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For cover letter: Funding request for 2018:
For participation in the 2018 European Macrophage and Dendritic Cell Conference in Verona, Italy and in the 15th International Symposium on Dendritic Cells in Aachen, GermanyI request a support of € 920.-
At these conference I will meet up with several committee members and with several colleagues, who make relevant contributions to nomenclature issues.

Preamble

List of the current Committee Members
Loems Ziegler-Heitbrock (Germany)
Petronela Ancuta (Canada)
Suzanne Crowe (Australia)
Marc Dalod (France)
Veronika Grau (Germany)
Derek N. Hart (Australia)
Pieter J.M. Leenen (The Netherlands)
Yong-Jun Liu (USA)
Gordon MacPherson (UK)
Gwendalyn J.Randolph (USA)
Juergen Scherberich (Germany)
Juergen Schmitz (Germany)
Ken Shortman (Australia)
Silvano Sozzani (Italy)
Herbert Strobl (Österreich)
Marek Zembala (Poland)
Jonathan M. Austyn (UK)
Manfred B. Lutz (Germany)

Summary of the activities of the Committee during 2017:
LZH has participated in the EMDS conference in Madrid, Spain (September 21-13, 2017) and in the Meeting “Macrophage Biology in the single cell era” in Ghent, Belgium (October 26-27, 2017) and has discussed nomenclature issues with various colleagues.

Preparation of a nomenclature up-date workshop
In 2017 LZH has had intense e-mail and telephone exchange with the different Committee Members regarding new nomenclature developments and the prospect of a 2019 up-date workshop. The favourite site for the workshop is alongside the 2019 EMDS conference in Marseille, France but alternative sites are still being discussed.
Meanwhile LZH has managed to obtain funding for the event based on a call by the journal “ONOCTARGET” (Impact Journals LLC). The amount of 12 500 € has been transferred to the EMDS account. With an estimate of a total of 53 000 €, we currently look into additional support for the event.

An extension and up-date of the nomenclature is necessary since over the last couple years there have been several major developments regarding additional cell populations and the precursor cells in blood and bone marrow.
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?
f) omics to define subsets versus different versions of a subset
g) single cell sequencing: How many subsets are there?
h) man, mouse and other species
i) markers in health and disease

For this the nomenclature committee plans on a meeting of 35 leading experts in September 2019. 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.

Since not all potential participants have been contacted, the list of planned participants is not given in the present document.

The meeting works towards agreeing on an extension and up-date of the nomenclature of blood monocytes and dendritic cells. The up-date is targeted to be published in 2020.

Report on the Nomenclature of Monocytes and Dendritic Cells in Blood 2017

Use of the 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 November 30st 2017 this paper has been referenced more than 800 times (see www.researchgate.net/publication/45185387).
A search in the "www.monocyte.eu" webpage in 08 2016 gave 45 papers, which use the term "intermediate monocyte" in the abstract. This demonstrates that the definition of these previously overlooked cells in the nomenclature proposal has had direct impact on research.

A pubmed search using the terms "classical monocytes", "nonclassical monocytes" and "intermediate monocytes" resulted in 3.050, 740 and 1250 publications. This confirms that the monocyte nomenclature is being widely used even without citing the original paper in Blood.

As of November 2017 the term "CD1c myeloid DC" was used in 80 publications and "CD1c DC" was used in 456 publications.
The high number of references to the original nomenclature paper and these additional indicators of use of the nomenclature terms indicate that the new nomenclature is now well established for monocytes, while for blood DCs it is still in flux.

Promoting the nomenclature
The nomenclature had been initially published **in Blood.2010; 116: e74-e80**.
In Wikipedia.org in the section monocytes the paragraph "monocyte subpopulations" was introduced and under the dendritic cell section we added a paragraph with the title "dendritic cells in blood". This was done along with the relevant references. Please see http://en.wikipedia.org/wiki/Monocyte and http://en.wikipedia.org/wiki/Dendritic_cell.
The nomenclature was also promoted in three review articles covering monocytes:
Ziegler-Heitbrock, L, Hofer, TPJ: Toward a refined definition of blood monocytes, Frontiers in Inflammation, 4: 23, 2013
Ziegler-Heitbrock, L: Monocyte subsets in man and other species Cellular Immunology 289 (2014) 135–139)
Ziegler-Heitbrock, L: Blood monocytes and their subsets: established features and open questions in (August 2015 | Volume 6 | Article 423)

Updating the nomenclature: Monocytes

6-sulfo LacNAc+ (slan+) cells

The monocyte nature of slan+ blood cells has been pointed out already early on Siedlar, Immunobiology, 2000. For the 6-sulfo LacNAc+ (slan+) cells a few reports still address these cells as dendritic cells (Dobel, Blood, 2013 , Micheletti, JLB, 2013, Bsat, J Leuk Biol, 2015, Thomas Neurol Neuroimmunol Neuroinflamm. 2014, Rutella, J Transl Med. 2014, Brunner J Allergy Clin Immunol. 2013, Jähnisch, Cancer Lett. 2013) albeit they do not provide evidence regarding the nature of the slan+ cells.

