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Soluble CD14 acts as a DAMP in human macrophages: origin and involvement in inflammatory cytokine/chemokine production

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Short title: sCD14 in human macrophages

29 **ABBREVIATIONS**

30

31 Abbreviations used in this article: sCD14, soluble form of CD14; CF, cystic fibrosis; DAMPs,
32 danger-associated molecular patterns; ER, Endoplasmic Reticulum; FEV1, Force Expiratory
33 Volume in one second; NLRs, NOD-like receptors; PAMPs, pathogen-associated molecular
34 patterns; PI-PLC, phosphatidylinositol-phospholipase C.

35

36 **ABSTRACT**
37
38 The innate immune system is able to detect bacterial lipopolysaccaride (LPS) through the pattern
39 recognition receptor CD14, which delivers LPS to various TLR signaling complexes that
40 subsequently induce intracellular pro-inflammatory signaling cascades. In a previous study, we
41 showed the overproduction of the soluble form of CD14 (sCD14) by macrophages from patients
42 with cystic fibrosis (CF). CF is an autosomal recessive disorder that is caused by mutations in the
43 gene that encodes the CFTR protein and characterized by persistent inflammation. Macrophages
44 play a significant role in the initial stages of this disease due to their inability to act as suppressor
45 cells leading to chronic inflammation in CF. In this work, we investigated the origin of sCD14 by
46 human macrophages and studied the effect of sCD14 on the production of inflammatory
47 cytokine/chemokine. Our data indicate that sCD14 stimulate pro-inflammatory
48 cytokine/chemokine production in a manner that is independent of LPS but dependent on the
49 TLR-4/CD14 membrane complex, NF-κB and the inflammasome. Therefore sCD14,
50 overproduced by CF macrophage, originates primarily from the endocytosis/exocytosis process
51 and should be considered to be a DAMP. In fact, this elucidation of the origin and inflammation-
52 induced mechanisms associated with sCD14 contributes to our understanding of maintained
53 tissue inflammation.
54
55 **Key words:** inflammation, cystic fibrosis, NF-κB, inflammasome
56
57
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59

60 **INTRODUCTION**

61

62 CD14 was first characterized as a membrane-associated GPI-anchored protein and a cell surface
63 differentiation marker that is present on the surface of monocytes, macrophages, dendritic cells
64 and neutrophils (1–5). CD14 acts as a receptor for bacterial LPS in cooperation with TLR-4 and -
65 2 (5, 6) and can bind various bacterial ligands and receptors on phagocytes, thereby mediating the
66 phagocytosis of bacteria and the clearance of apoptotic cells (4, 7–9). CD14 also exists as two
67 soluble forms: a form with a low molecular mass of 48-50 kDa and a form with a high molecular
68 mass of 53-56 kDa. The mechanisms of CD14 production have been reported to be linked to
69 neosynthesis, proteolytic or GPI-tail cleavage by phospholipases or endocytosis/exocytosis (2, 3,
70 10–18). In cells that express membrane CD14, including mononuclear cells, high concentrations
71 of sCD14 are able to inhibit LPS-mediated functional responses (19, 20). In cells that lack
72 membrane CD14, including most epithelial and endothelial cells, sCD14 enables these cells to
73 respond to LPS (8, 21). At the local site of infection, pro-inflammatory signals that result from
74 sCD14 can be protective, leading to local clearance of invading bacteria. However, widespread
75 infection and activation of this pro-inflammatory signaling pathway can cause fatality through
76 sepsis (22, 23). Furthermore, significant levels of sCD14 were detected in both serum and
77 bronchoalveolar lavage fluid from patients with acute respiratory distress syndrome (24). In
78 addition, it was demonstrated in a murine model that *Streptococcus pneumoniae* uses sCD14 in
79 the bronchoalveolar space to cause invasive respiratory tract infections (25). Overall, sCD14
80 appears to act as a key component during pulmonary inflammation/infection.

81

82 Chronic bacterial airway infection and subsequent intense neutrophilic inflammation with the
83 release of intracellular proteases are considered to be the main contributors to bronchiectasis and
84 end-stage lung disease CF patients. Recent research highlighted the worsening role of
85 inflammation and immune responses in CF airway disease (26). Macrophages are antigen-
86 presenting phagocytes that secrete pro-inflammatory mediators and antimicrobial factors in
87 response to challenge by extracellular pathogens. Recently, several studies showed that specific
88 features of macrophage activation in CF patients play an important role during the CF disease
89 process (27–29). In our previous work, we observed an increase in sCD14 secretion by peripheral
90 monocyte-derived macrophages from stable adult CF patients (29). However, the origin and

91 involvement of sCD14 has not been studied. In this work, we examined the mechanisms involved
92 in the release of sCD14 by peripheral monocyte-derived macrophages from adult with CF and the
93 inflammatory signaling pathways that are induced by sCD14 in non CF cells.

94

95 **MATERIALS AND METHODS**

96

97 **CF patients**

98 The experiments were conducted according to the Good Clinical Practice guidelines (Kong,
99 1997) and approved by the Ethics Committee for human subjects of Rennes University Hospital
100 (France, Ethics No. 11/38-827). All patients included in this study gave written informed consent.
101 Sixty stable adult patients with CF were recruited at the '*Centre de Ressources et de*
102 *Compétences pour la Mucoviscidose*' of Rennes University Hospital (France). The CF patients
103 considered for inclusion were Caucasian and included 27 males and 33 females, who were aged
104 between 18 and 52 years (mean age: 30 ± 9). The CF diagnosis was based on typical clinical
105 manifestations of the disease and confirmed by positive sweat tests and by CFTR gene mutation
106 detection. Stable patients were defined by the absence of changes in symptoms in the 3 months
107 prior to the study. All patients with CF received medication at the time of blood collection,
108 including azithromycin (27%), aerosol DNAse (48%), inhaled corticosteroids (72%) and azole
109 therapy (47%). Oral corticosteroid therapy at the time of blood collection was an exclusion
110 criterion, as this therapy may influence inflammatory phenotypes. Patients with the G551D
111 mutation were not treated with ivacafor at the time of their participation in the study (except for
112 patient 19, supplemental Table 1). The clinical features of the patients are reported in
113 supplemental Table 1. According to the FEV1 values (% predicted), the majority of our patients
114 (41/60) had mild to moderate lung disease (FEV1 values ≥ 55%). In their sputum samples, 32
115 patients had microbiological evidence of *Pseudomonas aeruginosa,* and *Staphylococcus aureus*
116 and *Aspergillus fumigatus* were detected in 45 and 29 patients, respectively. The CF genotypes
117 were representative of the French CF population, with 57% of participants having
118 F508del/F508del mutations, 36% of patients having F508del/other mutations and 7% of patients
119 having no F508del mutations (30). Blood monocyte counts were within the normal range, with a
120 median number of 0.679×10^9/L (range 0.23-1.12×10^9/L).

121

122 **Cell cultures and treatments**

123 Leukocytes were isolated by Ficoll gradient centrifugation, as described previously (31).
124 Peripheral blood mononuclear cells from healthy non-CF subjects (written consent for the use of

125 blood samples for the research protocol was obtained, according to the regulation for blood
126 transfusion of the French blood organization EFS, Rennes) were seeded according to the specific
127 blood count of each subject. Monocytes, which were selected *via* a 1-hour adhesion step, were
128 differentiated for 6 days using GM-CSF (400 UI/ml, Genzyme, Lyon, France) in RPMI 1640
129 medium supplemented with 2 mM glutamine, antibiotics and 10% FBS (Lonza, Levallois Perret,
130 France). Before treatment, the macrophages were placed in medium without serum for 24 hours.
131 To study proteolytic origin of sCD14, non-CF macrophages were treated by e64d (1 µM, Sigma-
132 Aldrich, Saint-Quentin Fallavier, France), pepstatin A (1 µM, Sigma-Aldrich, Saint-Quentin
133 Fallavier, France), EDTA (0.1 mM, Sigma-Aldrich, Saint-Quentin Fallavier, France) or aprotinin
134 (0.3 µg/ml, Sigma-Aldrich, Saint-Quentin Fallavier, France) for 24 hours. To study GPI-tail
135 cleavage by phospholipase, PI-PLC (1 U/ml, Life Technologies, ThermoFisher Scientific, Saint
136 Aubin, France) was added to non-CF macrophages for 2 hours in the absence or presence of the
137 PI-PLC inhibitor U-73122 (12.5 µM, 1-hour pretreatment, Bertin Pharma, Montigny le
138 Bretonneux, France). In parallel, macrophages from CF patients were treated with U-73122 for
139 24 hours. To study membrane trafficking pathways, the cells were supplemented with water-
140 soluble cholesterol (15 to 30 µg/ml, 1-hour pretreatment, followed by 24 hours, Sigma-Aldrich,
141 Saint-Quentin Fallavier, France) or with brefeldin A (100 ng/ml, 1-hour pretreatment, followed
142 by 2 h, Cell Signaling Technology, Ozyme, Saint Quentin en Yvelines, France). To study
143 inflammatory signaling pathways, human recombinant sCD14 protein (500 ng/ml, <0.1 EU/µg
144 endotoxin, Sigma-Aldrich, Saint-Quentin Fallavier, France) was added to non-CF macrophages
145 for 24 hours in the absence or presence of Bay 11-7082 (1 and 10 µM, 1-hour pretreatment,
146 Calbiochem, Merck Millipore, Molsheim, France) or the caspase inhibitor Z-WEHD-FMK (1 to
147 30 µM, 30-min pretreatment, Calbiochem, Merck Millipore, Molsheim, France). To study TLR
148 involvement in the inflammatory effect of sCD14, sCD14 was added to non-CF macrophages in
149 the presence of the TLR-4 antagonist LPS-RS (LPS from the photosynthetic bacterium
150 *Rhodobacter sphaeroides*, 2 µg/ml, InvivoGen, Toulouse, France), a CD14-neutralizing antibody
151 (anti-hCD14 IgA, 10 µg/ml, 1-hour pretreatment, InvivoGen, Toulouse, France), a TLR4-
152 neutralizing antibody (anti-hTLR4 IgA, 10 µg/ml, 1-hour pretreatment, Invitrogen, ThermoFisher
153 Scientific, Saint Aubin, France) or control IgG2a (10 µg/ml, 1-hour pretreatment, InvivoGen,
154 Toulouse, France). At the doses used, the treatments did not affect the viability of the
155 macrophages (data not shown). Viability was measured using the CellTiter 96® AQueous One

156 Solution Cell Proliferation Assay (Promega, charbonnières les bains, France) according to the
157 instructions provided by the manufacturer.
158

159 **Gene expression**

160 RNA expression was analyzed using RT-qPCR assays. Briefly, total RNA was isolated from CF
161 macrophages using NucleoSpin® RNA XS (Macherey Nagel, Hoerdt, France) and from non-CF
162 macrophages using the PureLink RNA Mini Kit (Ambion®, ThermoFisher Scientific, Saint
163 Aubin, France). The total RNA (1 µg) was reverse transcribed into cDNA using the High
164 Capacity cDNA Reverse Transcription Kit (Applied Biosystems ThermoFisher Scientific, Saint
165 Aubin, France). Real-time PCR was performed using the Power SYBR Green PCR Master kit
166 (Applied Biosystems ThermoFisher Scientific, Saint Aubin, France) according to the
167 manufacturer's instructions and an ABI Prism 7300 detector (Applied Biosystem ThermoFisher
168 Scientific, Saint Aubin, France). The gene-specific primers for 18S, CD14, IL-1β, IL-8 were
169 Quantitect®Primer Assay primers obtained from Qiagen (Hilden, Germany). The gene-specific
170 primers for 18S, TNF, MMP-9, MMP-12, Cathepsin B, Cathepsin D, Cathepsin F, Cathepsin K,
171 Cathepsin S, ADAM-9, ADAM-10, ADAM-12, ADAM-17, and PLCγ were purchased from
172 Eurogentec (Liège, Belgium). A list of the primer sequences used for the RT-qPCR analysis is
173 reported in supplemental Table 2. The amplification curves of the PCR products were analyzed
174 with the ABI Prism SDS software using the comparative cycle threshold (CT) method. Relative
175 gene expression was calculated by comparing the number of thermal cycles that were necessary
176 to generate threshold amounts of product (CT). The CT was calculated for the each gene and for
177 the housekeeping gene 18S. For each cDNA sample, the 18 S CT was subtracted from the CT for
178 each gene to yield the ΔCT, thus normalizing the initial amount of RNA used. The amount of
179 mRNA was calculated as $2^{-\Delta\Delta CT}$, where the ΔΔCT is the difference between the ΔCT of the two
180 cDNA samples to be compared. The data from CF samples are expressed relative to the mRNA
181 level found in non-CF samples. The data from the treatment samples are expressed relative to the
182 mRNA level found in the controls.
183

