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The lipopolysaccharide co-receptor CD14 is present and functional in seminal plasma and expressed on spermatozoa

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SUMMARY

CD14 is a 54 000-molecular weight (MW) glycolipid-anchored membrane glycoprotein, expressed on myeloid cells, which functions as a member of the lipopolysaccharide (LPS) receptor complex. Soluble forms of CD14 have been reported in plasma, cerebrospinal fluid, amniotic fluid and breast milk. In plasma and breast milk, soluble CD14 has been implicated as a regulator of T- and B-cell activation and function. Expression of CD14 in the male reproductive system has not previously been investigated. We here show that soluble CD14 is present in seminal plasma at levels comparable to those in serum. Spermatozoa expressed CD14 on their membranes, as demonstrated by fluorescence microscopy and flow cytometry. Post-vasectomy, the levels of seminal plasma CD14 (spCD14) were much reduced, implying an origin distal to the point of transection of the vas deferens. Ultracentrifugation analyses demonstrated that spCD14 was not associated with lipid complexes, indicating that it lacks the glycolipid anchor. Purified spCD14 mediated activation by LPS of CD14-negative cells. These findings suggest that CD14 may play a hitherto unexplored role in immune defence and cell activation in the male reproductive tract.

INTRODUCTION

CD14 was initially described as a glycosyl phosphatidylinositol (GPI)-anchored membrane glycoprotein expressed predominantly on cells of the myelomonocytic lineage (monocytes and macrophages) and, at lower levels, on neutrophils and B lymphocytes.[1,2] On these cells, CD14 forms part of the lipopolysaccharide (LPS) receptor complex by acting as a co-receptor. Efficient cell activation induced by bacterial LPS requires the interaction of LPS with CD14, an interaction that is facilitated by the activity of a serum protein, LPS-binding protein (LBP), which catalyses the transfer of LPS monomers to CD14. Subsequently, the CD14–LPS complex triggers cell activation, predominantly via the cell-surface signalling molecule Toll-like receptor 4 (TLR4) and its associated molecule, MD-2.[3,4] It has become clear that CD14 is critical for an efficient recognition not only of LPS but also of a number of other bacterial cell wall components from diverse organisms, heat shock proteins and some viruses.[5]

The presence in plasma of a soluble form of CD14 (sCD14) was first noted in 1986.[6] Later work showed that normal plasma concentrations of sCD14 are in the range of 2–6 μg/ml,[7] although higher levels have been reported in infectious and inflammatory diseases.[8,9] Two distinct forms of sCD14, termed sCD14α (48 000 MW) and sCD14β (56 000 MW) are present in plasma.[10,11] Monocytes are the source of both forms of plasma sCD14; it has been suggested that sCD14α is generated by cleavage of the GPI-anchored membrane CD14, whereas sCD14β is a secreted molecule derived from a common precursor of the GPI-anchored form. Plasma sCD14 retains the ability to bind LPS and mediate activation of CD14-negative and CD14-positive cells.[10,12] These activation events, like those triggered through membrane CD14, are signalled predominantly via TLR4.[13]

Soluble forms of CD14 have also been reported in cerebrospinal fluid (CSF), amniotic fluid, synovial fluid and breast milk.[14–17] In normal CSF, sCD14 is present at a concentration of 0·19 μg/ml and is elevated in meningitis, apparently as a consequence of local biosynthesis.[14] In breast milk, sCD14 levels are 20–100 μg/ml, 10-fold higher than the levels in plasma.[16] In each of these fluids, sCD14 has been implicated as a sensor for bacterial LPS and is capable of rapidly mediating local cell activation in response to bacterial invasion.

