The CD14+ CD16+ blood monocytes: their role in infection and inflammation

Loems Ziegler-Heitbrock[1]

Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, UK; and Clinical Cooperation Group, Inflammatory Lung Diseases, GSF and Asklepios-Kliniken, Gauting, Germany

Abstract: Blood monocyte subpopulations have been defined in man initially, and the two major types of monocytes are the CD14++ CD16– and the CD14+ CD16+ monocytes. These cells have been shown to exhibit distinct phenotype and function, and the CD14+ CD16+ were labeled proinflammatory based on higher expression of proinflammatory cytokines and higher potency in antigen presentation. The current review describes these properties, including the relationship to dendritic cells, and summarizes the host of publications about CD14+ CD16+ monocytes in inflammation and infectious disease in man, all of which suggest a crucial role of these cells in the disease processes. The review also covers the more recent description of homologues of these cells in other model species, which is expected to better define the role of monocyte subsets in disease. *J. Leukoc. Biol.* 81: 584–592; 2007.

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INTRODUCTION

Monocyte subsets in man and other mammals

The existence of different populations of monocytes in blood of man is now well established. In addition to the classical monocytes, which are strongly positive for the CD14 cell surface molecule (CD14++ CD16– monocytes), we discovered a population of monocytes, which coexpresses CD16 and low levels of CD14 antigens. The first description of these cells dates back to 1988, when we identified these cells by two-color flow cytometry using mAb [1]. A following detailed description of phenotype and function [2] demonstrated that the CD14+ CD16+ cells are a unique type of monocyte. These cells have some characteristic patterns of cell surface molecules when compared with the classical monocytes, and this includes high HLA-DR, epidermal growth factor module-containing mucin-like receptor 2 (EMR2), Ig-like transcript 4 (ILT-4), CD43, and CD45RA expression [3–7] and expression of MDC8 on a fraction of CD14+ CD16+ monocytes [7]. Also, several cell surface molecules were found absent from the CD14+ CD16+ monocytes. A selection of characteristic cell surface molecules of the CD14+ CD16+ monocytes is compiled in **Table 1**. The pattern of surface antigens in many respects resembles the pattern seen in tissue macrophages [2], and in vitro maturation studies showed that macrophages expressing CD16, along with low CD14, can be developed from CD14++ monocytes [8]. This indicates that the CD14+ CD16+ monocytes are more mature than the CD14++ monocytes.

There was a later approach to subdivide monocyte subsets based on expression of CD64 [9], but it turned out that the CD64-negative CD14low cells consisted of the CD14+ CD16+ monocytes plus a CD14+ CD16– subpopulation [5]. Therefore, the use of CD64 for definition of a blood monocyte subset offers little advantage. The minor subset of CD14+ CD16– monocytes has been shown to have high HLA-DR expression [5] and to have high APC activity [10].

An additional subset of CD56+ monocytes was reported (originally in ref. [5]). When studied in more detail, the CD56+ monocytes showed high allostimulation of T cells [11]. Based on their high expression of CD14, these cells appear to be distinct from the CD14+ CD16+ monocytes and rather form a subset of the classical monocytes. More work is, however, needed to clearly define the CD56+ monocytes.

Taken together, staining for CD14 and CD16 can define subsets of human monocyte populations, and the most prominent populations are the classical CD14++ CD16– and the CD14+ CD16+ monocytes.

Using the human CD antibodies, the same subsets of monocytes have been defined and analyzed in nonhuman primates such as Cynomolgus and Rhesus monkeys [12–14].