184 **Cytokine/chemokine, sCD14 and NFκB p65 level quantification**

185 IL-1β, IL-8, TNF-α and sCD14 levels were measured in the supernatants of the cell cultures and
186 in plasma from CF patients using a Duoset® ELISA kit (R&D system, Abingdon, United
187 Kingdom), according to the instructions provided by the manufacturer. The NFκB p65 level was
188 measured in the nuclear fraction of cell cultures using TransAM® NFκB p65 (Active Motif, La
189 Hulpe, Belgium), according to the instructions provided by the manufacturer.
190
191 **Neosynthesis measurement**
192 Soluble CD14 neosynthesis was measured using Click-IT ® technology (Thermo-fischer
193 scientific). Macrophages were placed in RPMI 1640 medium without methionine (Gibco,
194 ThermoFisher Scientific, Saint Aubin, France). After 30 min incubation (37°C, 5% CO_2),
195 macrophages were pulse-chase labeled in the presence of Click-IT® L- Azidohomoalanin
196 (50µM; Molecular Probes, ThermoFisher Scientific, Saint Aubin, France) in RPMI 1640 medium
197 without methionine for 3 hours. Afterwards, medium was changed by RPMI 1640 with
198 methionine and macrophages were incubated for 21 hours (37°C, 5% CO_2). Then supernatants
199 were recovered and analyzed for sCD14 level quantification using Duoset® ELISA kit (R&D
200 system, Abingdon, United Kingdom). . To measure neosynthesis, biotin-coupled detection
201 antibody was substituted by Click-IT® Biotin DIBO Alkyne (Molecular Probes, ThermoFisher
202 Scientific, Saint Aubin, France), for copper-free click chemistry.
203
204 **Cholesterol level quantification**
205 Total lipids were extracted with 200 µl $CHCl_3$/MeOH/NP-40 (7:11:0.1; v/v). The sample was
206 centrifuged for 10 min at 14,000 rpm. The chloroformic layer was evaporated to dryness under a
207 nitrogen stream. Then, the free cholesterol levels was determined using Cholesterol/Cholesteryl
208 Ester Quantitation Kit (Merck Millipore, Molsheim, France), according to the instructions
209 provided by the manufacturer. Cholesterol is oxidized by cholesterol oxidase to yield H_2O_2. The
210 resulting H_2O_2 interacts with a sensitive cholesterol probe to produce resorufin, which can be
211 detected by spectrophotometrically 570 nm. Total protein concentrations were determined using a
212 BCA protein assay kit (Thermoscientific, Saint Aubin, France) to express cholesterol based on
213 the protein concentration for each sample.
214

215 **Zymography assay**

216 The gelatinolytic activity of secreted MMP-9 was analyzed using gelatin zymography, as
217 described previously (32). The samples were subjected to electrophoresis via 10% SDS-PAGE
218 containing 1 mg/ml of gelatin (Sigma Aldrich, Saint-Quentin Fallavier, France) under non-
219 reducing conditions. After electrophoresis, the gels were washed twice with 2.5% Triton X-100,
220 rinsed with water, and incubated at 37°C overnight in reaction buffer (50 mM Tris, 5 mM $CaCl_2$,
221 and 2.1 mM $ZnCl_2$, pH 8.0). The gels were stained with Coomassie brilliant blue (Sigma Aldrich,
222 Saint-Quentin Fallavier, France) and de-stained in a solution of 25% ethanol and 10% acetic acid.
223 Gelatinase activity appeared as clear bands against a blue background. The molecular weights of
224 the gelatinolytic bands were estimated using a prestained protein ladder (10 - 250 kDa,
225 Euromedex, Souffelweyersheim, France). Images of the zymograms were acquired using the Gel
226 Doc 1000 Gel Documentation System (Bio-Rad laboratories, Marnes-La –Coquette, France), and
227 both the surface and intensity of the lysis bands were quantified by densitometry using the
228 MultiGauge software (Fujifilm, Tokyo, Japan).

229

230 **Gene silencing by siRNA**

231 ON-TARGETplus siRNAs against NLRC4 or NLRP3 were synthesized and annealed by
232 Dharmacon RNAi Technologies (ThermoFisher Scientific, Saint Aubin, France). siRNA
233 duplexes were transfected using the Lipofectamine RNAiMAX Reagent (Life Technologies,
234 ThermoFisher Scientific, Saint Aubin, France). The transfection reagent and siRNAs were mixed,
235 and complex formation was allowed to proceed for 5 min at room temperature before the
236 complex was added to the macrophages. After 72 hours, the cells were treated or not treated with
237 sCD14 (500 ng/ml, 24 hours) before being harvested for analysis.

238

239 **Immunoblotting**

240 Membrane-bound proteins were extracted from the macrophages by lysing the cells with RIPA
241 lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton-X100,
242 12 mM deoxycholate, 2 mM NaF, 1 mM Na_3VO_4, 2 mM PMSF, cOmplete™ EDTA-free
243 Protease Inhibitor Cocktail (Roche, Bâle, Switzerland) and PhosSTOP Phosphatase Inhibitor
244 Cocktail (Roche, Bâle, Switzerland). The macrophages were incubated at 4°C for 30 minutes in
245 lysis buffer with vortexing for 30 seconds every 10 minutes. The supernatant containing proteins

246 was obtained by subsequent centrifugation at 10,000 g for 10 minutes at 4°C, and the protein
247 concentration was determined using a BCA protein assay kit (ThermoFisher Scientific, Saint
248 Aubin, France). Proteins were separated via SDS-PAGE and transferred to a nitrocellulose
249 membrane. Then, the membrane was subjected to western blotting using a rabbit anti-IκBα, rabbit
250 anti-P-IκBα, rabbit polyclonal anti-Caspase 1, mouse anti-IL-1β, rabbit anti-NLRP3, rabbit anti-
251 NLRC4 (Cell Signaling Technology, Ozyme, Saint Quentin en Yvelines, France), rabbit anti-
252 CD14 (Epitomics, Burlingame, CA, US) or mouse anti-HSC70 (Santa Cruz, Heidelberg,
253 Germany) antibodies. Horseradish peroxidase-conjugated goat anti-rabbit (Cell Signaling
254 Technology, Ozyme, Saint Quentin en Yvelines, France) or goat anti-mouse (Dako, Les Ulis,
255 France) antibodies were used as secondary antibodies, and proteins were detected using enhanced
256 chemiluminescence. The images were scanned with the Fujifilm LAS-3000 imager (Fujifilm,
257 Tokyo, Japan) and analyzed with the MultiGauge software (Fujifilm, Tokyo, Japan) for
258 densitometry. The intensity of the bands was normalized based on HSC-70.
259
260 **Statistical analysis**
261 The number of subjects and experiments used in each group is stated in the respective figures.
262 Statistical significance was evaluated by using the GraphPad Prism software v.6.0 (GraphPad
263 Software Inc., San Diego, CA, US). A non-parametric Mann-Whitney test was used to assess the
264 statistical significance of differences between non-CF and CF groups. A parametric ratio
265 Student's t-test was used to assess statistical significance between treatments. For each analysis, a
266 p-value <0.05 was considered to be significant.
267

268 **RESULTS**

269

270 **Origin of sCD14 in human CF macrophages**

271

272 We previously observed the overproduction of sCD14 in the supernatant of macrophages from
273 stable adult patients with CF (29). However, plasma levels of sCD14 do not differ between stable
274 adult CF patients and healthy subjects (Fig. 1*A*).

275

276 To elucidate the molecular mechanisms that underlie the production of the soluble form of CD14
277 in human CF macrophages, we studied several hypotheses based on the literature. It was reported
278 that sCD14 could be generated by different mechanisms, including the endocytosis of mCD14,
279 the cleavage of the GPI anchor by PI-PLC, direct proteolytic cleavage from the cell surface and
280 neosynthesis.

281

282 To explore the neosynthesis hypothesis, we examined the regulation of CD14 production at the
283 mRNA level in CF and non-CF macrophages. We found that CD14 mRNA levels were similar
284 between CF and non-CF macrophages (Fig. 1*B*). Furthermore, pulse chase experiments with
285 Click-IT® L- Azidohomoalanin showed no differences in neosynthesis of sCD14 between non-
286 CF and CF macrophages whereas we demonstrated an increase of total sCD14 production in
287 supernatant of CF macrophages (Fig. 1*C*). Thus, neosynthesis can be ruled out as a mechanism.

288

289 sCD14 may be formed by direct proteolytic cleavage from the cell surface. However, the
290 inhibitors of metalloproteases (EDTA, Fig. 2*A*), cysteine proteases (e64d, Fig. 2*D*), aspartic
291 proteases (pepstatin A, Fig. 2*E*) or serine proteases (aprotinin, Fig. 2*F*) did not influenced sCD14
292 production. Furthermore, the gene expression of the metalloproteases MMP-9 (Fig. 2*B*), MMP-12
293 (Supplemental Fig. 1*A*), ADAM-9, -10, -12, 17 (Supplemental Fig. 1*B* to 1*E*) and cathepsins B,
294 D, F, K and S (Supplemental Fig. 1*F* to 1*J*) in CF macrophages is decreased. Moreover, the
295 latent form of the MMP-9 protein and supernatant MMP-9 activity, as measured by zymography,
296 are decreased in CF *vs.* non-CF macrophages (Fig. 2*C*). The protease cleavage hypothesis is
297 excluded by these results.

298

299 The main hypothesis concerning sCD14 generation is the cleavage of the GPI tail by PI-PLC. We
300 investigated this hypothesis by exposing non-CF macrophages to PI-PLC (1 U/ml) with or
301 without its inhibitor, U-73122 (12.5 μM). We observed that PI-PLC significantly increased the
302 production of sCD14 by non-CF macrophages and that this effect was inhibited by U-73122 (Fig.
303 3*A*). However, the treatment of CF macrophages with U-73122 did not inhibit the increased
304 secretion of sCD14 into their supernatant (Fig. 3*B*). Furthermore, the expression of the PLC gene
305 does not differ between non-CF and CF macrophages (Supplemental Fig. 1*G*). Indeed, PI-PLC
306 participates in the production of sCD14 in non-CF macrophages but has no role in sCD14
307 overproduction from CF macrophages.
308
309 Cholesterol has been reported to increase sCD14 secretion through the endocytosis and
310 subsequent cleavage of mCD14. Furthermore cholesterol is increased in CF macrophages (Fig.
311 4*A*). Thus, we investigated the effect of water-soluble cholesterol supplementation in non-CF
312 macrophages. The results presented in Fig. 4*B* show that sCD14 production by non-CF
313 macrophages is increased by supplementation with water-soluble cholesterol (15 μg/ml). By
314 modulating membrane lipid composition, cholesterol could be involved in the production of
315 sCD14 in CF macrophages.
316 The fungal metabolite brefeldin A blocks forward protein transport between the ER and the Golgi
317 complex but not retrograde transport (33). Incubation with brefeldin A decreased sCD14
318 secretion in non-CF and CF macrophages, implicating the secretory ER/Golgi pathway in sCD14
319 secretion in CF (Fig. 4*C*).
320
321 **Function of human recombinant sCD14 in human macrophages**
322
323 To evaluate the inflammatory role of sCD14 in human non-CF macrophages, we used human
324 recombinant sCD14 (hr-sCD14), which did not affect the viability of the cells (data not shown).
325
326 We had to check the effect of human recombinant sCD14 on the NFκB activation, which is
327 required for cytokine/chemokine production. In fact, the mean concentration of sCD14 in CF
328 macrophage supernatants was 14.68 ng/ml per $2x10^6$ cells/well (Fig. 3*B*), which corresponds to
329 220 ng/ml per $30x10^6$ cells, in comparison to the mean level of sCD14 observed in non-CF