Seminal plasma is known to contain immunoregulatory activities that restrict infection of the genital tract.[18–21] The molecular bases of these activities remain poorly understood. We undertook to examine whether seminal plasma contained

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a soluble form of CD14 that might contribute to its immunoregulatory properties. We here show that seminal plasma from normal donors contains soluble CD14 (spCD14) at levels comparable to those in blood plasma. spCD14 mediated cell activation in assays that have previously been used to characterize soluble CD14 from other sources. Post-vasectomy, spCD14 levels are much reduced, suggesting that spCD14 is derived in part from testis and/or seminal vesicles. Finally we show that spermatozoa express a membrane-bound form of CD14, the roles of which remain unexplored.

MATERIALS AND METHODS

General reagents and antibodies

All reagents were sourced from Fisher Scientific (Loughborough, UK) unless stated otherwise. The anti-human CD14 monoclonal antibody (mAb) MEM-18 (IgG1) has been described previously.[6] The rabbit anti-CD14 polyclonal antiserum was generated in-house by immunization with purified CD14 and was adsorbed with human IgG before use. The isotype-control antibody, 6D1, was generated in-house. BRIC229 anti-CD59 was from IBGRL (Bristol, Avon, UK). Horseradish peroxidase (HRP)-conjugated secondary antibody against rabbit immunoglobulin (minimal cross-reactivity) was from Jackson Immunoresearch (West Grove, PA). Phycoerythrin (PE)-conjugated secondary antibodies against mouse and rabbit immunoglobulin were from DAKO (High Wycombe, UK) and Sigma Aldrich (Poole, Dorset, UK), respectively. Fluorescein isothiocyanate (FITC)-conjugated secondary antibodies against mouse and rabbit immunoglobulin were from DAKO. The human astrocytoma cell line, U373MG, was obtained from the American Type Culture Collection (ATCC) (Rockville, MD).

Seminal plasma and spermatozoa

Seminal plasma samples were obtained fresh from the Infertility Clinic of the University Hospital of Wales, either from normospermic individuals (normals) or postvasectomy. For the majority of samples, cells were immediately removed by centrifugation (1000 ***g***, 5 min) and the cell-free seminal plasma was stored at -70° until use. Prostasome-free seminal plasma was obtained as previously described,[21,22] by subjecting fresh cell-free seminal plasma to ultracentrifugation (100 000 ***g***, 1 hr) in a Beckman benchtop ultracentrifuge. Motile spermatozoa were obtained free of contaminating cells using a modification of the 'swim-up' technique. Fresh seminal plasma samples were allowed to liquefy at room temperature for 30 min in a plastic tube, then carefully overlayed with an equal volume of sterile normal saline at room temperature. The tube was placed at 37° for 1 hr, the saline carefully removed using a glass pipette and cells harvested from the saline by gentle centrifugation (500 ***g***, 15 min). Spermatozoa obtained by swim-up were essentially free of other cells.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting of seminal plasma

Samples for analysis (1 ml) were diluted 1 : 3 in phosphate-buffered saline (PBS) and incubated for 2 hr at room temperature with MEM-18 anti-CD14 immobilized on cyanogen bromide (CNBr)–sepharose (Amersham Pharmacia, Little Chalfont, UK) at 1·4 mg of IgG/ml of the gel (0·2 ml of packed gel). The immunosorbent was washed five times in PBS, twice in PBS containing 0·5 M NaCl and then eluted with 0·1 ml of non-reducing Laemlli sample buffer. The sample was split and half was diluted in sample buffer containing β-mercaptoethanol to reduce. Samples were boiled for 90 seconds and loaded on 10% or 12·5% SDS–polyacrylamide gels. Gels were either silver stained to identify proteins or electroblotted onto nitrocellulose. Blots were blocked in PBS containing 1% non-fat milk and then incubated sequentially with primary antibody (polyclonal anti-CD14, 1 : 4000 in PBS/milk; overnight at 4°) and secondary antibody (HRP-anti-rabbit IgG, 1 : 2000 in PBS/milk; 1 hr at room temperature). Blots were developed using the enhanced chemiluminescence (ECL) system (Pierce, Chester, UK).