In the pig, monocytes can be defined using antihuman CD14 antibodies [15]. Sanchez et al. [16] analyzed the porcine monocyte subpopulations and identified the homologue of the human CD14+ CD16+ monocytes. These cells are CD14low CD16high DRhigh, and they were shown to be CD163+. This would be in line with these cells being more mature, as CD163, a scavenger receptor binding haemoglobin:haptoglobin, is expressed on many tissue macrophages [17]. The expression of CD163 is in contrast to man, where CD163 is low-to-absent on the CD14+ CD16+ monocytes [18] (Adam K. A. Wright and L. Ziegler-Heitbrock, unpublished). Still, this finding does not invalidate the concept that the CD14+ CD16+ monocytes are akin to tissue macrophages [8], as some tissue macrophages

[1] Correspondence: Department of Infection, Immunity and Inflammation, University of Leicester, Medical Sciences Building, University Road, Leicester, LE1 9HN, UK. E-mail: lzh1@le.ac.uk

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TABLE 1. Differential Expression of Cell Surface Molecules in CD14+ CD16+ Monocytes As Compared with CD14++ Monocytes

	Monocyte population	
Surface marker	CD14+ CD16+	CD14++
CD14	+	++
CD15	–	+
CD16	+	–
CD33	+	++
CD43*	+++	++
CD38	–	++
CD45Ra	++	+
CD62L	–	++
CD64	–	+
CD163*	–	++
CCR2	–	++
DR	++	+
MDC8	+	–
EMR2	++	+
ILT-4	++	+

* L. Ziegler-Heitbrock, unpublished.

such as the marginal zone macrophages in the spleen are CD163– [17]. In spite of this difference in expression of CD163 between man and pig, the bulk of evidence indicates that the porcine CD14low CD16high cells are the homologue of the respective human monocyte subset.

For rat monocytes, a series of mAb has been generated [19]. Ahuja et al. [20] reported that rat monocytes can be subdivided into CD43+++ and CD43++ subpopulations, and the CD43++ cells are the major subset. Although no direct combination of CD43 and CD62 ligand (CD62L) antibodies was applied, it appears that there are CD43++ CD62L+ and CD43+++ CD62L– subpopulations [21]. Among these, the CD43+++ CD62L– monocytes formed a major subset [21, 22]. The high percentage of CD43+++ monocytes in the latter two studies may be a result of the use of perfusates, which include monocytes from the marginal pool (see below). In addition it has been shown that rat CD43+++ cells show a lower level of CCR2 and higher expression of CX3CR1 [23]. These findings suggest that these CD43+++ monocytes are the rat homologue of the human CD14+ CD16+ monocytes.

Murine homologues of the CD14+ CD16+ monocytes have been described using different approaches. This includes the Ly6Clow cells (described in ref. [24]) and the CX3CR1high cells (defined in ref. [25]). Data from Sunderkotter et al. [24] suggest that the Ly6Clow cells are more mature, and this is based on depletion of blood monocytes, which saw the Ly6Clow cells appear late and after the resurgence of the Ly6Chigh cells, suggesting that the Ly6Chigh cells may give rise to Ly6Clow cells. These Ly6Clow cells at the same time were found CD43high. Functional studies of mouse and rat monocyte subsets, with respect to phagocytosis, antigen presentation, and cytokine production, are not available as yet.

Taken together, it appears that the high CD43 and low CCR2 expression may be common features of the CD14+ CD16+ monocytes in man and its homologues in other mammalian species.

FUNCTIONAL PROPERTIES OF THE CD14+ CD16+ MONOCYTES

Cytokine production

Cytokine production in response to TLR ligation is one of the prominent features of monocytes and macrophages. Using cell-sorter purification and RT-PCR, we demonstrated that similar levels of LPS induced TNF mRNA for the CD14+ CD16+ and the CD14++ CD16– monocytes [26], and IL-10 mRNA was low-to-absent in the CD14+ CD16+ monocytes. When using intracellular cytokine staining of monocytes, we then showed higher levels of TNF protein in the CD14+ CD16+ monocytes after stimulation of whole blood with the TLR-4 ligand LPS and with the TLR-2 ligand Pam3Cys [27]. These findings of high levels for the proinflammatory TNF and low levels for the anti-inflammatory IL-10 led us to coin the CD14+ CD16+ cell proinflammatory monocytes.

