Biocompatibility of hemodialysis (HD) membranes: The role of endocellular mediators in HD-associated neutropenia

C. Tetta, G. Segoloni, G. Camussi, S. Piva, A. Vercellone
Laboratorio di Immunopatologia, Cattedra di Nefrologia, Divisione di Nefrologia e Dialisi, Ospedale Maggiore S. G. Battista - Molinette, Torino, Italy.

In its early years, hemodialysis (HD) posed the difficult task of overcoming numerous, often life-threatening complications. The development of advanced technologies has turned HD into a safe mode of treatment. As a result, a great effort is nowadays being made in order to optimize it. In this light, the search for an ever higher degree of biocompatibility of artificial membranes has become a leading issue in contemporary HD.

From the biological point of view, biocompatibility of a biomaterial is the result of complex interactions at the interface involving circulating cells and plasma components [1]: it requests the absence of thrombogenic, toxic-allergic-inflammatory properties, no effect on circulating cells, plasma proteins and on the immune system.

Among the earliest events occurring in HD with cuprophan membranes, is the acute granulocytopenia which was first described by Kaplow and Goffinet [2] as a marked, transient drop of circulating leucocytes occurring within 2-15 min after the start of HD. These authors made the essential observations that this event is due to the contact of blood with the dialyser membrane and related it to the sequestration of neutrophils in the pulmonary capillary network rather than in the dialyser [2].

For many years, however, the pathogenesis of HD-associated neutropenia remained obscure. In 1977, Craddock et al. [3] first showed that infusion of autologous plasma incubated with dialyser membranes of the cuprophan type in the experimental animal reproduced the acute granulocytopenia occurring in HD and implicated the activation of the complement system in the pathogenesis of the intravascular alterations and pulmonary dysfunction. Further *in vitro* studies implicated C5a, a potent anaphylatoxin [4-6] generated following contact of plasma with cuprophan membranes, as the mediator of the neutrophil aggregating activity [7]. C5a was later shown by Chenoweth et al. [8] to interact with specific receptors present on human neutrophils, which rapidly internalize and degrade this ligand.

A number of studies have dealt with the basic mechanisms of complement activation and the biological properties of the cascade products (as reviewed in ref. 9). Cuprophan membranes activate the complement system via the alternate pathway according to a sequential multistep process (as reviewed in ref. 10): 1) binding of metastable C3b to activating surfaces (i.e. those which restrict binding of regulatory proteins such as Factor H and I); 2) the formation of properdin-stabilized C3bBb complex following binding of Factor B to C3b (C3 convertase); 3) further binding of C3b to C3 convertase generates C5 convertase, capable of releasing C5a.

Aside from their complement activating properties, membranes may also differ as to their ability to absorb large amounts of C3a and C5a, there by removing circulating anaphylatoxins from the blood, and neutralizing their biological potential [11, 12]. C5a rather than C3a is active on neutrophils leading to aggregation *in vitro* [7, 13], to leukoembolization and enhanced adherence to pulmonary endothelium *in vivo* [13-15]. However, in plasma C5a is rapidly desarginated to C5a desArg by a carboxypeptidase B [16] which loses chemotactic activity but acquires a more potent neutrophil aggregating effect than its native molecule [13]. The recent availability of the radioimmunoassays [17] specific for C3a desArg and C5a desArg have further confirmed that complement activation does occur in clinical HD and allowed to monitor the production of anaphylatoxin by different types of membranes [18].

Aside from the activation of complement, a number of studies have implicated other mechanisms in the pathogenesis of HD-associated neutropenia and the related respiratory dysfunction. Although the severity of neutropenia related to the degree of complement activation, the latter occurs in the absence of any detectable drop in circulating neutrophils [19, 20]. Woodward et al. showed that complement inhibition does not prevent neutropenia induced by reinfusion of showed that neutropenia occurs following transfusion of products from disintegrated leukocytes [22].

