*Kidney International, Vol. 61, Supplement 80 (2002), pp. S89–S93*



# Cell apoptosis and hemodialysis-induced inflammation

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**Cell apoptosis and hemodialysis-induced inflammation.** Hemodialysis patients exhibit a defective immune response leading to an increased susceptibility of infections and neoplasms. Far from being helpful, dialytic therapy per se also may be responsible for this acquired immunodeficiency. Dialysis membranes and bacterial products present in dialysis water may trigger and even perpetuate an abnormal mononuclear cell activation. Upon contact with cellulosic dialysis membranes, monocytes display an increased expression of surface markers of cell activation, such as adhesion molecules CD18, CD49, CD54 and the lipopolysaccharide (LPS) ligand (CD14). Moreover, proinflammatory cytokines as IL-1β and TNF-α are released both in vivo and in vitro when monocytes are exposed to cellulosic membranes. Of special interest is the fact that end-stage renal disease patients undergoing hemodialysis exhibit an increased mononuclear cell apoptosis. This apoptosis is directly related to the degree of biocompatibility of the dialysis membrane. Apoptosis is activated when monocytes enter in contact with the cellulosic dialysis membrane through cell surface receptors linked to G-proteins. In early steps of apoptosis signaling, pertussis toxin-sensitive G proteins are coupled to protein kinase C (PKC)-dependent phosphorylative mechanisms. Furthermore, recent evidence support that the execution phase of apoptosis is mediated by a caspase-3 dependent pathway. Finally, very recent available data support that monocytes subjected to repeated activation suffer a process of accelerated senescence, as demonstrated by the senescent phenotype (CD14 and CD32) expressed and their shortened telomeric length. This senescent profile may generate a defective cellular response in acute stress situations, explaining (at least in part) the altered immune response observed in hemodialysis patients.

Patients with ESRD show a deterioration in their immune response that is reflected in a greater susceptibility to bacterial and viral infections, diseases of the autoimmune system, and neoplasms [1–4]. The immunologic alteration associated with ESRD is not a common immunodeficiency. Paradoxically, it can be observed that not only does the immune response of sufferers from ESRD fail to improve with hemodialysis treatment but, in the majority of cases, such treatment produces an activation of immunocompetent cells that is more obvious in mononuclear cells and may intensify the immunodeficiency response of such patients [5–10]. In this connection, for the past 15 years, our group has been studying the immunomodulatory effects induced by hemodialysis.

**Key words:** mononuclear cells, dialysis membrane, biocompatibility, apoptosis.



## CELLULOSE MEMBRANES INDUCE ACTIVATION OF MONONUCLEAR CELLS

Since our initial studies, we have demonstrated how the degree to which the immune response is affected is not only associated with hemodialysis per se, but also depends on the dialysis membrane used [1]. Our first results suggested that the mononuclear cell recognized the hemodialysis membrane as a foreign element and thus, in accordance with the immunologic concept, activated itself to produce a specific cellular response. In order to confirm this hypothesis, we placed mononuclear cells in contact with different hemodialysis membranes and observed how the interaction with cellulose membranes produced an increase in the degree of phosphorylation of cellular proteins [11], a result that was not observed with noncellulose membranes. Following the induction of phosphorylation, the interaction of the cells with the cellulose membranes induced an increase in the expression of adhesion molecules such as CD18, CD49, and CD54; an increase in the expression of activation markers such as the LPS (CD14) receptor; or the formation of cellular aggregates [11–13]. Similarly, the effect induced by the cellulose membrane was dependent on the interaction between the cellular membrane and the dialysis membrane [14]. The incubation of the dialysis membrane with albumin or cellular adhesion proteins prevented such activation, indicating that the potential bioincompatible structures were capable of becoming saturated, and confirming the results obtained by other groups [15]. In addition to these results, various groups have demonstrated how the activation induced by hemodialysis is related to the presence of various complement factors [16–20] or by the presence of bacterial endotoxins [16, 17], thus amplifying its effects on the process of cellular activation.