Enzyme-linked immunosorbent assay (ELISA) for spCD14 in seminal plasma samples

The concentration of spCD14 in seminal plasma samples was measured using a commercial ELISA (IBL, Hamburg, Germany), according to the manufacturer's instructions. The working range of the assay was 5–100 ng/ml. Samples from 35 normal donors and 20 postvasectomy donors were measured. All samples were run in duplicate, at a dilution of 1 : 50 in assay diluent buffer. Results were calculated from the standard curve.

Purification of spCD14

Pooled, cell-free seminal plasma (10 ml) was diluted in an equal volume of PBS containing 0·1% 3-[(cholamidopropyl)dimethyl-ammonio]-1-propane-sulfonate (CHAPS), centrifuged to remove debris and applied first to a precolumn containing 10 ml of sepharose 4B (Amersham Pharmacia) in PBS/0·05% CHAPS and then to an immunoaffinity column comprising 5 ml of MEM-18 anti-CD14 mAb immobilized on CNBr–sepharose at 1·4 mg of IgG/ml of the gel (described above). The immunoaffinity matrix used for these studies was new and had not been exposed to any other possible source of CD14. The immunoaffinity column was washed with 10 column volumes of PBS/CHAPS, then with 10 column volumes of PBS containing 0·5 M NaCl and finally eluted in PBS containing 50 mM diethylamine, pH 11·5. The eluted protein was pooled, neutralized by addition of a 1/10 volume of 1 M Tris-HCl, pH 7·0, and dialysed extensively into PBS. Protein was concentrated in an Amicon ultrafiltration cell (Amicon Ltd, Stonehouse, UK) prior to storage in aliquots at -20°. Protein concentration was measured using the Coomassie protein assay (Pierce).

Cell activation assays using purified spCD14

To test the biological activity of spCD14, 90% confluent cultures (in 96-well plates) of the human astrocytoma cell line, U373MG, were washed and cultured for an additional 20 hr in Dulbecco's modified Eagle's minimal essential medium (DMEM) (Gibco BRL, Paisley, Strathclyde, UK) supplemented with 20 mM glutamine and different amounts of purified spCD14, with or without LPS at 100 ng/ml (from *Escherichia coli*, strain 055 : B5; Sigma). For some control experiments, the spCD14 preparation was preincubated (30 min, room temperature) with blocking mAb against CD14 (MY4; Beckman Coulter, High Wycombe, UK) or its isotype-matched control (MOPC141; Beckman Coulter) before adding to the cultures. The 20-hr culture supernatants were

collected and assayed for interleukin (IL)-6 and IL-8 by ELISA (R & D Systems, Abingdon, UK). The cells were harvested and stained with anti-CD54 (intracellular adhesion molecule-1 [ICAM-1]) mAb or its isotype-matched control (Diaclone/IDS, Tyne and Wear, UK), followed by a PE-conjugated rabbit anti-mouse immunoglobulin (Sigma), and then analysed on a fluorescence-activated cell sorter (FACScalibur; Becton-Dickinson, San Jose, CA).

Flow cytometry analysis

Spermatozoa obtained by swim-up were washed three times in PBS by centrifugation and resuspended in FACS buffer (1% bovine serum albumin [BSA] and 0·2% NaN_3 in PBS) at a concentration of 10^6/ml. All staining steps were conducted on ice. Cells (50 µl) were incubated with the same volume of primary antibody (MEM-18; 10 µg/ml) or control antibodies (6D1 or BRIC229; 10 µg/ml) for 40 min on ice, washed twice with FACS buffer and incubated for 40 min on ice with a PE-conjugated secondary antibody diluted 1 : 150 in FACS buffer. Cells were washed twice in FACS buffer and fixed with 1% paraformaldehyde in FACS buffer. Fluorescence was measured using a FACScalibur flow cytometer (Becton-Dickinson).

