Krick G, Ronco C (eds): On-Line Hemodiafiltration: The Journey and the Vision.
Contrib Nephrol. Basel, Karger, 2011, vol 175, pp 110–116

Evolution of the Concept of Biocompatibility and the Cardioprotective Effect of On-Line Hemodiafiltration

Rafael Ramírez · Alejandro Martín-Malo · Pedro Aljama

Nephrology Department, Hospital Reina Sofia, University of Cordoba, Cordoba, Spain

Abstract

Chronic inflammation presented by chronic kidney disease patients is accompanied by an increase in the percentage of CD14+/CD16+ monocytes. The inflammatory activity produced by these cells provokes the activation and death of endothelial cells, which suggests that these cells may play an important role in the coupling of inflammation and endothelial damage in these patients. Therefore, the reduction in CD14+/CD16+ inhibitory monocytes may constitute a therapeutic goal in the treatment of chronic kidney disease patients. The use of the most effective hemodialysis techniques such as on-line hemodiafiltration appears to be a suitable tool for achieving this objective.

Copyright © 2011 S. Karger AG, Basel

Patients suffering from chronic kidney disease (CKD) present a chronic inflammatory state of moderate intensity (low-grade inflammation) that appears to play an important role in the development of endothelial damage, arteriosclerosis and other cardiovascular changes, which are the principal causes of morbidity and mortality in these patients [1].

Monocytes and Disease

In the natural course of the immune response, immunocompetent cells (mainly monocytes) generate inflammation as a rapid and efficacious response to pathogens. These cells express surface molecules such as the CD14 receptor which,

Downloaded by:
UCSF Library & CKM
169.230.243.252 - 9/15/2014 2:24:34 AM

once they have combined with the antigen, activate the inflammatory response controlled by cytokines and other soluble factors released by activated immune cells [2]. When the antigenic stimulus ceases, the production of inflammatory cytokines also finishes, generating an anti-inflammatory response in the course of which the activated immunocompetent cells die by apoptosis [3]. This biological mechanism guarantees a controlled response that limits the perpetuation of potentially injurious cells. However, when the antigenic stimulus persists (as occurs in CKD patients with the uremic toxins and/or the immune stimulation associated with the hemodialysis therapy), the inflammatory response is perpetuated to become a chronic inflammatory response that is damaging [4].

The inflammatory profile observed in CKD patients differs from the standard inflammation and seems to be largely dependent on mononuclear cell activity [5–7]. Chronic inflammation in these patients resembles the inflammatory response described in elderly. This inflammatory response is characterized by maintaining a moderate increase in circulating proinflammatory cytokines and the presence of a subset of monocytes with a decreased expression of the CD14 receptor and the expression of the low-affinity receptor for the immunoglobulin Fc fraction, FcyRIII (CD16) [8–10].

Chronic Inflammation and CD14+/CD16+ Monocytes

In healthy subjects, peripheral blood monocytes are a heterogeneous population of cells characterized by their high level of expression of the CD14 molecule. This molecule is the receptor of the bacterial lipopolysaccharide, which is expressed in more than 90% of peripheral blood monocytes. However, under pathological situations, a different population of monocytes increase in peripheral blood, and these irregular cells are characterized to present a variable expression of the CD14 molecule and co-express the CD16 molecule. According to the levels of expression of these molecules, at least three different subsets of monocytes have been described (fig. 1): (1) monocytes with a high level of expression of CD14, which do not express CD16 (CD14++/CD16–) that are the usual monocytes found in healthy subjects; (2) CD14++/CD16+ monocytes, a population of cells found at higher levels in patients with cardiovascular pathology (stroke, infarcts, etc.) [11], and (3) inflammatory CD14+/CD16+ monocytes that are found in patients with diseases associated with chronic inflammation including CKD patients [8–10].

