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The mononuclear phagocyte system in organ transplantation

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mononuclear phagocyte system (MPS)

dendritic cells (DCs

plasmacytoid (pDCs),

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common DC progenitors (CDP)

Mononuclear Phagocytes (MPs)

MHC class II (MHC-II)

local complement C3 (C3)

hypoxia inducible factor-1alpha, HIF-1α),

macrophage/DC precursor, MDP)

myeloid DCs type 1, MDC1

myeloid DCs type 2, MDC2

NK, natural killer

Abstract

The mononuclear phagocyte system (MPS) comprises monocytes, macrophages and dendritic cells (DCs). Over the past few decades, classification of the cells of the MPS has generated considerable controversy. Recent studies into the origin, developmental requirements and function of MPS cells are beginning to solve this problem in an objective manner. Using high-resolution genetic analyses and fate mapping studies, three main mononuclear phagocyte lineages have been defined, namely: macrophage populations established during embryogenesis, monocyte-derived cells that develop during adult life and DCs. These subsets and their diverse subsets have specialized functions that are largely conserved between species, which justifies the introduction of a new, universal scheme of nomenclature and provides the framework for therapeutic manipulation of the immune responses in the clinic. Here, we comment on the implications of this novel MPS classification in solid organ transplantation.

Introduction

Organ transplantation generally involves the replacement of a failing organ with an allograft bearing non-self antigens. The immune system is able to recognize and mount an immune response against non-self protein and carbohydrate antigens, which results in allograft rejection unless immunosuppressive treatment is administered. While the progress of immunosuppressive therapy

has dramatically improved the short-term results of organ transplantation, no immunosuppressive agent is devoid of side effects, and despite multiple studies have been performed, no alternative regimen has seriously challenged the almost universal use of these non-specific immunosuppressive drugs.

Research in transplantation immunology is focused on developing novel regulation-inducing protocols using different experimental models that attempt to use the current available immunosuppressive agents in a less toxic manner. Historically, transplant immunologists have attempted to develop such protocols by targeting adaptive immune response mechanisms, based on the early observation in by Jacques Miller, which proved that T cells mediate allograft rejection (1). However, the induction of long-term allograft survival cannot be fully explained by mechanisms that target only the adaptive immunity such as deletion of effector T cells (2, 3), suggesting that additional levels of immune regulation need to be studied.

Recent advances in our understanding of how the alloimmune response is influenced by variety of antigen non-specific responses have highlighted the critical role of the innate immune system in shaping the adaptive immune response towards immunity or tolerance. Elegant studies from Lakkis and colleagues demonstrated that the innate immune system is able to mount an immune response against allogeneic non-self that initiates allograft rejection independently of lymphocytes (4, 5). Consistent with these studies, Xian Li and colleagues reported that phagocytic macrophages mediate rejection of allogeneic non-self in presensitized hosts (6). On the other hand, tissue resident and monocyte-derived macrophages have been reported to promote prolonged allograft survival by preventing alloreactive T cell immunity and promoting Treg expansion (7-10). This suggests that a more accurate understanding of the regulatory mechanisms that govern the innate immune system may provide novel therapeutic targets and make organ transplantation a more effective procedure. Among these novel therapeutic strategies that target the innate immune response is manipulation of cells of the mononuclear phagocyte system (MPS).

The MPS includes different subsets of monocytes, macrophages and dendritic cells (DCs) at different stages of differentiation and activation. These cells participate in ischemia reperfusion injury, vascular trauma, tissue repair, antigen presentation, rejection and tolerance induction. In organ transplantation, macrophages and DCs within tissues originate from two sources: graft-resident

macrophages and DCs that are transferred to the recipient as passenger leucocytes at the time of transplantation; and recipient monocytes and DCs that migrate into transplanted organ. A better understanding of the MPS immune biology will therefore open new avenues for therapeutic intervention, either by inhibiting their function or by enhancing their immune protective properties. The efficacy and success of mononuclear phagocyte immune therapy depends on our ability to manipulate specific cell subsets, but finding a phenotypic profile that characterizes each mononuclear phagocyte subset and elucidating their developmental needs is complicated. Using surface markers to separate resident DCs and macrophages from recruited monocyte-derived cells in the transplanted organ under inflammatory conditions can be confusing. A new MPS classification proposal attempts to resolve this problem by paying careful attention to ontogeny as the first basis of classification (11). Here, in light of this new MPS classification, we provide a historical overview of the MPS in the context of solid organ transplantation, and suggest comprehensive list of phenotypic markers that may be used to identify major subsets that define the MPS.

Historical Background

Ralph van Furth and Zanvil Cohn first described the origin and kinetics of the mononuclear phagocytes by demonstrating that the promonocytes proliferate and differentiate in the bone marrow after which they enter the circulation as monocytes and migrate to the tissues where they are called macrophages (12). These studies led to the classification of the mononuclear phagocyte system (13), which initially included macrophages, monocytes, and their precursor cells based on the morphology, function, and kinetics of these cells (14). With the identification by Ralph Steinman and Zanvil Cohn of a novel cell type with the functional ability to stimulate T cells in an allogeneic mixed leukocyte reaction (15, 16), the MPS nomenclature DCs (17). The classification of the MPS generated considerable controversy over several decades, as confusion arose from the use of nonspecific cell surface markers, such as CD11c or F4/80, as a definitive surrogate marker for lineage of mononuclear phagocytes in lymphoid and non-lymphoid organs under steady state and inflammatory conditions. The functional plasticity of mononuclear phagocytes facilitates their adaptation to environmental factors present in the tissue, but results in marked alteration of their cell surface antigens, which makes their phenotypic characterization a difficult task, especially under inflammatory conditions. In addition, there has been a tendency to oversimplify descriptions of the MPS. Historically, researchers classified monocyte-derived macrophages as inflammatory/classical/M1 versus anti-inflammatory/alternative/M2 macrophages based on the

effects of IFNγ and/or LPS versus IL-4 on macrophage gene expression and their ability to determine the immunological outcome by promoting a Th1- or Th2-type response (18, 19). However, recent data indicate that this bipolar system ignores much greater complexity in macrophage activation (20). As a result, consensus on which markers should be used to identify the MPS, and unified guidelines to analyze DCs, monocyte-derived cells and macrophages are of critical importance to experimental biology and clinical studies.