The low-to-absent production of IL-10 in the CD14+ CD16+ monocytes was also confirmed in cell sorter-purified, LPS-stimulated monocyte subsets [28]. In the study, they showed undetectable levels of IL-10 mRNA for the CD14+ CD16+ monocytes, as assessed by RT-PCR, and lower production of IL-10 protein. There was also preferential production of hemoxygenase-1 in the CD14+ CD16+ monocytes, an enzyme, which depending on the system, may have anti- and proinflammatory properties [29, 30].

A similar pattern as seen for LPS-stimulated monocytes was observed after stimulation of monocyte subsets with tumor cells in vitro. This led to higher TNF and IL-12 production and lower IL-10 production in the CD14+ CD16+ monocytes as compared with the CD14++ CD16– cells [31]. Also, in the same study, inducible NO synthase and NO production were found higher in the CD14+ CD16+ cells. Conversely, production of reactive oxygen was essentially absent in CD14+ CD16+ monocytes stimulated with tumor cells [31].

The CD163+ pig homologue of the CD14+ CD16+ monocytes also showed high levels of TNF mRNA and IL-10 low-to-absent [16]. Also, IL-1 expression was higher in the CD163+ monocytes. This is in keeping with the concept that these cells are proinflammatory monocytes. Such functional studies into cytokine gene expression in the monkey, mouse, and rat monocyte subsets are still outstanding. Taken together, based on the pattern of cytokine production, the CD14+ CD16+ monocytes and their pig homologue may be labeled proinflammatory monocytes.

Antigen presentation

Antigen presentation to CD4 T cells involves uptake of foreign antigen, which is digested and presented in the context of MHC Class II. The CD14+ CD16+ monocytes show a higher level of HLA-DR [2], and this would predict a higher APC activity in these cells. For presentation of protein antigen to T cells, the CD14++ and CD14+ CD16+ monocytes were shown to have similar capacity with respect to tetanus toxoid [10].

Using inactivated CMV as an antigen, we found a three-times higher level of T cell stimulation for the CD14+ CD16+ monocytes (M. Frankenberger and L. Ziegler-Heitbrock, un-

published). When comparing CD64+ CD16– and CD64– CD16+ monocytes, the CD64– CD16+ monocytes, i.e., the CD14+ CD16+ monocytes, were found to induce a greater-than-threefold higher response to purified protein derivative and influenza antigen [32].

The MDC8+ antigen is expressed on ~40% of CD14+ CD16+ monocytes [7], and it could be demonstrated that these cells have a higher APC activity for a primary T cell response to keyhole limpet hemocyanin when compared with the MDC8-negative cells [33].

In the pig, the CD14+ CD16+ homologue, i.e., the CD163+ cells, shows higher activation of allogeneic T cells [16] and a better APC activity in a secondary T cell response to hen egg lysozyme when compared with the CD163– cells [34].

Monocytes can give rise to macrophages or dendritic cells (DC), and the latter pathway has received much attention recently. For CD16+ monocytes, development of DC with preferential induction of TH2 cells was demonstrated [35, 36]. In a model of spontaneous reverse transmigration, Randolph et al. [37] showed the CD14+ CD16+ monocytes to preferentially migrate and to develop into DC with superior allostimulatory capacity. Also, when CD16+ monocytes were cultured for 2 days in the presence of TLR2 ligands, they developed preferentially into CD1b+ DC with high antigen-presenting capacity [38]. Taken together, these data support the idea that the CD14+ CD16+ monocytes and their DC progeny are superior APC.

Many of these functional studies, however, need to be taken with a cautionary note, as in most reports, cells were isolated by positive selection with mAb against cell surface molecules such as CD16. The interaction of the antibody with the respective receptor may lead to some degree of activation, and this may differentially influence the functional activity of the cells. Hence, although it appears that the CD14+ CD16+ monocytes are superior APC, confirmation is required using negative (no-touch) isolation procedures that use markers exclusively expressed on one and not the other monocyte subset.

