.

Single cell sequencing (SCS) of human cells by Villani et al (*Science* 2017) picked up two populations within the intermediate gate. One population, termed Mon3, expressed CD16, CXCR1 and mincle (CLEC4e) and this was confirmed to represent intermediate monocytes (Günther et al *BioRxiv*, Jun. 3, 2019; doi: <http://dx.doi.org/10.1101/658179>). The other population, termed Mon4, expressed CD2 and perforin and was shown to be an artefact, since it was a contamination by NK cells (Günther, *BioRxiv* 2019).

SCS of intermediate mouse monocytes (Mildner et al, *Immunity*. 2017) has uncovered two cell clusters, one that is in between classical and non-classical monocytes for all genes and one that has unique features with high class II, CD74 and DC-SIGN. This would mean that within this gate we are dealing with cells that are merely in transition and with cells that form a unique population.

The data demonstrate the power of single cell sequencing and the open issues we still face with these early studies. Ultimately this approach should be able to determine how many subsets of monocytes there are. It is expected that we will have solid data and consensus on intermediate monocytes in the next 1 or 2 years.

Tie2-expressing monocytes

Tie2-expressing monocytes have been defined earlier in man and mouse (Da Palma 2005) and here Tie2 expression was found in CD16-positive monocytes (Venneri Blood, 2007) and more specifically in the intermediates (Murdoch JIM 2007). In a recent study high TIE2-expressing monocytes were reported for CLL patients, but it remained unclear whether this increase was due to an increase of CD16+ monocytes in the samples (Wos, Cancers, 2021). Since expression of Tie2 is weak it is difficult to define clearly positive and clearly negative cells and the question is whether TIE2-expressing monocytes are a distinct cell population.

Classical monocytes

The definition of classical monocytes is straightforward in man and in the mouse. The human cells can be defined based on the CD14++CD16- pattern and the mouse cells are Ly6C++CD43+ and this has not changed since the 2010 proposal.

A group of cardiologists (European Society of Cardiology) has suggested a variation of the monocyte nomenclature in calling the classical monocytes Mon1 (see Weber et al Thrombosis Hemostasis, 2016). Given that the 2010 nomenclature for monocytes is well established, this unfortunately only adds confusion. The use of the ESC nomenclature has waned and papers by members of the author team of that paper now publish using the 2010 IUIS nomenclature.

Single cell sequencing has shown these classical monocytes to be homogenous (Milder Immunity 2017, Villani, Science, 2017). Still there have been some attempts to define subsets within the classical monocytes.

Using SCS Kobayashi (J Autoimmun 2020) described non-classicals and 6 additional clusters in the context of systemic sclerosis. Jiang (JCI insight, 2020) observed, in addition to the non-classical monocytes, four clusters related to classical monocytes, but this was only in ARDS without data on apparently healthy controls. Wen (Wen, Annals Transl Med, 2020) reported a non-classical cluster and 5 clusters related to classical monocytes. Also, Wilk (Nature Medicine, 2020) identified 14 clusters and pointed out that there are resting and activated cell states both for classical and non-classical monocytes. Also, Guo (Nature Communications, 2020) analysed two cases of COVID-19 and found the non-classical monocyte cluster plus 3 classical related clusters. One cluster, which was expanded in severe COVID-19 was characterized by high transcript levels for cytokines and chemokines.

Furthermore, Lu (Research Square, 10.21203/rs.3.rs-416878/v1) found non-classicals and 5 additional clusters, including “antigen presenting monos, with high HLA-DR transcript levels and “inflamed monos” with high IL-1b and chemokine transcripts.

In the context of the Vogt-Koyanagi-Harada autoimmune disorder, Hu et al (PNAS 2020) have described the non-classical monocytes and three clusters related to classical monocytes. Also Schulte-Schreping in the context of COVID-19, found non-classical, classical and three additional clusters related to classical monocytes (Cell, 2020). While it is difficult to align these clusters across the studies, it is possible that the S100A12 cluster of Hu and the HLA-DRlo S100Ahi cluster of Schulte-Schreping overlap. The same is possible for the HLA monocyte cluster of Hu and the HLA-DRhi CD83hi cluster of Schulte-Schreping and the “antigen presenting monos” cluster of Lu et al. These HLA-DRhi cells might well represent the intermediate monocytes, which are known to have highest levels of this type of MHC genes. Also, activated monocytes of Wilk, the “inflamed mono” cluster of Lu and the “pro-inflammatory monocyte” cluster of Hu are likely to match. The cells of this latter cluster might represent transient cell states, activated via TLR-ligands.