Lipid mediators are now recognized in the

Correspondencia: C. Tetta, MD.
Laboratorio di Immunopatologia.
Cattedra di Nefrologia, Università di Torino.
Divisione di Nefrologia e Dialisi.
Corso Polonia 14, 10126 Torino. Italia.

pathogenesis of pulmonary hypertension developed in the swine injected with cuprophane activated plasma [23] or of the hypoxia and pulmonary hypertension in sheep infused with zymosan activated plasma [24].

In the present review, we will describe studies performed in this laboratory and elsewhere showing: neutrophil aggregation *in vitro* after challenge with soluble immune complexes (IC), inflammatory constituents released from neutrophils were found to be potent aggregating substances of these cells *in vitro* and caused a marked transient neutropenia, when injected systemically in the rabbit.

In the present review, we will describe studies performed in this laboratory and elsewhere showing 1) the role of endocellular mediators (namely neutrophil - derived cationic proteins, NCP, and platelet - activating factor, PAF) in the aggregation of immunologically and non-immunologically stimulated neutrophils; 2) the relevance of these mediators in the pathogenesis of experimental and clinical HD.

Mediators of neutrophil aggregation: Role of endocellular mediators

Soluble immune complexes (IC) aggregate neutrophils in the absence of plasma. Cuprophan membranes were first shown by Camussi et al. to be a potent non-immunological stimulus for neutrophils [25]. Short-term (10 min, at 37° C) exposure of neutrophils to cuprophan membranes triggered the generation of potent neutrophil and platelet aggregating activities in the supernatants [25]. The neutrophil aggregating activity was purified by sequential ion-exchange and gel permeation chromatography in a group of neutrophil-derived proteins highly cationic in nature (isoelectric point > 8.5) (NCP) with a molecular weight ranging from 21,000 to 29,000 D. First described by Ranadive and Cochrane [26], NCP were later purified and characterized by Olsson and Venge [27] from leukemic leukocytes. NCP display several biological activities as they increase vascular [28] and glomerular [29] permeability, activate the complement cascade with generation of anaphylatoxin activity in serum [30], have a bactericidal capacity [31], aggregate neutrophils *in vitro*, cause neutropenia *in vivo* [13, 28]. Furthermore, NCP are actively released from immunologically stimulated neutrophils [13, 28].

Using inhibitors with selective effects on cell metabolism, C5a, C5a desArg, NCP, NCP desArg appeared to act on neutrophils via similar metabolic steps that were independent from cyclooxygenase-derived metabolites, but partially dependent on lipoxygenase-derived products [13]. Studies using a specific antiserum to NCP, showed that C5a-triggered neutrophil aggregation was to a large extent dependent upon the release of NCP, as the antiserum markedly inhibited the aggregation [32]. NCP can be regarded as an amplifying mechanisms of C5a triggered aggregation [33]. Both NCP and C5a appear to interact with the same receptors on the neutrophil membrane as shown in cross specific desensitization studies with NCP and C5a [32]. The transient nature of HD-associated neutropenia may be considered as due to down-regulation of receptors not only for C5a [34] but also for NCP. Purified NCP as well as C5a mimick the acute neutropenia observed after infusion of IC or cuprophan incubated plasma [13, 14].

Neutrophil granule proteins (e.g. lactoferrin [35]) other than NCP may also contribute to neutrophil aggregation both *in vitro* and *in vivo*. Raised serum concentration of lattoferrin and eosinophil cationic proteins during dialysis with cuprophan were observed by Hallgren et al. [36]. Very recently, using sensitive assays, Hoerl et al. have convincingly shown granulocyte activation and degranulation in HD patients despite the complete absence of circulating C3a and C5a antigens [37]. The platelet-aggregating activity, recovered in the supernatants from plasma-free neutrophils incubated with cuprophan membranes [25] was identified in a lipid mediator, the platelet-activating factor (PAF) which has been later purified and characterized as a 1 - 0 - alkyl - 2 - acetyl - sn - glyceryl - 3 - phosphorylcholine [38]. PAF is now known to be one of the most potent inflammatory mediators capable of a wide spectrum of diverse biological activities (for review see ref. 39). Chemotactic stimuli induce the release of PAF [40, 41] and PAF *per se* promotes the aggregation of neutrophils and the release of their granule constituents [42, 43].