A large number of studies have shown an increase in the percentage of CD14+/CD16+ monocytes in CKD patients (these may make up as much as 30% of circulating monocytes in these patients) as occurs in other pathological situations. It has been suggested that the increase in CD14+/CD16+ monocytes plays a principal role in the chronic inflammatory disease associated with CKD, in contrast to that observed in the usual CD14++/

Downloaded by:
UCSF Library & CKM
169.230.243.252 - 9/15/2014 2:24:34 AM

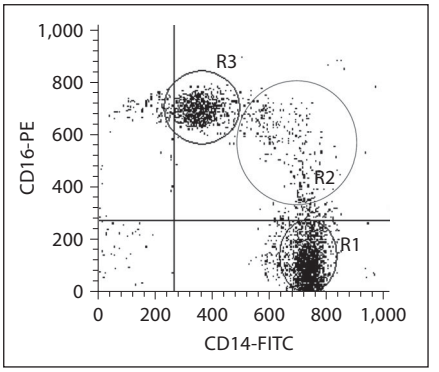

Fig. 1. Representative flow cytometry analysis of CD14 monocytes in a CKD patient. The CD14++/CD16– cells (R1) were defined as monocytes expressing CD14, but not CD16 (lower right quadrant). The CD14++/CD16– cells (R2) were defined as monocytes expressing CD16 and either high levels of CD14 (upper right quadrant). The CD14+/CD16+ cells (R3) were defined as monocytes expressing CD16 and either low levels of CD14 (upper left quadrant). For determination of CD14/CD16 cells, monocytes, were gated and labeled using 2-color fluorescence phycoerythrin (PE)-conjugated CD16 antibody, and fluorescein isothiocyanate (FITC)-conjugated CD14. R1 = CD14++/CD16–; R2 = CD14++/CD16+; R3 = CD14+/CD16+.

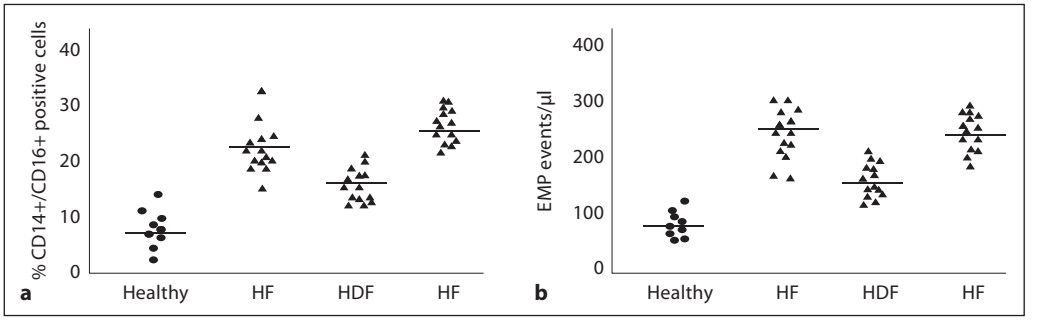

Fig. 2. Percentage of CD14+/CD16+ monocytes (**a**) and levels of endothelial microparticles (**b**) were studied by flow cytometry in 10 healthy controls and 14 CKD patients. Patients were dialyzed following the sequence: HF (basal), HDF (4 months), and HF-HD (4 months), and samples were obtained at the end of period. HF = High-flux hemodialysis; HDF = on-line hemodiafiltration.

CD16– monocytes or in CD14++/CD16+ monocytes (fig. 2). CD14+/CD16+ are activated cells that have phenotypical characteristics associated with monocyte activation, such as high expression of HLA-DR antigens or adhesion molecules, and contain high levels of preformed inflammatory cytokines in their cytoplasms [12, 13].

Downloaded by:
UCSF Library & CKM
169.230.243.252 - 9/15/2014 2:24:34 AM

Why Are Inflammatory CD14+/CD16+ Cells Produced?

