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Editorial

# Editorial: **Monocyte subpopulations and lentiviral infection**

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HIV is a lentivirus of global importance, which infects CD4 T cells efficiently, leading to their depletion. Today, we have effective treatments for the infection, and the antiretroviral therapy can result in immune restoration. However, the virus cannot be eliminated completely from the body, as it persists in tissues and cellular reservoirs including monocytes/macrophages. Monocytes are bone marrow-derived cells that circulate in blood for 1–3 days. They then enter various tissues and develop into different types of macrophages. Early in vitro and in vivo studies demonstrated that blood monocytes and tissue macrophages can be infected by HIV [1, 2].

Blood monocytes can be divided into subsets based on the expression of CD14 and CD16 antigens [3]. The classical monocytes express high levels of CD14 and no CD16 ($CD14^{++}CD16^{-}$) cells, and the nonclassical monocytes express low CD14 and high CD16 ($CD14^{+}CD16^{++}$). In addition, there is a minor population of intermediate monocytes ($CD14^{++}CD16^{+}$; **Fig. 1**). The latter cells have received little attention thus far, but phenotypic studies have confirmed that their expression of various cell-surface molecules is intermediate between the classical and nonclassical monocytes [4]. These cells can expand during M-CSF therapy and in inflammatory diseases, including sarcoidosis and asthma.

Kim et al. [5] have examined monocyte subsets in macaque monkeys infected with SIV and have shown that there are dynamic changes that change with stage of disease. In their paper (this issue of *JLB*), they analyze all three monocyte subsets, i.e., the classical (termed $CD14^{high}$ $CD16^{-}$ in their paper), the nonclassical (termed $CD14^{low}CD16^{high}$), and the intermediate (termed $CD14^{high}$ $CD16^{low}$) populations. They provide the first analysis of the gene expression profile of the intermediate subset of monocytes, which demonstrates 63 genes to be more than fourfold differentially expressed by the two $CD16^{+}$ monocyte populations as compared with the classical monocytes, and 18 genes were selectively, differentially expressed by the intermediate monocytes. These data demonstrate unique features for the intermediate monocytes in rhesus monkeys at the mRNA level. In their analyses of cytoplasmic markers, Kim et al. [5] show clear distinctions in maturation between the two $CD16^{+}$ subpopulations at the protein level, and only the $CD14^{+}CD16^{++}$ cells are $CD68^{+}$ $MAC387^{-}$. In addition to these maturation markers, the general phenotype of the $CD14^{++}CD16^{+}$ monocyte population (CD11b, CD31, CD64, CCR2, and others) suggests that these cells are indeed an intermediate population between the classical and nonclassical subsets. Although transcriptome and proteome studies of these two human monocyte subsets have been published [6, 7], studies about the transcriptome and proteome of the intermediate monocytes in human blood are still to be reported.

Kim et al. [5] then have analyzed a large cohort of 54 infected rhesus macaques compared with 48 uninfected control animals for the impact on SIV infection on monocyte subsets.

The effect of HIV infection on the composition of human blood monocyte subsets has been studied earlier, showing an increase in CD16 expression on monocytes [8]. These studies were done in patients with chronic HIV infection, as kinetics of the monocyte subsets in early HIV infection in man is difficult to analyze, as the time of infection is often estimated, and when known, access to material from patients with acute infection is limited. Therefore, animal models of HIV infection using SIV, where infection of T cells and monocytes has been demonstrated, can be particularly useful. These nonhuman primates have the advantage that leukocyte subsets can be defined with the help of many of the CD markers used in man. Following the first description of the $CD16^{+}$ subset in cynomolgus monkeys nearly two decades ago (*Macaca fascicularis*), an increase of $CD14^{+}CD16^{++}$ monocytes in cynomolgus monkeys on Day 10 postinfection with SIVmac239 has been observed. Increases in $CD16^{+}$ monocytes have also been reported in rhesus monkeys (*Macaca mulatta*) in the context of lentiviral encephalitis [9].

The paper by Kim et al. [5] describes the characteristics of monocyte subsets in various phases of SIV infection in these monkeys, i.e., acute postinfection phase, chronic phase

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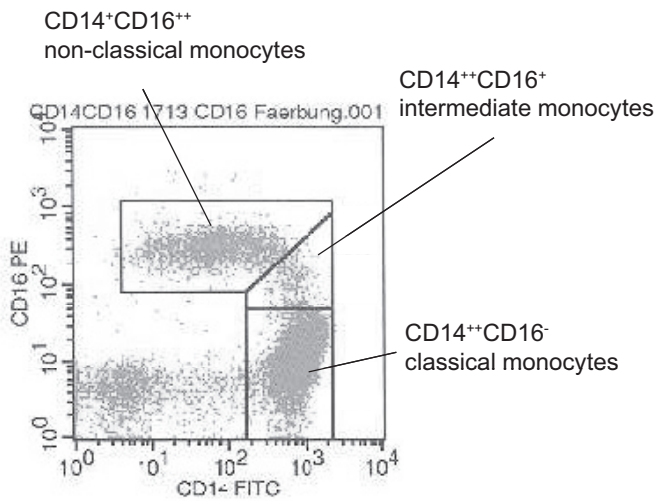


