Research Letter

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Soluble CD14 is a nonspecific marker of monocyte activation

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Soluble CD14 is associated with morbidity and mortality in HIV disease. It is a co-receptor for lipopolysaccharide (LPS) that is released from monocytes upon activation. We demonstrate here, that inflammatory cytokines can induce the release of sCD14 in peripheral blood mononuclear cell cultures from healthy donors, and that TLR ligands other than LPS can cause a decrease in the monocyte cell surface expression of CD14. Thus, sCD14 is a marker of monocyte activation, not restricted to activation by LPS.

Elevated plasma levels of soluble CD14 (sCD14) are associated with poor prognosis in HIV-infected patients and are a strong predictor of morbidity and mortality [1–4]. Increased plasma sCD14 levels can persist even in treated HIV-infected patients, with durable control of viremia [5,6], and this is associated with diminished $CD4^+$ T-cell restoration [5]. Additionally, plasma levels of sCD14 have been associated with cardiovascular disease in HIV infection [7], as well as in HIV-uninfected patients [8]. Elevated plasma levels of sCD14 are also observed in pediatric inflammatory lung diseases [9], in chronic hepatitis C virus (HCV) and hepatitis B virus (HBV) infections [10,11], and in rheumatoid arthritis [12].

CD14 is a myeloid differentiation marker found primarily on monocytes and macrophages, although low levels are also found on neutrophils. CD14 can exist as a glycophosphatidylinositol (GPI)-anchored membrane protein or as one of two soluble isoforms that can be generated either by cleavage from the surface of the cell or released from intracellular pools [12–16]. CD14 is a co-receptor for lipopolysaccharide (LPS) – a cell wall component of gram-negative bacteria [17,18] – and it has been suggested that high levels of sCD14 in the plasma are reflective of LPS exposure [10,19]. Yet, CD14 does not bind only LPS; it can also bind Gram-positive cell wall components, lipoarabinomannan (LAM) and heat shock protein-60 (HSP 60) [20,21], as well as endogenous lipids [22,23].

Upon cell activation, CD14 surface expression decreases on monocytes and sCD14 is released. Although release of sCD14 is often accompanied by a decrease in surface expression of CD14, this is not always the case. This suggests that sCD14 can be generated by a mechanism other than cleavage from the cell surface [14]. Although LPS is a very potent activator of monocytes and induces protease-dependent generation of sCD14, we demonstrate here that a variety of Toll-like receptor (TLR) ligands can cause a down-modulation of CD14 on monocytes. We stimulated peripheral blood mononuclear cells (PBMCs) from healthy donors overnight with medium alone or medium supplemented with LPS (20 ng/ml; Sigma, St Louis, Missouri, USA), flagellin (1 μg/ml; InvivoGen, San Diego, California, USA), Cytosine-phosphate-guanine (CpG) oligodeoxynucleotides (CpG 2395 3 μg/ml; InvivoGen), or a combination of all three TLR ligands. The following day, the cells were washed and stained with anti-CD14-phycoerythrin (PE) antibodies (BD, San Jose, California, USA). Events were acquired on an LSRII flow cytometer and analyzed for expression of CD14. The fluorescence of CD14 expression after stimulation is shown in Fig. 1a. Stimulation with each individual TLR ligand caused a decrease in CD14 surface expression, and the greatest decrease was seen when all three ligands were combined.

Recognizing that the activation of monocytes by LPS induces inflammatory cytokines, we stimulated PBMCs from healthy donors with LPS (100 ng/ml; Sigma), recombinant human interleukin (IL)-6 (100 ng/ml; R&D Systems, Minneapolis, Minnesota, USA), or recombinant human IL-1β (10 ng/ml; R&D Systems). After 48 h of stimulation, we collected the culture supernatant and measured the concentration of sCD14 by ELISA (R&D Systems). We show that PBMCs stimulated with either IL-6 or IL-1β can induce sCD14 at levels comparable to, or only slightly less than concentrations induced by LPS (Fig. 1b). Similar findings were reported by Bas *et al.* [12] after stimulation of the human hepatoma cell line, HepG2. Though the levels they measured were lower than what we demonstrated, they saw the release of sCD14 from HepG2 cells after stimulation with IL-6, but saw slight decreases in sCD14 levels in HepG2 hepatoma cultures stimulated with IL-1β [12]. Marcos *et al.* [9] also saw increased culture supernatant levels of sCD14 after 40 h stimulation of PBMCs with various TLR ligands, including LPS and CpG oligodeoxynucleotides. Bas *et al.* examined plasma levels of sCD14, IL-6, and C-reactive protein from arthritis patients, and found elevated plasma levels of sCD14 not only in patients with infection-mediated arthritis but also in patients with both crystal-mediated and rheumatoid arthritis [12]. This suggests that, *in vivo*, a number of inflammatory stimuli may cause elevated plasma levels of sCD14 and the presences of LPS is not required.

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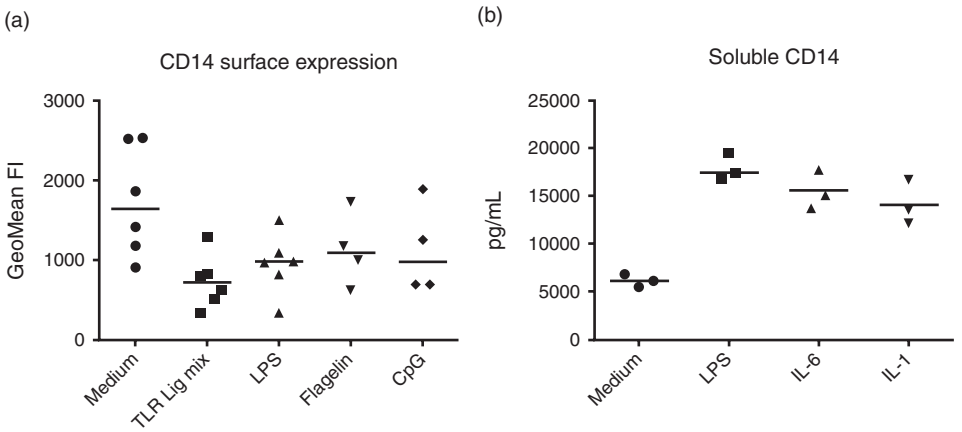

Fig. 1. Soluble CD14 is generated by monocyte activation, not only by lipopolysaccharide stimulation. (a) PBMCs from healthy donors were stimulated overnight with medium alone or medium supplemented with LPS (20 ng/ml), flagellin (1 μg/ml), CpG-2395 (3 μg/ml), or a combination of the three TLR ligands. Samples were stained and then acquired by flow cytometry, and expression of surface CD14 is shown as the geometric mean fluorescence intensity. (b) PBMCs from healthy donors ($n = 3$) were stimulated for 48 h with medium alone or medium supplemented with LPS (100 ng/ml), human IL-6 (100 ng/ml), or IL-1β (10 ng/ml). After 48 h, supernatant was measured for sCD14 levels by ELISA. IL, interleukin; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell.

In conclusion, although LPS is a potent monocyte activator that binds to CD14 and induces cellular release of sCD14, other TLR ligands such as flagellin and CpG oligodeoxynucleotides can also induce release of sCD14 as can inflammatory cytokines such as IL-6 and IL-1β. Therefore, sCD14 should be considered a marker of monocyte activation that does not necessarily reflect monocyte activation by LPS.

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Conflicts of interest

The authors have no conflicting interests to declare.

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