In addition to CKD patients, an increased number of CD14+/CD16+ cells are present in the elderly and in patients with chronic inflammation. So the question arises why such cells are produced. Recent studies performed by our group have demonstrated that CD14+/CD16+ monocytes are a population that have differentiated from CD14++/CD16– monocytes [14]. CD14+/CD16+ monocytes are generated when monocytes are repeatedly stimulated and maintained in an environment rich in proinflammatory cytokines, and these differentiated CD14+/CD16+ monocytes exhibit characteristics of senescent cells (low telomeres and expression of senescence associated enzymes such as the β-galactosidase). These data have led us to postulate that CD14+/CD16+ monocytes are activated cells that in the presence of maintained elevated levels of inflammatory cytokines (albeit moderately elevated) do not undergo apoptosis, prolong their survival and become senescent cells with a modified phenotype and functional activity.

Role Played by CD14+/CD16+ Monocytes in Endothelial Damage

Unlike what occurs with CD14++/CD16+ monocytes, whose activity is related to cardiovascular disease, inflammatory CD14+/CD16+ monocytes appear to be involved in the endothelial damage which is usually presented by elderly people and CKD or other chronic inflamed patients [15, 16]. In vitro the inflammatory response promoted by CD14+/CD16+ monocytes induced increased oxidative stress and apoptosis in endothelial cells [13]. In CKD patients, CD14+/CD16+ correlated with endothelial damage markers such as the plasma levels of endothelial microparticles or the number of reparative endothelial progenitor cells [16–18]. Additionally, the CD14+/CD16+ inflammatory monocytes exhibit high expression of adhesion molecules or chemokines such as CX3CR1, CCRS and CCR7, which facilitate the interaction between these inflammatory monocytes and the endothelium to generate a local inflammatory response that induces increased oxidative stress and apoptosis of endothelial cells [14, 19].

CD14+/CD16+ Inflammatory Monocytes in CKD Patients

As indicated above, several studies have reported that hemodialysis patients presented a higher percentage of proinflammatory CD14+/CD16+ monocytes than the healthy subjects. However there is little information about the percentage of these cells in other CKD populations such are CKD4–5 patients or CKD patients dialyzed with other techniques such as peritoneal dialysis (PD). In a recent study [19] we observed that, compared with healthy subjects, CKD4–5

Downloaded by:
UCSF Library & CKM
169.230.243.252 - 9/15/2014 2:24:34 AM

patients have an increased percentage of CD14+/CD16+ monocytes (3.9 ± 1.1 in controls versus 7.7 ± 1.2% in CKD). It is important to highlight that the percentage of these cells in patients who initiate hemodialysis increased to 18.9 ± 3.5%. Interestingly, the percentage of CD14+/CD16+ cells in PD patients (3.6 ± 1.3%) was similar to that observed in healthy subjects, and no significant differences were observed when PD patients were analyzed according to their renal residual function (2.2 ± 1.5% in PD-RRF >1 and 4.3 ± 2.1% in PD-RRF ≤1).

Cardioprotective Effect of On-Line Hemodiafiltration

These data suggest that at least partially the increase in CD14+/CD16+ cells observed in CKD patients may be associated with the hemodialysis treatment. In the course of the past few years, significant advances have been made in hemodialysis therapy, particularly, after the introduction of convective therapies, such as on-line HDF, and different studies have been performed in order to analyze whether the beneficial effect of on-line HDF might be at least partially mediated by modulating the inflammatory response associated to CD14+/CD16+ monocytes. These reports showed a reduction in the percentage of CD14+/CD16+ monocytes in patients treated with on-line HDF [20–23]. Furthermore, we have reported that the reduction in CD14+/CD16+ monocytes observed in patients treated with on-line HDF is associated with a decrease in markers of endothelial damage [21], in a process that seems clearly dependent on the convective transport, and when these patients return to the high-flux techniques, the numbers of CD16 + CD14 + rise [20, 21]. Therefore, the beneficial effect of the on-line HDF seems to be related with the convective transport, and not with the biocompatibility of the dialysis membrane [16, 21].