Finally, the metabolic steps involved in the aggregation of neutrophils challenged by C5a desArg and NCP are those known to be involved in PAF synthesis [43]. The aggregation induced by C5a desArg and NCP is quantitatively and temporally correlated with the amount and kinetics of PAF released in the supernatants [43]. These data suggest that PAF may be the final common mediator of neutrophil aggregation [43]. At the nadir of the neutropenia induced after infusion of C5a and NCP, PAF is released in the circulation [43]. The infusion of synthetic PAF produces a neutropenic response that closely mimicks that seen during HD and is associated with profound cardiovascular changes [43, 44].

Experimental model of HD

Dissection of the complex events of neutrophil activation in whole blood or in the presence of plasma is not feasible in *in vivo* conditions. For this reason, we developed an experimental model of HD with cuprophan dialyser without having the patient on the circuit under controlled dialysate temperature and composition [45].

A suspension of plasma-free human neutrophils was recirculated and, at different time intervals, supernatants were obtained and the release of NCP,

lysozyme, beta-glucoronidase, lactic dehydrogenase and PAF were evaluated. As early as 1 to 5 min after start of recirculation, neutrophils formed aggregates which could be shown to firmly adhere to the dialyser membranes. Concomitantly, NCP and PAF were released in the supernatants. These events were due to active processes dependent on Ca^{++} and Mg^{++} ions in the buffer. These data are in full agreement with those of Danielson et al. [46], who showed that, in a similar model of experimental dialysis using fresh, heparinized whole blood, neutrophil and eosinophil degranulation occur and are not dependent upon complement activation. However, the latter is for some reason blunted in experimental model of HD when recirculating recalcified banked human serum in the absence of cells [47]. As an explanation, apart from inadequate procedures in handling the serum, one may speculate that the lack of endocellular mediators released from activated cells may be at least in part responsible for the much slower rise in C3a and C5a levels than one normally sees in clinical HD. Preliminary evidence in our laboratory confirm that C3 split products are markedly increased in plasma added with autologous leucocytes or platelets in respect to fresh normal plasma after incubation with cuprophan membranes (Tetta C, Rotunno M, unpublished observations).

The direct neutrophil-membrane interaction: role of the electric charges

Among the various factors involved in the response to the surface of biopolimers, the general role of the electrical properties of HD membranes in contact with blood (charge transfer, other electrochemical phenomena) are extremely important [48]. Ionic groups or strong hydrogen bonding functionalities can trigger platelet activation [49]. Whether surface electric charges occur in the HD membranes currently in use and can somehow be held responsible for «bioincompatible» effects when coming into contact with blood, is a difficult matter to assess. Furthermore, as Merrill suggested in 1977 [50], surface mobility of polimer molecules may profoundly condition protein adsorption and cell interactions, so that the polymer surface must be considered highly dynamic when coming into contact with blood.

Polymethylmethacrylate (PMMA) is a biopolimer that is produced with methylmethacrylate (MMA). PMMA offers the unique opportunity to study negative or positive electric charges in respect to biological interactions depending on whether MMA is copolymerized with hydroxymethylmethacrylate or quaternized dimethyl aminoethyl methacrylate, respectively. Using PMMA of different net electric charge (anionic, neutral, cationic), we recently showed that only cationic PMMA membranes trigger neutrophil aggregation, release of PAF and depletion of the intracellular content of NCP and elastase [38]. These events were quantitatively similar to those observed in the presence of cuprophan membranes. No neutrophil aggregation nor release of PAF or lysosomal constituents were observed in the presence of anionic or neutral PMMA. Modification of the net electric charge, therefore, can turn PMMA, known as a «biocompatible» material, into a highly reactive material with plasma-free neutrophils.