There are now two main issues with the MPS as originally conceived: that DCs are an independent lineage to monocytes, and that tissue-resident macrophages do not require monocytes for homeostasis. The recognition of different DCs and monocyte subpopulations and the necessity to characterize these subsets phenotypically led to a nomenclature proposal under the auspices of the International Union of Immunological Societies and the World Health Organization (21). This classification focused on DCs and monocytes present in human blood under steady state conditions, dividing blood DCs into plasmacytoid (pDCs), CD1c+ myeloid, and CD141+ myeloid DCs, and monocytes into classical, intermediate, and non-classical monocytes based on their surface markers. A different nomenclature that focused on macrophages proposed eight different macrophage categories based on three principles: the source of macrophages, the culture conditions to generate them in vitro, and the collection of phenotypic markers that describe their activation state (22). This classification aimed at establishing a common macrophage nomenclature and provided recommendations for experimental standards to define macrophage polarization. The latest nomenclature proposal includes DCs, monocyte-derived cells and macrophages and is based on a two-stage system, proposing that mononuclear phagocytes should first be defined based on their ontogeny (level one), and that these cells can be subsequently defined based on their function, location and/or morphology (level two). A guideline to define new mouse and human subsets without loosing flexibility is also provided (11). This classification reflects recent advances in the immune biology of human MPS, which revealed that the human and murine MPS have functionally and phenotypically similar populations (23-26). Thus, it was proposed that the cells of human and mouse MPS should be categorized in the same fashion and named in unified strategies, based primarily on their origins and further subcategorized based on their transcriptional dependencies for their differentiation and localization (11). Guilliams and colleagues contend that the cells of MPS should be categorized into three groups, based primarily on the ontogeny of the each population: macrophages derived from embryonic precursors; monocyte-derived cells differentiated from common monocyte progenitors; and DCs derived from common DC progenitors (CDP) (11). DCs are

further subdivided into three groups based on their transcriptional dependencies: BATF3-dependent conventional DC (cDC1), best described as $XCR1^+CD141^+$ DC in human; IRF4-dependent cDC2, best described as $CD1c^+$ DC in human; and E2-2-dependent pDC. The division of DCs into cDC1 and cDC2 has functional implications because cDC1 are specialized to cross-present antigens to CD8+T cells and have a higher capacity to stimulate Th1-mediated immune responses (23), while cDC2 are required for Th2 and Th17-mediated immunity (27, 28). These differences are not absolute and may be modified by TLR stimulation (29) and the type of pathogens. Macrophages are more flexibly subcategorized based on their localization, phenotype, and functions, in addition to some historical nomenclature, such as Kupffer cells in the liver (30). This nomenclature reclassifies some populations; for instance, Langerhans cells in epidermis are now categorized as macrophages because of their origin arising from fetal liver monocytes (31, 32), despite their migratory and potent antigen-presenting capacity, which is normally associated with the DC lineage (32, 33). In addition, embryonic-derived macrophages and monocyte-derived macrophages now represent two different lineages. Here, we comment on the implications of this novel nomenclature proposal in solid organ transplantation. While it may be difficult to reclassify previous transplantation studies in accordance with Guilliams' classification, as limited phenotypic information was originally reported, we provide suggestions to reclassify future studies according to the recent advancements in the recognition of the different lineages that constitute the MPS.

Donor Mononuclear Phagocytes (MPs)

Macrophages and DCs are normal constituents of tissue stroma (34), where they serve vital functions in maintaining tissue homeostasis by eliminating necrotic cells and preventing inflammatory responses against innocuous stimuli (35). Under steady-state conditions in the absence of inflammation, tissue-resident DCs continuously migrate to the lymphoid tissues via afferent lymphatics where they contribute to the maintenance of peripheral T cell tolerance of self and other non-harmful antigens (36, 37).

Early work from Hart and Fabre characterized MHC class II $(MHC\text{-}II)^+$ expressing DCs in the rat tissue. Using immunofluorescence and histochemical analysis, they demonstrated that $MHC\text{-}II^+$ DCs were widely distributed in the interstitial connective tissues (heart, liver, pancreas, thyroid, skin, skeletal muscle, kidney, ureter, and bladder) except for the brain (38). Shortly afterwards, DCs and

macrophages isolated from different rat tissues were distinguished by nonspecific esterase staining, phagocytic capability, expression of MHC-II and Fc receptors, and their ability to stimulate T cells *in vitro* (39). Jonathan Austyn further characterized DCs in mouse hearts and kidneys using two-color flow cytometry, demonstrating that MHC-II$^+$ cells co-stained for macrophage antigens CD68, CD32 (FcγRII) and F4/80, and the restricted DC markers CD11c, CD205 (DEC-205) or DCIR2 (33D1) (40). These cells exhibited immunostimulatory properties as measured by oxidative mitogenesis and stimulation of allogeneic mixed leukocyte responses; hence they would likely have contained CD11b$^+$ cDC2 or monocyte-derived DCs in non-inflamed tissues (41). More recently, Ginhoux et al reported that tissue-resident CD11c$^+$MHC-II^{hi} DCs consist of two main subsets (CD103$^+$ and CD11b$^+$ DC) with different origin, developmental requirements, and antigen presenting function (42). As described in the classification by Guilliams' classification (11), the CD103$^+$ cell is now known to be a member of the cDC1 family of classical (43) or myeloid (human) DCs, dependent upon Batf3 and IRF8, and expressing XCR1. The identity of the CD11b$^+$ DCs is more complex as CD11b is also expressed on monocyte-derived cells and some resident macrophages. Several recent detailed analyses have succeeded in resolving CD11b$^+$ DCs from monocytes and macrophages using additional markers such as CD64, CCR2 and MerTK (Immunological Genome Project-ImmGen.org) (30, 41). Most previous work using CD11b alone to profile DCs or macrophages requires some degree of re-interpretation based on these results.

Characterization of tissue-resident DC subsets has also been documented in humans. Early characterization of myeloid cells in the human liver led to the differential phenotypic characterization of DCs (44), while characterization of human renal DCs led to the identification of two main subsets: myeloid CD1c$^+$DC-SIGN$^{+/-}$HLA-DR$^+$ and CD303$^+$HLA-DR$^+$ (45). Using the new recommended nomenclature the myeloid CD1c$^+$ cDC2 subset would be classified as DC-SIGN$^+$ monocyte-derived cells, as the existence of cDC1 is difficult to infer in the absence of specific markers because some cells also co-express CD1c. Lymphoid DCs expressing CD303$^+$HLA-DR$^+$ would be classified as pDC. Moreover, the new classification recognizes the subset of myeloid DCs dedicated to cross-presentation identified by CD103 in the mouse, CD141 in the human and by expression of XCR1 in all species studied so far.