Despite the improvement in microinflammation and endothelial damage observed in these patients, the levels of CD14+/CD16+ cells in these patients do not return to the values showed by healthy subjects. Recent studies, such as the one presented by Hung et al. [24] using anakinra, showed that these therapeutics drugs may modulate the inflammatory activity mediated by monocytes in CKD patients. Furthermore, we have preliminary data suggesting that therapeutic agents such as the angiotensin II receptor antagonists may prevent at least in vitro the differentiation of CD14+/CD16+ cells. Whether the combination of high convective transport with these drugs may eventually normalize CD14+/CD16+ cells in CKD patients constitutes a matter which remains unresolved.

Acknowledgments

This work was supported by grants from Fondo de Investigación Sanitaria, Instituto de Salud Carlos III (FIS PI10/00960, PI08/1038, RETICs Red Renal RD06/0016/0007),

Downloaded by:
UCSF Library & CKM
169.230.243.252 - 9/15/2014 2:24:34 AM

Junta de Andalucía (JA0227/2009, JA0797-2010, P08-CTS-3797, P010-CTS-6337), and Fundación Nefrológica.

References

1 Foley RN, Parfrey PS, Sarnak MJ: Epidemiology of cardiovascular disease in chronic renal disease. J Am Soc Nephrol 1998;9:S16–S23.

2 Tacke F, Randolph GJ: Migratory fate and differentiation of blood monocyte subsets. Immunobiology 2006;211:609–618.

3 Maskrey BH, Megson IL, Whitfield PD, Rossi AG: Mechanisms of resolution of inflammation: a focus on cardiovascular disease. Arterioscler Thromb Vasc Biol 2011;31:1001–1006.

4 Meuwese CL, Stenvinkel P, Dekker FW, Carrero JJ: Monitoring of inflammation in patients on dialysis: forewarned is forearmed. Nat Rev Nephrol 2011;7:166–176.

5 Carracedo J, Ramirez R, Pintado O, Gomez-Villamandos JC, Martin-Malo A, Rodríguez M, Aljama P: Cell aggregation and apoptosis induced by hemodialysis membranes. J Am Soc Nephrol 1995;6:1586–1591.

6 Carracedo J, Ramírez R, Martin-Malo A, Rodríguez M, Aljama P: Nonbiocompatible hemodialysis membranes induce apoptosis in mononuclear cells: the role of G-proteins. J Am Soc Nephrol 1998;9:46–53.

7 Martín-Malo A, Carracedo J, Ramírez R, Rodriguez-Benot A, Soriano S, Rodriguez M, Aljama P: Effect of uremia and dialysis modality on mononuclear cell apoptosis. J Am Soc Nephrol 2000;11:936–942.

8 Nockher WA, Scherberich JE: Expanded CD14+/CD16+ monocyte subpopulation in patients with acute and chronic infections undergoing hemodialysis. Infect Immun 1998;66:2787–2790.

9 Scherberich JE, Nockher WA: Blood monocyte phenotypes and soluble endotoxin receptor CD14 in systemic inflammatory diseases and patients with chronic renal failure. Nephrol Dial Transplant 2000;15:574–578.

10 Carracedo J, Ramírez R, Martin-Malo A, Rodríguez M, Aljama P: The effect of LPS, uraemia, and haemodialysis membrane exposure on CD14 expression in mononuclear cells and its relation to apoptosis. Nephrol Dial Transplant 2002;17:428–434.

11 Rogacev KS, Seiler S, Zawada AM, Reichart B, Herath E, Roth D, Ulrich C, Fliser D, Heine GH: CD14++CD16+ monocytes and cardiovascular outcome in patients with chronic kidney disease. Eur Heart J 2011;32:84–92.

12 Ramirez R, Carracedo J, Berdud I, Carretero D, Merino A, Rodríguez M, Tetta C, Martín-Malo A, Aljama P: Microinflammation in hemodialysis is related to a preactivated subset of monocytes. Hemodial Int 2006;10(suppl 1):S24–S27.