These published clusters need to be aligned as exemplified above and importantly their biological meaning and medical relevance is to be determined. For this the cells within the clusters need to be

isolated based on discriminating markers in order to study various immune functions. Only with this type of information a cluster can become a defined cell type or cell state.

There is however diagnostic potential for gene expression analysis even at the early cluster analysis even without further characterization of the cells, which the clusters represent. This is exemplified by clusters within classical or non-classical monocyte, which show high level cytokine transcripts. This information can be used to document the degree of inflammation and the cell groups involved, like T cells or monocytes. Still, a complete characterization of a given cluster up to the level of a defined cell type will increase the diagnostic potential substantially.

CD14+DR^{low} monocytes (“myeloid-derived suppressor cells”)

Myeloid-derived suppressor cells (MDSC) are a mixture of cells mainly characterized by their ability to suppress T cell response more so in cancer patients than in healthy controls. They were then subdivided into monocyte-type and neutrophil-type MDSC. The monocyte type cells are defined as CD14^{high} DR^{low/-} cells (Bronte Nature Comm, 2016). These cells are found increased in cancer and in sepsis and they may represent immature cells or deactivated cells (see Volk, Intensive Care Med, 1996)

Problems that the M-MDSCs face are that the cut-off value for DR is not clearly defined, that cells of this phenotype can be suppressive or not, that studies on side by side comparison with other monocyte subsets are scarce and that comparative transcriptomics are not available (but may be in the pipeline). Also the few single cell sequencing studies no “MDSC” cell type has been detected (Villani, Science. 2017;356(6335), Mildner, Immunity. 2017 May 16;46(5):849-862).

While the term “monocytic myeloid derived suppressor cells” gives 1.100 publications in pubmed in October 2021 there is still serious doubt whether these cells represent a cell type or a cell state or only an entity defined as the low end of a MHC class II cell surface expression distribution.

Taken together the current evidence does not support inclusion of MDSC into a blood monocyte subset nomenclature at this point in time.

CD319+ monocytes, CD141+ monocytes

Cell surface expression of SLAMF7 = CD319 is prominent on CD14+ CD16- monocytes in myelofibrosis, but there is hardly any signal in healthy controls (Maekawa Blood 2019). Also expression of CD141 has been reported on classical monocytes in myelodysplasia (Leeuwen-Kerkhoff, Haematologica, 2019). These studies highlight a distinct area of monocyte and dendritic cell biology in that it covers changes in abnormal hematopoiesis. As to whether these changes deserve the status of unique subsets may largely depend on whether they can be detected in healthy individuals or whether they represent the result of somatic mutations only. Of note, CD319 mRNA apparently can be induced in GM-CSF, IL-4-treated monocytes following stimulation by lipopolysaccharide (Guo, J Immunol, 2021).

Updating the nomenclature: Dendritic Cells

General remarks

The 2010 nomenclature proposal suggested a subdivision into two myeloid DC-populations and one pDC population with the markers CD141, CD1c and CD303. The myeloid nature of the former cell types was based on the expression of CD33. On the other hand CD68 a macrophage associated molecule, is clearly expressed by pDCs. So it is unclear whether the term myeloid is appropriate and whether it should be replaced or can be substantiated with newer data.

Also the terms DC1 and DC2 have been widely used for CD141+ DCs and CD1c+DCs, respectively. Some also use the terms cDC1 and cDC2 with “c” mean variously conventional or classical. The “c” can be justified in juxtaposition to the “p” of pDCs. Given the possibility that pDCs may lose their DC status, the “c” may not be necessary any more.