Tissue-resident macrophages develop from the yolk sac (brain), from the fetal liver (lung, spleen, kidney, liver heart, intestine, and skin), or from both origins (epidermal Langerhans cells) (31, 32). However, some of them will be replaced after birth at different kinetics by adult bone marrow monocytes (intestine, skin, and heart) (33, 46-48). Under homeostatic conditions, murine tissue

resident macrophages can be widely characterized by CD11b, F4/80, CD68, and low levels of MHC-II on their cell surface. Likewise, most human tissue-resident macrophages express CD11b, CD33, CD68 and MHC-II, whereas expression of CD163, CD163L1, CD169, factor XIIIA, LYVE-1, CD204, CD206, CD209 and MARCO is restricted to particular subsets or non-activated forms only (25, 49, 50). Similar to DCs, tissue-resident macrophages also contribute to the maintenance of peripheral a suppressive function by suppressing T cell activity under steady state, and several reports have documented the inhibitory function of brain microglia (51), lung alveolar macrophages (52), liver Kupffer cells (53) and lamina propria macrophages (54). Figures 1 and 2 summarize the donor MPS present in the organ before transplantation.

The percentage of tissue resident macrophages and DC subsets varies depending on the tissue under study, which suggests a different MP distribution and function following transplantation (33, 42). Tables 1 and 2 summarize the tissue distribution of mouse resident macrophages and DC subsets that are present in the donor organ at the time of transplantation. These tables also provide information about the half life and ontogeny of the resident macrophages and DC subsets, which suggest that differences in immunogenecity of the transplanted organs may be related, in part, to differences in the distribution of MPs subsets in the tissue. The gut and the skin are the most immunogenic organs and, unlike embryonic-derived tissue macrophages that seed the kidney, liver and lung, intestinal and skin macrophages derive directly from bone marrow circulating monocytes that continuously seed the intestine and the skin. Therefore, it is possible that differences in MPs subsets present in the donor tissue, together with the influence of the microbiota on the activation of MPs (55, 56), may account for the overall immunogenicity of the transplanted organ. This supports the hypothesis that differences in immunogenicity of transplanted organ may in part related to the origin and subset distribution of the MPs the donor tissue.

Activation of Donor MPs

Macrophages and DCs in tissues are exquisitely sensitive to inflammatory signals from their environment, even in the absence of infection, which drive their maturation to an immunogenic state (57). Ischemia reperfusion injury (IRI) following organ harvest has a profound effect on MPs present in the donor tissue at the time of transplantation. In an experimental murine model of IRI, Halloran and colleagues determined that MHC-I and MHC-II together with several cytokines

including IFN-γ, TGF-β, GM-CSF, IL2 and IL10, were upregulated in the kidney during sterile injury (58). In addition, Griffin and colleagues reported alterations to the kinetic and phenotypic characteristics of renal DCs that led to activation of antigen-specific T cell that mediate pathogenicity (59). This process is also mediated by TNFα, which is upregulated by renal DCs following ischemia reperfusion (60). The authors went on to demonstrate that DCs expressing $CD11c^{+}F4/80^{+}CD68^{+}MHC\text{-}II^{+}$ cells are a potent source of TNFα, IL-6, MCP-1, and RANTES during IRI. Therefore, ischemic episodes alone lead to the activation and accumulation of myeloid cells in the injured tissue (61). DC activation and migration to the lymph nodes where these inflammatory DCs prime T cells during IRI depends on TLR4 signaling (62). DCs exposed to IRI do not mature in the absence of TLR4, which also results in reduced neutrophil infiltration and increased cytoprotective heme oxygenase-1 (HO-1) (63). In a series of very elegant studies using CSFR1-GFP reporter mice, Duffield and colleagues determined that resident macrophages represent 30-40% of inflammatory macrophages present in kidneys damaged by ureteric obstruction (64).

Since macrophages play an injurious role during IRI (65), therapeutic approaches that prevent the inflammatory macrophages include the use of proinflammatory cytokine IL-18 inhibitors, which reduce production of CXCL1 in the injured kidney (66). Depletion of macrophages prior to ischemia-reperfusion injury of kidneys also reduces infiltration and preserves renal function in the short-term (67). Increasing HO-1 or its catabolic components decreases inflammatory mediators in the allografts and prolongs survival associated with reduced infiltrating $ED1^{+}$ macrophages during IRI (68-70). Elegant studies by Sacks and colleagues demonstrated that local complement C3 (C3) deficiency in the donor allograft prevented acute rejection (71), which suggest that impairing the complement system decreases donor MPs activation and Th1 priming. These findings are consistent with other studies demonstrating that complement deficiency or pharmaceutical blockade of complement activation could improve graft survival, especially by preventing donor MPs activation by complement components C3a and C5a (72-74). Other studies suggest that hypoxia-differentiated DCs overexpress hypoxia inducible factor-1alpha (HIF-1α), which induces CD40 expression and IFNγ secretion, leading to activated T cells causing an inflammatory response in the organ (75). Interestingly, pharmacological HIF-1α preconditioning of recipients, but not the cardiac allograft, results in anti-inflammatory effects with decreased influx of $ED1^{+}$ macrophages into cardiac allografts, leading to prolonged allograft survival (76). The precise role of HIF-1α and TLR4 during hypoxia and its putative beneficial role remain to be delineated, since these pathways may be

needed by suppressive macrophages to exert their immune protective role in organ transplantation (77, 78).