13 Ziegler-Heitbrock L: The CD14+ CD16+ blood monocytes: their role in infection and inflammation. J Leukoc Biol 2007;81:584–592.

14 Merino A, Buendia P, Martin-Malo A, Aljama P, Ramirez R, Carracedo J: Senescent CD14+CD16+ monocytes exhibit proinflammatory and proatherosclerotic activity. J Immunol 2011;186:1809–1815.

15 Li G, Kim YJ, Mantel C, Broxmeyer HE: P-selectin enhances generation of CD14+CD16+ dendritic-like cells and inhibits macrophage maturation from human peripheral blood monocytes. J Immunol 2003;171:669–677.

16 Ramirez R, Carracedo J, Merino A, Nogueras S, Alvarez-Lara MA, Rodríguez M, Martin-Malo A, Tetta C, Aljama P: Microinflammation induces endothelial damage in hemodialysis patients: the role of convective transport. Kidney Int 2007;72:108–113.

Downloaded by:
UCSF Library & CKM
169.230.243.252 - 9/15/2014 2:24:34 AM

17 Merino A, Nogueras S, García-Maceira T, Rodríguez M, Martin-Malo A, Ramirez R, Carracedo J, Aljama P: Bacterial DNA and endothelial damage in haemodialysis patients. Nephrol Dial Transplant 2008;23:3635–3642.
18 Merino A, Nogueras S, Buendía P, Ojeda R, Carracedo J, Ramirez-Chamond R, Martin-Malo A, Aljama P: Microinflammation and endothelial damage in hemodialysis. Contrib Nephrol. Basel, Karger, 2008, vol 161, pp 83–88.
19 Merino A, Portolés J, Selgas R, Ojeda R, Buendia P, Ocaña J, Bajo MA, del Peso G, Carracedo J, Ramírez R, Martín-Malo A, Aljama P: Effect of different dialysis modalities on microinflammatory status and endothelial damage. Clin J Am Soc Nephrol 2010;5:227–234.
20 Ramírez R, Carracedo J, Merino A, Soriano S, Ojeda R, Alvarez-Lara MA, Martín-Malo A, Aljama P: CD14+CD16+ monocytes from chronic kidney disease patients exhibit increased adhesion ability to endothelial cells. Contrib Nephrol. Basel, Karger, 2011, vol 171, pp 57–61.
21 Carracedo J, Merino A, Nogueras S, Carretero D, Berdud I, Ramírez R, Tetta C, Rodríguez M, Martín-Malo A, Aljama P: On-line hemodiafiltration reduces the proinflammatory CD14+CD16+ monocyte-derived dendritic cells: a prospective, crossover study. J Am Soc Nephrol 2006;17:2315–2321.
22 Rogacev KS, Seiler S, Zawada AM, Reichart B, Herath E, Roth D, Ulrich C, Fliser D, Heine GH: CD14++CD16+ monocytes and cardiovascular outcome in patients with chronic kidney disease. Eur Heart J 2011;32:84–92.
23 Kim HW, Yang HN, Kim MG, Choi HM, Jo SK, Cho WY, Kim HK: Microinflammation in hemodialysis patients is associated with increased CD14CD16+ pro-inflammatory monocytes: possible modification by on-line hemodiafiltration. Blood Purif 2011;31:281–288.
24 Hung AM, Ellis CD, Shintani A, Booker C, Ikizler TA: IL-1β receptor antagonist reduces inflammation in hemodialysis patients. J Am Soc Nephrol 2011;22:437–442.

Pedro Aljama
Nephrology Department, Hospital Universitario Reina Sofía
Avda Menendez Pidal S/N, Córdoba 14004 (Spain)
Tel. +34 011 34 957 010143
E-Mail pedro.aljama.sspa@juntadeandalucia.es

Downloaded by:
UCSF Library & CKM
169.230.243.252 - 9/15/2014 2:24:34 AM