CD141+ DCs

Among dendritic cells the CD141+ myeloid blood DCs have been further characterized and the expression of markers like XCR1, Clec9A and the transcription factor IRF8 have strengthened the view that these DCs and the mouse CD8-positive DCs are homologous cells and share the property to cross-present exogenous antigen to CD8 T cells (Contreras J Immunol. 2010;185:3313-25. Crozat K. J Exp Med. 2010;207: 1283, Bachem A J Exp Med. 2010;207: 1273). Of note, in blood the respective mouse DCs do not express the CD8 antigen on the cell surface. XCR1 has been recently identified as a conserved universal marker for these DCs across tissues and species (Vu Manh, Frontiers Immunology, 2015).

Bachem has generated an anti-mouse monoclonal against XCR1 in a knock-out mouse showing expression on 80% mouse CD8+ DCs cells in the spleen (Bachem Frontiers Immunology 2012). Recently, a mouse anti-human XCR1 monoclonal antibody has been patented (Anti-human XCR1 antibodies US 9371389 B2, 2016).

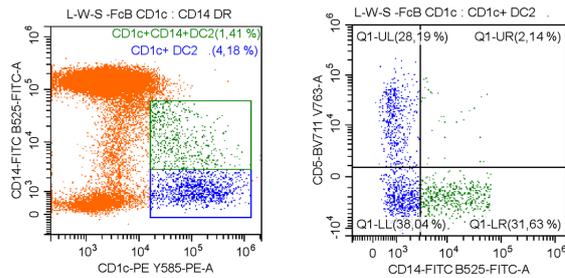
CD1c+ DCs

For the CD1c+ myeloid DC in human blood it had been noted earlier that there are CD14- and CD14^{low} cells (see the BLOOD-2010 Nomenclature proposal) and these have not been characterized further. A recent study (Bakdash, de Vries Cancer Research, 2016) has described CD1c+ CD14+ cells to be expanded in melanoma patients. The cells were shown to express high levels of PD-L1 and to reduce T cell proliferation more efficiently compared to CD1c+CD14- and to M-MDSC. Transcriptome analysis comparing the CD1c+CD14+ cells to CD1c+CD14- cells and to classical monocytes found them to be in between. Comparison to a comprehensive set of blood DCs and monocytes is required in order to define the position of these cells and their assignment to monocytes or DCs.

More recently in a 2017 study the CD1c cells were subdivided into CD5^{low} and CD5^{high} (Yin et al J Immunol. 198: 1553, 2017). The CD5^{high} cells were more potent in antigen presentation and in that are similar to the CD1c+ CD14- cells. Thus we may be dealing two populations of CD1c+ cells a) CD14-CD5^{hi} and b) CD14+ CD5^{lo}. This still needs to be formally shown. The concept of two cell types is supported by recent SCS data (Villani, Science. 2017; 356: 6335), who falsely claimed being the first to describe this subdivision of CD1c cells. The study identified one cell type expressing CD14, S100A9 and S100A8 and one cell type with higher Class II expression. With some more data consolidating this subdivision the Nomenclature Committee may discuss to officially recognize two subtypes of CD1c+ cells. The open question then remains whether the CD1c+CD14+ cells are dendritic cells or monocytes. Comparative transcriptomics with a large set of prototypic monocytes and dendritic cells may help in making that decision.

This will have to take into account the possibility of three subsets of CD1c+ DC2 cells, since Alcantara et al (Immunity, 47(6), 1037-1050 e6 (2017)) have shown that there are CD172^{high} CD163^{low} and CD172^{low} CD163^{med} and CD172^{high} CD163^{high} DCs. Also, we have confirmed three subsets, i.e. CD14+ CD5- cells, CD14- CD5+ cells and CD14-CD5- cells (see figure below, taken from Heger Front Immunol, 2020).

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An important nomenclature issue is that the CD14+CD5-CD1c+ cells have been called DC3 in the SCS paper by Villani, (Science, 2017). Work by three independent groups (Dutertre Immunity, 2019, Bourdely, Immunity, 2020, Cytlak, Immunity, 2020) have described the unique features of these cells. The latter study has shown that this subset, but not the CD14- CD1c+ cells, can be generated from CD33+ GMP and that they segregate with monocytes. Taken together, these cells have low APC and are potent cytokine producers, such that they likely represent a novel subset of monocytes.