Activated Donor MPs in Transplant Recipients

Activated donor MPs initiate the acute inflammatory cascade that results in secondary tissue injury (79). Some activated donor DCs alter their expression of cytokines, costimulatory receptors and adhesion molecules then migrate into lymphoid tissues to stimulate T cells (80). MPs present in the donor organ migrate rapidly into the recipient secondary lymphoid organs (lymph nodes and spleen) shortly after transplantation, activating immune mechanisms through the direct pathway of allorecognition (81-84). These donor "passenger leukocytes" that presumably exit the transplanted organ through transendothelial migration (85) contribute to initiation of allograft rejection (86). Due to the potential immunogenic properties of direct allorecognition mediated by donor passenger leukocytes, several studies reported the benefits of depleting myeloid cells in donor organs before transplantation (87-89). Using $CD11c^{GFP\text{-}DTR}$ mice, Abdi and colleagues demonstrated that donor DCs express high levels of MHC-II and the costimulatory molecule CD86 in the spleen of recipient mice early after transplantation, rendering them potent initiators of alloimmune responses (90). Using donor/recipient MHC-I/MHC-II mAb, Matsuno's laboratory described three $MHCII^{+}CD103^{+}$ DC subsets of donor DCs in the transplanted liver in rats: $CD11b^{-}SIRP\alpha^{+}CD86^{+}$ DCs that stimulate systemic CD8 T cell responses; a large radio-resistant $CD11b^{+}SIRP\alpha^{+}CD86^{+}CD25^{+}$ DC subset thought to induce CD8 T cell responses in the allograft; and a small $CD11b^{+}SIRP\alpha^{-}$ subpopulation (91). Paradoxically, although elimination of donor-derived MPs prolongs allograft survival, deletion of passenger MPs also prevents induction of allograft tolerance (92-94). Using $CX3CR1^{GFP\text{-}DTR}$ mice, Abdi and colleagues reported that depletion of CX3CR1 expressing donor MPs prevents the induction of tolerance to cardiac allografts despite tolerogenic treatment with anti-CD40L mAb (95). This suggests a necessary role for donor myeloid cells in the establishment of transplantation tolerance. More recent work from Terry Strom and colleagues discovered a protective role for tissue-resident macrophages in transplantation. In a series of very elegant experiments it was demonstrated that tissue resident macrophages migrate to the lymph nodes, where they exhibit immunoregulatory properties that support prolonged allograft survival (7). This suggests that particular MPs subsets may be involved in the induction of tolerance, and mice deficient for macrophage and DC subsets, such as CSF1/CSF2, BATF3 (cDC1), IRF4 (cDC2), and E2-2 (pDC), have been generated and may be used in future to answer this question. Another important aspect of donor MPs is that they may

persist in tolerant recipients and participate in immune regulatory responses that protect the allograft as some MPs display a long half –life (tables 1 and 2). Flow cytometry data supports this view and demonstrated presence of donor derived $CD11c^{+}GFP^{+}$ cells in the spleen of islet allograft recipients one month after islet transplantation in mice (90). In humans, donor-derived Langerhans cells expressing CD207, CD1a, and S100 proteins are present after >4 years following hand transplantation with no signs of rejection (96). Tissue resident pre-DCs expressing $CD45^{+}MHCII^{-}$ $CD11c^{+}Flt3^{hi}SIRP\text{-}\alpha^{-}$ have the ability to differentiate into both $CD11b^{+}$ DC and $CD103^{+}$ DC in the liver and kidney, which indicates myeloid cells of donor origin may be present in the transplanted organ long after engraftment (33, 42). This indicates that presence of donor MPs in transplant recipients may be associated with better clinical outcome. Consistent with this hypothesis, Abdi and colleagues reported that depletion of CX3CR1-expressing donor MPs worsens chronic rejection of heart allografts indicated by increased interstitial infiltration, smooth muscle necrosis/inflammation, and intimal thickening (95). This view, is also supported by pioneer studies by Hart and Fabre that reported the presence of donor $MHC\text{-}II^{+}$ cells in long surviving rat kidney allotransplants (97), arguing that donor cells present in donor organs may promote macrochimerism and lower rejection rates without graft versus host disease (98). Figure 3 summarizes the activated donor MPS "passenger leukocytes" after transplantation.

Recipient MPs

Surgical trauma associated with vascular anastomosis of an ischemic donor organ leads to MP bone marrow mobilization and infiltration in the transplanted tissue (9). Once in the inflamed transplanted organ, these recipient MPs secrete proinflammatory cytokines that damage the tissue leading to DCs activation and donor-specific immune responses. Recipient monocytes, DCs, and their precursors are recruited from the blood, bone marrow and the spleen (99, 100) to the transplanted organ where they differentiate into macrophages and DCs. Bone marrow-derived cells are recruited to transplanted organs and play a key role in determining the outcome of rejection or tolerance. Direct evidence of the ontogeny of macrophages and DCs in organ transplantation is hard to find. In contrast to the bone marrow common macrophage/DC precursor (MDP) that gives rise to DCs and monocytes, the CDP gives rise to DCs but not monocytes (101-103), and we reported the differential roles of MDP versus CDP in the induction of transplantation tolerance. In CCR2-deficient recipients in which tolerance cannot be induced despite tolerogenic treatment with anti-CD40L mAb, adoptive transfer of MDPs, but not CDPs, gave rise to $CSF1R^{+}$ monocytic cells that rescued indefinite allograft

survival (9). Under the new proposed classification, it is recommended to separate monocyte-derived cells from DCs originating from pre-DCs. In addition, when the literature refers to 'macrophages', the bulk of these cells are probably monocyte-derived but it may be hard to distinguish between monocyte-derived macrophages and tissue-resident macrophages in the inflamed organ.

Approaches to distinguish between monocyte-derived macrophages and tissue-resident macrophages have used CD45.1/CD45.2 and GFP reporter mice that can differentiate between donor and recipient cells. As described above, Duffield and colleagues investigated the phenotype and function of CSF1R (CD115)-expressing myeloid cell subsets that are recruited to the injured kidney. Interestingly, the authors distinguished between tissue-resident macrophages and monocyte-derived macrophages to report at least three different subsets of monocyte-derived macrophages ($CD115^+$ $Ly6C^{hi}$, $CD115^+$ $Ly6C^{int}$, and $CD115^+$ $Ly6C^{lo}$) accumulating in the inflamed tissue (64). It is also important to acknowledge that monocyte-derived cell accumulation within injured organ occurs very rapidly. In a rodent model of kidney IRI, CCR2- and CX_3CR1-dependent infiltration by macrophages was observed, starting 30 minutes after insult and peaking within 24-48 hours (104). This rapid accumulation of macrophages is the first step in a stereotypical reaction that leads to further tissue damage through release of reactive oxygen and nitrogen intermediates, production of matrix-degrading enzymes, release of pro-inflammatory cytokines, and recruitment of other leucocytes (105). A similar contribution of macrophages to IRI has been demonstrated in lung (106) and liver (107). However, in these studies it is appropriate to question the use of the terms DC or macrophage, based solely on a small number of surface markers. The use of donor CD45.1 grafts transplanted into recipient CD45.2 mice or the use of reporter mice, such as GFP or RFP as either donors or recipients, would allow us to distinguish between recipient inflammatory MPs from donor tissue-resident MPs. Recent characterization of distinct DC precursors in the bone marrow together with the identification of the genetic reprogamming of pDC, cDC and monocyte-derived DCs under inflammatory conditions may allow to further characterize the specific MP subsets that infiltrates the allograft (108-110). Mouse inflammatory DCs are MHC-II^+, $CD11c^{int}$, and $CD11b^+$ and are phenotypically difficult to distinguish from steady-state $CD11b^+$ DCs and activated recruited monocytes, although the expression of CD64 and the high affinity IgE receptor FcεR1 has been recently used to identify inflammatory DCs in the tissue (111). Two distinct types of murine inflammatory DCs have been described in the lymph nodes: Tip-DCs and $CD209^+CD206^+$ DCs (112, 113). In humans, inflammatory DCs have been reported to express HLA-DR^+, $CD11c^+$, $CD11b^+$, $CD1c^+$,

$CD1a^+$, $CD14^+$, $CD206^+$, $Fc\varepsilon R1^+$, and $SIRP\alpha^+$ (109, 114, 115). These are likely to be monocyte derived-DCs.