On the other hand expression of slan on CD16 monocytes has been confirmed (Dutertre, Blood. 2012). In a comprehensive study slan was shown to define non-classical monocytes versus intermediate monocytes and hierarchical clustering experiments show that they are distinct from CD1c+ myeloid DCs (Hofer et al, BLOOD, 2015). An additional transcriptomic analysis has shown that the slan+ cells are distinct from both CD1+ DCs and CD141+DCs (Leeuwen-Kerkhoff, J Leuk Biol 2017).

The evidence regarding the allocation of slan DCs to the non-classical monocytes will be presented at the next Committee Meeting for discussion. Also the question is as to whether slan can be used to define human non-classical monocytes.

Non-classical monocytes

Using the slan marker a subdivision of the CD16+ monocytes has been suggested (Hofer Blood 2015, see Figure) and it is proposed that the slan+ cells may be the proper non-classical monocytes.

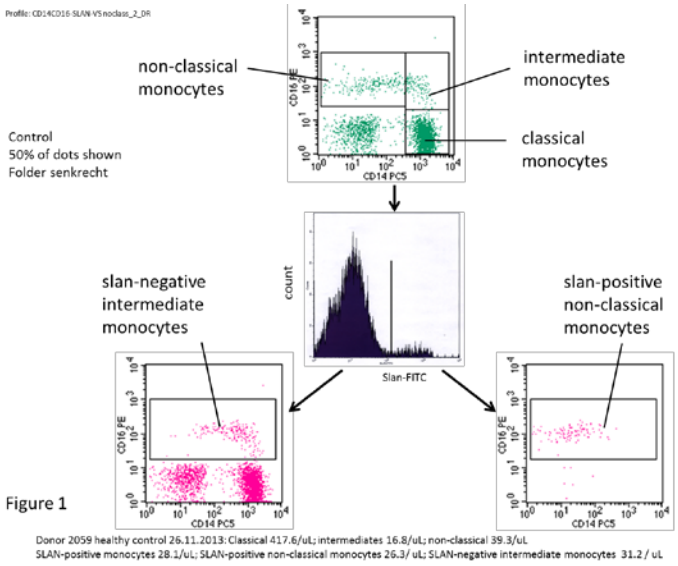


Figure 1

The study also showed a selective increase of the CD14++CD16+slan-negative intermediate monocytes in sarcoidosis and a selective depletion of the CD14+CD16+slan+ non-classical monocytes in the neurodegenerative disease HDLS (Hofer, Blood, 2015).

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) and in the mouse 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. This included transcriptome studies which show unique signatures (Wong, Blood, 2011, Zawada, Blood, 2011) and several reports on their increase in clinical settings and a study of their induction by TLR7/8 ligands in non-human primates (Kwissa Blood, 2012). 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 definition of intermediate monocytes is easy with respect to CD16 expression since here an isotype control can be used to define the cut-off versus the classical monocytes. The cut-off versus the non-classical monocytes is to some extent arbitrary and it often cuts right through what appears to be a homogenous cell population (see Figure)

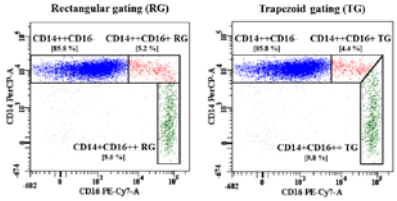


Figure 1

When an oblique line is used to dissect the non-classical and intermediates then this can slightly improve the predictive power of the intermediates (Zawada, Cytometry A. 2015 Aug; 87(8):750-8). However, the difference is too small in order to suggest this as the appropriate strategy.

An improvement of the definition of intermediate monocyte may come with the use of an additional marker as suggested by Wong et al (Immunological Research, 2012).

An approach that uses of 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 recent study has shown a clear separation of these cells from the non-classical monocytes 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 expressing the CSF3R (a typical neutrophil gene) and one expressing the IL2R beta chain, CD2 and CD3zeta (typical T cell genes). The nature of these cells still needs to be determined.