**Figure 1. The human blood monocyte subpopulations and the nomenclature used in this editorial.** Whole blood was stained with CD14 and CD16 antibodies and analyzed by flow cytometry.

with and without viral RNA in plasma, and terminal phase. In addition, these investigators address all three subsets of blood monocytes. Their data show that acute infection is accompanied by an increase in intermediate and nonclassical monocytes, and the classical monocytes decreased 2 weeks after infection. A similar increase of both $CD16^+$ monocyte subsets (intermediate and nonclassical) was observed in animals with chronic infection and with viremia. As chronically infected animals without viremia have near-normal values for these monocyte subsets, it appears that virus replication and the resultant viral load are driving forces for the expansion of the $CD16^+$ monocytes. Microbial translocation, with endotoxemia, may provide an alternative hypothesis to viremia. Animals in the terminal phase of SIV infection, which show highest levels of viral RNA in plasma, are heterogeneous with respect to expansion of CD16-positive monocytes, and some animals show very high levels and others very low, a finding probably caused by additional pathophysiological mechanisms leading to the eventual death of the animals.

With respect to virus infection and expression by monocyte subsets, Kim et al. [5] analyzed the monocyte subsets for evidence of SIV infection (levels of DNA) and evidence of viral replication (levels of RNA) in acutely infected animals. Their data relating to SIV infection in macaques generally concur with, although differ in part to, results published by other groups, including our own. One of the important contributions of this paper is the examination of three monocyte subpopulations for evidence of infection, rather than looking only at classical and nonclassical monocytes, as has been done for most human studies [10]. The study of the intermediate monocytes in humans is particularly challenging because of the very low number of these cells present in blood.

Kim and colleagues [5] report that the intermediate $CD14^{++}CD16^+$ monocyte subset contains the highest levels of SIV RNA, and they found SIV DNA levels to be equivalent in all monocyte subpopulations. This contrasts to Ellery et al. [10], who found that $CD16^+$ monocytes (not purified into the nonclassical and the intermediate monocytes) had significantly higher levels of HIV DNA compared with classical $CD14^{++}CD16^-$ monocytes.

These differences might be a result of host species and lentivirus type. Also, animals studied by Kim et al. [5] were untreated, although virologic suppression may not influence findings as shown in other studies. Low-level T cell contamination (up to 5% in the Kim et al. [5] study) may also mask differences in levels of viral DNA among the three monocyte populations. Finally, viral infection and expression are reported for acute infection in the simian model, and studies in man have analyzed infection of monocytes in the chronic phase.

Regarding explanations for the higher SIV expression in the intermediate monocytes, the authors suggest that SerpinB2 (also known as plasminogen activator inhibitor-2), which they report to be expressed strongly and specifically in $CD14^{++}CD16^+$ monocytes, may be involved, as this has been reported to promote HIV replication. Another candidate molecule that might explain the differential expression of SIV in the intermediate monocytes is APOBEC3G. $CD16^+$ human monocytes (not purified into nonclassical and intermediate monocytes) contain two forms of APOBEC3B, one of low molecular mass, which restricts HIV replication, as well as several forms with higher molecular mass similar to T cell lines that are permissive for HIV replication [10]. Whether the higher molecular mass forms are found exclusively in the $CD14^{++}CD16^+$ population, thus rendering these cells more permissive for HIV and SIV replication, is unknown. Infection of intermediate $CD14^{++}$ $CD16^+$ monocytes (and of nonclassical $CD14^+CD16^{++}$ monocytes) has been reported for hepatitis C virus [11], demonstrating that the intermediate monocytes may be important in this viral disease as well.

When discussing monocyte subsets, we need to keep in mind that differences in excitation, amplification, and compensation settings of the flow cytometer can lead to different representations of cells in the two-color plots. Also, the gates set to define the subsets may differ. This is especially true for the intermediate monocytes, which can be separated unequivocally from the classical monocytes based on isotype controls but not from the nonclassical monocytes. Until selective markers for the intermediate monocytes are available, it therefore will

remain difficult to compare data about these cells generated by different laboratories.

Overall, this exciting body of work by Kim et al. [5] demonstrates that expansion of the $CD16^+$ monocytes is associated with viral replication. It shows a unique pattern of gene expression in the intermediate $CD14^{++}$ $CD16^+$ simian blood monocytes, and it demonstrates that these cells show the highest level of SIV replication. Similar studies are needed in man to confirm and expand these intriguing findings, which suggest a unique role of the intermediate monocytes in lentivirus infection.

## DISCLOSURE

We confirm this has not been published or submitted elsewhere. There are no competing financial interests.

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