Another important aspect of monocyte-derived cell recruitment into the transplanted organ is that monocyte mobilization and infiltration seem to be independent of the allogeneic differences between donor and recipient, as syngeneic grafts are infiltrated by myeloid subsets at similar rates. Using two-color immunohistochemistry, Penfield and colleagues described the increase of MHC Class II ($OX6^+$)-expressing $CD103^+$ ($OX62^+$) DCs and $CD163^+$ ($ED2^+$) macrophages following syngeneic renal transplantation in rats (116, 117), indicating that inflammation associated with the vascular anastomoses during the surgical procedure triggers myeloid cell mobilization into the transplanted organ. Very elegant studies from Matthias Nahrendorf and colleagues in a murine heart transplant model confirmed the above results and further extended these findings by comparing syngeneic versus allogeneic myeloid subsets in the transplanted graft (118). The same study examined development of different myeloid subsets to demonstrate that, while the ratio of $Ly6C^{lo}/Ly6C^{hi}$ $CD11b^+$ myeloid cells in the transplanted syngeneic grafts was 70/30%, opposite results were found in the transplanted allogeneic grafts with a $Ly6C^{lo}/Ly6C^{hi}$ $CD11b^+$ myeloid cell ratio of 30/70. Our unpublished observations further suggest that the ratio of $Ly6C^{lo}/Ly6C^{hi}$ $CD11b^+$ myeloid cells in the transplanted allogeneic grafts treated with tolerogenic anti-CD40L mAb was similar to the syngeneic grafts, in which $Ly6C^{lo}/Ly6C^{hi}$ $CD11b^+$ myeloid cells represent 70% (8). In a rat kidney transplant model, Steiniger and colleagues demonstrated that both syngeneic and allogeneic kidney transplantation dramatically increased the number of monocytes that infiltrate the grafts. Phenotypic analyses revealed two distinct populations of $CD68^+SIRP\alpha^+$ ($ED1^+Ox41^+ED9^+$) macrophages that include a large and activated $CD11a^{int}CD18^{int}CD4^{neg}CD62L^{int}MHC\text{-}II^{hi}CD161^{int}CD43^{lo}$ macrophage subset and $CD11a^{hi}CD18^{hi}CD4^{int}CD62L^{neg}MHC\text{-}II^{neg}CD161^{neg}CD43^{hi}$ macrophages. The study also revealed that activated monocytes express CD161 and CD8 molecules and are able to phagocyte opsonized paramagnetic beads (119).

Some therapeutic approaches to prevent IRI include blockade of the CD80/86 costimulatory pathway using CTLA4-Ig that results in the significant inhibition of macrophage infiltration into the ischemic tissue (120). Depletion of macrophages prior to IRI of kidneys reduces macrophage infiltration and preserves renal function in the short-term (67). However, the cost of preventing early macrophage injury is impaired tissue remodeling and poorer later kidney function (121).

With regards to allostimulatory function of MPs in the transplantation setting, Gershwin and colleagues described four different populations that were $CD11c^+NK1.1^-$ ($B220^+CD4^+$ $CD11b^-$, $B220^+CD4^-CD11b^-$, $B220^-CD4^+CD11b^-$, and $B220^-CD4^-CD11b^-$) with different allogeneic T cell stimulation capabilities as measured by *in vitro* mixed leukocyte reaction (122). In a murine liver transplant model the authors reported that $CD11c^+B220^+$ (presumable pDC) failed to induce detectable proliferation of allogeneic $CD4^+$ T cells, while both subsets of $CD11c^+B220^-$ (which also expressed higher levels of MHC-II, CD80/86 and CD40) were shown to be equally potent stimulators. With regards to the alloinhibitory function of MPs in the transplantation setting we recently described that monocyte-derived recipient DC-$SIGN^+$ macrophages that inhibit $CD8^+$ T cell-mediated immunity and promote $CD4^+Foxp3^+$ Treg cell expansion are necessary for the induction of tolerance under costimulatory blockade with anti-CD40L mAb (8).

In the clinical setting, monocyte-derived cells are a prominent component of inflammatory infiltrates in acutely rejecting transplants. Glomerular, vascular and tubulointerstitial infiltration by monocyte-derived cells is a hallmark of acute kidney transplant rejection in patients (123). Moreover, the total number of infiltrating macrophages is correlated with the severity of kidney transplant rejection (124) and the density of glomerular infiltration by macrophages predicts worse clinical outcomes, independently of peritubular capillary C4d deposition (125). Infiltration of kidney allografts by macrophages within 10 days of transplantation is associated with worse clinical outcomes (126, 127). In human kidney transplants, Hancock and colleagues reported the presence of macrophages in severe rejection (60% of graft infiltrating cells), mild rejection (52%), and moderate rejection (38%) using the human leukocyte-common antigen PHM1 and the mononuclear phagocyte PHM2 and FMC32 (CD14) (128). The study indicated that macrophages represent a major cell subset that mediates acute allograft rejection and the findings were confirmed shortly afterwards using a different monocyte/macrophage antibody (OKM-1, CD11b) (129). Further characterization of the macrophage subsets present in human kidney grafts was possible using the MAX.1, MAX.2, MAX.3, and MAX.11 monoclonal antibodies (130). Interestingly MAX.1 staining correlated with the severity of rejection and its expression was not detected in Cyclosporin A-treated recipients. Using a modified avidin-biotin peroxidase complex (ABD) immunohistochemical approach CD11b, CD14, and CD68 (in addition to CD36 and HAM56) further identified $CD68^+$ macrophages as effector cells in acute kidney rejection (131). The clinical importance of $CD68^+$ cells (which represent approximately 50% of the total infiltrating cells) was confirmed using the histologic Banff scores showing a trend for higher infiltration of $CD68^+$ cells in more severe rejection episodes (132). Expression of allograft

inflammatory factor-1 (AIF-1) together with number of activated macrophages was significantly increased in the infiltrate of clinical rejection biopsies, distinguishing clinical from subclinical kidney graft rejection. Microarray studies conducted by Halloran and colleagues corroborated these histological observations by showing that macrophage-associated transcripts (especially "M2-associated" markers) were indicative of T cell-mediated rejection (133, 134).