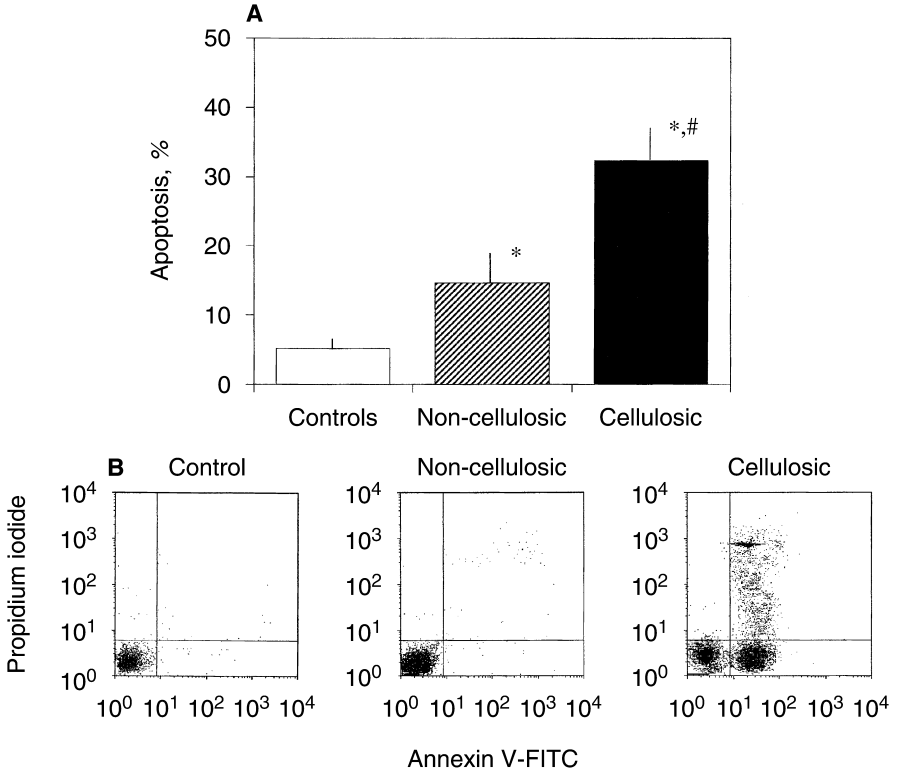


**Fig. 1. Peripheral blood mononuclear cells from patients dialysed with cellulosic membranes show an increased apoptosis after 48 hours of culture in vitro.** (*A*) Mean apoptosis rate in cells cultured during 48 hours in complete medium. The percent of apoptosis increased significantly in monocytes from patients with cellulosic membranes (* $P < 0.001$ vs. controls, # $P < 0.05$ vs. non-cellulosic). (*B*) Determination of apoptosis by flow cytometry (annexin-V/propidium iodide method). Cells were regarded as apoptotic when they were marked by annexin-V-FITC (X-axis) and excluded propidium iodide (Y-axis).

## PROINFLAMMATORY ACTIVITY INDUCED BY CELLULOSE MEMBRANES

The cellular activation induced by hemodialysis membranes has certain functional consequences. Mononuclear cell activation is characterized as being accompanied by cytokine secretions. After 24 hours, cells cultured with cellulose membranes liberate proinflammatory cytokines such as IL-1β and TNF-α into the cell culture medium. These data confirm the results produced by other groups [5–10], which have demonstrated rises in the levels of these cytokines in the serum of patients undergoing hemodialysis.

## CELLS THAT HAVE BEEN IN CONTACT WITH CELLULOSE MEMBRANES ARE MORE LIKELY TO UNDERGO SPONTANEOUS APOPTOSIS

Cellular apoptosis regulates the homeostasis of immunocompetent cells. The cells that interacted with cellulose membranes were more liable to die by apoptosis [13]. It has also been demonstrated that patients with renal insufficiency who are undergoing hemodialysis are more likely to display spontaneous apoptosis [21, 22]. Our experiments have shown that the degree of spontaneous apoptosis is related to the degree of biocompatibility of the hemodialysis membrane (Fig. 1).