Migration

Migration of monocytes into sites of inflammation has been shown in various studies to be governed by the CCL2 chemokine. In early in vitro migration studies, we noted that the CD14+ CD16+ monocytes failed to migrate in response CCL2, consistent with the absence of CCR2 on these cells [39]. There is evidence that in the absence of CCL2 action, i.e., in CCR2−/− mice, monocytes can immigrate into sites of *Listeria monocytogenes* infection in the spleen [40], thus indicating that other chemokines are involved. Along these lines, studies by Ancuta et al. [41] showed that the CD14+ CD16+ monocytes will migrate in response to CX3CL1 and CXCL12. It was also noted, however, that the CD16+ monocytes adhere to activated endothelium more strongly [42], and this was suggested to be mediated in part by CX3CL1 expressed on the cell surface of the endothelial cells (EC). Such firm adherence to CX3CL1-expressing EC did lead to a reduced transmigration in response to CX3CL1 in vitro. It remains to be shown whether such adhesion of CD14+ CD16+ monocytes to the EC may also lead to enhanced transmigration when triggered by additional cytokines, as has been shown for CD8+ T cells [43].

A role of CX3CL1 has been implicated in rheumatoid arthritis (RA), atherosclerosis, and HIV infection [44], and CD14+ CD16+ monocytes may be involved in the pathophysiology of these diseases after recruitment by this chemokine.

Monocyte migration in the mouse was studied mainly in vivo, in that phenotypes of cells recruited to various sites were determined. Sunderkoetter et al. [24] showed that the Ly6Cmed/high are recruited to the inflamed peritoneum, and the Ly6Clow cells, i.e., the murine homologue of the CD14+ CD16+ cells, did not migrate into this site. Also, the study of Geissmann et al. [25] suggests that the GR-1-negative CX3CR1high monocytes are not recruited to inflammatory sites, based on the inability of such adoptively transferred cells to be recruited to the peritoneum upon thioglycolate injection. Similar results were obtained for CD43high cells in the rat [45]. Conversely, Maus et al. [46] demonstrated recruitment of GR-1-negative monocytes to the alveolar space in mice treated with CCL2 or with CCL2 and LPS. Also, it was shown that CX3CR1+ monocytes can migrate into tissue in a CX3CL1-dependent manner, as shown in a murine myositis model [47]. This in vivo model does demonstrate that CX3CR1+ cells are not always prevented by endothelial CX3CL1 from migrating into tissue. Hence, whether the subset of CD62L– GR-1– CD43+++ cells is recruited to sites of inflammation appears to depend on the experimental model used.

As summarized by Leon et al. [48], murine CD11c+ MHC Class II-negative DC precursors can give rise to splenic DC, and this can happen in response to injection and phagocytosis of bacteria. Also, such CD11c+ MHC Class II-negative cells can be recruited into the granulomateous liver. Based on the extensive phenotypic similarity of the GR1– monocytes and the CD11c+ MHC Class II-negative DC precursors, Leon et al. [48] suggested that it may be the GR-1– blood monocytes that go into tissue under inflammatory conditions. This interesting hypothesis awaits proof by experimental approaches that define the murine blood DC precursors using CD62L, GR-1, and CD43 markers.

ROLE IN INFECTION AND INFLAMMATION

There is a host of reports about increases and decreases in the number of CD14+ CD16+ monocytes in various diseases. Although most of these studies use the CD14 and CD16 antibodies for detection of the cells, there are differences in instrument setting and gating that may impact on the results.

We now use a single-platform, lyse-no-wash procedure, which involves CD14, CD16, and DR plus flow count beads for direct determination of absolute numbers (see example in **Fig. 1**). This whole-blood procedure reduces manipulation of the sample to a minimum, thereby reducing variability.