Changes observed in human solid organ transplants appear to be reflected by changes in peripheral blood monocyte and DC subsets. A prospective multicenter clinical study measured blood circulating myeloid DCs type 1 (MDC1, correspond to $CD1C^+$ cDC2) and type 2 (MDC2, correspond to $CD141^+$ cDC1) and pDCs ($CD303^+$) of patients receiving standard immunosuppression at time points before transplantation, 2 weeks and 1-3-6 months after transplantation. Four-color flow cytometry with CD303 (BDCA-2), CD1c (BDCA-1), and CD141 (BDCA-3) was used to report that higher MDC1/cDC2 levels occur two weeks after transplantation (135). These studies complement previous immunocytometric studies in which van Kooten and colleagues quantified three different myeloid DC subtypes characterized by $CD1c^+$DC-$SIGN^+$, $CD1c^+$DC-$SIGN^-$, and $CD303^+$DC-$SIGN^-$ in cryosections of human renal transplanted tissue (45). Recent human studies that investigated the phenotype, activation status and cytokine production capacity of classical ($CD14^{++}CD16^-$), intermediate ($CD14^{++}CD16^+$) and non-classical ($CD14^+CD16^{++}$) monocytes in blood of kidney transplant patients reported that the percentage of $CD14^{++}CD16^+$ and $CD14^+CD16^{++}$ blood circulating monocytes increased in transplant recipients with time, while their HLA-DR cell surface expression tended to decrease. Interestingly, the levels of intracellular cytokine production in monocytes revealed increased levels of TNFα, IFNγ and IL10 three months after transplantation (136). Another marker to bear in mind is CD163, which was reported to distinguish non-inflammatory blood-circulating monocytes in kidney transplant patients (137). Figure 4 summarizes the recipient MPS that infiltrate the organ after transplantation.

Donor versus Recipient MPs in Allogeneic Responses

Solid organ transplantation is a special immunological circumstance since mononuclear phagocytes within grafted tissues may originate from either donor or recipient. The immunogenicity of macrophages and DCs is critical to their fate in allogeneic recipients and their role in transplant pathology. Early experiments demonstrated the importance of donor-derived, "passenger"

leucocytes for initiating acute rejection of fully mismatched allografts (138). By passively depleting grafts of DCs, or by adoptively transferring donor-derived DCs to naïve recipients, Lechler and Bachelor showed that donor-type DCs were sufficient to prime naïve T cells and initiate acute allograft rejection (139, 140). Priming of naïve T cells depended upon migration of donor-type DCs via the lymph to lymph nodes, or via the blood to spleen (141); hence, "direct" recognition of MHC alloantigens on donor DCs by recipient T cells was presumed sufficient for initiation of allograft rejection (142). The subsequent discovery that minor-mismatched and DC-depleted fully-mismatched organs underwent delayed acute rejection challenged this view. Evidence that the lifespan of donor-derived DCs after migration to secondary lymphoid tissues is limited by NK cells (143), and the capacity of cell-free donor alloantigen to sensitize naïve recipients, suggested recipient-derived antigen presenting cells alone were capable of initiating alloimmune responses. Experiments using CD11c-DTR mice confirmed that depletion of recipient DCs prevented cardiac allograft rejection, whereas depletion of donor-type DCs had limited impact (144). Accordingly, "indirect" presentation of alloantigens by recipient DCs appeared to be necessary and sufficient for initiating acute transplant rejection. The finding that intact MHC complexes can be transferred from donor to recipient DCs (145), which can then prime donor-restricted alloreactive T cells via the "semi-direct" pathway, cast doubt about the importance of direct allo-recognition to priming of alloimmune responses. There has been much argument about the relative contribution of the direct, indirect and semi-direct pathways to the initiation of alloimmune responses. It is generally accepted that indirect allorecognition is sufficient to drive acute and chronic transplant rejection, and that indirect allorecognition becomes increasingly important for responses occurring late after transplantation. Adoptive transfer of T cells recognizing intact donor MHC can certainly cause acute transplant rejection, although dissecting the roles of direct and semi-direct presentation in priming such T cells has proven controversial. Two classic experiments are often cited as *in vivo* evidence of direct allorecognition. Firstly, Lee *et al*. showed that MHC class II-deficient recipients were capable of rejecting wildtype allografts (146). Secondly, Pietra *et al*. showed that grafts from MHC class II deficient donors were not rejected by $CD4^+$ T cell reconstituted $Rag^{-/-}$ recipients (147). Unfortunately, neither experiment distinguishes between direct and semi-direct presentation. In principle, CD11c-DTR mice allow separation of the antigen-presenting activity of donor and recipient DCs; however, it is unclear whether complete systemic depletion of DCs is a fair way of interfering with the activity of those DCs responsible for allo-stimulation. Ochando *et al*. tracked donor DCs to draining lymph nodes and spleen, where they persisted for at least 7 days; further, they visualized the interaction of donor DC and recipient $CD4^+$ T cells in the LN and spleen of mice undergoing rejection (83). Thus, although donor DCs certainly interact with recipient T cells in secondary lymphoid tissues, the

pathological consequences of this interaction cannot be easily assessed *in vivo*. Ultimately, proving that direct allorecognition alone is sufficient to initiate an alloimmune response would require a reliable means to block semi-direct presentation. As we describe below, a growing appreciation of the graft-protective potential of mononuclear phagocytes has reignited discussion about DC and macrophage allogenicity. This is especially true in the field of tolerogenic DC therapy.

Therapeutic Intervention and Future Perspectives

MPs with demonstrated suppressive activity are present in transplant recipients (148). Given the influence of DCs and macrophages over innate and adaptive immune responses against allografts, therapeutic strategies to bias their activities towards tissue repair and transplant acceptance are very attractive. In principle, there are three possible approaches to manipulating the balance of graft-damaging and graft-protective myeloid cell subsets, namely, eliminating or excluding immunogenic forms from the graft, forcing their differentiation into suppressor cells, or adoptively transferring graft-protective subsets.