Fluorescence microscopy

Spermatozoa obtained by swim-up were stained with primary antibodies (MEM-18 anti-CD14 mAb, polyclonal anti-CD14, BRIC229 anti-CD59 mAb) essentially as described for flow cytometry, except that FITC-conjugated secondary antibodies were used. Controls included cells incubated with the isotype-control mAb, an irrelevant polyclonal antiserum, or no first antibody. The stained cells were fixed with paraformaldehyde, washed once with deionized water and dried onto glass slides. Coverslips were applied using Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and the stained cells were viewed using a Nikon inverted fluorescence microscope. Images were captured and processed using the Openlab image analysis system (Improvision, Coventry, UK).

RESULTS

Western blotting demonstrates the presence of sCD14 in seminal plasma

Immunoprecipitates from cell-free seminal plasma samples were separated by SDS–PAGE (on 10% gels) under reducing or non-reducing conditions, blotted onto nitrocellulose and probed with polyclonal anti-CD14. Two major bands of apparent M_r 45 kDa and 50 kDa under non-reducing conditions, and 48 kDa and 53 kDa under reducing conditions, were present in all seminal plasma samples tested (Fig. 1a, 1b). Serum, run as a positive control, gave an essentially identical banding pattern, although the bands were consistently 1–2 kDa larger (Fig. 1a, 1b). Silver staining of spCD14 immunoprecipitated from seminal plasma showed the same two bands with only trace contaminants present (Fig. 1c). Prostasome-free seminal plasma was also run for comparison and appeared to be identical to unfractionated, cell-free seminal plasma (not shown).

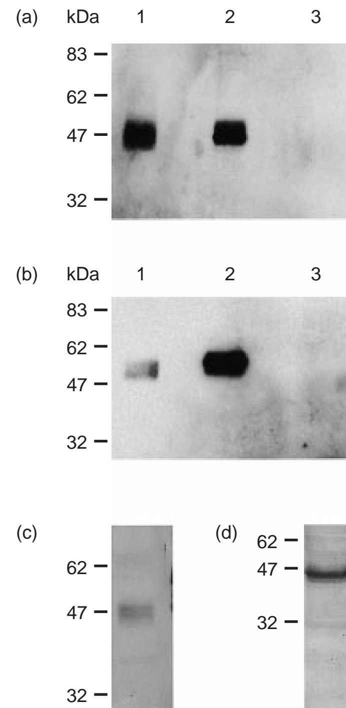

Figure 1. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blot analysis of soluble CD14 in seminal plasma (spCD14). Cell-free seminal plasma and serum were immunoprecipitated using a monoclonal anti-CD14 antibody and the immunoprecipitates were subjected to SDS–PAGE and Western blotting. Samples were run under nonreducing (a) or reducing (b) conditions: lane 1, seminal plasma; lane 2, serum; lane 3, blank immunoprecipitate using the solid-phase incubated with phosphate-buffered saline (PBS). (c) Silver-stained SDS–polyacrylamide gel of immunoprecipitated seminal plasma. (d) Silver-stained SDS–polyacrylamide gel of immunoaffinity-purified spCD14. The gels in (c) and (d) were run under non-reducing conditions.

ELISA confirms that spCD14 is present in seminal plasma and decreased postvasectomy

In 35 samples of cell-free seminal plasma from normospermic individuals, the mean concentration of spCD14 was 1·89 µg/ml (standard deviation [SD] 0·97; range 0·25–4·36 µg/ml) (Fig. 2). In 20 samples of cell-free seminal plasma from vasectomised males, the mean concentration of spCD14 was 0·68 µg/ml (SD 0·38; range 0·24–1·4 µg/ml). The difference between these two groups was highly significant ($P < 0·0001$; Student's unpaired *t*-test, two-tailed with Welch correction applied).