With excessive exercise, the number of CD14+ CD16+ monocytes can increase up to fourfold, and it was suggested that these cells are mobilized from the marginal pool in a cathecolamine-dependent manner [49, 50]. Hence, with increases or decreases in the number of cells in peripheral blood, there is always the question of whether this is a

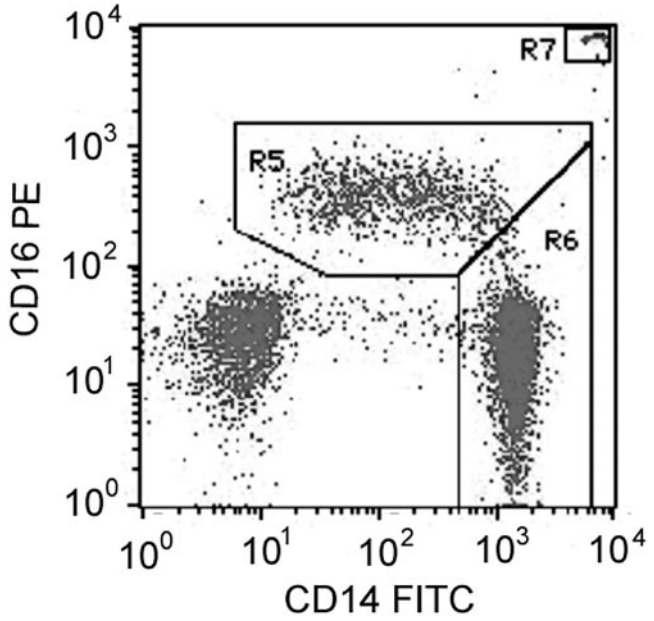

Fig. 1. Monocyte subpopulation analysis. Shown is an example of a staining for identification of monocyte subsets using antibodies to CD14 and CD16 in a blood sample from an apparently healthy volunteer. Analysis was done by gating on DR+ cells to exclude CD16+ NK cells (not shown), and the absolute count was determined with reference to standard beads [population of event in upper right corner (R7)]. The absolute number of CD14++ CD16− monocytes in R6 was 207 cells/ul in this example, and CD14+ CD16+ double-positive cells (R5) were at 46 cells/ul.

transient compartment shift or a net increase or decrease of the cells in the vasculature. This question, however, is difficult to address in man, as excessive exercise studies in the face of infection and inflammation may bear risks to the patient. Here, studies in experimental animals will be crucial.

CD14+ CD16+ MONOCYTES IN INFLAMMATION

In rheumatoid arthritis (RA)

Baeten et al. [51] noted an increase in HCgp-39+ monocytes in patient blood, and these cells were found to be CD14+ and CD16+. This increase was found to correlate with erythrocyte sedimentation rate and C-reactive protein. Others confirmed these findings and found the rise to be most pronounced in active disease [52–54]. The numbers of CD14+ CD16+ monocytes were found lower in response to therapy, which may be a result of selective depletion of these cells by glucocorticoids [55, 56]. Of note, one study found lower levels of the CD14+ CD16+ monocytes in a cohort of RA patients, but therapy effects might have contributed to this finding [57].

TGF-β is one of many cytokines expressed in synovial fluid, and this mediator was shown to induce CD16 on blood monocytes [58, 59]. Macrophages in synovial fluid also express CD16 [52, 58] and so do macrophages in the synovial membrane-lining layer [59–61].

In a rat model of adjuvant arthritis, inhibition of heme oxygenase-1 (HO-1) was shown to reduce inflammation in the joints [30]. As CD14+ CD16+ monocytes have been shown to preferentially express HO-1 [28], it is tempting to speculate that it is a rat homologue of these cells that drives the inflammation in this model via expression of HO-1. Experiments to identify the cells expressing HO-1 in the rat model are required to substantiate this hypothesis.

Although one might speculate that it is the CD16+ blood monocytes that give rise to CD16+ synovial macrophages in arthritis, it remains unclear whether the CD16+ macrophages in the inflamed joint tissue derive from immigrating blood CD14+ CD16+ monocytes. Alternatively, the CD16+ macrophages in RA joints may develop locally from immigrated CD14++ CD16− classical monocytes under the influence of cytokines such as TGF-β.