CD303+ pDCs

Plasmacytoid DCs have been characterized in man by expression of CD303 and also by high level CD123. Differential CD2 staining has been noted within the population of pDCs with the CD2 high cells expressing lysozyme and higher levels of IL-12 (Matsui T, J Immunol. 2009). Also in the rat subsets of pDC have been described and a closer look at heterogeneity of these cells in man is warranted. An indication of such heterogeneity is given in Guilliams (Immunity 45, 669–684, September 20, 2016) where differential expression of CD2, CD5, CD14 and CD39 is shown for two subsets of pDCs in human blood (see Fig 6 in Guilliams 2016).

pDCs are characterized by high IFN α production. Given that pDCs are not very good at antigen presentation when compared to CD1c+ and CD141+ DCs it can be questioned whether it is justified to call these cells DCs. The DC nature of these cells has been questioned in several recent papers. Cabeza-Cabrero et al, for example, have decided to call these cells PCs (plasmacytoid cells) (see Annual Review Immunol 2021).

The question has arisen whether these cells can be assigned to another lineage like for instance the innate lymphocytes cells. LZH has written, together with Committee Member Ken Shortman and others, an opinion paper on the nature of pDCs. This is meant to serve as a basis for discussion of a revised nomenclature.

SCS

Villani et al (Science, 2017) have searched human blood leukocytes for subsets of DCs and they described the pDCs, the CLEC9A+ cells (CD141+, DC1), the two types of CD1c+ cells mentioned under CD1c+DCs (termed DC2 and DC3 by Villani et al). Many of the mistakes and problems of the Villani study have been experimentally refuted by other groups (Calzetti, JACI, 2018, Guenther, BioRxiv, 2019). DC4 are CD16-positive and are non-classical monocytes contaminating the DC-preparation of cells. A novel population are cells called AS DC, which are positive for genes AXL and SIGLEC6 are, in fact, DC precursors (see Günther, BioRxiv 2019). All of this needs confirmation by independent studies but it is expected that over the next couple years, we will definitely know, how many subsets of DCs there are in human and mouse blood.

Nomenclature covering precursors of macrophages and dendritic cells

Monocyte Precursors

After the common monocyte progenitors (cMoPs) had been described as CD117+CD115+CD135^{low} in the mouse (Hettinger, *Nature Immunology*, 2013) more recently a CLEC12A^{hi}CD64^{hi} cMoP has been described in man (Kawamura, *Immunity* 2017). In addition, a cell termed pre-monocyte has been described in the mouse, (Chong, *J Exp Med*, 2017), a cell characterized by high CXCR4 expression. Similar CXCR4^{hi} cells were detected in human samples. Hence, a model emerges with GMPs giving rise to cMoPs that give rise to pre-Monos, which in turn develop into classical monocytes (GMP→cMoPs→pre-monocytes→ classical monocytes). When these precursors and their properties have been confirmed by independent studies then their inclusion in a nomenclature proposal can be discussed by the committee. This will have to consider new data, which indicate that monocytes can derive from MDPs and from GMPs (Yanez *Immunity*, 2017)

DC Precursors

In the mouse, a macrophage dendritic cell precursor (MDP) has been postulated. However, the existence of these cells has been disputed (Sathé, *Immunity*, 41: 104, 2014). More data with clonal analysis and fate mapping are required until a solid consensus can be reached in this field of precursors. This topic also includes the precursors for CD1c⁺ and CD141⁺ DCs that have been covered above.

A novel blood subset belonging to the human DC family has been described (Breton, *J Exp Med*, 212: 401-13, 2015). What they have described is a DC precursor for both the CD141⁺ DCs and the CD1c⁺ DCs. This may be an important new finding and a confirmation of these data by other teams is awaited.