## INTRACELLULAR SIGNALS MODULATED BY CONTACT WITH CELLULOSE MEMBRANES

Following interaction with bioincompatible structures, the surface molecules of mononuclear cells transduce the activation signals to the interior of the cell. The biochemical route depending on the PKC activity is probably the principal cellular mediator implicated in the activation and differentiation of immunocompetent cells. Both cellular activation and apoptosis induced by cellulose membranes increase as a result of co-stimulation of PKC-inductive agents, and they are inhibited when this biochemical route is blocked [13]. Furthermore, there exists a family of intracellular proteins (G proteins) that regulate many of the biochemical signals transmitted to the cell interior via the surface molecules [23–25]. In order to determine whether the G proteins are implicated in the transmission of signals by cellulose membranes, we utilized various bacterial toxins with modulatory activity on G proteins. Pertussis toxin (PTX), which is capable of inhibiting signals that depend on G proteins, inhibited apoptosis induced by cellulose membranes, confirming the hypothesis that mononuclear cells recognize and interact with the bioincompatible structures of cellulose membranes [11].

The execution phase of apoptosis is mediated by a family of proteases with cysteine residues, known as Caspases. The activity that depends on caspase-3 plays a fundamental role in the spontaneous apoptosis of mono-

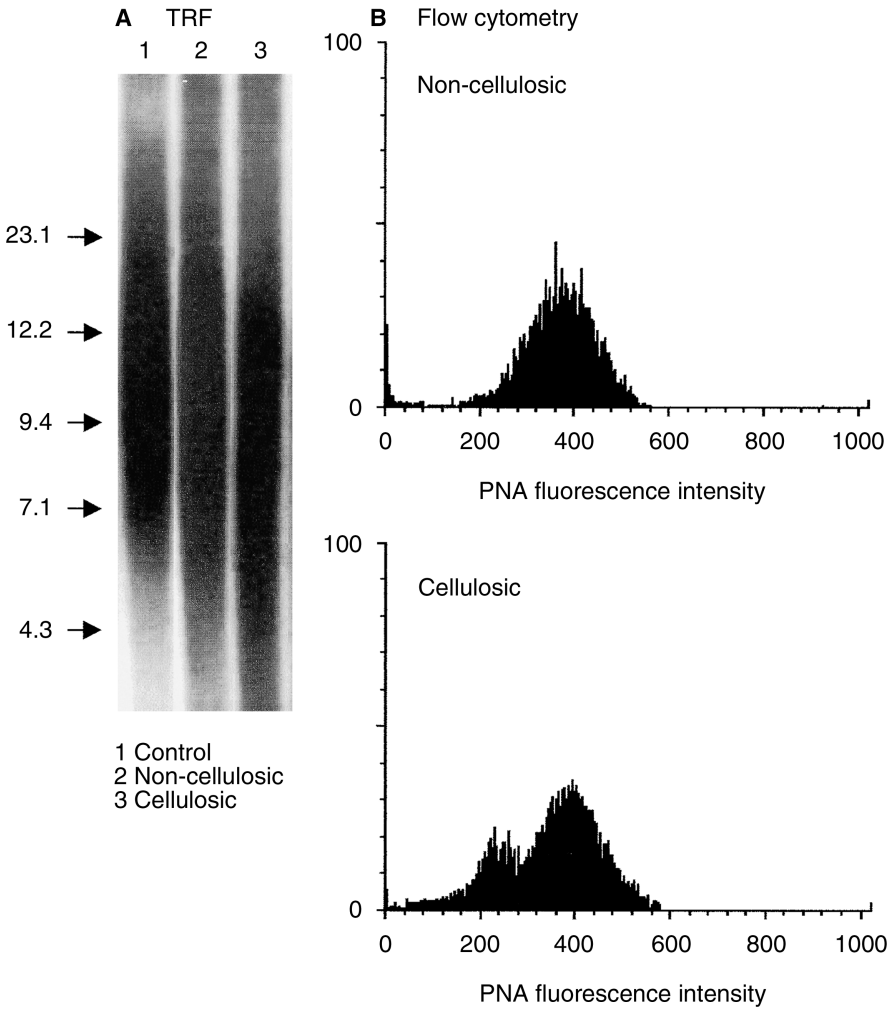


**Fig. 2. Measurement of telomere length in peripheral blood mononuclear cells from patients dialyzed with non-cellulosic or cellulosic membranes.** Measurement of telomere length was performed by Terminal Restriction Fragment (TRF) assay (*A*) or flow cytometry using a PNA telomeric specific probe (*B*). A) Cells from patients dialyzed with cellulosic membranes have a shorted telomere as compared with cells from controls or patients undergoing hemodialysis therapy with non-cellulosic membranes. B) Telomere length in mononuclear cells from two patients dialyzed with non-cellulosic and cellulosic membranes. After 48 hours of cell culture, a subpopulation with shortened telomeres can be observed in the cellulosic-membrane-treated patient.