Diabetes

Diabetes was studied in the NOD mouse model, where increases of the Ly6Clow cells in murine blood were noted [62]. As the higher numbers were observed in prediabetic animals, the increase was suggested not to be secondary to inflammation but rather, to contribute to the development of the autoimmune disease in this model. By contrast, CD14+ CD16+ monocytes did not show an increase in Type 1 and Type 2 diabetes in men [63, 64], suggesting that the mechanism of disease may be distinct from what is happening in NOD mice. Of note, Type 1 diabetes in man has not been studied for monocyte populations in the early stages when immune destruction of the islet cells occurs. Otherwise, blood monocyte subsets have received little attention in murine models of inflammatory disease, and there is a huge potential for informative studies about the role of these cells in such disease models.

Hemodialysis and extracorporal cardiopulmonary bypass systems

Hemodialysis and extracorporal cardiopulmonary bypass systems are based on direct contact of patient blood with artificial membranes to allow for clearance of toxic metabolites and for gas exchange, respectively.

Exposure of leukocytes to such membranes can induce an inflammatory response with increased cytokine production. Nockher and Scherberich [65] showed increased numbers of the CD14+ CD16+ monocytes in hemodialysis patients without signs of infection, and this was confirmed [66]. When following patients during hemodialysis, Nockher et al. [67] also noted a preferential depletion of CD14+ CD16+ monocytes in blood early on within the first hour of hemodialysis with recovery of cell numbers within 4 h, and this was also seen in ref. [68]. A similar depletion was reported in patients on cardiopulmonary bypass, which involved exposure of blood to membranes in the oxygenator [69, 70]. Although cardiac surgery as such may also have an effect on monocyte subsets, the data suggest preferential adhesion of the CD14+ CD16+ monocytes to artificial membranes. This adhesion indicates that membranes are not entirely biocompatible, and testing for transient depletion of the proinflam-

matory monocytes may prove to be a sensitive test for biocompatibility [71]. This is of substantial importance, as such adhesion may also trigger activation and cytokine release by these monocytes contributing to the posthemodialysis inflammatory syndrome [72].

Atherosclerosis

Atherosclerosis has been linked to altered lipid profiles, to infection, and more recently, to inflammation of the arterial wall. In the early stages of the disease, the atherosclerotic lesions show an inflammatory infiltrate, which is predominated by macrophages. These macrophages have been shown to express CD16 [73]. The study of the respective blood monocytes revealed a correlation of CD14+ CD16+ monocyte percentages and lipids [5], in that in patients with hypercholesterolemia, low high-density lipoprotein cholesterol were associated with high CD14+ CD16+ cells. In a large study involving more than 200 patients with coronary artery disease (CAD), increased percentages of the CD14+ CD16+ monocytes were seen in the patient cohort [74]. Furthermore, patients with highest percentages (upper quartile) of CD14+ CD16+ monocytes had an odds ratio of 4.7 for CAD, even after adjusting for other confounding factors such as diabetes, hypertension, and lipid profile [74]. This indicates that the increase in the proinflammatory monocytes is an independent risk factor for CAD, suggesting that these cells might be involved in the disease process. Of note, it has been shown that in addition to immigration of monocytes into the arterial wall, blockade of emigration of these cells from the vessel wall can determine the fate of the lesion [75]. It remains unclear at this stage whether the higher migratory potential of subendothelial CD14+ CD16+ monocytes [37] contributes to the level of macrophage infiltration of the atherosclerotic plaque.

Kawasaki disease

Kawasaki disease is an acute inflammatory disease of the arteries, which affects children and can lead to formation of aneurysms of the coronary arteries. In the acute phase of the disease, an expansion of the CD14+ CD16+ monocytes has been described [28, 76, 77]. Of note, treatment of the disease involves infusion of high doses of Ig, the mechanism of action of which has been linked to blockade of FcRs [78]. It remains to be analyzed whether such infusion will act on the CD14+ CD16+ monocytes and dampen their activity.