330 macrophage supernatants, which was 2.35 ng/ml per 30x10^6 cells/well (Fig. 4*B)*. Indeed, we used
331 increasing concentrations of human recombinant sCD14 (1, 10, 100 and 500 ng/ml on 30x10^6
332 non-CF cells/well) to evaluate its inflammatory role in macrophages.
333
334 To determine whether sCD14 has an inflammatory effect, we investigated the production of pro-
335 inflammatory cytokine/chemokine by non-CF macrophages exposed to sCD14. Treatment with
336 sCD14 significantly increased IL-1β, IL-8 and TNF-α gene expressions at 6 hours and secreted
337 levels at 24 hours in a dose-dependent manner (Fig. 5*A* and 5*B*). The effects on gene expression
338 were maintained at 24 hours (Fig. 5*A*). These results show that sCD14 could participate in
339 chronic inflammation by promoting pro-inflammatory cytokine/chemokine secretion.
340 Furthermore, sCD14 potentiates LPS-induced cytokine/chemokine production by non-CF
341 macrophages (Supplemental Fig. 2).
342
343 The NF-κB pathway is one of the pathways involved in cytokine expression. We studied NF-κB
344 activation after sCD14 treatment in non-CF macrophages. The results showed the
345 phosphorylation of IκBα at 10 min, concomitant with a decrease in the IκBα protein and a time-
346 dependent translocation of the NFκB p65 subunit to the nucleus (Fig. 6*A* and 6*B*). Indeed, the
347 NF-κB pathway is activated by sCD14 in non-CF macrophages. To determine whether this
348 activation is related to the expression and secretion of inflammatory cytokines, we pretreated
349 non-CF macrophages with an inhibitor of NF-κB, Bay 11-7082. Bay 11-7082, which is a
350 potential anti-inflammatory agent, is an irreversible inhibitor of IKKα and cytokine-inducible
351 IκBα phosphorylation that does not affect constitutive phosphorylation (34). A significant
352 decrease in IL-1β, TNF-α and IL-8 gene expressions and secreted levels was observed with Bay
353 11-7082 (Fig. 6*C* and 6*D*). These results show that the NF-κB pathway participates in the pro-
354 inflammatory cytokine/chemokine production induced by non-CF macrophages sCD14 treatment.
355
356 Unlike other pro-inflammatory cytokines, IL-1β production is tightly regulated by a unique two-
357 signal mechanism. The primary signal induces the expression of pro-IL-1β and is mediated in
358 part by NF-κB activation. The secondary signal activates the NLR-subset inflammasome
359 intracellular signaling complexes, which are composed of NLRs, pro-caspase 1, and the adaptor

360 protein ASC. The formation of the inflammasome complex results in the proteolytic cleavage of
361 pro-caspase 1 to yield active caspase 1, which in turn cleaves pro-IL-1β, producing mature and
362 active IL-1β. To determine if caspase 1 could be involved in IL-1β processing in response to
363 sCD14, we investigated caspase 1 expression and activation. Macrophage exposure to sCD14
364 induced early caspase 1 cleavage starting after 5 min through the early production of pro-caspase
365 1 and the appearance of cleaved caspase 1 (Fig. 7*A*). This cleavage is followed by the subsequent
366 secretion of IL-1β by macrophages starting after 120 min (Fig. 7*B*). To investigate the
367 involvement of caspase 1 in IL-1β secretion after sCD14 treatment, we pre-incubated non-CF
368 macrophages with the caspase 1 specific inhibitor Z-WEHD-FMK. The caspase 1 inhibitor
369 abolished IL-1β secretion by non-CF macrophages (Fig. 7*C*). All of these results showed that IL-
370 1β production in response to sCD14 involves the caspase 1 pathway.
371
372 Caspase 1 pathway activation requires a specific platform called the inflammasome complex.
373 However, various inflammasome complexes are formed depending on the stimulus. Concerning
374 the inflammasome complex in macrophages, the most frequently involved stimuli are flagellin or
375 LPS. We investigated the NLRC4 and NRLP3 inflammasome complexes by using RNA
376 interference. The efficiency of transfection of NLRC4 siRNA and NLRP3 siRNA was assessed
377 via western blotting of the total protein fraction from non-CF macrophages. Specific siRNAs
378 directed against NLRC4 and NLRP3 turned off NRLC4 and NLRP3 protein expression in
379 macrophages, respectively (Fig. 8*A* and 8*B*). Furthermore, while transfection with NLRP3 siRNA
380 or NLRC4 siRNA had no significant effect on IL-1β secretion, in macrophages treated with
381 sCD14, transfection with each siRNA significantly decreases IL-1β secretion (Fig. 8*C*). These
382 results suggested that both inflammasome platforms (NLRC4 and NLRP3) participate in the
383 induction of IL-1β secretion in response to sCD14 exposure.
384
385 In view of the mechanisms described above, the TLR-4 receptor and its signaling pathways
386 should be involved in the effects induced by sCD14 in macrophages. To efficiently neutralize the
387 biological activity of sCD14, non-CF macrophages were pre-incubated with a CD14-neutralizing
388 antibody, resulting in decreased IL-8, TNF-α and IL-1β levels in the cell supernatants (Fig. 9).
389 Moreover, the pretreatment of non-CF macrophages with an antagonist of TLR-4 (LPS-RS)

390 strongly inhibited the TNF-α, IL-8 and IL-1β production induced by sCD14 (Fig. 9). Finally to
391 neutralize TLR4, non-CF macrophages were pre-incubated with a TLR4-neutralizing antibody,
392 resulting in decreased IL-8 and TNF-α levels in the cell supernatants. Treatment with sCD14 did
393 not modify the membrane expression of TLR-4, as measured by flow cytometry, showing that
394 TLR-4 is not endocytosed after activation (data not shown). In this context, TLR-4 should be
395 involved in the inflammatory effect of sCD14.

396 **DISCUSSION**

397

398 Macrophages coordinate inflammatory responses by secreting cytokines and chemokines that are
399 responsible for diverse physiological effects that are important in the pathogenesis of CF and
400 result in chronic infection/inflammation (35–37).

401

402 In a previous study, we observed an increase in cytokine/chemokine production in CF
403 macrophage supernatants and particularly strong secretion of sCD14 (29). Elevated
404 concentrations of sCD14 were also found in serum from patients with bacterial infections (38, 39)
405 or pneumonia (40), as well as in other chronic inflammatory diseases, such as lupus
406 erythematosus (41). However, we did not observe elevated sCD14 levels in plasma from stable
407 adult CF patients, showing that sCD14 is not a systemic marker in CF, as previously observed in
408 the plasma of children with CF (40).

409

410 It has been previously shown that sCD14 could be generated by several mechanisms, which
411 include neosynthesis, direct proteolytic cleavage from the cell surface, cleavage of the GPI
412 anchor by phospholipases and endocytosis/exocytosis (2, 3, 10–18). In our study, CD14 gene
413 expression was the same in CF and non-CF macrophages. Moreover, no neosynthesis of sCD14
414 was observed in CF macrophage. Indeed, the elevated sCD14 levels observed in CF macrophage
415 supernatants cannot be explained by an increase in gene expression and synthesis. Proteases,
416 metalloproteases and/or cathepsins have been demonstrated to be present in large amounts in CF
417 airways (42). However, the gene expression of MMPs, ADAMs as well as cathepsins is lower in
418 CF macrophages than in non-CF macrophages. Furthermore, MMP-9 and its activity are also
419 decreased in CF macrophage supernatants. Regarding these data, the origin of sCD14 in human
420 CF macrophages was not direct proteolytic cleavage from the cell surface, as reported previously,
421 explaining the increase in plasma sCD14 that occurs during sepsis (43). Cleavage of the GPI
422 anchor by phospholipases is also suggested to explain sCD14 release. In our study, PI-PLC
423 induced increased production of sCD14 in non-CF macrophage supernatants. The PI-PLC
424 inhibitor reversed this effect but not the sCD14 overproduction observed in CF macrophages.
425 Thus, PI-PLC participates in the production of sCD14 in non-CF macrophages but has no role in
426 sCD14 overproduction in CF macrophages. Finally, the hypothesis that involves the

427 endocytosis/exocytosis of CD14 was considered. The depletion of cholesterol from the plasma
428 membrane has been shown to affect endocytosis and protein sorting (44). As an example,
429 cholesterol perturbation by filipin led to the redistribution of GPI-GFP from the cell surface to the
430 Golgi complex (45), and the supplementation of the medium with cholesterol allowed the
431 transport of secretory membrane proteins from the ER to the Golgi (46). In our study, cholesterol
432 level is higher in CF than in non-CF macrophages and cholesterol supplementation in non-CF
433 macrophages increased sCD14 secretion by macrophages. Furthermore, other work has shown
434 that the inhibition of cholesterol synthesis by lovastatin decreased sCD14 production in RAW
435 264.7 macrophages (47). Endocytosis/exocytosis appears to be a suitable hypothesis because the
436 use of brefeldin A to block forward transport between the ER and the Golgi complex decreases
437 sCD14 secretion in CF macrophages.
438
439 It is well established that sCD14 alone is not sufficient to promote inflammation, except in cells
440 that do not express mCD14, but the presence of sCD14 can enhance the sensitivity of monocytes
441 and macrophages to LPS or lipopeptide by several orders of magnitude (48). In our study, we
442 show that treatment with sCD14 alone enhances the induced inflammatory response and the
443 effects of LPS by stimulating the secretion of IL-8, TNF-α and IL-1β in non-CF macrophages.
444 However, one question remains to be answered: how can sCD14 induce the production of these
445 inflammatory cytokines and chemokines?
446
447 Therefore, CD14 is assumed to function as a multi-ligand pattern recognition receptor by
448 recognizing and binding to different PAMPs and DAMPs (49). In combination with the TLRs
449 proteins, CD14 is an important mediator of innate immune responses to infection (5, 8).
450 Membrane CD14 is a co-receptor for endotoxin through TLR-4 trans-membrane signaling, both
451 at the cell surface and within the endosomal compartment. TLR-4 has been shown to contribute
452 to the PAMP response of human cells, and its activation results in a pathway based on the
453 activation of TIR-containing adaptor molecules: the rapid TIRAP/MyD88 pathway, which
454 activates NF-κB and MAPK, resulting in the expression of pro-inflammatory cytokines and
455 chemokines (50). In human monocyte-derived macrophages, sCD14 promoted the
456 phosphorylation of IκBα, allowing the activation of NF-κB. Bay 11-7082, which is a potential
457 anti- inflammatory agent and an irreversible inhibitor of IKKα and IκBα phosphorylation,

458 diminished sCD14-elicited IL-8, TNF-α and IL-1β expression and secretion, suggesting a role for
459 NFκB signaling in sCD14-dependent macrophage inflammation. Furthermore, macrophage
460 activation with sCD14 induces the activation of pro-caspase 1, leading to the cleaved and active
461 form of caspase 1. Next, pro-IL-1β is cleaved by caspase 1, and IL-1β mature is secreted in
462 macrophage supernatants; this effect is inhibited by Z-WEHD-FMK, which is a specific caspase
463 1 inhibitor. The activation of caspase 1 involves inflammasome complex formation, which
464 requires NLRs. NLRs are pattern recognition receptors and play key roles in the regulation of the
465 innate immune response in cooperation with TLRs. Among the NLRs, NLRC4 and NLRP3
466 appear to be the most involved in this response. The infection of macrophages with several
467 Gram-negative bacteria, including *Pseudomonas aeruginosa,* which currently infects CF patients,
468 activates caspase 1 through NLRC4 (51). The activation of caspase 1 through NLRP3 is induced
469 by DAMPs, such as K^+ efflux, ATP *via* P2XR7, ROS, and cathepsin D from lysosome damage
470 (52). We turned off NRLC4 and NLRP3 protein expression using specific siRNAs directed
471 against NLRC4 or NLRP3. The treatment of macrophages with NLRP3 siRNA or NLRC4
472 siRNA abolished IL-1β secretion, suggesting that both inflammasome platforms (NLRC4 and
473 NLRP3) induce IL-1β secretion in response to sCD14 exposure. Altogether, our data suggest that
474 sCD14 acts as a DAMP in macrophages. In fact, extracellular sCD14 can bind membrane
475 proteins or phospholipids (53) that could be responsible for inflammatory cytokine production.
476 An anti-CD14 neutralizing antibody is able to inhibit the sCD14-induced synthesis of TNF-α, IL-
477 8 and IL-1β. To explore the action of sCD14 *via* the TLR-4 receptor, we used a potent antagonist
478 of this receptor LPS-RS and an anti-TLR4 antibody, which decreases partly sCD14-induced
479 cytokine production in macrophages.Thus, part of the effect of sCD14 appears to be mediated by
480 the CD14/TLR-4 complex. However, further investigations are needed to completely understand
481 the molecular mechanisms involved in sCD14 signaling pathway in macrophages.
482
483 In conclusion, numerous mechanisms concerning the origin of sCD14 were considered in this
484 work, but in CF macrophages, the endocytosis/exocytosis process appears to be the main
485 mechanism involved in sCD14 production. We propose that the overproduction of sCD14
486 previously observed in macrophages from CF patients (29) should be considered to be a DAMP
487 that can induce pro-inflammatory cytokine/chemokine production independent of LPS.