Purification and functional characterization of spCD14

spCD14 was purified to near-homogeneity following a single passage over the anti-CD14 mAb immunoaffinity column (Fig. 1d). The yield from 10 ml of pooled seminal plasma, estimated using the Coomassie protein assay, was 120 µg, an unanticipated result given that the mean seminal plasma concentration of spCD14 measured in the ELISA was <2 µg/ml.

spCD14 mediates cell activation induced by endotoxin

To test whether spCD14, like soluble CD14 from other sources, functioned as a mediator of LPS activation of cells that lack

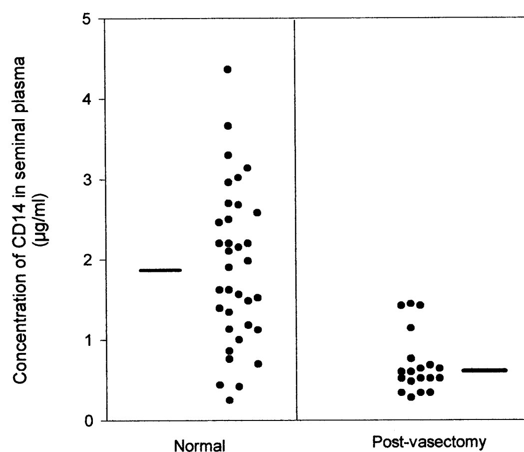

Figure 2. Measurement of soluble CD14 in seminal plasma (spCD14) from normal and postvasectomy males. Samples were measured in duplicate in a commercial enzyme-linked immunosorbent assay (ELISA) for CD14. Individual samples are represented by dots, and horizontal bars indicate the means for each population.