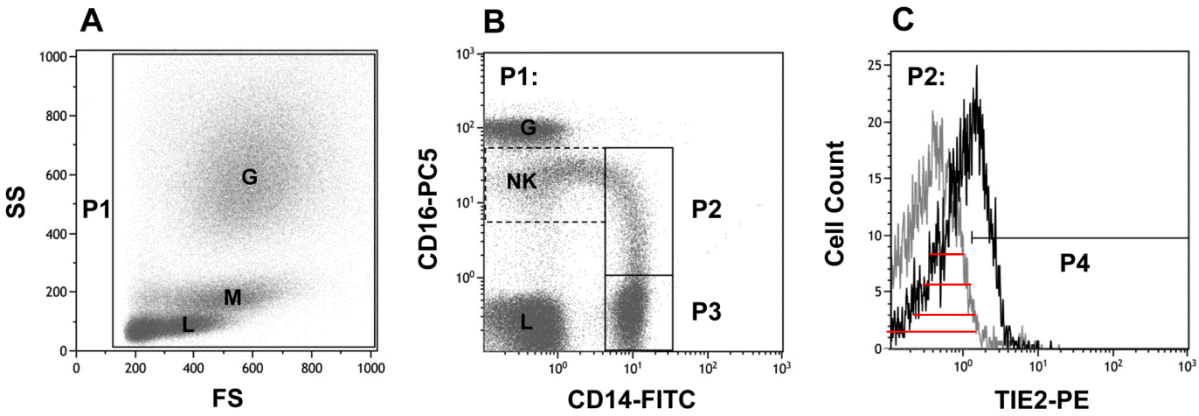
SCS of intermediate mouse monocytes (Mildner et al, Immunity. 2017) has uncovered two cell clusters, one that is in between classical and non-classical monocytes for all genes and one that has unique features with high class II, CD74 and DC-SIGN. This would mean that within this gate we are dealing with 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). The recent study by Zawada et al has shown that the intermediate monocytes are the cells with a higher tie2 protein expression.

Recently several additional papers on the Tie2 cells have appeared (Schauer, Plosone 7: e4450, 2012; Matsubara, Hepatology, 57:1416, 2013; Goede, Cancer Invest, 30:225, 2012, Patel, EMBO Mol Med, 5:856, 2013). Schauer (Plosone 7: e4450, 2012) noted that intermediate monocytes and not TIE2+ monocytes can be used for diagnostics of colon cancer. Here TIE2 expression was determined within the intermediates and only the cells, which localize to the right of the isotype control, were taken as positives (see below). This is, however, a debatable approach since also the cells overlapping with the isotype control are positive (see red shaded area).



Along these lines Fiocari et al reported that ibrutinib can induce TIE2 expression on CD14+ monocytes (Fiocari , Oncotarget 2016) but there again was a weak staining of all monocytes and no distinct population in patient blood. Also, the few recent 2017 studies on TIE2 do not describe a discrete population of monocytes (Wang et al, Br J Cancer, 117, 1371, 2017, Mao et al PLoS One. 2017; 12(9): e0183880). These data confirm the notion that TIE2-expressing monocytes are not a distinct cell population. This is to be discussed by the Committee.

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.

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.

Menezes et al (Immunity 2017) looking at mouse bone marrow analyzed CD115+Ly6Chi cells, which, however, included some Ly6C medium cells (Fig1A of that paper).They describe an additional monocyte subset i.e. CD11c- FLT3+ cells (R2 and Cluster3) that is also found in blood at low levels. Of note, the CD11c- FLT3+ cells (R2) are FLT3L independent and they express DC-SIGN transcripts and after GM-CSF they express DC-SIGN protein. What has not been addressed is whether the R2 cells are intermediate monocytes.

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 (see Fig 2, Jakubzick, Immunity, 2013). Whether the differential Class II expression helps in defining additional subset of monocytes remains to be analyzed.

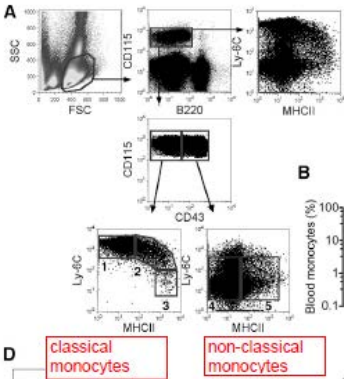


Fig2, Jakubzick, Immunity, 2013

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 CD14high DRlow/- 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).

Of note in recent single cell sequencing studies using blood myeloid cells no cell type with the properties of “MDSC” has been detected (Villani, Science. 2017;356(6335), Mildner, Immunity. 2017 May 16;46(5):849-862). Taken together the current evidence does not support inclusion of MDSC into a blood monocyte subset nomenclature at this point in time.