Clinical HD

The role of the direct cell-membrane interaction and of the attendant release of endocellular mediators such as PAF and NCP is strongly suggested. The mechanisms involving complement and cell activation, however, are intimately interwoven so that they are mutually at work (fig. 1). The exact importance of PAF and NCP in clinical HD is under evaluation. In some patients undergoing HD with cuprophan membranes, a neutrophil aggregating activity was detectable in the plasmas coincidently with the maximal drop in circulating neutrophils [32]. This activity is totally antagonized by preincubation with an antiserum specific for NCP but only partially with an anti-C5 serum. The release of small amounts of PAF in the effluent (venous) line after the dialyser has been obtained in a limited number of patients. Failure to detect PAF in a higher number of patients and amounts may find various reasons. First, the release of PAF appears to be an early event and often occurs before connecting the venous line to the patient. As this phase of HD is usually done with blood pump at low speed, the time necessary for filling the dialyser with blood may be crucial. Furthermore, as PAF is bound to albumin, which is absorbed onto membranes, this mediator may be rapidly removed from the extracorporeal circulation. Preliminary experiments in this laboratory support this interpretation as PAF can be extracted from HD membranes. Small amounts of PAF released from neutrophils may remain undetectable in the venous line or degraded by the specific plasma PAF-acylhydrolase [51]. The inhibitory serum potential of PAF biologic activity is intact and even tends to increase in the course of HD [32].

Acknowledgements

Hoechst-Toray, Japan, kindly provided PMMA membranes of different net electric charges. The studies presented have been supported by Consiglio Nazionale delle Ricerche, Rome, Gruppo Nazionale: «Terapie delle grandi insufficienze d'organo». Area A: Rene, from 1980 to 1986.