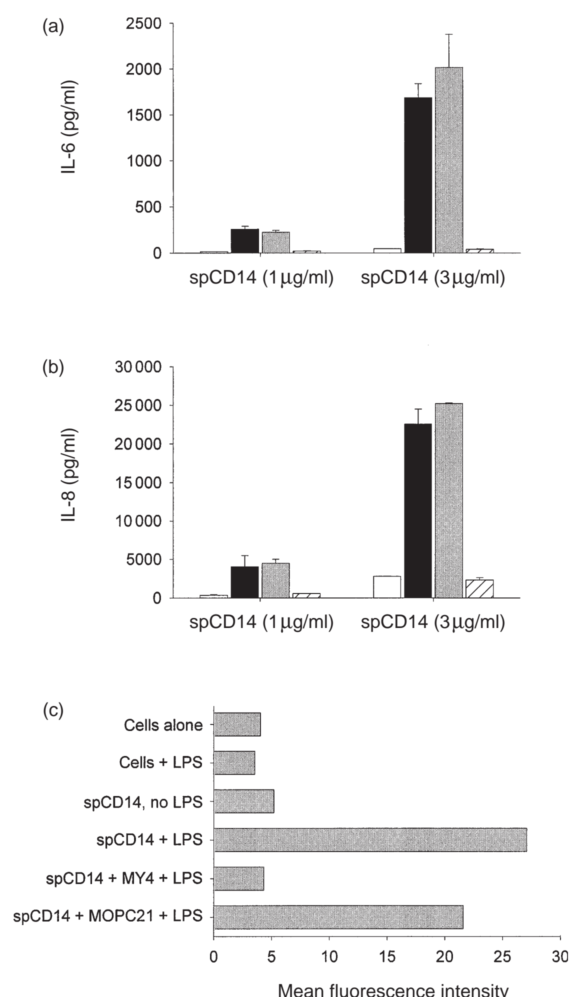

Figure 3. Functional characterization of CD14 purified from seminal plasma (spCD14). U373 cells were incubated for 20 hr with lipopolysaccharide (LPS) and/or spCD14. In some experiments the spCD14 was preincubated with either a blocking monoclonal antibody (mAb) against CD14 (MY14) or an isotype-control mAb (MOPC141). (a) and (b) Open bars represent spCD14 (no LPS); black bars, spCD14 + LPS; dotted bars, spCD14 pretreated with control mAb + LPS; hatched bars, spCD14 pretreated with anti-CD14 mAb plus LPS. (a) Interleukin-6 (IL-6) production. Supernatants were collected from triplicate wells for each experimental condition and measured in the IL-6 enzyme-linked immunosorbent assay (ELISA). Bars represent the mean value ± SD of triplicate wells and the result is representative of three separate experiments. (b) Interleukin-8 (IL-8) production. Supernatants were collected from triplicate wells for each experimental condition and measured in the IL-8 ELISA. Bars represent the mean value ± SD of triplicate wells and the result is representative of three separate experiments. (c) Surface expression of intracellular adhesion molecule-1 (ICAM-1) (CD54). Cells were harvested and stained (as described in the Materials and methods) prior to analysis by flow cytometry. The results are representative of three separate experiments.

membrane CD14, we examined the effects of spCD14, with or without LPS, on the CD14-negative cell line, U373. spCD14 mediated LPS-dependent IL-6 and IL-8 production by U373 in a dose-dependent manner (Fig. 3a, 3b), and also induced expression of ICAM-1 (CD54) in these cells (Fig. 3c). An anti-CD14-specific mAb (MY-4) abrogated all observed changes, confirming the CD14 dependence of these events.

Flow cytometric analysis demonstrates that spermatozoa express CD14

Spermatozoa stained with MEM-18 anti-CD14 gave a homogeneous population of positive cells (Fig. 4). Cells stained with secondary antibody alone, or with an irrelevant, isotype-matched mAb (6D1, anti-rat CD59), were all negative. Cells stained with BRIC229 anti-human CD59 (positive control) were all strongly positive.

Fluorescence microscopy reveals homogeneous distribution of CD14 on spermatozoa

Spermatozoa stained for CD14 using either the mAb MEM-18 (Fig. 5c) or the polyclonal anti-CD14 antiserum (Fig. 5d) exhibited a global membrane staining for CD14, which was stronger on the tail and neck than on the head of the cell. Cells stained with mAb anti-CD59 as a positive control gave a granular staining on all parts (Fig. 5b), as previously described.[21] Controls in which the primary antibody was omitted (Fig. 5a, inset), or was substituted for an irrelevant mAb or polyclonal antiserum, were negative.

DISCUSSION

Understanding of the innate mechanisms operating to protect the host from bacterial invasion has increased greatly in recent years.[5,23] Biological fluids contain an arsenal of agents that can directly or indirectly target and destroy pathogens. The contribution made by CD14 to innate immunity is now well recognized. CD14 belongs to a family of receptors, termed pattern recognition receptors, which are involved in innate immune recognition of pathogen-associated molecular patterns.[24] Membrane-bound CD14 on monocytes, macrophages and neutrophils binds LPS and other bacterial wall products, enabling these cells to 'sense' the presence of pathogens.

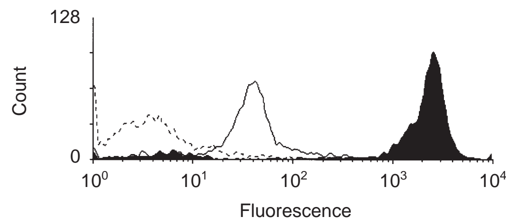

Figure 4. Expression of CD14 on human spermatozoa. Spermatozoa, purified by 'swim-up', were stained for CD14 (MEM-18 monoclonal antibody [mAb]; solid line), CD59 (BRIC229 mAb; shaded profile) or without primary antibody (dashed line), as described in the Materials and methods. Staining was analysed on a fluorescence-activated cell sorter (FACsCalibur flow cytometer).