488 Moreover, sCD14 can also enhance the LPS response. Both of these effects are dependent on the
489 TLR-4/CD14 membrane complex, NF-κB and the inflammasome. The characterization of the
490 origin and inflammatory function of sCD14 presented in this paper is an important step in
491 understanding chronic infection/inflammation. Furthermore, this study provides a novel approach
492 to the development of anti-inflammatory therapeutics in CF.
493

494 **REFERENCES**

495

496 1. Bazil, V., Horejsi, V., Baudys, M., Kristofova, H., Strominger, J. L., Kostka, W., and
497 Hilgert, I. (1986) Biochemical characterization of a soluble form of the 53-kDa monocyte
498 surface antigen. *Eur. J. Immunol.* **16**, 1583–1589
499 2. Haziot, A., Chen, S., Ferrero, E., Low, M. G., Silber, R., and Goyert, S. M. (1988) The
500 monocyte differentiation antigen, CD14, is anchored to the cell membrane by a
501 phosphatidylinositol linkage. *J. Immunol.* **141**, 547–552
502 3. Simmons, D. L., Tan, S., Tenen, D. G., Nicholson-Weller, A., and Seed, B. (1989)
503 Monocyte antigen CD14 is a phospholipid anchored membrane protein. *Blood* **73**, 284–289
504 4. Wright, S. D. (1995) CD14 and innate recognition of bacteria. *J. Immunol.* **155**, 6–8
505 5. Wright, S. D., Ramos, R. A., Tobias, P. S., Ulevitch, R. J., and Mathison, J. C. (1990)
506 CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein.
507 *Science* **249**, 1431–1433
508 6. Schütt, C. (1999) CD14. *Int. J. Biochem. Cell Biol.* **31**, 545–549
509 7. Gegner, J. A., Ulevitch, R. J., and Tobias, P. S. (1995) Lipopolysaccharide (LPS) Signal
510 Transduction and Clearance. Dual roles for LPS binding protein and membrane CD14. *J.*
511 *Biol. Chem.* **270**, 5320–5325
512 8. Pugin, J., Heumann, D., Tomasz, A., Kravchenko, V. V., Akamatsu, Y., Nishijima, M.,
513 Glauser, M. P., Tobias, P. S., and Ulevitch, R. J. (1994) CD14 Is a pattern recognition
514 receptor. *Immunity* **1**, 509–516
515 9. Schiff, D. E., Kline, L., Soldau, K., Lee, J. D., Pugin, J., Tobias, P. S., and Ulevitch, R. J.
516 (1997) Phagocytosis of gram-negative bacteria by a unique CD14-dependent mechanism. *J.*
517 *Leukoc. Biol.* **62**, 786–794
518 10. Bazil, V. and Strominger, J. L. (1991) Shedding as a mechanism of down-modulation of
519 CD14 on stimulated human monocytes. *J. Immunol.* **147**, 1567–1574
520 11. Bufler, P., Stiegler, G., Schuchmann, M., Hess, S., Krüger, C., Stelter, F., Eckerskorn, C.,
521 Schütt, C., and Engelmann, H. (1995) Soluble lipopolysaccharide receptor (CD14) is
522 released via two different mechanisms from human monocytes and CD14 transfectants. *Eur.*
523 *J. Immunol.* **25**, 604–610

524 12. Durieux, J.-J., Vita, N., Popescu, O., Guette, F., Calzada-Wack, J., Munker, R., Schmidt, R.
525 E., Lupker, J., Ferrara, P., Ziegler-Heitbrock, H. W. L., and Labeta, M. O. (1994) The two
526 soluble forms of the lipopolysaccharide receptor, CD14: Characterization and release by
527 normal human monocytes. *Eur. J. Immunol.* **24**, 2006–2012
528 13. Stelter, F., Pfister, M., Bernheiden, M., Jack, R. S., Bufler, P., Engelmann, H., and Schütt,
529 C. (1996) The Myeloid Differentiation Antigen CD14 is N- and O-Glycosylated. *Eur. J.*
530 *Biochem.* **236**, 457–464
531 14. Thieblemont, N. and Wright, S. D. (1999) Transport of bacterial lipopolysaccharide to the
532 Golgi apparatus. *J. Exp. Med.* **190**, 523–534
533 15. Kirkland, T. N. and Viriyakosol, S. (1998) Structure-function analysis of soluble and
534 membrane-bound CD14. *Prog. Clin. Biol. Res.* **397**, 79–87
535 16. Bazil, V., Baudys, M., Hilgert, I., Stefanová, I., Low, M. G., Zbrozek, J., and Horejsí, V.
536 (1989) Structural relationship between the soluble and membrane-bound forms of human
537 monocyte surface glycoprotein CD14. *Mol. Immunol.* **26**, 657–662
538 17. Coyne, C. P., Howell, T., Smodlaka, H., Willetto, C., Fenwick, B. W., and Chenney, E.
539 (2002) Alterations in membrane-associated CD14 expression and the simultaneous
540 liberation of soluble CD14 fragment in adherent macrophages mediated by a leukocyte
541 carboxyl/aspartate protease. *J. Endotoxin Res.* **8**, 273–283
542 18. Labeta, M. O., Durieux, J.-J., Fernandez, N., Herrmann, R., and Ferrara, P. (1993) Release
543 from a human monocyte-like cell line of two different soluble forms of the
544 lipopolysaccharide receptor, CD14. *Eur. J. Immunol.* **23**, 2144–2151
545 19. Haziot, A., Rong, G. W., Bazil, V., Silver, J., and Goyert, S. M. (1994) Recombinant
546 soluble CD14 inhibits LPS-induced tumor necrosis factor-alpha production by cells in
547 whole blood. *J. Immunol.* **152**, 5868–5876
548 20. Schütt, C., Schilling, T., and Krüger, C. (1991) sCD14 prevents endotoxin inducible
549 oxidative burst response of human monocytes. *Allerg. Immunol. (Leipz.)* **37**, 159–164
550 21. Tapping, R. I. and Tobias, P. S. (2000) Soluble CD14-mediated cellular responses to
551 lipopolysaccharide. *Chem. Immunol.* **74**, 108–121
552 22. Opal, S. M., Scannon, P. J., Vincent, J.-L., White, M., Carroll, S. F., Palardy, J. E., Parejo,
553 N. A., Pribble, J. P., and Lemke, J. H. (1999) Relationship between Plasma Levels of

554 Lipopolysaccharide (LPS) and LPS-Binding Protein in Patients with Severe Sepsis and
555 Septic Shock. *J. Infect. Dis.* **180**, 1584–1589
556 23. Salomao, R., Brunialti, M. K. C., Rapozo, M. M., Baggio-Zappia, G. L., Galanos, C., and
557 Freudenberg, M. (2012) Bacterial sensing, cell signaling, and modulation of the immune
558 response during sepsis. *Shock Augusta Ga* **38**, 227–242
559 24. Martin, T. R., Ruhenfeld, G., Steinberg, K. P., Hudson, L. D., Raghu, G., Moriarty, A. M.,
560 Leturcq, D. J., Tobias, P. S., and Ulevitch, R. J. (1994) ENdotoxin, endotoxin-binding
561 protein, and soluble cd14 are present in bronchoalveolar lavage fluid of patients with adult
562 respiratory distress syndrome. *Chest* **105**, 55S – 56S
563 25. Dessing, M. C., Knapp, S., Florquin, S., de Vos, A. F., and van der Poll, T. (2007) CD14
564 Facilitates Invasive Respiratory Tract Infection by Streptococcus pneumoniae. *Am. J.*
565 *Respir. Crit. Care Med.* **175**, 604–611
566 26. Ratner, D. and Mueller, C. (2012) Immune Responses in Cystic Fibrosis. *Am. J. Respir. Cell*
567 *Mol. Biol.* **46**, 715–722
568 27. Cifani, N., Pompili, B., Anile, M., Patella, M., Diso, D., Venuta, F., Cimino, G., Quattrucci,
569 S., Di Domenico, E. G., Ascenzioni, F., and Porto, P. D. (2013) Reactive-Oxygen-Species-
570 Mediated P. aeruginosa Killing Is Functional in Human Cystic Fibrosis Macrophages. *PLoS*
571 *ONE* **8**, e71717
572 28. Del Porto, P., Cifani, N., Guarnieri, S., Di Domenico, E. G., Mariggiò, M. A., Spadaro, F.,
573 Guglietta, S., Anile, M., Venuta, F., Quattrucci, S., and Ascenzioni, F. (2011) Dysfunctional
574 CFTR Alters the Bactericidal Activity of Human Macrophages against Pseudomonas
575 aeruginosa. *PLoS ONE* **6**, e19970
576 29. Simonin-Le Jeune, K., Le Jeune, A., Jouneau, S., Belleguic, C., Roux, P.-F., Jaguin, M.,
577 Dimanche-Boitre, M.-T., Lecureur, V., Leclercq, C., Desrues, B., Brinchault, G., Gangneux,
578 J.-P., and Martin-Chouly, C. (2013) Impaired Functions of Macrophage from Cystic
579 Fibrosis Patients: CD11b, TLR-5 Decrease and sCD14, Inflammatory Cytokines Increase.
580 *PLoS ONE* **8**, e75667
581 30. Bellis, G., Lemonnier, L., and Sponga, M. (2012) Registre Français de la Mucoviscidose.
582 31. Grevenynghe, J. van, Rion, S., Ferrec, E. L., Vee, M. L., Amiot, L., Fauchet, R., and Fardel,
583 O. (2003) Polycyclic Aromatic Hydrocarbons Inhibit Differentiation of Human Monocytes
584 into Macrophages. *J. Immunol.* **170**, 2374–2381

585 32. Martin-Chouly, C. A. E., Astier, A., Jacob, C., Pruniaux, M.-P., Bertrand, C., and Lagente,
586 V. (2004) Modulation of matrix metalloproteinase production from human lung fibroblasts
587 by type 4 phosphodiesterase inhibitors. *Life Sci.* **75**, 823–840
588 33. Miller, S., G., Carnell, L., and Moore, H.-P., H. (1992) Post-Golgi membrane traffic:
589 brefeldin A inhibits export from distal Golgi compartments to the cell surface but not
590 recycling. *J. Cell Biol.* **118**, 267–283
591 34. Pierce, J. W., Schoenleber, R., Jesmok, G., Best, J., Moore, S. A., Collins, T., and Gerritsen,
592 M. E. (1997) Novel Inhibitors of Cytokine-induced I B Phosphorylation and Endothelial
593 Cell Adhesion Molecule Expression Show Anti-inflammatory Effects in Vivo. *J. Biol.*
594 *Chem.* **272**, 21096–21103
595 35. Douglas, T. A., Brennan, S., Gard, S., Berry, L., Gangell, C., Stick, S. M., Clements, B. S.,
596 and Sly, P. D. (2009) Acquisition and eradication of P. aeruginosa in young children with
597 cystic fibrosis. *Eur. Respir. J.* **33**, 305–311
598 36. Eickmeier, O., Huebner, M., Herrmann, E., Zissler, U., Rosewich, M., Baer, P. C., Buhl, R.,
599 Schmitt-Grohé, S., Zielen, S., and Schubert, R. (2010) Sputum biomarker profiles in cystic
600 fibrosis (CF) and chronic obstructive pulmonary disease (COPD) and association between
601 pulmonary function. *Cytokine* **50**, 152–157
602 37. Tang, A., Sharma, A., Jen, R., Hirschfeld, A. F., Chilvers, M. A., Lavoie, P. M., and
603 Turvey, S. E. (2012) Inflammasome-Mediated IL-1β Production in Humans with Cystic
604 Fibrosis. *PLoS ONE* **7**, e37689
605 38. Lin, B., Noring, R., Steere, A. C., Klempner, M. S., and Hu, L. T. (2000) Soluble CD14
606 Levels in the Serum, Synovial Fluid, and Cerebrospinal Fluid of Patients with Various
607 Stages of Lyme Disease. *J. Infect. Dis.* **181**, 1185–1188
608 39. Nockher, W. A., Wick, M., and Pfister, H.-W. (1999) Cerebrospinal fluid levels of soluble
609 CD14 in inflammatory and non-inflammatory diseases of the CNS: upregulation during
610 bacterial infections and viral meningitis. *J. Neuroimmunol.* **101**, 161–169
611 40. Marcos, V., Latzin, P., Hector, A., Sonanini, S., Hoffmann, F., Lacher, M., Koller, B.,
612 Bufler, P., Nicolai, T., Hartl, D., and Griese, M. (2010) Expression, regulation and clinical
613 significance of soluble and membrane CD14 receptors in pediatric inflammatory lung
614 diseases. *Respir. Res.* **11**, 32