A new candidate has emerged with cells showing high CD14 and no CCR2, i.e. CD14++CD16- CCR2- monocytes (Gama et al JLB, 2012). These cells increase with immunodeficiency virus infection and they are functionally distinct from the CD14++CD16- CCR2+ monocytes. The study by Gama has been referenced 25 times by 2017 but there are no further experimental studies on these cells. They may overlap with what is termed myeloid-derived suppressor cells.

FcepsilonRI+ monocytes

Regarding the FcepsiolonRI+ monocytes a substantial number of these cells (2.5% of all monocytes) was noted to occur in children (Dehlink, E, PLoSOne 5:8, e12204 (2010)) and these cells were shown to be involved in clearance of serum IgE (Greer AM, J Clin Invest (2014) **124**:1187–98, Shin&Greer, Cellular and Molecular Life Sciences, 2015, 72: 2349-2360). While there is little activity in this field the FcepsilonRI+ monocyte is consolidating and its inclusion among the monocyte subsets can be discussed at the next Committee meeting and nomenclature update. For this, the enumeration of these cells in a large cohort of cases and controls with a standardized flow cytometry approach and the molecular characterization of these cells by e.g. transcriptome analysis would be desirable. In this context LZH has contacted Dr. J.S. Shin from UCSF, San Francisco, CA).

Updating the nomenclature: Dendritic Cells

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

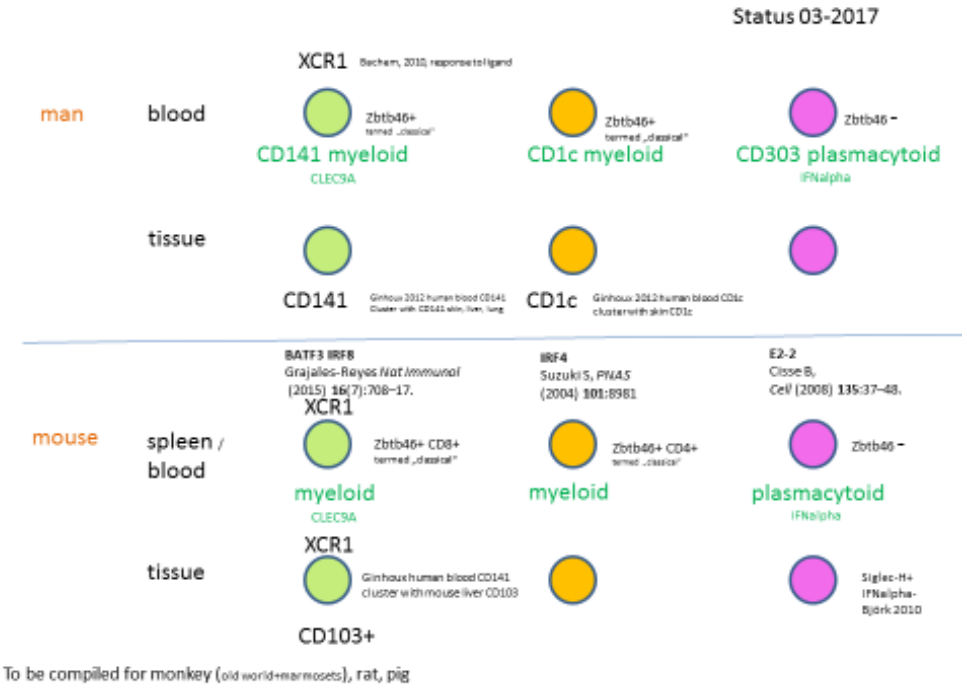
For the CD1c+ myeloid DC in human blood it had been noted earlier that there are CD14- and CD14low cells (see the BLOOD-2010 Nomenclature proposal) and these have not been characterized further. A recent study (Bakdash, de Vries Cancer Research, 2016) has described CD1c+ CD14+ cells to be expanded in 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 CD5low and CD5high (Yin et al J Immunol. 198: 1553, 2017). The CD5high 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-CD5hi and b) CD14+ CD5lo. 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. The expression of MAFB by these cells would support a monocyte nature. Comparative transcriptomics with a large set of prototypic monocytes and dendritic cells may help in making that decision.

CD303+ pDCs

Plasmacytoid DCs have been characterized in man by expression of CD303 and also by high level CD123.

Since in the rat subsets of pDC have been described, a closer look at heterogeneity of these cells in man is warranted. An indication of such heterogeneity is given in Guilliams (Immunity, 2017) where expression of CD2 and CD5 is shown on a subset of pDCs in human blood (see Fig 6 in Guilliams 2017). pDCs are characterized by high IFNalpha production. When subsets of pDCs fail to produce IFNalpha then the question is whether this disqualifies them as pDCs. In other words what needs to be discussed is whether phenotype or function dominates in the assignment to pDCs.