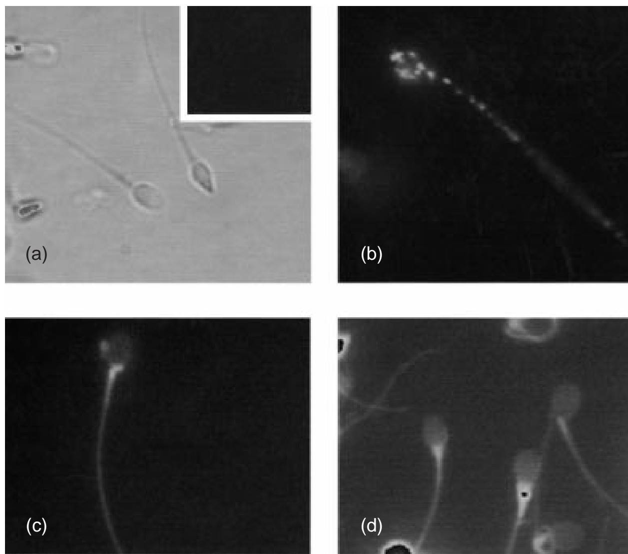

Figure 5. Distribution of CD14 on human spermatozoa. (a) Phase-contrast image of spermatozoa, prepared as described in the Materials and methods. The inset panel is a fluorescence image of stained cells but with the primary antibody omitted (negative control) (original magnification $\times 500$). (b) Spermatozoa stained for CD59. The granular staining pattern is apparent on all parts of the cell (original magnification $\times 500$). (c) Spermatozoa stained for CD14 using the monoclonal antibody (mAb) MEM-18 (original magnification $\times$ 500). (d) Spermatozoa stained for CD14 using the polyclonal anti-CD14 antiserum (original magnification $\times 500$).

Interaction of the LPS–LBP–CD14 complex with TLR4 and associated molecules on the membrane causes the cell to respond appropriately.[3,4] Soluble forms of CD14, present in plasma and other biological fluids, retain the capacity to bind LPS, and the complex formed can bind and activate both CD14-positive and CD14-negative cells, again via interactions with TLR4, providing a second pathway for cell activation.[4,13] LBP, while not essential for association of sCD14 with LPS, accelerates assembly of the complex and thus enhances cell activation.[25] The importance of this sCD14-mediated cell-activation pathway in immune surveillance in biological fluids, and the immunoregulatory capacity of sCD14, has only recently been recognized, first in plasma,[26–27] and, even more recently, in breast milk.[16,17]

Seminal plasma is a highly specialized fluid that is rich in immunomodulatory activities, including prostaglandins and numerous inhibitors of the complement system.[18–21,28] A delicate balance must be achieved in seminal plasma between the need to maintain powerful immune surveillance to ward off infection and the requirement to protect spermatozoa in the potentially hostile environment of the female genital tract. Because of the important immunoregulatory roles now being ascribed to sCD14, we set out to examine whether CD14 was expressed in seminal plasma and on its constituent cells.

Western blot analysis demonstrated that seminal plasma contained protein bands reactive with antibodies against CD14. spCD14, like the serum protein, comprised two major bands of approximately equal intensity; however, the band sizes had a consistently smaller M_r than those in serum: 48 and 53 kDa versus 50 and 56 kDa for serum sCD14 under reducing conditions. Although we have yet formally to investigate the reason for this difference in molecular mass, it is likely to be a consequence of differences in glycosylation patterns in this heavily glycosylated glycoprotein. CD14 is GPI anchored on cells and some GPI-anchored molecules in seminal plasma, for example, CD59 and CD55, reside predominantly or exclusively on prostasomes (which are vesicular structures present in abundance in seminal plasma).[21] We examined, in two ways, the possibility that spCD59 was GPI anchored. First, ultracentrifugation to remove prostasomes demonstrated that sCD14 was not prostasome-associated, in that identical bands of similar intensity were present in prostasome-free seminal plasma (data not shown). Second, gel filtration of cell-free seminal plasma showed that all the CD14-reactive material was present in late fractions, compatible with GPI anchor-free protein, whereas CD59 was present predominantly in early fractions, confirming its association with prostasomes (results not shown).