Various other inflammatory diseases showed expansions of the CD14+ CD16+ monocytes, and this includes hemophagocytic lymphohistiocytosis [79], asthma [80], sarcoidosis [81], peridontitis [82], atopic eczema [83], pancreatitis [84], and alveolar proteinosis [85]. The increase of CD14+ CD16+ monocytes, in spite of immunosuppressive therapy in kidney transplant patients, suggests that they may be involved in a persistent, allograft-induced inflammatory reaction [86].

Also, increased numbers of CD14+ CD16+ monocytes were found in uremic patients on peritoneal dialysis [87] and in obesity [88].

Although the increase of CD14+ CD16+ monocytes in blood would suggest a potential role of these cells in the inflammation, in an alternative scenario, these cells may just expand in response to the inflammatory milieu in the body. In this scenario, they would not be drivers of inflammation but indicators of the extent of inflammation. This would reduce these cells to useful markers, the decrease of which would indicate response to therapy. The demonstration of a potential role of the CD14+ CD16+ monocytes in the various inflammatory conditions requires selective depletion of the cells in patients or experimental animals with inflammatory disease.

CD14+ CD16+ MONOCYTES IN INFECTION

Bacterial infections

Pronounced increases of CD14+ CD16+ monocytes from the average normal count of approximately 50 cells/ul to in excess of 500 cells/ul were seen in patients with severe bacterial sepsis [89]. Also, Nockher and Scherberich [65] reported on substantial rises of the proinflammatory monocytes in blood culture-positive sepsis in patients on hemodialysis. In a unique case of repeated, self-induced bacteraemia, we also saw a strong increase of these cells [90]. In this case, we noted a rise of fever and cytokines before the peak of the CD14+ CD16+ monocytes, suggesting that cytokines may induce the rise of these cells. Herra et al. [91] analyzed patients with systemic infections and found highest values of CD16+ monocytes in patients with blood cultures positive for gram-negative bacteria.

In neonatal sepsis, the percentage of CD16+ monocytes was found to increase with sepsis, and there was no such increase in trauma [92].

We then asked whether local infection would also go along with expansion of the CD14+ CD16+ monocytes and for this, analyzed a well-defined entity, i.e., erysipelas, a skin infection caused typically by infection with b-hemolytic streptococci Group A. Here, we noted increases in excess of 100 cells/ul in most patients, and this resolved with successful antibiotic therapy [93]. The expanded CD14+ CD16+ cells but not the classical monocytes were found to have reduced expression of TNF protein, and this selective tolerance may represent a protective mechanism that reduces potentially damaging, excessive cytokine production in the disease.

Also, the CD14+ CD16+ monocytes were found increased in children with hemolytic uremic syndrome (HUS), caused by infection with Shiga toxin-producing *Escherichia coli* bacteria [94]. The strongest increase was seen in the acute phase, but high cell numbers were still found several weeks after the acute illness. Similar to the findings reported for erysipelas above, the TNF production, after ex vivo stimulation with LPS, was reduced in HUS, again demonstrating that monocytes have become tolerant [94]. As in this study TNF was measured in supernatants of mononuclear cells, the tolerance could not be attributed to any one of the monocyte subpopulations.

In tuberculosis, an increase in CD14+ CD16++ monocytes was noted, an effect enhanced further by coinfection by HIV [95].

HIV infection

Increased percentages of CD14+ CD16+ monocytes in blood of HIV+ patients were noted by several groups [96–100]. Pulliam et al. [100] found the percentage of CD14+ CD16+ monocytes in the blood to be highest in patients with HIV-associated dementia.

Monocytes have been demonstrated to be productively infected by HIV in vivo [101–103], although a contribution by contaminating T cells cannot be excluded completely. With respect to the human CD14+ CD16+ monocytes, there are, however, no solid data available about integrated viral DNA.