cytes in healthy subjects [26]. The cellulose membranes induce caspase-3 activity in mononuclear cells, and this activity mediates spontaneous apoptosis in cellulose membranes (abstract; Carracedo et al, *J Am Soc Nephrol* 11: 259A, 2000.

## IS APOPTOSIS INDUCED BY CELLULOSE MEMBRANES AN IRREVERSIBLE PROCESS?

The results obtained demonstrated that mononuclear cells received a death signal after interacting with the cellulose membrane. However, these data do not permit us to confirm whether the spontaneous apoptosis observed in such cells is a consequence of a specific signal or the activation of physiologic apoptosis. In order to explore these two possibilities, we exploited an inherent characteristic of the mononuclear cell, the fact that such cells live longer when they are stimulated by proinflammatory cytokines or bacterial proteins such as LPS [27, 28]. Both the spontaneous apoptosis observed in the cells of patients dialyzed using cellulose membranes and that induced in vitro in cells from healthy subjects after contact with these membranes were prevented when the cells were stimulated with IL-1 or LPS, contradicting the hypothesis of a specific death signal.

Recent studies have demonstrated that somatic cells subjected to repeated activation suffer a process of accelerated senescence [29]. The results described using other models were compatible with the hypothesis that the mononuclear cells were suffering a process of repeated activation that induced the production of elevated levels of proinflammatory cytokines, which in turn had the effect of prolonging the survival of these cells in peripheral blood [28]. This hypothesis suggests that the cells of these patients were elderly cells and thus more susceptible to death by apoptosis when they were isolated from the proinflammatory environment in which they had existed. We are currently working on the studies needed to confirm this hypothesis, although we have already shown that mononuclear cells from patients dialyzed with cellulose membranes display an increase in their