615 41. Egerer, K., Feist, E., Rohr, U., Pruss, A., Burmester, G. R., and Dörner, T. (2000) Increased
616 serum soluble CD14, ICAM-1 and E-selectin correlate with disease activity and prognosis
617 in systemic lupus erythematosus. *Lupus* **9**, 614–621
618 42. Le-Barillec, K., Si-Tahar, M., Balloy, V., and Chignard, M. (1999) Proteolysis of monocyte
619 CD14 by human leukocyte elastase inhibits lipopolysaccharide-mediated cell activation. *J.*
620 *Clin. Invest.* **103**, 1039–1046
621 43. Arai, Y., Mizugishi, K., Nonomura, K., Naitoh, K., Takaori-Kondo, A., and Yamashita, K.
622 (2015) Phagocytosis by human monocytes is required for the secretion of presepsin. *J.*
623 *Infect. Chemother.* **21**, 564–569
624 44. Ikonen, E. (2001) Roles of lipid rafts in membrane transport. *Curr. Opin. Cell Biol.* **13**,
625 470–477
626 45. Nichols, B. J., Kenworthy, A. K., Polishchuk, R. S., Lodge, R., Roberts, T. H., Hirschberg,
627 K., Phair, R. D., and Lippincott-Schwartz, J. (2001) Rapid Cycling of Lipid Raft Markers
628 between the Cell Surface and Golgi Complex. *J. Cell Biol.* **153**, 529–542
629 46. Miwako, I., Yamamoto, A., Kitamura, T., Nagayama, K., and Ohashi, M. (2001)
630 Cholesterol requirement for cation-independent mannose 6-phosphate receptor exit from
631 multivesicular late endosomes to the Golgi. *J. Cell Sci.* **114**, 1765–1776
632 47. Frey, T. and De Maio, A. (2007) Increased Expression of CD14 in Macrophages after
633 Inhibition of the Cholesterol Biosynthetic Pathway by Lovastatin. *Mol. Med.* **13**, 592–604
634 48. Jersmann, H. P. (2005) Time to abandon dogma: CD14 is expressed by non-myeloid lineage
635 cells. *Immunol. Cell Biol.* **83**, 462–467
636 49. Schmitz, G. and Orsó, E. (2002) CD14 signalling in lipid rafts: new ligands and co-
637 receptors. *Curr. Opin. Lipidol.* **13**, 513–521
638 50. Dentener, M. A., Bazil, V., Asmuth, E. J. V., Ceska, M., and Buurman, W. A. (1993)
639 Involvement of CD14 in lipopolysaccharide-induced tumor necrosis factor-alpha, IL-6 and
640 IL-8 release by human monocytes and alveolar macrophages. *J. Immunol.* **150**, 2885–2891
641 51. Franchi, L., Eigenbrod, T., Muñoz-Planillo, R., and Nuñez, G. (2009) The Inflammasome:
642 A Caspase-1 Activation Platform Regulating Immune Responses and Disease Pathogenesis.
643 *Nat. Immunol.* **10**, 241
644 52. Yu, H. B. and Finlay, B. B. (2008) The Caspase-1 Inflammasome: A Pilot of Innate Immune
645 Responses. *Cell Host Microbe* **4**, 198–208

646 53. Yu, B., Hailman, E., and Wright, S. D. (1997) Lipopolysaccharide binding protein and
647 soluble CD14 catalyze exchange of phospholipids. *J. Clin. Invest.* **99**, 315–324
648
649

AUTHOR CONTRIBUTIONS

M. Lévêque, K. Simonin-Le Jeune and C. Martin-Chouly designed research; M. Lévêque, K. Simonin-Le Jeune and C. Martin-Chouly performed the experiments; S; Moulis, C. Belleguic, G. Brinchault, B. Desrues, S. Le Trionnaire, J-P. Gangneux and S. Jouneau contributed new reagents or analytic tools; M. Lévêque and C. Martin-Chouly analyzed data; M. Lévêque, M-T. Dimanche-Boitrel and C. Martin-Chouly wrote the paper.

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659

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664

665 **FIGURE LEGENDS**

666

667 **Figure 1. Soluble CD14 level in plasma and CD14 gene expression in macrophages.** (*A*)
668 sCD14 levels in plasma from healthy subjects (non-CF, *n*=8) and CF patients (*n*=12, patients 1-
669 12, supplemental Table 1), as measured using an ELISA assay. Each symbol represents a single
670 individual, and the line is the mean. (*B*) CD14 gene expression in non-CF macrophages (*n*=14)
671 and CF macrophages (*n*=15, patients 6, 13-26; supplemental Table I). mRNA levels were
672 determined by RT-qPCR (supplemental Table 2). Each symbol represents a single individual, and
673 the line is the mean. (*C*) Neosynthesis (O.D.) and total (pg/ml) sCD14 in macrophage supernatant
674 from healthy subjects (non-CF, *n*=5) and CF patients (*n*=5, patients 6, 13-16, supplemental Table
675 1). The data are shown as the mean ± SEM. Mann-Whitney test: * p<0.05 *vs.* non-CF
676 macrophages.

677

678 **Figure 2. Altered CF macrophage phenotype is associated with markedly decreased**
679 **expression and activity of proteases.** (*A*) The inhibition of metalloproteases by EDTA (0.1 mM,
680 24 hours) did not influenced sCD14 secretion in non-CF macrophages (n=8) (*B*) MMP-9
681 expression is reduced in CF macrophages (*n*=6, patients 6, 13-16 and 32, supplemental Table 1)
682 *vs.* non-CF macrophages (*n*=9). Gene expression was determined by RT-qPCR (supplemental
683 Table 2). (*C*) In a representative gelatin zymogram, latent MMP-9 (92 kDa) is observed based on
684 proteolysis areas, which appeared as clear bands against a dark background. After scanning the
685 gels, both the surface and intensity of the lysis bands were analyzed by densitometry (bar graph)
686 and showed decreased expression of latent MMP-9 in CF macrophages (*n*=12, patients 33-44,
687 supplemental Table 1) *vs.* non-CF macrophages (*n*=11). The results were expressed *vs.* the
688 relative latent MMP-9 intensity observed in the supernatant of non-CF macrophages. (*D, E, F*)
689 The inhibition of cysteine proteases by e64d (*D*, 1 µM, 24 hours, n=10), aspartic proteases by
690 pepstatin A (*E*, 1 µM, 24 hours, n=4) and serine proteases by aprotinin (0.3 µg/ml, 24 hours, n=9)
691 did not influenced sCD14 secretion in non-CF macrophages. The data are shown as the mean ±
692 SEM. Mann-Whitney test: * p<0.05 and ** p<0.01 *vs.* non-CF macrophages.

693

694 **Figure 3. PI-PLC increases sCD14 production in non-CF macrophages.** sCD14 levels were
695 measured in supernatants from macrophages using an ELISA assay. (*A*) PI-PLC (1 U/ml, 2

696 hours) significantly increases sCD14 production by non-CF macrophages (*n*=11). The inhibition
697 of PI-PLC by U-73122 (12.5 μM, 1 hour pretreatment) inhibited PI-PLC-induced sCD14
698 secretion in non-CF macrophages (*n*=7). (*B*) U-73122 (12.5 μM, 24 hours) did not prevent
699 spontaneous sCD14 production by CF macrophages (*n*=6, patients 43, 45-49; supplemental Table
700 1). The data are shown as the mean ± SEM. Ratio Student's t-test: ## p<0.01 *vs.* untreated non-
701 CF macrophages; ** p<0.01 *vs.* PI-PLC-treated non-CF macrophages.
702
703 **Figure 4. Impact of intracellular protein transport on sCD14 production by non-CF and CF**
704 **macrophages.** (*A*) Free cholesterol level is increased in CF (*n*=8, patients 43, 47, 50-55;
705 supplemental Table 1) *vs* non-CF (*n*=4) macrophages (*B*) Water-soluble cholesterol (15 μg/ml; 24
706 hours) significantly increases sCD14 production by non-CF macrophages (*n*=5). (*C*) Brefeldin A
707 (Bref A; 100 ng/ml; 3 h) significantly decreased sCD14 production by non-CF (*n*=5) and CF
708 (*n*=5, patients 56-60; supplemental Table 1) macrophages. Levels in the supernatants were
709 measured using an ELISA assay. The data are shown as the mean ± SEM. Mann-Whitney test: **
710 p<0.01 *vs.* non-CF macrophages. Ratio Student's t-test: # p<0.05 *vs.* untreated non-CF
711 macrophages and § p<0.05 *vs.* untreated CF macrophages.
712
713
714 **Figure 5. Human recombinant sCD14 induces the expression and secretion of pro-**
715 **inflammatory cytokines in non-CF macrophages.** (*A*) IL-1β, IL-8 and TNF-α mRNA
716 expression was quantified by RT-qPCR in non-CF macrophages treated with hr-sCD14 (100 and
717 500 ng/ml, 6 hours and 24 hours). The data are shown as the mean ± SEM of four and five
718 independent experiments for 6 hours and 24 hours, respectively. (*B*) IL-1β, IL-8 and TNF-α
719 release were measured by using an ELISA assay in supernatants from non-CF macrophages
720 treated with hr-sCD14 (1 to 500 ng/ml, 24 hours). The data are shown as the mean ± SEM (*n*=6-
721 11, *n*=4 and *n*=3-6 for IL-1β, IL-8 and TNF-α, respectively). Ratio Student's t-test: * p<0.05; **
722 p<0.01; *** p<0.001 and **** p<0.0001 *vs.* untreated macrophages.
723
724 **Figure 6. NF-κB pathway is involved in the sCD14-induced production of pro-inflammatory**
725 **cytokines in non-CF macrophages.** (*A*) Cytosol P-IκBα and IκBα expression were determined

726 by Western blotting of the total protein fraction from non-CF macrophages treated with hr-sCD14
727 (500 ng/ml, 0 to 60 min, representative blot of three independent experiments). Equal protein
728 loading was controlled via HSC-70 detection. (*B*) NFκB p65 activation was measured using an
729 assay in non-CF macrophages that were treated or not treated with hr-sCD14 (*n*=5; 500 ng/ml, 24
730 hours). A positive control was used to establish the result. (*C*) IL-1β, IL-8 and TNF-α gene
731 expression was analyzed by RT-qPCR in non-CF macrophages treated or not treated with hr-
732 sCD14 (500 ng/ml, 6 hours) in the absence and presence of an NF-κB inhibitor (Bay 11-7082, 10
733 μM, 1 hour pretreatment, *n*=5) (*D*) IL-1β, IL-8 and TNF-α protein expression were quantified
734 using an ELISA assay in non-CF macrophages that were treated or not treated with hr-sCD14
735 (500 ng/ml, 24 hours) in the absence or presence of Bay 11-7082 (1 and 10 μM, 1 hour
736 pretreatment; *n*=4, *n*=4 and *n*=5 for IL-1β, IL-8 and TNF-α, respectively). The data are shown as
737 the mean ± SEM. Ratio Student's t-test: * p<0.05 and *** p<0.001 *vs.* untreated macrophages; #
738 p<0.05 and ## p<0.01 *vs.* hr-sCD14-treated macrophages.
739
740 **Figure 7. Caspase-1 is involved in the sCD14-induced production of IL-1β in non-CF**
741 **macrophages.** (*A*) Pro-caspase 1 and caspase 1 expression were determined by Western blotting
742 analysis of the total protein fraction from non-CF macrophages treated with hr-sCD14 (500
743 ng/ml, 0 to 60 min, a representative blot of three independent experiments). (*B*) IL-1β secretion
744 was measured using an ELISA assay in supernatants from non-CF macrophages treated with hr-
745 sCD14 (500 ng/ml, 0 to 1440 min). The data are shown as the mean ± SEM (*n*=7). (*C*) IL-1β
746 secretion was measured using an ELISA assay in supernatants from non-CF macrophages treated
747 or not treated with hr-sCD14 (500 ng/ml, 24 hours) in the absence or presence of the caspase 1
748 inhibitor Z-WEHD-FMK (1 to 30 μM, 1-hour pretreatment). The data are shown as the mean ±
749 SEM (*n*=4). Ratio Student's t-test: * p<0.05 and ** p<0.01 *vs.* untreated macrophages; # p<0.05
750 *vs.* hr-sCD14-treated macrophages.
751
752 **Figure 8. NLRC4 and NLRP3 inflammasome pathways are involved in IL-1β secretion in**
753 **non-CF macrophages treated with hr-sCD14.** (*A and B*) The efficiency of transfection with
754 NLRC4 siRNA or NLRP3 siRNA was assessed by Western blotting of the total protein fraction
755 from non-CF macrophages. The bar graph shows the results of densitometric analysis (*n*=6). The