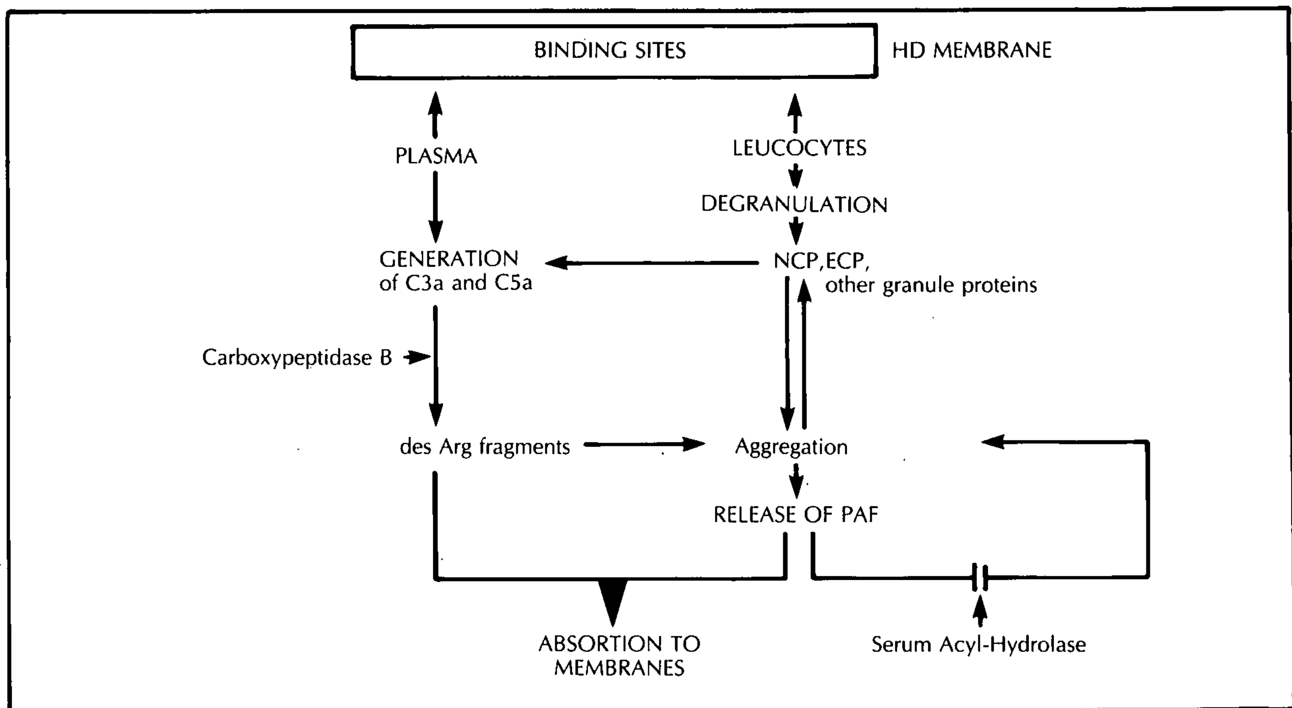

Fig. 1.—Schematic representation of the plasma and cellular mechanisms of neutrophil aggregation following interaction with cuprophan membranes. Underlined are the limiting factors of either complement or cellular activation induced by the contact with HD membranes. NCP: neutrophil - derived cationic proteins; ECP: eosinophil cationic proteins.

References

1. Klinkmann H, Wolff H and Schmitt L: Definition of biocompatibility. *Contr Nephrol* 37:104, 1984.
2. Kaplow LS and Goffinet JA: Profound neutropenia during the early phase of hemodialysis. *J Am Med Ass* 203:133, 1968.
3. Craddock PR, Fehr J, Brigham KL, Kronenberg RS and Jacob HS: Complement and leukocyte-mediated pulmonary dysfunction in hemodialysis. *New Engl J Med* 296:169, 1977.
4. Cochrane LG and Mueller-Eberhard HJ: The derivation of two distinct anaphylatoxin activities from the third and fifth components of human complement. *J Exp Med* 127:371, 1968.
5. Johnson AR, Hugli TE and Mueller-Eberhard HJ: Release of histamine from mast cells by the complement peptides C3a and C5a. *Immunology* 28:1067, 1975.
6. Fernández HN and Hugli TE: Primary structural analysis of the polypeptide portion of human C5a anaphylatoxin. *J Biol Chem* 253:6955, 1971.
7. Craddock PR, Hammerschmidt D, White JG, Dalmasso AP and Jacob HS: Complement (C5a)-induced granulocyte aggregation in vitro. *J Clin Invest* 60:260, 1977.
8. Chenoweth DE and Hugli TE: Demonstration of specific C5a receptor on intact human polymorphonuclear leukocytes. *Proc Nat Acad Sci USA* 75:3943, 1978.
9. Mueller-Eberhard HJ and Schreiber RD: Molecular biology and chemistry of the alternate pathway of complement. *Adv Immunol* 29:1, 1980.
10. Cheung AK and Henderson LW: Effects of complement activation by hemodialysis membranes. *Am J Nephrol* 6:81, 1986.
11. Amador A, Candi P, Sasdelli M, Massai G, Favilla S, Passalera A and Ricci M: Hemodialysis leukopenia and complement function with different dialysers. *Kidney Int* 24:775, 1983.