In a study by the Reis e Sousa team it was shown that the CD141⁺ Clec9A⁺ DCs can be generated from the common myeloid progenitor (CMP) and with high efficiency from multipotent lymphoid early progenitors (MLPs) (Helft, *Cell Reports*, 20, 529–537, 2017). Cells generated from the two progenitors show an identical transcriptome (convergent differentiation). The study by Breton et al has described a cell termed hpre-CDC-like which is found in human blood and bone marrow, has the phenotype Lin⁻CD34⁻ CD117⁺ CD135⁺ CD116⁺ CD115⁻. In clonal analysis 90% of these cells can give rise to CD1c⁺ (DC2) and CD141⁺ (DC1) myeloid DCs but only 10% lead to production of monocytes (Breton, *J Exp Med*, 212: 401-13, 2015). These studies also reported on CDPs (common dendritic cell precursors, which has similar features as the GMPs =granulocyte monocyte progenitor,) to give rise to pDCs and pre-CDCs which can generate DC1 and DC2. In addition, single cell sequencing analysis (See *et al.*, *Science* 2017) has proposed a separate pre-DC for DC1 and DC2 each. Here pre-DC1 (CD123^{lo}CD115⁺CD117⁺CD135⁺CD116⁺CD115⁻) and pre-DC2 (CD123^{lo}CD115⁺CD117⁺CD135⁺CD116⁺CD115⁻) that are committed to generate DC1 and DC2, respectively.

Other species: monocytes and DCs in blood of non-human primates

As mentioned in the original nomenclature document many of the anti-human reagents can be used directly for non-human primates and they give similar patterns for blood leukocytes in old world monkeys. The CD16-positive monocyte subset was originally reported for *Macaca fascicularis* by Munn et al (*Blood* 1990) and Otani et al (*AIDS Res Human Retro viruses* 1998) expanded on this in the context of retrovirus infection.

For *Macaca mulatta* the Williams team also reported on monocyte subsets and they identified and characterized the intermediate monocytes (Kim, *JLB*, 2011). Monocyte subsets could be properly identified based on CD14 and CD16 in rhesus macaques, cynomolgus macaques and pigtail macaques (Sugimoto, *J Immunol*, 2015). The same study using BrdU injection demonstrated consecutive appearance of label in classical, intermediates and non-classical monocytes, similar to the developmental sequence after bone marrow transplant (Dayyani, *JLB*, 2002), and after deuterium glucose injection in man (Patel, *JEM*, 2017). Also injection of the R848 TLR7/8 ligand into rhesus monkeys a strong increase of intermediate monocytes could be demonstrated (Kwissa, *Blood*, 2012).

The humanized anti-slan antibody REA1050 was recently shown to stain a population of CD16+ monocytes in marmosets (Hofer, *Frontiers in Immunology*, 2019) such that these animals can be used as model for the study of slan+ monocytes. Importantly, Sibley et al have shown that there are strong differences in subset composition when looking at Rhesus versus Cynomolgus, with the latter having a much higher proportion of CD16+ monocytes. (*Scientific Reports*, 2021). Cynomolgus monkeys show a strong increase of intermediate monocytes post vaccinia-virus injection (Rosenbaum, *Front Immunol*, 2021). In a SARS-CoV-2 infection model both Cynomolgus and Rhesus show an increase of CD16+ monocytes (Salguero, *Nature Communications*, 2021).

In a SCS study on Lewis et al, 2021 have described in rhesus macaques non-classical and intermediate monocytes and seven clusters within classical monocytes, including a S100A8hi subset (*Front Immunol*, 2021)

For DCs, it was noted that anti-human CD141 antibodies do not identify a CD141-high population in rhesus monkeys but stains at a low level all myeloid DCs and monocyte subsets (Autissier, *J Imm Methods*, 2010). Still the three DC subsets can be identified using antibodies against CD1c and CD123+for pDCs (Sugimoto, *J Immunol* 2015) and against CADM1 for the CD141+ homologue in rhesus macaques (Dutertre, *J Immunol*, 2014).

Also for new world marmosets cross-reactivity for CD14, CD1c and DR has been demonstrated. These findings support a suggestion to recommend the human nomenclature also in non-human primates, albeit different reagents may be needed at times.