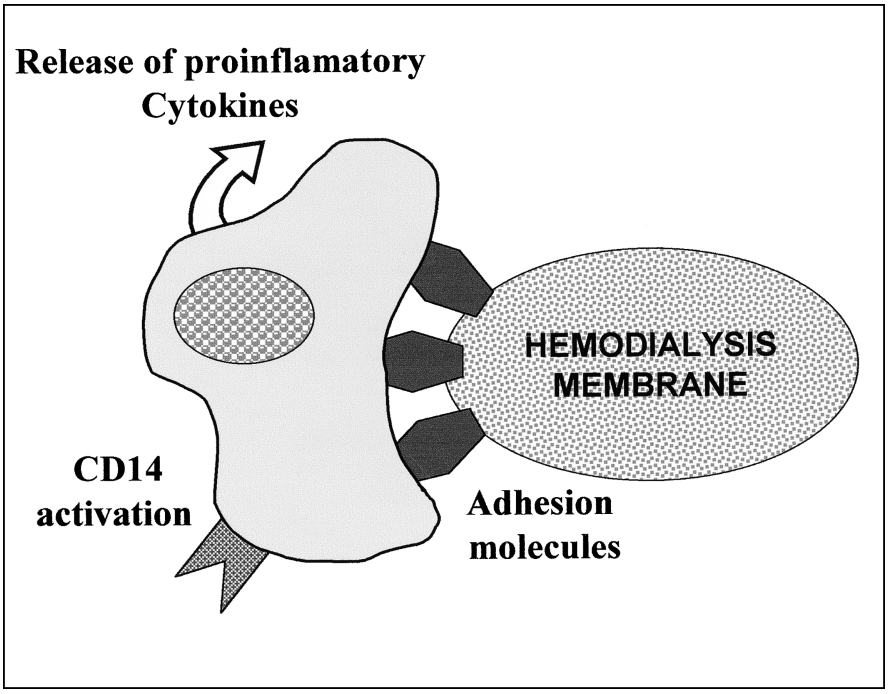


**Fig. 3. Mononuclear peripheral blood cells undergo a process of cyclic activation as a consequence of their interaction with hemodialysis membrane, producing proinflammatory cytokines, an increased expression of adhesion molecules such as CD18, CD49, and CD54 and activation molecules such as the LPS-receptor (CD14).**

expression of CD14 and CD32 molcules (a phenotype characteristic of senescent monocytes). This phenotype is accompanied by a shortened telomere such as defines cellular senescence (Fig. 2) [28, 30].

## CONCLUSIONS

It seems clear that as a consequence of interaction with the hemodialysis membrane, mononuclear cells suffer a process of cyclic activation. Furthermore, other factors such as fractions of the complement or contamination by endotoxins may amplify this process. As far as the immune system is concerned, the final result is an aggression in the face of which an inflammatory response is induced. As a consequence of this, the activated mononuclear cells produce proinflammatory cytokines, which act on their own cells in an autocrine manner, prolonging their survival in peripheral blood, although they are more likely to die by apoptosis when they are isolated from the inflammatory medium (Fig. 3). These "senescent" cells end up altering their production of cytokines and generate a modified response in the face of acute stress situations, which may explain the alterations in immune response of the patient in dialysis.

## ACKNOWLEDGMENTS

This study has been financed by the Fondo de Investigación Sanitaria (FIS 98/0423, 00/0788, 00/0701), the Junta de Andalucía (213/00 and 72/00), the Sociedad Española de Nefrología, and the Fundación Renal of Cordoba.

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## REFERENCES

1. Aljama P, Martin-Malo A, Garin JM, *et al*: Granulocyte adherence changes: An index of biocompatibility. *Kidney Int* 33:S68–S72, 1988
2. Cheung AK, Parker CJ, Janatova J, Brynda E: Modulation of complement activation on hemodialysis membranes by immobilized heparin. *J Am Soc Nephrol* 2:1328–1337, 1992
3. Hakim RM: Clinical implications of hemodialysis membrane biocompatibility. *Kidney Int* 44:484–494, 1993
4. Martin-Malo A, Velasco F, Rojas R, Torres A, *et al*: Fibrinolytic activity during hemodialysis: A biocompatibility-related phenomenon. *Kidney Int* 41(Suppl):S213–S216, 1993
5. Lonnemann G, Haubitz M, Schindler R: Hemodialysis-associated induction of cytokines. *Blood Purif* 8:214–222, 1990
6. Haeffner-Cavaillon N, Jahns G, Poignet JL, Kazatchkine MD: Induction of interleukin-1 during hemodialysis. *Kidney Int* 43(Suppl):S139–S143, 1993
7. Dinarello CA: Cytokines: Agents provocateurs in hemodialysis? *Kidney Int* 41:683–694, 1992
8. Herbelin A, Nguyen AT, Zingraff J, *et al*: Influence of uremia and hemodialysis on circulating interleukin-1 and tumor necrosis factor α. *Kidney Int* 37:116–125, 1990
9. Lonnemann G, Bingel M, Floege J, *et al*: Detection of endotoxin like interleukin-1-inducing activity during in vitro dialysis. *Kidney Int* 33:29–35, 1988
10. Laude-Sharp M, Caroff M, Simard L, *et al*: Induction of IL-1 during hemodialysis: Transmembrane passage of intact endotoxins (LPS). *Kidney Int* 38:1089–1094, 1990
11. Carracedo J, Ramirez R, Martín-Malo A, *et al*: Apoptosis induced in monocytes by hemodialysis membrane is inhibited by pertussis toxin. *J Am Soc Nephrol* 9:46–53, 1998
12. Tielemans CL, Delville JPC, Husson CP, *et al*: Adhesion molecules and leukocyte common antigens on monocytes and granulocytes during hemodialysis. *Clin Nephrol* 39:158–165, 1993
13. Carracedo J, Ramirez R, Pintado O, *et al*: Cell aggregation and apoptosis induced by hemodialysis membranes. *J Am Soc Nephrol* 6:1586–1591, 1995
14. Carracedo J, Ramirez R, Martin-Malo A, Aljama P: The role of adhesion molecules in mononuclear cell apoptosis induced by cuprophan hemodialysis membrane. *Nephron* 89:186–193, 2001