Quantification by ELISA showed that the levels of sCD14 in seminal plasma were similar to the levels reported in blood plasma. Seminal plasma consists of the products of multiple components of the reproductive tract: the seminiferous tubules, the epididymis, the seminal vesicles, the prostate and the bulbourethral glands. The fluid part is contributed chiefly by the prostate and seminal vesicles. Transection of the vas deferens (vasectomy) removes spermatozoa and components contributed by the seminiferous tubules and epididymis. Post-vasectomy, levels of sCD14 in seminal plasma were reduced to 36% of those in nonvasectomy samples, indicating that the bulk of the spCD14 was derived from testis, seminiferous tubules and/or epididymis.

Purification of spCD14 by immunoaffinity chromatography generated pure protein at a yield several fold greater than predicted from the levels measured by ELISA in seminal plasma. The probable explanation of this finding is that the ELISA underestimates the spCD14 concentration because of interfering components in seminal plasma. The purified spCD14 was tested in assays of cell activation utilizing the CD14-negative astrocytoma line, U373, as a target, as described previously.[12,16] spCD14 mediated the LPS-induced activation of the cells in a dose-dependent manner, as measured by three different activation parameters: secretion of IL-6; secretion of IL-8; and induction of expression of ICAM-1. The demonstration that spCD14 can mediate LPS-induced cell activation introduces the possibility that the protein may act as

a sensor for LPS *in vivo*, activating cells in seminal plasma to respond to infection.

Expression of CD14 on the membranes of spermatozoa has not previously been reported. We examined expression of CD14 on spermatozoa, obtained by swim-up to eliminate contaminating leucocytes, by flow cytometry and fluorescence microscopy. One hundred per cent of spermatozoa stained for CD14 using specific mAb, and staining was distributed globally over the cell. The distribution of CD14 on spermatozoa was homogeneous and differed markedly from that of another GPI-anchored molecule, CD59, which was distributed in clusters on the cell. Attempts to confirm GPI anchoring of CD14 by treatment of spermatozoa with phosphatidylinositol-specific phospholipase C (PIPLC) were unsuccessful because of the lability of the cells under conditions used for PIPLC treatment. CD14 is predominantly expressed on cells of the myelomonocytic lineage, although it has recently become apparent that other cell types can express this molecule. Human hepatocytes, gingival fibroblasts and airway epithelia have all been show to express CD14, although its functional relevance at these sites is unclear.[29–31]

The function of CD14 on human spermatozoa is uncertain. We propose that spermatozoal CD14 may have a complementary role to that of spCD14 in permitting sermatozoa to respond to LPS and other bacterial products. Spermatozoa have been shown to release reactive oxygen species (ROS) and cytokines in response to a variety of stimuli.[32,33] Incubation of spermatozoa with LPS caused little change in the production of ROS, whereas production of ROS by seminal plasma-derived neutrophils was increased.[34] Importantly, LPS caused increased secretion of IL-6 from purified human spermatozoa.[35] The mechanism of this activation was undefined. Taken in the context of our data it is probable that bacterial LPS can have both direct (via surface-expressed CD14) and indirect (via spCD14) activating effects on spermatozoa. LPS will also, directly or indirectly, activate neutrophils present in normal seminal plasma in small numbers ($<10^5$/ml) but much increased during infection.[36] We suggest that these effects may be important in the defence against infection in the male genital tract *in vivo*.

In this study we have examined spCD14 expression only in normospermic and vasectomised males. It will be of interest to examine spCD14 expression in seminal plasma and on spermatozoa in males with abnormalities of spermatozoal number, structure or function, and in those with infertility of unknown aetiology. Results of such studies will give further clues to the physiological role of CD14 in the male reproductive system.

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