In a Cynomolgus monkey model of experimental infection with SIV, an early rise of CD16+ monocytes was noted on Day 10 postinfection [13]. In a similar model in Rhesus monkeys, Williams and co-workers [14, 104] showed an early peak of the CD14+ CD16+ monocytes in blood. This was at 1–2 weeks when viral DNA became detectable in the brain, and it was coincident with evidence of brain damage, as determined by magnetic resonance spectroscopy in these animals.

Williams et al. [104] also demonstrated that the CD14+ CD16+ cells were consistently positive for SIV DNA, albeit there was variation over time. They suggested that these cells may function as Trojan horses, which carry the virus into the brain [104, 105]. Earlier studies in such monkeys could show perivascular macrophages to be positive for SIV RNA and gp120 protein [106].

In patients with HIV-associated encephalopathy, such perivascular macrophages in the brain were found to be CD14+ and CD16+ and to express HIV p24 protein indicative of productive infection [107].

The phenotypic similarity of CD14+ CD16+ monocytes and perivascular macrophages in the brain would support the concept that the CD14+ CD16+ monocytes transmigrate into the brain to differentiate further into perivascular macrophages. Being infected with HIV or SIV, they may carry the retrovirus into the brain, eventually leading to encephalopathy. Conversely, classical CD14++ CD16– monocytes can up-regulate CD16 in vitro [8, 108, 109], and an alternate scenario would suggest that the classical monocytes, which can also be infected by HIV, replenish the pool of perivascular macrophages. To resolve this question, the migration of CD14+ CD16+ monocytes into the perivascular space needs to be demonstrated formally. In this context, Ancuta et al. [42] have shown in an in vitro model that CD14+ CD16+ monocytes can migrate across brain microvascular EC in response to low doses of fractalkine. When such EC are, however, activated, then they may express membrane-bound fractalkine, which in vitro, can lead to firm adhesion of CD14+ CD16+ monocytes and failure to transmigrate under the experimental conditions [42]. The firm adhesion to fractalkine-expressing EC might, however, also lead to enhanced transmigration, as seen for human CD8+ lymphocytes that showed enhanced migration to MIP-1β when adhered to fractalkine-expressing EC [43]. Taken together, it is still open at this point in time as to whether in HIV infection, the CD14+ CD16+ monocytes will transmigrate to become perivascular macrophages.

Also, it was noted that the number CD16+ monocytes can increase in CSF fluid of patients with HIV infection and that the percentage of these CSF cells correlated with the viral load in this compartment [110]. Again, it remains to be analyzed whether these CD16+ cells in CSF are CD14+ CD16+ monocytes transmigrated from blood or whether they develop this phenotype locally.

With respect to infection of T cells by HIV, Ancuta et al. [111] have shown that the CD16+ monocytes do support T cells replication of the virus much stronger as compared with the classical CD14++ monocytes. The same group then went on to show that higher production of chemokines CCL2 and CCL24 was responsible for the higher replication of HIV in CD16+ monocyte/T cell conjugates [112]. Taken together, these studies suggest another, indirect role of CD16+ monocytes in promotion of HIV infection, in that these cells and their macrophage progeny provide signals for stimulation of HIV production by T cells.

CONCLUDING REMARKS

The finding that the CD14+ CD16+ monocytes and their mammalian homologues are potent producers of TNF but show no-to-low IL-10 production supports the concept that they are proinflammatory cells. Also, these cells and their DC progeny show a higher antigen-presenting capacity. At this point, there are conflicting data about the propensity of these cells to go into inflammatory tissues, and this may be dependent on the type and site of inflammation studied.

The increase in number of these cells has been reported for a host of inflammatory and infectious diseases in man, suggesting a role for the CD14+ CD16+ monocytes in such processes. The extent of the contribution of cells from the marginal pool to such increases remains unclear at this point, and this can best be addressed in animal models.

Although it appears that the CD14+ CD16+ monocytes can be crucial players in infection and inflammation, the formal demonstration of their role in these processes will require selective elimination of these cells in vivo.

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