Depletion or exclusion of myeloid cell subsets from allografts. Experiments in rodents have shown that appropriately timed depletion of monocytes, macrophages and DCs can preserve allograft function and promote graft survival prolongation (144, 149). In the clinical setting, there are currently no antibodies for selective depletion of DC and macrophage subsets (150). In the future, anti-CSF1/anti-CSF1R or FLT3L antibodies may prove useful for depriving monocytes/DC of tonic CSF1/FLT3L signaling necessary for their survival (151, 152). An alternative strategy is to prevent recruitment of inflammatory monocytes into allografts using antibodies or small molecules that interfere with CCR1 (153), CCR2 and CCR5 (154), or P-selectin (155, 156).

Manipulating the activation state of mononuclear phagocytic cells. Angus Thomson's laboratory used the hematopoietic growth factor FLT3L to increase the numbers of immature and pDCs *in vivo*. Flt3L-induced murine DCs *in vivo* produced no inflammatory IL-12, expressed low levels of co-stimulatory molecules, and promoted the generation of $CD4^+CD25^+IL10^+$ regulatory T cells *in vitro*, while failing to prime allogeneic T cells *in vivo* (157). When infused systemically into recipient mice before transplantation they prolonged cardiac allograft survival without immunosuppressive

therapy. Several immunosuppressant drugs used in current clinical practice influence the activation state of macrophages and DCs, including glucocorticoids (158), mTOR inhibitors (159) and MMF (160). Intravenous immunoglobulin (IVIg) treatment affects macrophage function by stimulating FcγRIIb, which up-regulates IL-10 production and other suppressive pathways (161-163). Angiotensin II type 1 (AT1)-receptor antagonists may be useful in preventing chronic allograft fibrosis because angiotensin II, which is produced by activated fibroblasts and macrophages, stimulates TGF-β secretion and induces differentiation of collagen-producing myofibroblasts (164). A number of exciting agents that affect maturation and polarisation of myeloid regulatory cells are currently in clinical development, including TLR4 antagonists (165) and inhibitors of TGF-β (166).

Adoptive transfer of suppressive mononuclear phagocytic cells. From the earliest discovery that transplantation tolerance could be transferred from tolerant to non-tolerant individuals with populations of several suppressive myeloid cells, it was suggested that the same principle could be applied therapeutically in man. While adoptive transfer has become a common experimental technique, its clinical translation has been problematic. Critically, most technical challenges and elementary safety concerns of manufacturing and administering pharmaceutical-grade cell preparations have been overcome (167). Several suppressive myeloid cell types of donor and recipient origin are now entering early-phase clinical trials in solid organ transplantation. As cell-based immunosuppressive treatments, myeloid suppressor cells in arrested states of immaturity have received most attention (168). Generally, such cell products are generated from peripheral blood monocytes obtained by elutriation or CliniMACS sorting of $CD14^+$ cells. Diverse treatments have been used to stabilise immature myeloid suppressor cells, including IL-10 (DC10 cells), rapamycin (Rapa-DCs), low amounts of GM-CSF (Tol-DCs) and dexamethasone plus vitamin D. Recent work from Angus Thomson's laboratory demonstrated the allograft protective potential of rapamycin-treated, donor-derived DCs in a nonhuman primate kidney transplant model (169). In humans, Cuturi and colleagues are currently trialling recipient-derived DCs generated with low GM-CSF concentrations as a therapy in kidney transplantation (Clinicaltrials.gov: NCT02252055). Work from Hutchinson and colleagues to develop a donor-derived suppressive myeloid cell-based medicinal product for use in transplantation revolves around a type of activation-induced myeloid regulatory cell, called regulatory macrophages (Mregs) (170). Human donor-derived Mregs reflect a distinct state of macrophage differentiation, which is characterized by a unique marker profile and potent suppression of T cell proliferation through IFN-γ-induced indoleamine 2,3-dioxygenase (IDO) activity (171). In addition, human Mregs mediate contact-dependent deletion of activated T cells and

drive the development of regulatory T cells from naive non-Tregs. Crude preparations of donor-derived Mregs have already been preoperatively administered to two living-donor kidney transplant recipients without adverse effect. Both patients are now more than 6 years posttransplantation with stable renal function and are maintained on very low-dose tacrolimus monotherapy. A further clinical trial of Mregs as an adjunct immunosuppressive therapy in 16 living-donor kidney transplant recipients is currently ongoing (Clinicaltrials.gov: NCT02085629).

In summary, although preclinical experiments have shown that donor- and recipient-derived MPS cells are potentially important therapeutic targets in transplantation, there are still no pharmacological agents targeting specific subsets of resident macrophages (CSF1/2 dependent), DCs (FLT3 dependent) and monocyte-derived cells in current clinical use. The use of the terms DC or macrophage as trivial drug names for various monocyte-derived cellular therapy products adds to confusion in the field. More robust definitions would describe all these cell types as monocyte-derived (11) with appropriate objective descriptions of the conditions or gene expression programs activated (20, 22). Developing drugs to target graft-destructive MP cells is challenging because macrophages and DCs serve critical functions in many tissues, both in health and disease. A more rigorous, unified definition of MPS cell subsets, and the markers they express, is therefore essential to future identification of suitable pharmacological targets. In an attempt to standardize protocols and methodological approaches to define cells of the MPS we suggest different flow cytometry panels to characterize monocytes, macrophages, and DCs in mice and humans (Figure 1).

Conclusion

Monocytes, macrophages and DCs constitute a fundamental branch of the immune system, which is responsible for the initiation of the immune response against transplant antigens. Acting within transplants, and at distant sites, cells of the MPS influence the development of transplant pathology through both innate and adaptive mechanisms (172, 173). Early after transplantation, DCs and macrophages react to IRI by mounting rapid, stereotypical responses that lead to secondary tissue damage. Myelomonocytic cell infiltration of solid organ allografts during acute rejection episodes contributes to tissue damage and amplifies T cell reactions. Later, macrophages and DCs serve as key mediators of chronic inflammation and fibrosis. Importantly, though, not all processes mediated by mononuclear phagocytes are detrimental to allografts. After transplantation, tissue-reparative

macrophages help to reestablish tissue homeostasis by suppressing inflammation, eliminating necrotic material and producing tissue-trophic factors. Both donor- and recipient-derived DCs are critical for induction and maintenance of transplantation tolerance. Given their pivotal role in shaping immune response to solid organ transplants, identification and characterization of myeloid populations is necessary to better appreciation of their origin, developmental requirements, and functions. Ultimately, a more complete understanding of MPS may suggest ways of manipulating their activities in the transplant setting to promote graft acceptance (174).