15. Vallar L, Rivat C: Regenerated cellulose-based hemodialyzers with immobilized proteins as potential devices for extracorporeal immunoadsorption procedures: An assessment of protein coupling capacity and in vitro dialysis performances. *Artif Organs* 36:257–265, 1989
16. Craddock PR, Fehr J, Dalmaso AP, *et al*: Hemodialysis leukopenia: Pulmonary vascular leukostasis resulting from complement activation by dialyzer cellophane membranes. *J Clin Invest* 59:879–888, 1977
17. Cheung AK, Henderson LW: Effects of complement activation by dialysis membranes. *Am J Nephrol* 6:81–91, 1986
18. Cheung AK, Parker CJ, Wilcox LA, Janatoba J: Activation of complement by hemodialysis membranes: Polyacrylonitrile binds more C3a than cuprophan. *Kidney Int* 37:1055–1059, 1990
19. Bingel M, Lonnemann G, Shaldon S, *et al*: Human interleukin-1 production during hemodialysis. *Nephron* 43:161–163, 1986
20. Shindler R, Lonnemann G, Shaldon S, *et al*: Transcription, not synthesis of interleukin-1 and tumor necrosis factor by complement. *Kidney Int* 37:85–93, 1990
21. Heidenreich S, Schmidt M, Bachmann J, Harrach B: Apoptosis of monocytes cultured from long-term hemodialysis patients. *Kidney Int* 49:792–799, 1996
22. Martín-Malo A, Carracedo J, Ramírez R, *et al*: Effect of uremia and dialysis modality on mononuclear cell apoptosis. *J Am Soc Nephrol* 11:936–942, 2000
23. Freissmuth M, Casey PJ, Gilman, AG: G proteins control diverse pathways of transmembrane signaling. *FASEB J* 3:2125–2131, 1989
24. Ramirez R, Carracedo J, Zamzami N, *et al*: Pertussis toxin inhibits activation-induced cell death of human thymocytes, pre-B leukemia cells and monocytes. *J Exp Med* 180:1147–1152, 1994
25. Carracedo J, Ramirez R, Marchetti P, *et al*: Pertussis toxin-sensitive GTP-binding proteins regulate activation-induced apoptotic cell death of human natural killer cells. *Eur J Immunol* 25:3094–3099, 1995
26. Fahy RJ, Doseff AI, Wewers MD: Spontaneous human monocyte apoptosis utilizes a caspase-3-dependent pathway that is blocked by endotoxin and is independent of caspase-1. *J Immunol* 163:1755–1762, 1999
27. Heidenreich S, Schmidt M, August C, *et al*: Regulation of human monocyte apoptosis by the CD14 molecule. *J Immunol* 159:3178–3188, 1997
28. Heidenreich S: Monocyte CD14: A multifunctional receptor engaged in apoptosis from both sides. *J Leukoc Biol* 65:737–743, 1999
29. Pawelec G, Effros RB, Caruso C, *et al*: T cells and aging (update February 1999). *Front Biosci* 13:69–77, 1997
30. Mangan DF, Wahl SM: Differential regulation of human monocyte programmed cell death (apoptosis) by chemotactic factors and pro-inflammatory cytokines. *J Immunol* 147:3408–3412, 1991