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 can be refuted or substantiated with newer data. Please note, mouse DCs will be detailed further below.

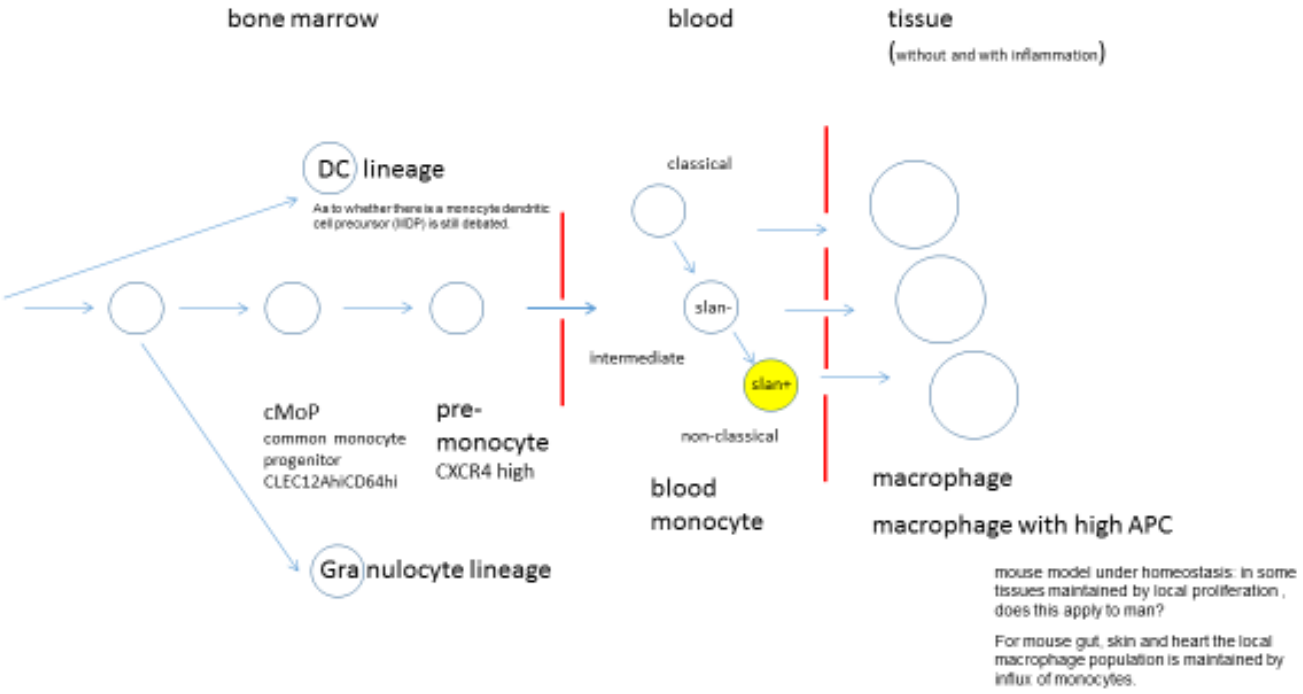
SCS candidates

Villani et al (Science, 2017) have search 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). DC4 are CD16-postive and likely are non-classical monocytes contaminating the DC-preparation of cells. A novel population are cells called AS DCs (DC5), which are positive of genes AXL and SIGLEC6. All of this needs confirmation by independent studies but it is expected that over the next 1-2 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+CD135low in the mouse (Hettinger, Nature Immunology,2013) more recently a CLEC12AhiCD64hi 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.