12. Cheung AK, Chenoweth DE, Otsuka D and Henderson LN: Compartimental distribution of complement activation products in artificial kidneys. *Kidney Int* 30:74, 1986.
13. Camussi G, Tetta C, Bussolino F, Caligaris-Cappio F, Coda R, Masera C and Segoloni G: Mediators of immune complex-induced aggregation of polymorphonuclear neutrophils. I. C5a anaphylatoxin, neutrophil cationic proteins and their cleavage fragments. *Int Archs Allergy Appl Immunol* 62:1, 1980.
14. Hammerschmidt DE, Harris PD, Wyland JH, Craddock PR and Jacob HS: Complement-induced granulocyte aggregation in vivo. *Am J Pathol* 102:146, 1981.
15. O'Flaherty, Craddock PR and Jacob HS: Effect of intravascular complement activation on granulocyte adhesiveness and distribution. *Blood* 51,4:731, 1978.
16. Bokisch VA and Mueller-Eberhard HY: Anaphylatoxin inactivator of human plasma: its isolation and characterization as a carboxypeptidase. *J Clin Invest* 49:2427-2436, 1970.
17. Hugli TE and Chenoweth DE: Biologically active peptides of complement: techniques and significance of C3a and C5a measurements. In Nakamura, Dito, Tucker, *Immunoassays: Clinical, Laboratory techniques for the 1980's,* NY, 1980.
18. Chenoweth DE, Cheung AK and Henderson LW: Anaphylatoxin formation during hemodialysis: effects of different dialyzer membranes. *Kidney Int* 24:764, 1983.
19. Aljama P, Bird PAE, Ward MK, Feest TG, Walker W, Tanbago H, Sussman M and Kerr DNS: Haemodialysis-induced leukopenia and activation of complement: effects of different membranes. *Proc Eur Dial Transplant Ass* 15:144, 1979.
20. Devinuesa SG, Resano M, Luño J, Gonzalez C, Baml G, Junco E and Valderrábano F: Leukopenia, hypoxia and complement activation in haemodialysis. Three unrelated phenomena. *Proc Eur Dial Transplant Ass* 19:159, 1982.
21. Woodward J and Brubaker LH: Production of neutropenia-overshoot cycle in sheep by reinfusion of cellophane exposed blood. *Clin Res* 21:56, 1973.
22. Weisberg AS, Heinle RW and Hannah RT: Transfusion of leukocytes and products of disintegrated leukocytes. *Proc Soc Exp Biol Med* 10:149, 1949.

