Blood monocyte phenotypes and soluble endotoxin receptor CD14 in systemic inflammatory diseases and patients with chronic renal failure

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Introduction

Patients with end-stage renal failure undergoing chronic renal replacement therapy reveal several signs of an impaired humoral and cellular immune response [1,2]. As a consequence patients are more susceptible to bacterial infections, exhibit defective phagocytosis, altered B-T-cell and disturbed killer cell activity, low response rate to hepatitis B vaccine, and increased risk for cancer. On the other hand, secondary immunodeficiency in chronic uraemia is masked by dialysis-related blood-

membrane interactions which show chronic signs of complement and monocyte activation. In addition, changes in proinflammatory cytokine synthesis and secretion (IL1, IL6, TNF-α) and rise in acute phase reactants revealing inadequate ‘immunostimulation’ apparently contribute to progressive atherosclerotic lesions and increased cardiovascular events [3,4].

Monocytes are closely involved in antigen presentation, T- and B-cell cooperation and secretion of various monokines, and play a pivotal role not only as targets in biocompatibility but are also responsible for microinflammation and the dysbalance of the immune system observed in chronically uraemic patients [5,6].

The present paper will focus on data supporting the view that monocytes contribute to the immunoincompetence addressed above. In addition, recent clinical
work of our group on major monocyte differentiation and activation antigens is summarized. The data stress the importance of distinct blood monocyte populations and the soluble and membrane-bound monocyte endotoxin receptor CD14 as biological markers for screening and monitoring inflammatory disease activity [7].

**CD14, a major monocyte receptor antigen**

Peripheral blood monocytes carry a glycoprotein, the myeloid differentiation antigen CD14, with a molecular weight of 53 kDa, which is attached to the cell membrane by a glycosylphosphatidylinositol anchor. The CD14 molecule is not only a major surface receptor for lipopolysaccharide (LPS, endotoxin) but also for lipoteichoic acid, peptidoglycans and phospholipids from Gram-positive bacteria, thus indicating that CD14 acts as a multifunctional molecule. Furthermore, the CD14 antigen is involved in recognition and phagocytosis of apoptotic cells. In cooperation with a LPS-binding protein (LBP), LPS upregulates CD14 expression, whereas immunosuppressive drugs down-modulate the antigen [8,9]. Activated monocytes secrete various cytokines, enzymes and free oxygen radicals. Patients suffering from non-infectious diseases, e.g. systemic vasculitis, or from aberrant immunoregulatory diseases disclosed an activation of blood monocytes via CD14, which is also released from the cells in a soluble form (sCD14) at an increased rate. Furthermore, a subset of CD14+ monocytes coexpresses CD16, a Fcγ-receptor type III, and selectively expands under acute and chronic inflammatory clinically defined conditions [10,11].

**Soluble CD14 (sCD14)**

We analysed the serum concentrations of sCD14 in patients with systemic lupus erythematosus [12]. The following three clinical groups were assessed for sCD14, soluble IL-2-receptor (sIL-2r), IL-1β, anti-nuclear antibodies (ANA), anti-ds-DNA antibodies, and complement C3: (i) 65 healthy volunteers, (ii) 35 patients with an inactive phase of systemic lupus erythematosus (SLE) and (iii) 17 patients with SLE relapses. Disease activity was scored according to the sensitive activity index reported by Isenberg [for details see ref. 12]. In healthy controls sCD14 levels were 2.18±0.46 mg/l (median 2.1 mg/l). They were significantly higher in patients with inactive SLE (median 4.1 mg/l, P<0.0001 vs controls). Patients with clinically active SLE revealed even higher serum concentrations of sCD14 (median 6.9 mg/l, P<0.001). SCD14 levels did not correlate with the amount of blood monocytes and IL-1β. A close correlation emerged between SLE activity score and sCD14, C3 and sIL-2r, but not with IL-1β and anti-ds-DNA titters. Longitudinal studies revealed that serum sCD14 paralleled the clinical course as defined by the above activity score. In a similar manner, as an example for chronic viral disease, compared to 78 normal controls sCD14 (median 2.2 mg/l) the mean sCD14 serum concentration was significantly higher in 115 HIV patients and depended from CDC-classification [13].

In patients with end-stage renal failure who were treated by intermittent haemodialysis sCD14 was significantly augmented (5.5 mg/l vs. 2.2 mg/l, P<0.0001) [6,7]. In dialysis patients with either acute or chronic infections a further rise in sCD14 levels emerged [8,11]. These data suggest suggest that increased serum levels of sCD14 reflect an activated status of monocytes or macrophages and result from increased membrane shedding.

**CD14+/CD16+ monocytes: the proinflammatory subset**

Blood monocytes represent a heterogeneous cell population [10]. At least two populations can be distinguished depending on the expression pattern of membrane surface antigens, release of cytokines and phagocytic activity. Most, if not all, blood monocytes express the endotoxin receptor antigen CD14, which differs in its cell surface density.