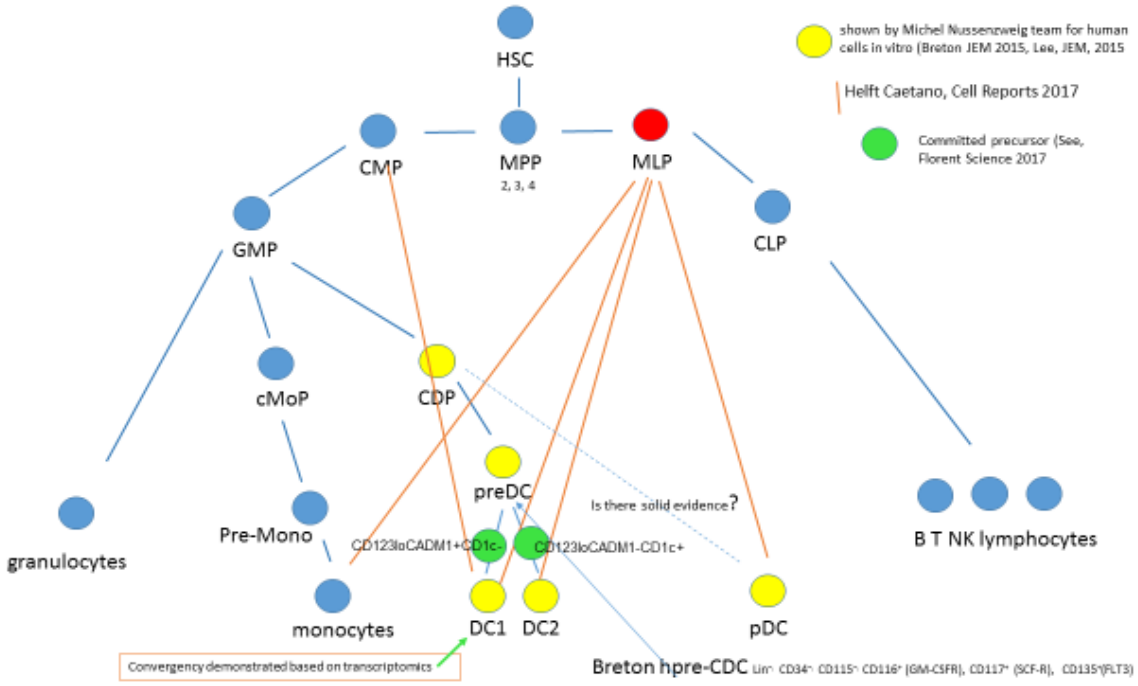
DC Precursors

In the mouse, a macrophage dendritic cell precursor (MDP) has been postulated. However, the existence of these cells has been disputed (Sathe, 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 by the team of Michel Nussenzweig (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). Cell generated from the two progenitors show an identical transcriptome (convergent differentiation).The study by Breton et al has described 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* 10.1126/science.eaag3009 (2017) has proposed a separate pre-DC for DC1 and DC2 each. Here pre-DC1 (CD123loCADM1+CD1c-) and pre-DC2 (CD123loCADM1-CD1c+) that are committed to generate DC1 and DC2, respectively.
With more data being published the working scheme (see below) may clear up.



Other species: monocytes and DCs in blood of non-human primates
As mentioned in the original nomenclature document most 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-positve monocyte subset was originally reported for Macaca fascicularis by Munn et al (Blood 1990) and Otani (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).
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 mDCs 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). Since in man patrolling behaviour has been shown for classical monocytes, it has become clear that the “patrolling” nomenclature for non-classical monocytes is misleading and should not be used.

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).

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+ (Sirpalpha+, 70%) and CD172a- (Sirpalpha-, 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+XCR1high CD72alow cells (DC1) and CD11c+CD26+ XCR1low CD72ahigh cells (DC2) could be identified.

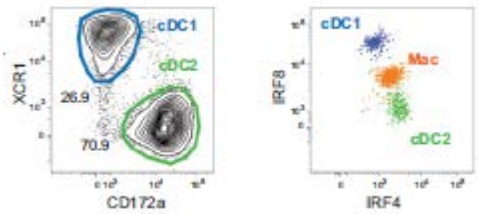


Fig 1 Guilliams et al 2017

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 $D11b^{lo}120G8^{hi}$ $CD172a^{int}$ $B220^{int}$ $Ly6C^{int}CD11c^{int}$ 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 below 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+CD43low classical and CD68+CD43high non-classical monocytes, with classicals being CD4 low and non-classicals CD4high 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 CD43lo His48hi. 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. 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 IFNalpha by pDCs. CD4+ and CD4- subsets were confirmed in spleen (Stojić-Vukanić Biogerontology, 2013).

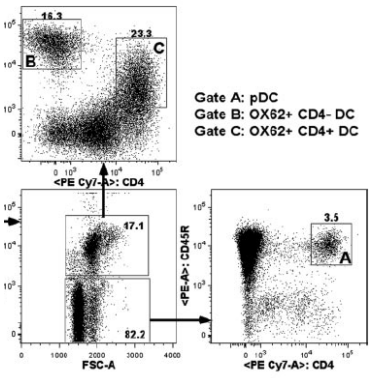


Fig 1, Hubert, J Immunol 2006

More recently a monoclonal antibody against rat pDCs (85C7) Anjubault (PLOSone, 2012) described a CD4low 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 CD4lo 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, Immunupathol, 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 CD172low CD4+ DCs and CD172low 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 (Vetenary 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.

Nomenclature covering macrophages and dendritic cells in **tissue** 2017-11-28

Given below are deliberations on aspects that have developed over the last couple years and that have been discussed with relevant colleagues including some members of the committee and this may result in a few general statements in the final document. However, a nomenclature for tissue cells is not the aim of the update workshop.