While providing a precise and consensual answer regarding how many monocytes, macrophages, and DC subsets there are may be a difficult task, it is of critical importance that immunologists and cell biologists find a minimum level of agreement on the markers that can be used to define widely accepted myeloid cell subsets. Equally importantly, the scientific community must design consensus protocols and methodological approaches to identify and characterize new myeloid cell subpopulations and these should be revised every few years. Pioneering work from Terry Strom and Nicholas Tilney first identified monocytes/macrophages in the transplanted organ by nuclear morphology and cytochemical staining with pararosaniline solution, which stains nonspecific esterases present on monocytes/macrophages (175, 176). While myeloid cell subsets have been historically defined according to morphology, cytochemistry and flow cytometry, the latest technological revolution in deep-sequencing and mass cytometry together with innovative biochemical and functional assays will enable us to identify novel myeloid cell subsets more rapidly and to understand their dynamic interaction with other cells of the immune system (177, 178). A consensus on the classification of existing myeloid cell subsets, as well as rules for defining novel subsets (ie. function, phenotype, and/or ontogeny) is urgently needed. As Norma Lang succinctly encapsulated, there is a need for a system that provides a nomenclature, language and classification to describe and organize data: "**If we cannot name it, we cannot control it, finance it, teach it, research it, or put into public policy**".

Figure legends

Figure 1: Phenotypic characterization of donor and recipient mononuclear phagocytes in the tissue. Figure suggests flow cytometry panels to identify and characterize monocytes, macrophages and dendritic cells in transplanted human and mice transplant recipients

Figure 2: Donor mononuclear phagocytes in the tissue. Image represents an overview of the donor mononuclear phagocytes that are present in the organ before transplantation. No pDC or monocytes are present in resident tissues (donor organ) under steady state.

Figure 3: Activated donor mononuclear phagocytes in the tissue. Image represents an overview of the activated donor mononuclear phagocytes (passenger leukocytes) that are present in the organ before transplantation.

Figure 4: Recipient mononuclear phagocytes in the tissue. Image represents an overview of the recipient mononuclear phagocytes that infiltrate the organ after transplantation. Classical monocytes convert into monocyte-derived macrophages. The contribution of recipient cDCs and non-classical monocytes is unknown.

Table legends

Table 1: Characterization of mouse tissue resident macrophages in the donor organ before transplantation. Table indicates the % of tissue resident macrophages among the total hematopoietic $CD45^+$ cells, origin, half-life and migratory properties of tissue resident macrophages.

Table 2: Characterization of mouse tissue resident DC subsets in the donor organ before transplantation. Table indicates the % of tissue DC subsets among the total hematopoietic $CD45^+$ cells, % of DC1 and DC2, and % of $CD103^+$ DC2. The table also indicates origin, half-life and migratory properties of tissue resident DCs.

Table 1

Tissue		% of CD45	Origin	Half Life	Migratory	Ref
Kidney		20-25%	Embryonic progenitors	Long, in situ proliferation	yes	(179)
Liver	Kupffer Cells	40-50%	Embryonic progenitors	Long, in situ proliferation	yes	(31, 47, 180)
Lung	Alveolar macrophages	40-50%	Embryonic progenitors	Long, in situ proliferation	yes	(181-183)
Skin	Epidermal LC	3%	Embryonic progenitors	Long, in situ proliferation	yes	(32)
	Dermal macrophages	10-15%	Monocyte	Short, no in situ proliferation	yes	(41)
Gut	Small intestine	20-25%	Monocyte	Short, no in situ proliferation	yes	(41, 184)
	Large intestine	10-15%	Monocyte	Short, no in situ proliferation	yes	(41, 184)
Heart		80-90%	Monocyte	Short, no in situ proliferation	ND	(46, 185)
Pancreas		80-90%	Embryonic progenitors and monocyte	Long, in situ proliferation	yes	(186)

Table 2

Tissue		% of CD45 (%DC1/DC2)	Origin	Half Life	Migratory	Ref
Kidney		20% (10/90%)	CDP/pre-DC	5-10days	yes	(42, 179)
Liver		1-5% (15/85%)	CDP/pre-DC	5-10days	yes	(42, 187)
Lung		10-15% (35/65%)	CDP/pre-DC	5-10days	yes	(42, 111)
Skin	Dermis	15-20% (10/90%)	CDP/pre-DC	ND	yes	(41, 42, 188)
	Migratory LC	10%	Embryonic progenitors	Long, in situ proliferation	yes	(32, 42, 188)
Gut	Small intestine	5-10% (25/75[40% CD103+]%)	CDP/pre-DC	ND	yes	(27, 33, 189)
	Large intestine	5-10% (40/60[10%	CDP/pre-DC	ND	yes	(189)

		CD103+]%)				
Heart		1-5% (30/70%)	CDP/pre-DC	ND	ND	(46, 185)
Pancreas		10-20% (20/80%)	ND (Likely CDP and pre-DC)	ND	yes	(190)

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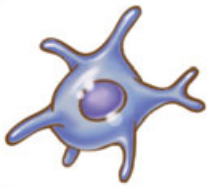
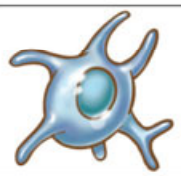
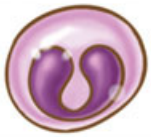
Cell		Human	Mouse
Dendritic Cells			
conventional DC1		**XCR1** **CLEC9A** CD11c CD141	**XCR1** **CLEC9A** CD11c CD103 / CD8 CD205 (DEC-205) Langerin
conventional DC2		**CD172 (SIRPα)** CD11c CD1c CD1a	**CD172 (SIRPα)** CD11b CD24 / CD4 ESAM
plasmacytoid DC		**CD123 (IL-3R)** CD303 (CLEC4C) CD304 (neuropilin)	**CD123 (IL-3R)** BST2 Siglec H B220
Monocyte-related cells			
classical monocyte		**CCR2 high** **S100A8/A9** CD14 CD11b	**CCR2 high** **S100A8/A9** Ly6C high Gr-1 high CD115
non-classical monocyte		**CX3CR1 high** CD16 CD14 low CD11b	**CX3CR1 high** Ly6C low Gr-1 low CD115
monocyte-derived macrophage		**CD209 (DC-SIGN)** CD14 CD11b Factor XIIIA	**CD209 (DC-SIGN)** CD11b CD64 MerTK
Macrophages			
resident macrophage		**CD11b** **CD64** CD163 CD14 Factor XIIIA LYVE-1	**CD11b** **CD64** F4/80 CD68 MerTK