756 results were expressed *vs.* the relative intensity observed in Control siRNA-treated non-CF
757 macrophages. (*C*) IL-1β release was measured by ELISA in supernatants from non-CF
758 transfected macrophages that were treated or not treated with hr-sCD14 (500 ng/ml, 24 hours;
759 *n*=6). The data are shown as the mean ± SEM. Ratio Student's t-test: ** $p<0.01$ *vs.* siControl-
760 treated macrophages; ## $p<0.01$ *vs.* untreated macrophages; § $p<0.05$ and §§ $p<0.01$ *vs.* hr-
761 sCD14-treated macrophages.
762
763 **Figure 9. sCD14-induced cytokine release by non-CF macrophages is reversed by**
764 **antagonists that target CD14 or TLR-4.** IL-1β, IL-8 and TNF-α secretion was measured by
765 ELISA in supernatants from non-CF macrophages that were treated or not treated with hr-sCD14
766 (500 ng/ml, 24 hours) in the absence or presence of an anti-CD14 neutralizing antibody (CD14-
767 ab, 10 µg/ml, 1-hour pretreatment), a TLR-4 antagonist (LPS-RS, 2 µg/ml, 1-hour pretreatment),
768 an anti-TLR4 neutralizing antibody (TLR4-ab, 10 µg/ml, 1-hour pretreatment) or the IgG2a
769 control (10 µg/ml, 1 hour pretreatment). The data are shown as the mean ± SEM (*n*=3-6, *n*=3-6
770 and *n*=3-6 for IL-1β, IL-8 and TNF-α, respectively). Ratio Student's t-test: * $p<0.05$ and **
771 $p<0.01$ *vs.* hr-sCD14-treated macrophages.