The major fraction of monocytes is strongly positive for CD14 (CD14+), however, it lacks coexpression with Fcγ-receptor III, the CD16 antigen. CD16, a low affinity IgG Fc-receptor, together with complement plays an important role in immune-complex mediated tissue injury. Leucocytes deficient for CD16 lack IgG mediated mast cell activation, show diminished Arthus reaction, resistance to cryoglobulin-associated vasculitis, and passive anaphylaxis. Furthermore, CD16 is involved in the onset of autoantibody mediated disease, and animals lacking the antigen do not develop immune-complex induced glomerulonephritis.

Blood monocytes expressing both the CD14+ and CD16+ antigen constitute a proinflammatory subtype, which exhibit features of tissue macrophages. CD14+CD16+ monocytes and sCD14 were highly increased in patients with infectious and non-infectious inflammatory diseases [7,8,11]. They also disclosed an augmented HLA-DR expression and phagocytic activity compared to CD14+/CD16- negative cells. CD14+ (and CD68+) cells accumulated up to 10-fold in kidneys of patients with progressive renal disease and in kidney allografts with chronic dysfunction, as shown by immunohistochemistry and image analysis [7]. As expected, in patients infected with HIV, the expression of CD14 on blood monocytes and the percentage of CD14+/CD16+ monocytes also increased significantly and paralleled disease state [13].

**Monocyte phenotypes in patients undergoing intermittent haemodialysis (HD)**

Blood monocytes are engaged in primary host defence against infections. We assessed the expression of the monocyte CD14 antigen as well as the presence of a minor subpopulation coexpressing low levels of CD14 together with CD16 in haemodialyzed patients [6,11]. Mean CD14 expression of total blood monocytes from HD patients was significantly lower compared to normal...
controls and uraemic patients not yet requiring dialysis treatment ($P<0.0001$). Since the amount of CD14 per cell is positively correlated with endotoxin (LPS) binding to the monocyte surface also processing of LPS and cytokine synthesis and release is altered in haemodialysis patients. Chronic intermittent 'stimulation' of monocytes by LPS or LPS-like substances during dialysis may finally result in low CD14-expression due to enhanced shedding of the antigen. Endotoxins and low molecular weight LPS-fragments, not detectable by conventional tests, can enter the blood across the haemodialysis membrane by diffusive and convective transfer [5] and prime cytokine release [14].

In healthy controls CD14+/CD16+ monocytes account for 8% of all CD14+ monocytes only. However, in stable HD patients the CD14+/CD16+ subpopulation was significantly elevated (14±3% or 66±28 cells/μl) while CD14++ monocytes remained constant [7,11]. In HD patients suffering from infection a further rise in CD14+/CD16+ monocytes was observed (128±71 cells/μl, $P<0.001$) and contributed to 24% of all blood monocytes. In contrast, numbers of CD14++ cells did not change compared to stable HD patients indicating that proinflammatory CD14+/CD16+ monocytes were selectively expanded. During acute infections, most of them being due to vascular access problems (shunt infections, infected jugular vein catheter) CD14+/CD16+ cells always are expanded ($P<0.0001$).

CD14+/CD16+ monocytes exhibited a significant higher phagocytosis rate for FITC-labelled than *Escherichia coli* CD14++/CD16 negative monocytes underlining their role during host defence. In addition, CD14+/CD16+ monocytes expressed higher levels of MHC class II antigens (HLA-DR, DP, DQ), and equal amounts of MHC class I. Thus, CD14+/CD16+ cells constitute a potent phagocytosing and antigen-presenting monocyte subpopulation, which is selectively expanded in HD patients, and further increases during infections. Ultrapure dialysate may improve monocyte alterations [15].

**Activated monocytes and drug response**

We monitored various monocyte markers in patients with acute vasculitis before and after pulse therapy with glucocorticoids [8,9]. Glucocorticoids caused rapid clinical improvement, that is, rise in glomerular filtration rate, decrease of proteinuria, drop of acute phase reactants etc. In parallel, elevated serum sCD14, monocyte expression of CD14 (~35%) and HLA-DR (~60%) decreased. Cultured monocytes from healthy people dramatically upregulated membrane CD14 and release of sCD14 in the presence of LPS [9]. LPS-binding capacity of FITC-labelled LPS from *E. coli* was directly correlated with monocyte CD14 expression ($r=0.89$, $P<10^{-4}$). Endotoxin-induced stimulation of CD14 and sCD14 synthesis was markedly but not completely abolished by glucocorticoids such as hydrocortisone, prednisolone and dexamethasone ($10^{-4}$–$10^{-9}$ M) following a sigmoid curve dose dependency. In addition, glucocorticoids significantly decreased the secretion of IL-1β of LPS activated monocytes. However, no changes of CD35, HLA-DR and CD33 expression were observed, whereas CD13 decreased, indicating specificity of glucocorticoid effect modulating the LPS-receptor antigen. Glucocorticoids selectively affected the CD14+/CD16− subpopulation of monocytes not only by down-regulating CD14-expression (and HLA-DR) and release of sCD14 but also by inducing a rapid decline of the amount of circulating proinflammatory cells [7]. In addition, 1,25-dihydroxyvitamin D3 exhibits various immunomodulatory properties, regulates the synthesis of immunoglobulins, cytokines and TNF-α, and the expression of HLA-DR. Calcitriol enhances monocyte expression of CD14 and release of sCD14 cultured monocytes in a dose dependent manner [18]. Uraemic ultrafiltrate contains factors that impair calcitriol activated function of monocytes. Calcitriol supplementation is capable of enhancing the phagocytic function of monocytes and may prevent spontaneous and allergic autoimmune diseases as well as graft rejection.