Ontogeny and Transcriptomics

There has been an interest in resolving some of the nomenclature issues for tissue macrophages and dendritic cells. Here a core group consisting of Florent Ginhoux, Shalin Naik and Martin Guilliams has suggested to consider ontogeny, i.e. to distinguish cells based on the different precursors they derive from (see Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, Segura E, Tussiwand R, Yona S., Nat Rev Immunol. 2014;14:571-8.)

As suggested by Pablo Engel, I have started discussions with these colleagues via detailed e-mail exchange **(**Florent Ginhoux,(e-mail 01.11. 2014), Marc Dalod (e-mail 22.10. 2014), Martin Guilliams (e-mail 10.11. 2014)**)**.

In parallel several articles were written (altogether 17) by the lead scientists and additional colleagues and published in Frontiers in Immunology under the title: "*Dendritic Cell and Macrophage Nomenclature and Classification*". LZH has contributed a paper with the title "*Blood monocytes and their subsets: established features and open questions*".
At this stage this initiative has not resulted in a consensus document but only in an editorial summary of the individual contributions.
However, in the exchange with Ginhoux, Naik, Dalod and Guilliams we have made substantial progress.

Important issues in these discussions:

One helpful notion that has emerged is that monocyte-derived dendritic cells need to be separated from bona fide dendritic cells.

Also it was discussed that if ontogeny is important for the eventual function of a cell then this needs to materialize in the gene expression of a cell and this can be determined via omics like the transcriptomics. Therefore transcriptomics is considered the adequate tool for cell classification by many contributors of "Dendritic Cell and Macrophage Nomenclature and Classification" topic in FRONTIERS IN IMMUNOLOGY.

One discussion point is that much of the concept is based on mouse data and before a nomenclature can be agreed there is an absolute need to confirm it in man or non-human primates. I have pointed out that there are many examples of an entirely different biology between man and mouse and have introduced into the discussion the slogan "avoid the mouse trap". Scientists who restrict themselves to mouse studies but extrapolate the findings to all mammalian species are caught in the mouse trap. I will continue to make a strong point of this.

When we want to use ontogeny as a central point in the nomenclature then we have to keep in mind that a common progenitor is not sufficient to assign common names to the progeny. An informative example is the megakaryocyte-erythrocyte-progenitor (MEP) cell, which gives rise to either megakaryocytes and their platelet progeny or to erythroblasts and their red blood cell progeny (Debili, Blood 88 (1996) 1284-96. Klimchenko, Blood 114 (2009) 1506-17). Megakaryocytes and erythroblasts have a distinct transcriptome (Macauley, Blood 109 (2007) 3260-9) and they are involved in distinct

functions, i.e. in blood clotting and oxygen transport, respectively. Therefore, although having a common ontogeny, these cells belong to clearly separate lineages.

On the other hand different precursors may give rise to similar progeny (convergence) and it may not be relevant to distinguish such cells. With respect to macrophages in various tissues the question is whether an embryonic/fetal origin of these cells as opposed to a derivation from adult monocytes will make a relevant difference. Several studies in the mouse model now have shown the embryonic or adult origin has little contribution:

Lavin et al (Cell, 2014) did show after eradication of embryonic derived macrophages in mice with lethal irradiation followed by replacement with adult bone marrow that the adult bone marrow-derived new macrophages show very similar enhancer landscapes compared to the embryonic-derived macrophages (see lung and peritoneum Fig 6 in that paper).

Gibbing et al (Blood, 2015) showed that after clodronate depletion of lung macrophages new alveolar macrophages, which derived from adult bone marrow, have 98% identical transcriptomes to the resident alveolar macrophages, which are of embryonic origin with a few exceptions (MARCO being much stronger on embryonic derived AM).

van den Laar et al, Immunity, 2017, in elegant studies have transferred yolk sac macrophages, fetal liver monocytes and adult monocytes into the lungs of neonatal CSF2rb-/- mice. Macrophages developing from these different precursors were shown to exhibit an identical transcriptome. Hence, at least for alveolar macrophages the embryonic and the adult origin of precursors is not relevant to the development of these cells.

Along these lines, in man bone marrow transplantation in two patients with alveolar proteinosis has led to resolution of lung opacities and recovery of lung function, indicating that functional alveolar macrophages have developed from the transplanted bone marrow cells (Tanaka-Kubota et al, Int J Hematology, 2018).

Hence, within the monocyte/macrophage lineage ontogeny does not appear to be relevant to the properties of tissue macrophages. Macrophages derived from different progenitors converge in their transcriptome and function to become tissue specific cell types

On the other hand it appears that ontogeny may help in dissecting DC and macrophage lineage.