Figure 1

Fig 1

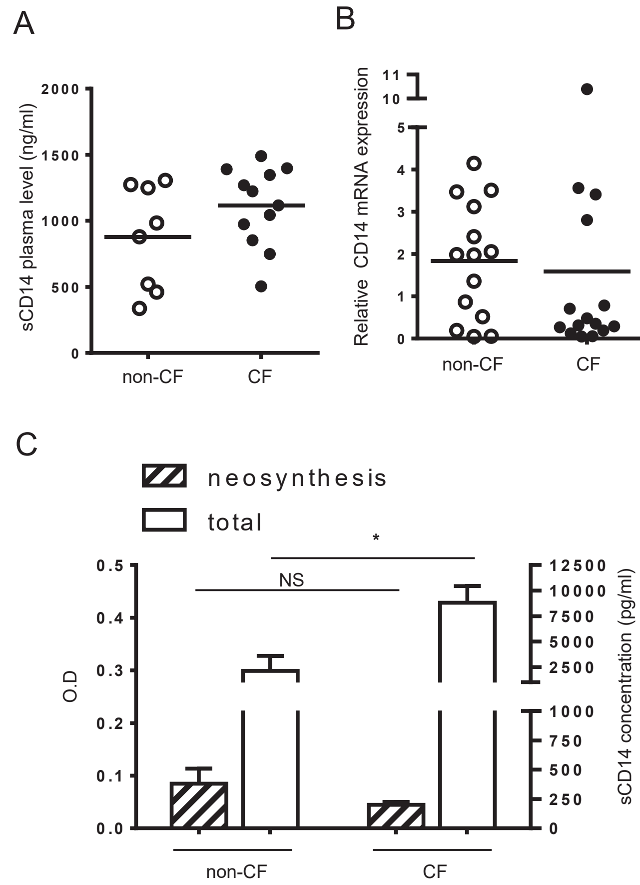

Figure 2

Fig 2

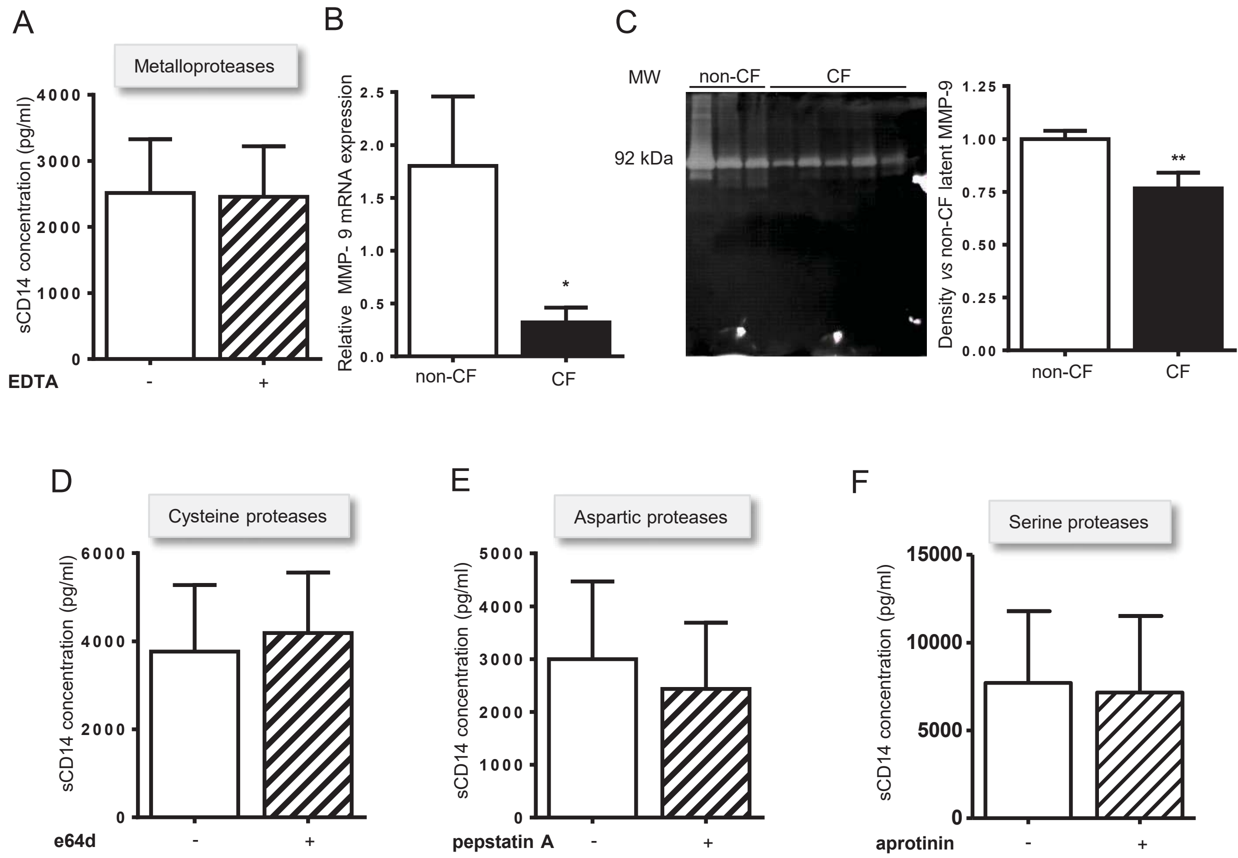

Figure 3

Fig 3

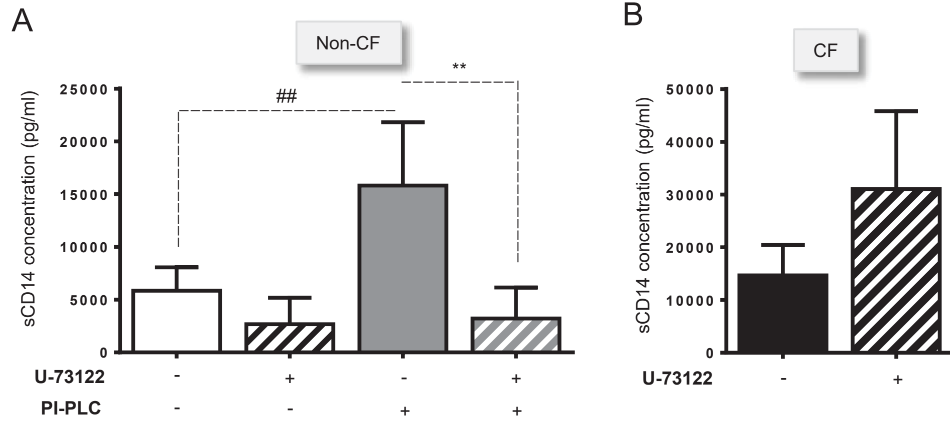

Figure 4

Fig 4

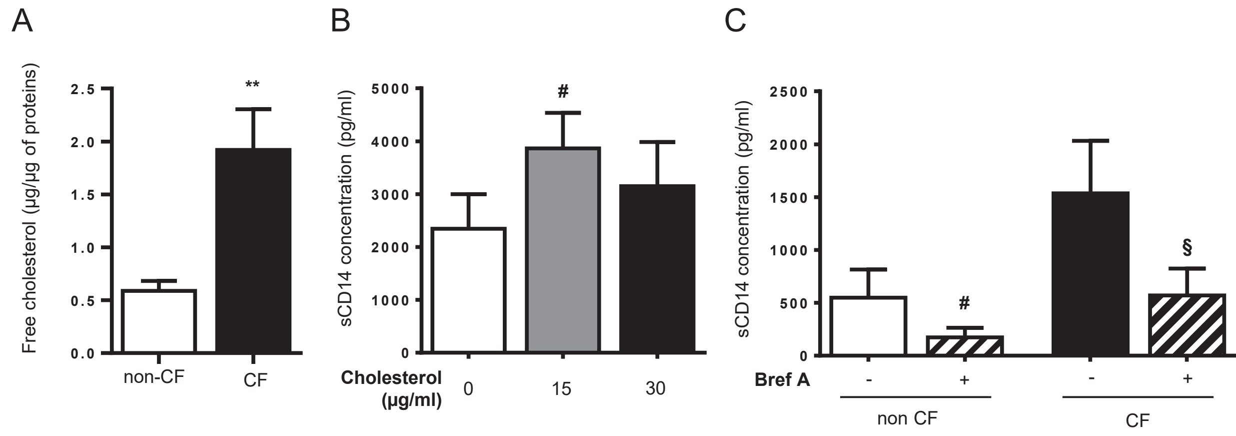

Figure 5

Fig 5

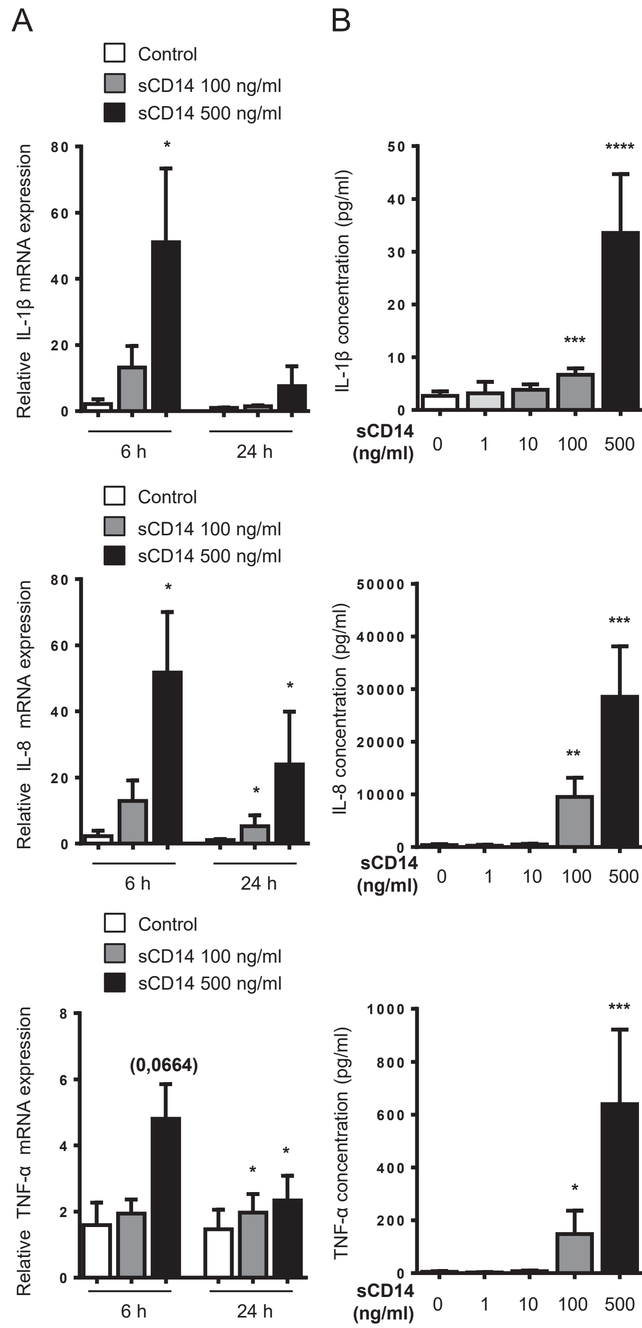

Figure 6

Fig 6

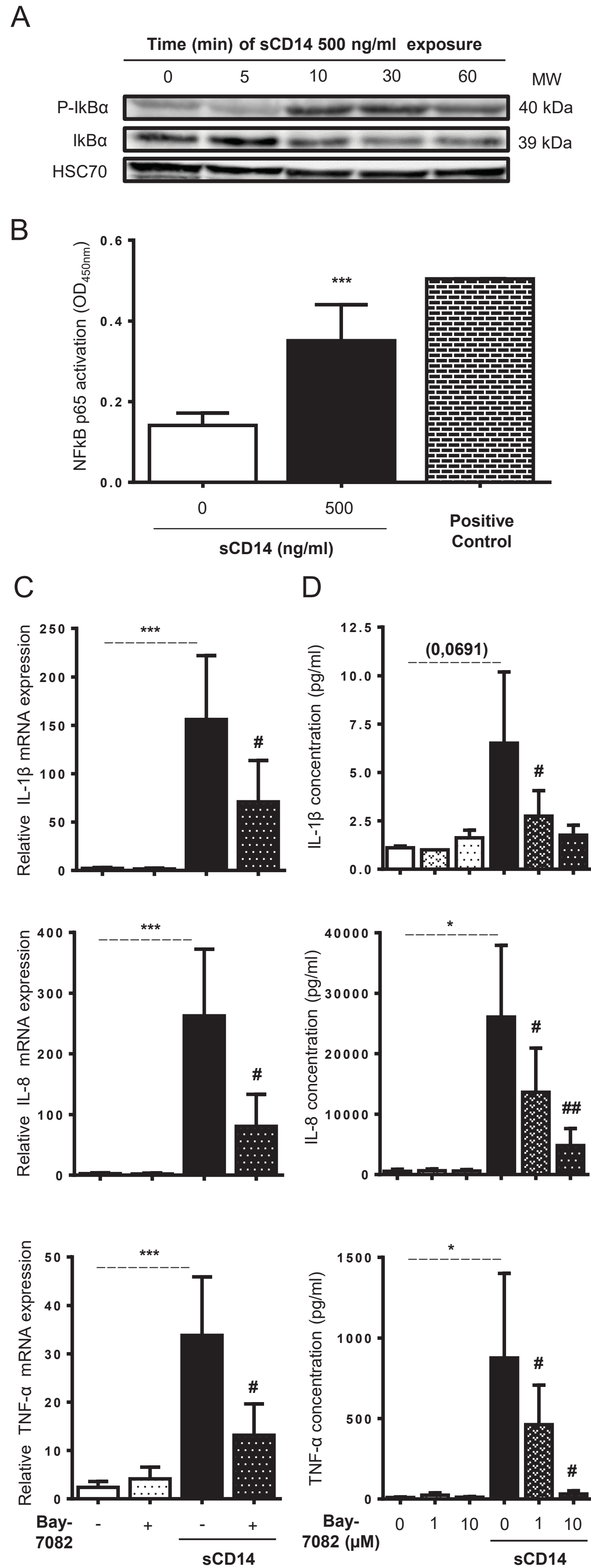

Figure 7

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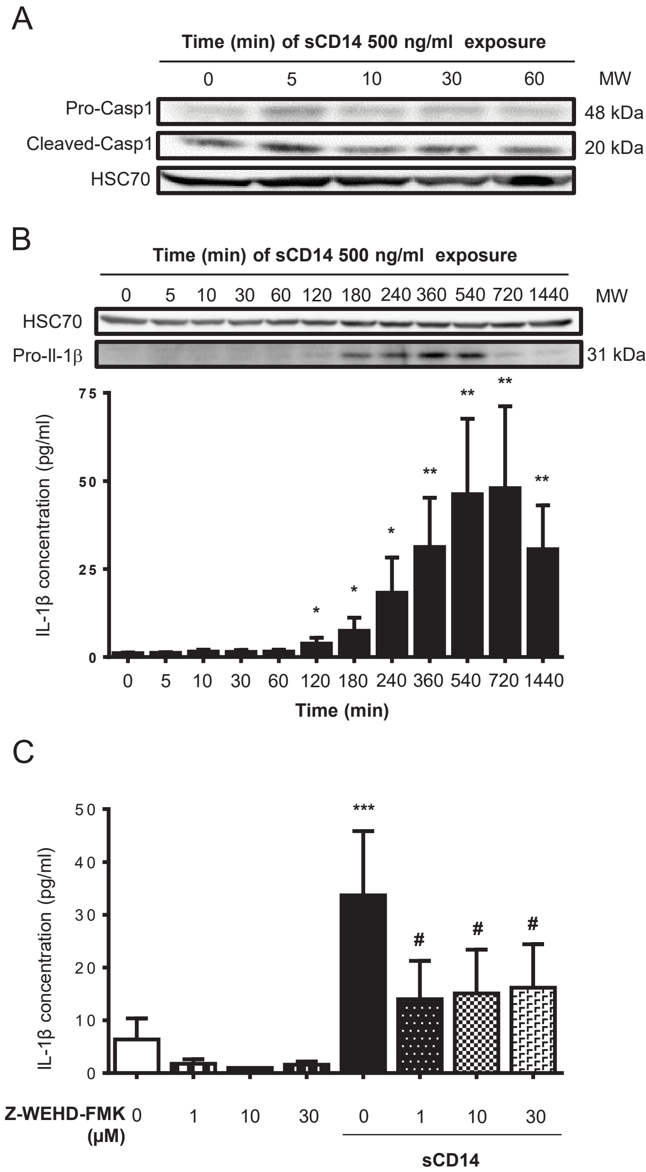

Figure 8

Fig 8

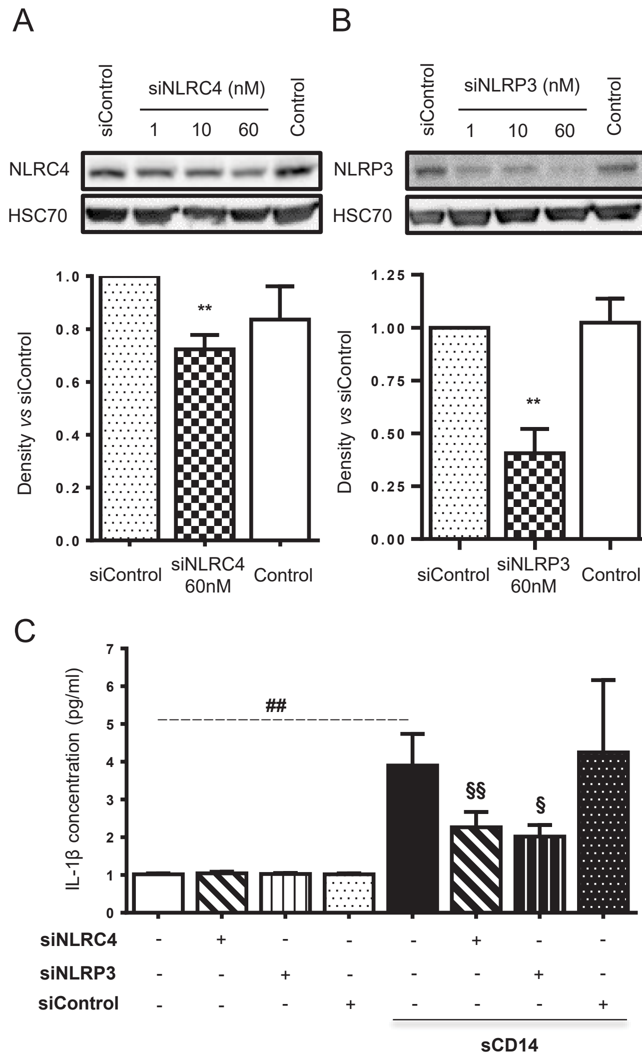

Figure 9

Fig 9

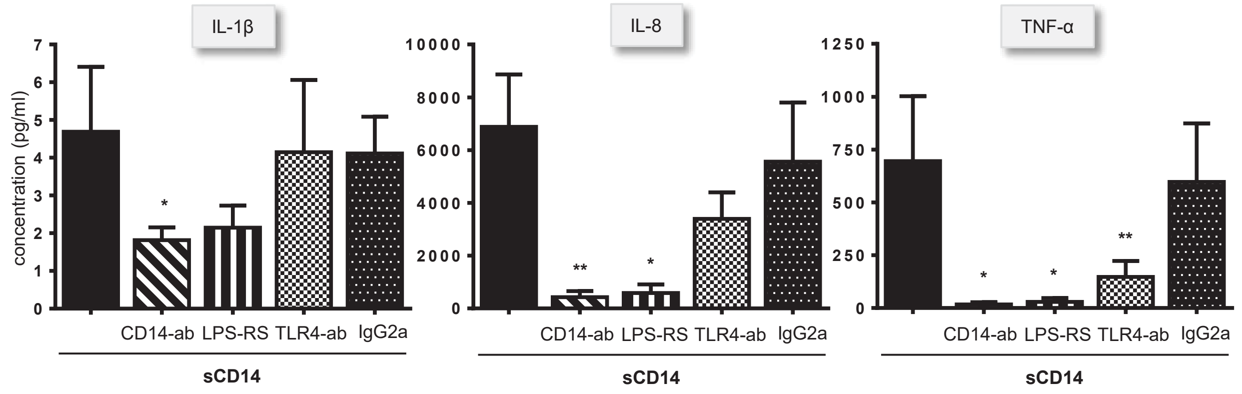

Table S1: Characteristics of CF patients

Patient	Age	Sex	Genotype	Microbiology	BMI	FEV1 % predicted
1	32	F	F508del / F508del	P.a / A.f / S.a	15	45
2	33	M	F508del / F508del	P.a / A.f / S.a	22,9	66
3	34	F	F508del / W486X	P.a / S.a	19,6	37
4	22	F	F508del / F508del	P.a / A.f / S.a	16,8	32
5	39	M	F508del / G551D	Af	22,1	40
6	32	M	F508del / F508del	P.a / S.a	20,1	82
7	20	M	F508del / F508del	P.a / A.f / S.a	19,9	66
8	20	M	F508del / F508del	S.a	23,2	98
9	39	F	F508del / 2789+5G→A	P.a / S.a	19,3	66
10	22	M	F508del / F508del	A.f / S.a	19,5	78
11	22	F	1248+1G→A	S.a	22,7	104
12	20	F	F508del / I507del	P.a / A.f	18,4	61
13	30	F	F508del / 1248+1G→A	P.a / S.a	18,3	33
14	31	F	F508del / N1303K	P.a / A.f / S.a	15,4	28
15	22	F	F508del / F508del	P.a / A.f / S.a	19,6	79
16	34	M	F508del / F508del	P.a / A.f / S.a	20,5	51
17	27	F	F508del / F508del	A.f / S.a	20,5	97,2
18	35	F	F508del / F508del	P.a / S.a	19,1	30,2
19	43	M	F508del / G551D	P.a	20,9	79,1
20	28	F	1248+1G→A	A.f / S.a	20,7	90,5
21	40	M	F508del / F508del	P.a / A.f	21,9	48,7
22	18	F	F508del / F508del	None	24,1	100,4
23	37	F	R600S / CFTRdup4-10	S.a	18,6	95
24	20	F	F508del / S945L	A.f / S.a	21,3	68,4
25	25	M	F508del / F508del	A.f / S.a	24,4	58,5
26	24	F	F508del / F508del	P.a / A.f / S.a	16,5	28,4
27	19	M	F508del / 2789+5G→A	S.a	22,23	94,7
28	40	F	F508del / S492F	P.a / S.a	27,82	55,3
29	53	F	F508del / 2789+5G→A	P.a / A.f	17,61	36
30	28	M	F508del / F508del	A.f	23,74	103
31	53	M	F508del / 2789+5G→A	S.a	22,14	96,2
32	20	M	F508del / F508del	A.f / S.a	22,3	62
33	19	M	F508del / F508del	P.a / A.f	20,4	91
34	26	M	F508del / F508del	S.a	20,2	58,7
35	37	M	F508del / F508del	S.a	21,1	77,4
36	23	F	F508del / F508del	S.a	17,1	54,5
37	49	F	F508del / 3272-26A→G	P.a / S.a	22,8	62,2
38	31	F	F508del / G91R	P.a / S.a	24,3	61,5
39	19	F	F508del / F508del	S.a	18,8	96
40	27	M	F508del / F508del	S.a	20,2	73,1
41	26	F	F508del / F508del	P.a / A.f / S.a	19,1	67
42	33	M	F508del / F508del	P.a	22,2	64,4
43	18	F	F508del / F508del	None	25,8	102,1
44	37	F	F508del / F508del	S.a	19,1	77,5
45	21	F	F508del / G551D	S.a	22,5	103,7
46	46	M	F508del / 2789+5G→A	A.f / S.a	22,2	96,6
47	35	F	F508del / F508del	P.a / S.a	19,3	35,2
48	25	M	F508del / F508del	A.f / S.a	23,5	55,3
49	39	M	F508del / F508del	P.a	21	49
50	18	F	F508del / F508del	P.a / A.f / S.a	19,1	62,3
51	23	M	F311L / N1303K	S.a	18,5	93,8
52	21	F	F508del / F508del	P.a / A.f / S.a	18,4	86,7
53	26	M	F508del / S1251N	P.a / S.a	18,81	41,1
54	23	F	F508del / F508del	P.a / S.a	16,86	33,7
55	38	F	F508del / F508del	None	18,2	41
56	30	M	F508del / 1749insTA	P.a / A.f / S.a	18,3	21,1
57	32	F	F508del / 4382delA	P.a / A.f / S.a	18	29,5
58	23	F	F508del / 1677delTA	P.a / A.f	18	49
59	52	M	F508del / 2789+5G→A	Af	22,5	107,9
60	38	M	F508del / 1078delT	P.a / A.f / S.a	21,7	63