**CD14 and apoptosis**

CD14 is engaged in the recognition and clearing of apoptotic cells without inducing inflammation [19]. The region of CD14 responsible for this interaction is closely associated with the LPS binding site. Up-regulation of CD14 (e.g. by LPS) prevents apoptosis, whereas down-modulation of CD14 (e.g. by IL-4) or shedding of CD14 promotes programmed cell death. Thus, this newly detected function of CD14 demonstrates that the 'LPS-receptor protein' is also involved in the regulation of monocyte senescence [20].

**Monocytes/macrophages and progressive renal disease:**

Under pathological conditions the influx of monocytes into the kidney and local proliferation of blood derived macrophages releasing proinflammatory and fibrogenic
cytokines contribute to structural and functional deterioration. Tubulointerstitial kidney lesions may result from maladapted cell interactions, paralleled by activation and proliferation of tubular epithelia, release of chemokines, increased extracellular matrix synthesis and lethal cell damage. Recent studies support a possible ‘crosstalk’ between activated monocytes and cytokine activated tubular cells, isolated from human kidney by immunomagnetic separation [21,22]. IL-1β, TNF-α and interferon-γ dramatically upregulated HLA-DR, adhesion molecule ICAM-1, and secretion of the chemokine RANTES in proximal and distal tubule cells. Thus, cytokine activated tubular epithelia are capable of attracting monocytes [21]. Immune-complexes, ischaemia, free oxygen radicals and cytokines activate tubule cells to synthesize chemokines MCP-1 and RANTES. Serum proteins ultrafiltered by glomeruli and internalized by tubular cells trigger the epithelial synthesis of chemokines MCP-1 and RANTES; thus, overload proteinuria may contribute to inflammatory cell recruitment [23]. Chemokine genes in kidneys of proteinuric animals were upregulated via activation of the transcription factor NF-κB.

Monokines, which stimulate tubule epithelia to over-express HLA-DR and adhesion molecules (ICAM-1) are released by ‘activated’ monocytes at an increased rate. LPS and chemically modified lipoproteins (and β2-microglobulin amyloid) transform blood monocytes (CD14+/CD16−) into the proinflammatory subtype now carrying the CD16 epitope. Such activated monocytes may pass the endothelial barrier, and accumulate (proliferate) within the glomeruli and the tubulo-interstitium. Experimental and clinical studies evidence the association of macrophage trafficking into the kidney and local tissue damage, or, depending on the macrophage subtype and cytokine profile, initiating repair and resolution. Release of proinflammatory cytokines and growth factors by activated monocytes/macrophages (IL-1, TNF-α, PDGF, prostaglandins, matrix-metallo-proteases type 1, 2, 3, and 9) amplifies cell injury, interstitial fibrosis, glomerulosclerosis and functional deterioration. Anti-inflammatory cytokines (IL4, IL10, IL13) and drugs such as glucocorticoids and cyclooxygenase II inhibitors positively interfere with the crosstalk of both cell types.

In patients with tubulointerstitial nephritis urine levels of sCD14 were significantly higher compared to those suffering from chronic glomerulonephritis and nephrosclerosis [24]. Patients with proliferative glomerulonephritis and haematuria excrete CD16 positive macrophages at an increased rate [25]. Urinary output of sCD14 and CD16 + macrophages may therefore be promising noninvasive markers to describe enhanced migration of leukocytes into the inflamed kidneys.

In summary, the data highlight monocytes as major effector and target cells for immune deficiency and chronic inflammatory alterations observed in patients with renal failure, where monocyte endotoxin receptor expression is downregulated. However, in these patients the amount of proinflammatory CD14 + cells newly coexpressing the Fc-receptor CD16 is enhanced and apparently associated with bioincompatibility, diagnosis water quality, β2-micro-globulin amyloidosis, atherogenesis and accelerated cardiovascular morbidity.

References