Here the Frontiers in Immunology Contribution by Durand and Segura (*The known unknowns of the human dendritic cell network*, Frontiers in Immunology: March 2015 | Volume 6 | Article 129 | 178) has pointed out that for several types of tissue cells, which are currently termed DCs, the transcriptomic analysis suggests that these are rather macrophages:

- CD14+CD1clow DCs in human skin are macrophages (McGovern,-Haniffa Immunity 2014)), here transcriptomics defines lineage
- CD103-CD172+ intestinal human DCs cluster with blood monocytes (Watchmaker, Nat Immunol 2014), here transcriptomics defines lineage.
- inflammatory DCs (human rheumatoid synovial fluid) similar to in-vitro monocyte-derived DCs (Segura, Immunity 2013) , here transcriptomics defines lineage.

Also for in-vitro generated progeny from monocytes transcriptomic analyses have shown that cells called monocyte-derived dendritic cells (MoDCs) have a transcriptome akin to monocytes and not to dendritic cells (Mabbott, Immunobiology. 2010 ;215:724-36). Defining MoDCs as high APC macrophages will be an important step and will settle much debate in the field.

A consensus to use for all monocyte-derived cells the term macrophages would already be a major step. This especially applies to the many monocyte-derived cells currently called DCs.

It has become clear that unbiased –omic data are an important tool for the definition of cells. What is currently used is transcriptomics and in addition proteomics, glycomics, metabolomics and epigenomics will have to be studied.

The M1 and M2 macrophage nomenclature

The M1 M2 concept has been challenged and was summarized in a consensus statement (Murray et al, Immunity,41: 14, 2014). M1 and M2 cells are macrophages that have been activated by IFNg and IL-4, respectively. As such they are not different macrophage cell types but just cells that have been activated by different signals.
In essence it has been agreed that M1 and M2 rather represent extremes of a spectrum of monocyte derived cells that have been stimulated with different sets of cytokines with the prototypic examples of IL-4 and IFNgamma. This consensus statement does not lead to a nomenclature that can be easily married with the above initiative to define tissue macrophages. Rather the endpoint will be that an IL-4 signature or an IFNgamma signature or an IL-10 signature can be detected with omic approaches in the various types of tissue macrophages.

Monocytes in tissue

When monocytes go into tissue then they differentiate into macrophages (or cells still addressed as DCs). One report has shown that in the steady state there are cells in the lymphnodes, which are akin to their blood monocyte progenitors (Jakubzick, Immunity, 2013). However there are 84 immune response genes that are >3 fold increased in these cells including CD1Q, CCR5, CCR7, C3AR1, CIITA,CD74, CD14, CD38, CD83, IFIT2, TLR1, osteopontin, IL1R2, and many chemokines (CCL2, CCL3, CCL5, CXCL2, CXCL9, CXCL10, CXCL13, CXCL16) . Similarly transcriptome analysis (2369 genes) of emigrated monocytes in the mouse skin showed clustering with blood monocytes (Tamoutounour, Immunity 2014). Still these cells showed up-regulated lysozyme and CD68, which is characteristic of macrophages. The question is whether the assignment to monocytes versus macrophages should be done based on PCA using all differential genes or whether a restricted set of genes and markers ought to be employed. Also, comparable analyses are needed in man. To be discussed by the committee but no recommendation planned since tissue is not within the scope of the committee.

There is the flawed concept that non-classical monocytes do not go into tissue: in the mouse these cells have been dubbed patrolling monocytes, which have as a main function the crawling on the endothelium in order to remove damaged endothelial cells (Auffray Science, 2017). The authors, who put forward this concept, have shown that a) in man classical monocytes also show crawling behavior and b) in the mouse non-classical monocytes go into the peritoneum within minutes of an inflammatory signal. Still, the crawling patrolling behavior was used to champion the concept that classical monocytes go into tissue and non-classical do not. This concept has been repeated in many reviews and is echoed in many publications as a fact. This scenario nicely demonstrates how a flawed nomenclature can direct our thinking, which then has a direct impact on what research is being done. Meanwhile a whole series of mouse studies have shown that non-classical monocytes do go into tissue without and with inflammation (Misharin, Cell Reports 2014, Saja, Cell Reports 2015, Olingy, Scientific Reports 2017).

There are several reports shown slan+ cells in various tissues (e.g Toma, Oncoimmunology 2015). The open question is: Are the slan+ cells in tissue derived from slan+ blood monocytes or do they represent cells that have up-regulated the slan-marker via induction of the enzyme CHST2?