Other species: monocytes and DCs in blood in the mouse

There are no new data on characterization of mouse monocyte subsets but rather on the biology of these cells. The subsets are still being defined via Ly6C and CD43 expression and CD115 or F4/80 are used to identify all monocytes. Based on an intermediate level of Ly6C intermediate mouse monocytes are being confirmed in several publications (e.g. Lessard *Cell Reports*, 2017, Makinde, *JIM*, 2017, Wolf, *JEM*, 2017).

Non-classical monocytes had been called “patrolling” and it was proposed that their main function is to monitor and clean up the endothelium (Auffray, *Science*, 2007) rather than going into tissue as is the standard fate of monocytes. However, already in the Auffray publication it was shown that these very cells rapidly go into tissue after injury, but disregarding this many follow up papers maintained that it is the classical monocytes that exclusively go into tissue. Recently several reports have shown that non-classical monocytes go into inflamed joint, normal mesenteric tissue and inflamed skin (Misharin, *Cell Reports*, 2014, Saja *Cell Reports*, 2015, Olingy, *Scientific Reports*, 2017).

While in man almost all monocytes are HLA-DR+, a large proportion of mouse monocytes does not express Class II cell surface antigen and this applies to both classical and non-classical monocytes (Jakubzick, *Immunity*, 2013). Whether the differential Class II expression helps in defining additional subset of monocytes remains to be analyzed.

Single cell sequencing has identified two types of mouse intermediate monocytes, one with intermediate expression of all DEGs and one with unique features like high class II, CD74 and DC-SIGN (Mildner, *Immunity* 2017 May 16;46(5):849-862).

Ym1+ monocytes

Expression of the chitinase 3 like protein 3 (also known as Ym1) has been reported for about 5% of mouse blood classical monocytes in homeostasis and 50% at 48 hs after LPS injection in mice (Ikeda, *Science Immunology* 3, eaat0207 2018). Since Ym1+ and – monocytes show only a few differential markers, the question arises whether the Ym1+ cells represent a novel cell type or a novel cell state under the influence of transiently expressed cytokines.

The intracellular protein is not readily accessible for flow cytometry and a human homologue is still to be defined.

Mouse blood DCs

For the PDCA-1-(CD317, BST2) positive pDCs two subsets (CD8⁺ CD8⁻) have been described (Sharma Infection Immunity, 2016). Also, it has reported that there are PDCA-1hi pDCs compared to PDCA-1lo CD11chi DCs in blood (Kim, Scientific Reports, 2016). Björk described CD9+Siglec-Hlo pDCs as the main IFNalpha producers (Björk, JIM, 2011). So subsets of pDCs in the mouse (and also in man) will have to be addressed.

For the human CD1c myeloid DCs the homologous blood DCs in the mouse appear to be the CD11c⁺CD11b⁺CD45RA⁻ cells, which are the CD8⁻ DCs in the spleen. Splenic CD8⁻ DCs can be subdivided into Clec12A^{high} DCIR2 (Clec4a4)^{low} and the Clec12A^{low} DCIR2(Clec4a4)^{high} (Kasahara, JLB, 91:437, 2012). Also, in mouse blood the CD11c⁺CD11b⁺CD45RA⁻ DCs can be subdivided into CD172a⁺ (Sirpalph⁺, 70%) and CD172a⁻ (Sirpalph⁻, 30%) DCs (Proietto PNAS 2008). Hence it appears that for mouse blood DCs a further subdivision emerges but better and selective markers are needed to support this.

Two papers (Meredith, JEM, 2012 and Satpathy, JEM, 2012) have described the Zbtb46 molecule as marker expressed by mouse CD4⁺ and CD8⁺ DCs but not by plasmacytoid DCs in blood and spleen. Interestingly this marker was also found with the same pattern in human blood DCs.

Here discussions are underway as to whether a consensus on the mouse blood DCs and their preferred markers can be reached. However, the Zbtb46 protein is a transcription factor and therefore not readily accessible by flow cytometry.

With a focus on DCs in tissue there is discussion on the use of surface markers other than CD8, like XCR1 and CLEC9A for the definition of what might be termed DC1 (vu Manh, Frontiers in Immunology, 2015).