Abbreviations : A.f.: *Aspergillus fumigatus;* P.a.: *Pseudomonas aeruginosa*; S.a.: Staphylococcus *aureus*
FEV1: Force Expiratory Volume in one second

Table S2: List of primer sequences used for RT-qPCR analysis in this study

Target genes	Primer	Sequence (5' to 3')
18S	Hs_RRN18S_1_SG	QT00199367
18S	Forward	CGCCGCTAGAGGTGAAATTC
	Reverse	TTGGCAAATGCTTTCGCTC
CD14	Hs_CD14_1_SG	QT00208817
IL-1β	Hs_IL1B_1_SG	QT00021385
IL-8	Hs_CXCL8_1_SG	QT00000322
TNFα	Forward	AACCTCCTCTCTGCCATC
	Reverse	ATGTTCGTCCTCCTCACA
MMP-9	Forward	TCTTCCCTGGAGACCTGAGA
	Reverse	ATTTCGACTCTCCACGCATC
MMP-12	Forward	ACACATTTCGCCTCTCTGCT
	Reverse	ATTGTCAGGATTTGGCAAGC
Cathepsin B	Forward	AGAATGGCACACCCTACTGG
	Reverse	GCCACCACTTCTGATTCGAT
Cathepsin D	Forward	GTACATGATCCCCTGTGAGAAGGT
	Reverse	GGGACAGCTTGTAGCCTTTGC
Cathepsin F	Forward	GCCTGTCCGTCTTTGTCAAT
	Reverse	TGGCTTGCTTCATCTTGTTG
Cathepsin K	Forward	CCGCAGTAATGACACCCTTT
	Reverse	GCACCCACAGAGCTAAAAGC
Cathepsin S	Forward	TGGGAGACATGACCAGTGAA
	Reverse	AGCAAGCACCACAAGAACCT
Adam 9	Forward	TGCAAATGTCTTCAGGGAAC
	Reverse	TGCATTAACCTCACCGATGAC
Adam 10	Forward	CATTGCTGAATGGATTGTGG
	Reverse	GAGCCTGGAAGTGGTTTACG
Adam 12	Forward	AACCTCGCTGCAAAGAATGTG
	Reverse	CTCTGAAACTCTCGGTTGTCTG
Adam 17	Forward	ACCTGAAGAGCTTGTTCATCGAG
	Reverse	CCATGAAGTGTTCCGATAGATGTC
PLCγ	Forward	AACCAGAAGTCCTTTGTCTTCATC
	Reverse	CTGGTTCTTCTCCCAGTACTTCAT

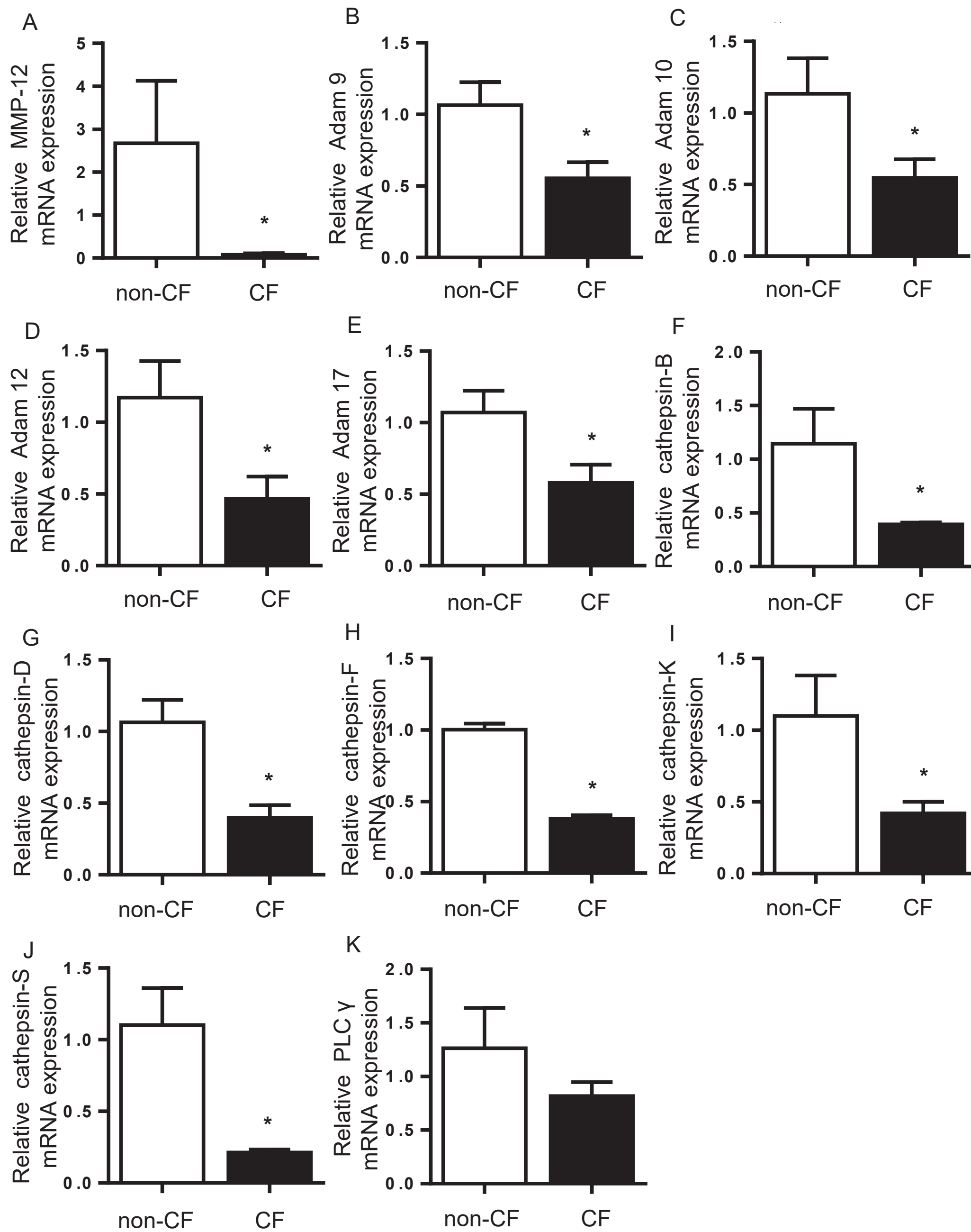

FIGURE S1. Proteases and phospholipase C-γ gene expressions in non-CF and CF macrophages. (*A*) MMP-12 (*n*=4, patients 6, 13, 15-16; supplemental Table 1), (B to E) Adam 9, Adam 10, Adam 12 (n= 6, patients 17-18, 20-22, 24; supplemental Table 1) and Adam 17 (n= 6, patients 17, 21-22, 24, 26, 41; supplemental Table 1), (*F* to *J*) cathepsin-B, cathepsin-D, cathepsin-F, cathepsin-K and cathepsin-S (*n*= 3-6, patients 15-16, 32; supplemental Table 1) and (*K*) phospholipase C-γ (PLCγ, *n=5-6*, patients 17, 18, 20, 22, 24; supplemental Table 1). Gene (mRNA) expression was quantified in CF and non-CF macrophages by RT-qPCR. The data are shown as the mean ± SEM. Mann-Whitney test: * $p<0.05$ *vs.* non-CF macrophages.

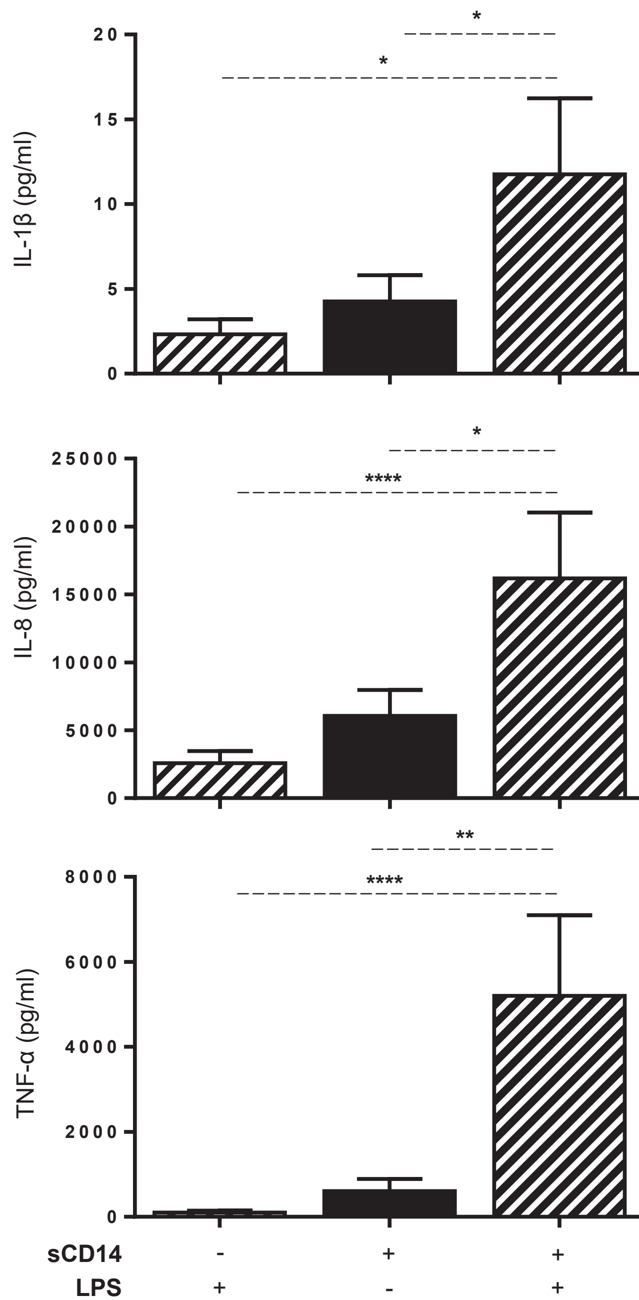

FIGURE S2. sCD14 potentiates LPS-induced inflammatory cytokines/chemokines production in non-CF macrophages. IL-1β, IL-8 and TNF-α levels were measured using an ELISA assay in non-CF macrophages incubated with LPS (from *P. aeruginosa*, 100 ng/ml) in the absence and presence of sCD14 (500 ng/ml) (n=8). Ratio Student's t-test: * $p < 0.05$; ** $p < 0.01$ and **** $p < 0.0001$ *vs* LPS-treated macrophages.