23. Cheung AK and Baranowski RL: The role of thromboxane in pulmonary hypertension (PHTN) induced by cuprophan-activated plasma (CAP) (Abstract). *Am Soc Nephrol,* Washington, p. 60A, 1984.
24. Del Maschio A, Corvazier E and Maclay J: Opsonized zymosan-induced human neutrophil leukotriene synthesis and degranulation are potentiated by thrombin activated platelets. *6th Int Conf on Prostaglandins and Related compounds,* p. 144, 1985.
25. Camussi G, Segoloni GP, Rotunno M and Vercellone A: Mechanism involved in acute granulocytopenia in hemodialysis. Cell-membrane direct interactions. *Int J Artif Org* 1,3:123, 1978.
26. Ranadive NS and Cochrane CG: Isolation and characterization of permeability factors from rabbit neutrophils. *J Exp Med* 128:605, 1966.
27. Olsson I and Venge P: Cationic proteins of human granulocytes. I. Isolation of the cationic proteins from the granules of leukaemic myeloid cells. *Scand J Haematol* 9:204, 1972.
28. Camussi G, Mencía-Huerta JM and Benveniste J: Release of platelet-activating-factor and histamine. I. Effect of immune complexes, complement and neutrophils on human and rabbit mastocytes and basophils. *Immunology* 33:523, 1977.
29. Camussi G, Tetta C, Segoloni G, Coda R and Vercellone A: Localization of neutrophil cationic proteins and loss of anionic charges in glomeruli of patients with Systemic Lupus Erythematosus glomerulonephritis. *Clin Immunol Immunopathol* 24:299, 1982.
30. Venge P and Olsson I: Cationic proteins of human granulocytes. VI. Effects on the complement system and mediation of chemotactic activity. *J Immunol* 115:1505, 1975.
31. Odeberg H and Olsson I: Antibacterial activity of cationic proteins from human granulocytes. *J Clin Invest* 56:1118, 1975.
32. Camussi G, Pacitti A, Tetta C, Bellone G, Mangiarotti G, Canavese C, Segoloni G and Vercellone A: Mechanisms of neutropenia in hemodialysis (HD). *Trans Am Soc Artif Int Organs* XXX:364, 1984.
33. Camussi G, Caligaris-Cappio F and Tetta C: Mechanisms of neutrophil aggregation. *New Engl J Med* 283-286, 1980.
34. Skubitz KM and Craddock PR: Reversal of hemodialysis granulocytopenia and pulmonary leukostasis: a clinical manifestation of selective down-regulation of granulocyte responses to C5a desArg. *J Clin Invest* 67:1383, 1981.
35. Boxer LA, Bjorksten B, Bjork J, Yang HH, Allen JM and Baehner RL: Neutropenia induced by sistemic infusion of lactoferrin. *J Lab Clin Med* 99:866, 1982.
36. Hallgren R, Venge P and Wikstrom B: Hemodialysis induced increase in serum lactoferrin and serum eosinophil cationic proteins as signs of local neutrophil and eosinophil degranulation. *Nephron* 29:233, 1981.
37. Hoerl WH, Riegel W, Schollmeyer P, Rautenberg W and Neumann S: Different complement and granulocyte activation in patients dialysed with PMMA dialysers. *Clin Nephrol* 25:304, 1986.
38. Tetta C, Camussi G, Segoloni G and Vercellone A: Direct interaction between polymorphonuclear neutrophils (PMN) and dialysis membranes: role of the electric charges (Abstract). *Kidney Int* 29:609, 1986.
39. Camussi G: The potential role of Platelet-activating factor in renal physiopathology (Editorial). *Kidney Int* 29:1, 1986.
40. Lotner GZ, Lynch JM, Betz SJ and Henson PM: Human neutrophil - derived platelet - activating factor. *J Immunol* 124,2:676, 1980.
41. Camussi G, Aglietta M, Coda R, Bussolino F, Piacibello W and Tetta C: Release of platelet-activating factor (PAF) and histamine. II. The cellular origin of human PAF: monocytes, polymorphonuclear neutrophils and basophils. *Immunology* 42:191, 1981.
42. Shaw JO, Ferrigni S, McManus L and Hanahan DJ: Activation of human neutrophils with 1-0-hexadecyl-octadecyl-2-aceyl-sn-glyceryl-3-phosphorylcholine (platelet-activating factor). *J Immunol* 127:1250, 1981.
43. Camussi G, Tetta C, Bussolino F, Caligaris-Cappio F, Coda R, Masera C and Segoloni G: Mediators of immune complex-induced aggregation of polymorphonuclear neutrophils. II. Platelet activating factor, as the effector substance of immune-induced aggregation. *Int Archs Allergy Appl Immunol* 64:25, 1981.
44. Montrucchio G, Alloatti G, Mariano F, Tetta C, Emanuelli G and Camussi G: Cardiovascular alterations in the rabbit infused with platelet-activating-factor (PAF): effect of Kadsurenone, a PAF-receptor antagonist. *Inst J Tissue Reactions* (in press).
45. Tetta C, Jeantet A, Camussi G, Thea A, Gremo L, Martini PF, Ragni R and Vercellone A: Direct interaction between polymorphonuclear neutrophils in a plasma-free model of dialysis. *Proc Eur Dial Transpl Ass* 21:150, 1984.
46. Danielson B, Hallgren R and Venge P: Neutrophil and eosinophil degranulation by haemodialysis membranes. *Contr Nephrol* 37:83, 1984.
47. Henderson LW, Chenoweth DE, Shinaberger JH, Miller J and Konstantin P: Preliminary report on complement activating potential of polycarbonate membrane. *Blood Purification* 4:14, 1986.
48. Andrade JD: Blood-materials interactions-20 years of frustration. *Trans Am Soc Artif Int Organs* XXVII:659, 1981.
49. Lindon JN, Collins REC, Coe NP, Jacoda A, Brier-Russel D, Merrill EW and Salamon EW: In vivo assessment in sheep of thromboresistant materials by determination of platelet survival. *Circ Res* 46:84, 1980.
50. Merrill EW: Properties of materials affecting the behaviour of blood at their surfaces. *Ann NY Acad Sci* 6:283, 1977.
51. Wardlow ML, Cox CP, Meng KE, Greene DN and Farr RS: Substrate specificity and partial characterization of the PAF-acylhydrolase in human serum that rapidly inactivates platelet activating factor. *J Immunol* 136,9:3441, 1986.