Guilliams (Immunity, 2016) has compared man and mouse for DC subsets. Looking at mouse spleen cells CD11c⁺CD26⁺XCR1^{high} CD72^{alow} cells (DC1) and CD11c⁺CD26⁺ XCR1^{low} CD72^{ahigh} cells (DC2) could be identified.

The former can, in addition, be defined as CADM1hi and the latter as CADM1low and the two subsets are characterized by mutually exclusive intracellular staining for IRF8 and IRF4 for what is termed DC1 and DC2 (Guilliams, Immunity 45, 669–684, 2017). Mouse pDCs are defined as D11blo120G8hi CD172aint B220int Ly6CintCD11cint in that study. In an IRF4 IRF8 staining the pDCs from mouse spleen overlap with DC1 cells.

These data still need to be confirmed for mouse blood samples.

Other species: monocytes and DCs in rat and pig and cow and horse

Rat:

For the rat monocyte subsets have been defined based on high or low expression CD43 expression in cells defined as monocytes via CD172a or CD68. Ahuja (Cell Immunol, 1995) demonstrated high CD4 on the CD43⁺⁺ cells. Infusion of IFNgamma into rats was shown to lead to a strong increase of CD43⁺ CD4^{low} monocytes (Scriba, JLB, 1997). The CD43⁺⁺ monocytes were then demonstrated to be low for CCR2 and high for CX3CR1 (Yrlid, J Immunol, 2006). Also the CD43⁺⁺ cells were low to negative for CD62L.

More recently the classical-non-classical nomenclature was applied to the rat by several groups. Work involving the Marijke Fass laboratory reported on CD172a⁺ CD43⁺ classical and CD172a⁺ CD43⁺⁺ non-classical monocytes and showed an increase of non-classical monocytes during pregnancy in the rat. (Melgert PLOSone, 2012, Groen, PLOSone, 2013, Spaans, Am J Reprod Immunol 2014).

Grad et al (Drug Deliv. and Transl. Res. 2017) described CD68⁺CD43^{low} classical and CD68⁺CD43^{high} non-classical monocytes, with classicals being CD4^{low} and non-classicals CD4^{high} confirming earlier work by Ahuja (Cell Immunol, 1995). Also Barnett (PLOSone 2016) has recently characterized CD43^{high} and low rat blood monocytes with the phenotypes CD43hi His48lo and

CD43^{lo} His48^{hi}. The two cell types were differential for CD4 and CD161. It appears that CD4 may assist in differentiation classical and non-classical monocytes in the rat. This is supported by a recent study that showed high expression of CD4 by the rat non-classical monocytes (Irvine, JLB, 2019). Taken together, while some homologies to man and mouse are evident the amount of knowledge on rat monocytes is still limited. Notably there are no comprehensive analysis of functional and molecular data without and with stimulation and the intermediate monocytes have not been delineated.

Dendritic cells

Hubert (J Immunol, 2006) has described CD103⁺ DCs in the spleen with a CD4⁺ and CD4⁻ subset. They noted a differential expression of TLRs and selective production of IFN α by pDCs. CD4⁺ and CD4⁻ subsets were confirmed in spleen (Stojić-Vukanić Biogerontology, 2013) More recently a monoclonal antibody against rat pDCs (85C7) Anjubault (PLOSone, 2012) described a CD4^{low} and a CD4^{high} subset. Such pDCs expressed E2-2 mRNA but CD103 DCs did not. Most of this is spleen data but it is shown that CD4^{lo} pDCs can be found in rat blood.

Pig: The original nomenclature proposal has not covered the pig. Here early work had shown that antihuman CD14 antibodies can stain pig monocytes (Ziegler-Heitbrock Scand J Immunol 1994; 40:509-14) and there were monocytes with high and low CD14.

A clear demonstration of monocyte subsets was provided by the Dominguez lab, which described CD14⁺CD163⁺ MHC Class II⁺ and CD14⁺⁺CD163⁻ MHC class II⁻ subsets (Sanchez, 162:5230, 1999). Also, Ondrackova et al (Vet. Res. (2010) 41:64) have found two subsets based on CD14 and CD163 and Fairbairn et al (JLB 89:855, 2011) have confirmed subdivision of porcine monocytes based on CD14 and CD163 with a CD14⁺CD163⁺ and a CD14⁺⁺CD163⁻ subset.

In functional analysis Sanchez et al (1999) reported for the CD163⁺ subset a higher antigen presenting activity, a higher production of TNF and a lack of IL-10 production. More recently the same team has demonstrated that the CD163⁺CD14⁺SLA-II⁺ monocyte subset lacks CCR2 and shows a preferential expression of CX3CR1 (Moreno, Veterinary Research, 2010), which further strengthens the homology to the non-classical monocytes in man.

Of note, the CD14⁺CD163⁺ MHC ClassII⁺ monocytes in the pig show the reverse expression pattern for CD163, since the non-classical monocytes in man are essentially CD163 negative. Also in the pig CD16 is expressed by both subsets albeit higher in the CD163⁺ cells. Still, all the other features studied show a similar pattern for the non-classical CD14⁺CD16⁺⁺ monocytes in man and the CD14⁺CD163⁺ MHC ClassII⁺ monocytes in the pig. There is also evidence for the existence of intermediate cells.

New data have been provided by the Dominguez team on staining with a new TLR4 antibody and here stronger signal was seen on the non-classical monocytes with intermediate levels on the intermediate monocytes (Alvarez, Vet Immunol, Immunopathol, 2013). Recently the monocyte subset definition was confirmed for porcine bone marrow (Fernandez, Dev Comp Immunol, 2017).

The impact of M-CSF on the development of non-classical monocytes has been reported for the pig (Sauter, Am J Physiol Gastrointest Liver Physiol.2016). Further characterization

A proposal for the use of the classical-intermediate-non-classical nomenclature for pig monocytes will be put on the agenda for the next meeting of the nomenclature committee.

Regarding blood dendritic in the pig CD172^{low} CD4⁺ DCs and CD172^{low} CD4⁻ interferon type I producing plasmacytoid DCs have been described (Summerfield, Immunology 2003, Balmelli, EJI 2005). Also, using a labelled XCR1 ligand (=XCL1) the respective DCs were detected in pig blood and skin (Deloizy, Dev Comp Immunol, 2016).

For horse monocytes a CD16 antibody has been described and was shown to identify CD16-positive blood monocytes (Noronha, Veterinary Immunol Immunopathol 146: 135, 2012).

Other species: monocytes and DCs in the cow

Hussen (Plosone 2015) and Corripio-Miyar (Veterinary Res, 2015) using CD14 and CD16 antibodies have defined the three monocyte subsets in Holstein cattle with high variation between individuals. The cell surface phenotype was similar to man and intermediate monocytes showed highest class II expression and non-classical monocytes lowest CD163. Also the non-classical monocytes showed highest levels of TNF and IL12 and lowest for IL-10 also matching the patterns in man. However, IL-1 was found lowest in non-classical monocytes by Hussen (2015). In addition DC-Sign may be informative in dissection of monocyte subsets in this species (Elnaggar, Vet Imm Immunopathol 2016).

A proposal for the use of the classical-intermediate-non-classical nomenclature for bovine monocytes will be put on the agenda for the next meeting of the nomenclature committee.

Looking at dendritic cells in cattle blood a homologue of the human CD141+DCs has been described as CD26+CADM1+CD205+MHCII+CD11b- cells, which express mRNA for XCR1 and for CLE9A and these cells migrate in response to XCL1 (Li et al PLOSone 0170575, 2017). CD209 cells have been described as DCs in the cow but with expression of CD14 it is also possible that these DC-SIGN+ cells are monocytes (Park PLOSone, 0165247, 2016). pDCs with the markers CD4 MHC II CD45RB CD172a CD32 were reported to produce high levels of IFN type I (Reid, J Virol, 85: 4297, 2011). It remains to be shown whether these cells can be further narrowed down with additional markers. Taken together there are indications for a similar subdivision of blood DCs in the cow, but a clearer definition of subsets is required.