Biocompatibility of Hemodialysis Membranes

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ABSTRACT
Exposure of blood to hemodialysis membranes results in numerous interactions between the blood elements and the membrane. Transformation and adsorption of plasma proteins (such as complement) and activation of blood cells (such as neutrophils and monocytes) have been studied most extensively by nephrologists in recent years. There is no consensus on the definition of biocompatibility for dialyzer membranes. An operational definition of biocompatibility is the lack of any perturbation of blood constituents. According to this "inert surface" definition, a membrane (for example, one that adsorbs β2-microglobulin) can be considered as bioincompatible and yet desirable. Because of the multitude of blood-membrane interactions that may occur during hemodialysis, multiple criteria for biocompatibility needs to be applied in the classification of membranes. A certain bioincompatible phenomenon can be further classified as beneficial or deleterious depending on its biological effects as well as its acute and chronic impacts on the dialysis patient.

Key Words: Hemodialysis membrane, biocompatibility, complement, neutrophil degranulation, monocytes, interleukins, protein adsorption

The primary purpose of hemodialysis membranes is to provide a semipermeable barrier for the transport of water and solutes from the blood compartment to the dialysate compartment. The membranes are, however, not inert. Contact of blood with these biomaterials per se induces interactions and subsequent alterations of the blood elements. In general, the topic "hemodialysis membrane biocompatibility" is concerned with these interactions. In this paper we shall first address the scope and definition of hemodialysis membrane biocompatibility. A novel concept to approach this complicated issue is proposed. A brief overview of membrane materials will then be given. This is followed by more detailed discussions on a few specific areas which have gained popularity in the past decade, including complement, neutrophil degranulation, cytokines, lymphocytes, β2-microglobulins and protein adsorption. Limitation in space precludes a broader coverage of this vast topic.

DEFINITION OF DIALYSIS MEMBRANE BIOCOMPATIBILITY

The scope of this topic and the definition of biocompatible membranes often vary. We have arbitrarily excluded solute transport from the discussion of biocompatibility because it is the designed function of the dialysis membranes. For example, the notion of transport of β2-microglobulin from the blood to dialysate compartment is in essence not different from the notion of transport of other known uremic toxins such as potassium, phosphorus, and urea. Adsorption of molecules to the dialysis membrane surface, however, will be included because this is usually not a designed function of the membrane. In addition, adsorption of proteins to the membrane surface can often result in further alterations in blood elements.

There is no uniform agreement on the definition of biocompatibility of hemodialysis membranes. Some investigators choose to define "biocompatible" as "beneficial." For example, adsorption of albumin to the membrane surface decreases the adhesion of platelets (1) and adsorption of β2-microglobulin may decrease the plasma concentrations of this amyloidogenic substance (2,3). These phenomena will therefore be considered as biocompatible by some. This definition, of course, assumes that we already understand which phenomena are beneficial and which ones are not.

An alternative definition of biocompatibility is the absence of any perturbations in the blood elements (4). In other words, there should be no adhesion or activation of blood cells. There should be no adsorption or transformation of proteins and nonproteinaceous substances. Mechanical shear at the blood-membrane interphase should be minimal, and there should be no leaching or spallation of substances into the blood stream. In essence, this definition describes an inert surface, which is extremely difficult to achieve for hemodialysis membranes. None-
theless, for the sake of discussion we shall use this definition in the present paper. It should also be emphasized the intact hemodialyzer also contains sterilsants, potting compounds, and other constituents, which may also have significant impacts on biocompatibility. These will not be addressed.

HEMODIALYSIS MEMBRANE MATERIALS

A concise overview of the materials used in the manufacturing of commercially available artificial kidney membranes has recently been published (5). These membranes are fabricated primarily from two classes of materials. One class is derived from cellulose. Cellulose is part of the plant cell wall matrix and is made up of chains of glucosan rings bearing free hydroxyl groups. Cuprophan® (Akzo, previously Enka), is the prototype of the cellulose membranes. Cellulose acetate differs from cuprophan in that it has most of the surface hydroxyl groups substituted by acetyl residues (6). Hemofan® (Akzo) is another type of cellulosic membrane in which only ~1% of the hydroxyl groups has been substituted with tertiary amino (DEAE) groups (7). There are a variety of other cellulosic membranes such as “saponified cellulose” and “regenerated cellulose.” Although the underlying structure of these membranes is that of cellulose, the manufacturing or regenerating processes may modify the transport and biocompatibility properties such that they are significantly different from those of cuprophan.

Another class of artificial kidney membranes is in general referred to as synthetic polymers. Polyacrylonitrile, AN69 (a copolymer of acrylonitrile and sodium methally sulfonate), polysulfone, polymethylmethacrylate (PMMA), and polyamide are some of the examples in this category. Synthetic membranes are in general more porous and are therefore more suitable for high-flux dialysis or hemofiltration purposes. They are also in general relatively hydrophobic, which tends to increase their protein adsorption capacity (6). It should, however, be emphasized that not all synthetic membranes are created equally. For example, some synthetic membranes (PMMA) have relatively low ultrafiltration coefficients and are designed for conventional hemodialysis. In contrast, certain cellulose acetate membranes are casted into a more porous configuration such that they are suitable for hemofiltration purposes.

Therefore, it is crucial to specify the exact material and the modifications when we discuss the biocompatibility of an artificial kidney membrane. Further, the porosity of the particular membrane should ideally be specified, because in some instances the markers for biocompatibility can be lost to the dialysate side and the plasma levels of these molecules would not accurately reflect their generation.

COMPLEMENT ACTIVATION BY HEMODIALYSIS MEMBRANES

The interest in complement in hemodialysis originated from the observation of dialysis-induced leukopenia, which was described more than 20 years ago (9). Craddock and colleagues convincingly demonstrated the activation of complement during hemodialysis using cellulosic membranes (10). We subsequently demonstrated the production of complement activation products C3a and C5a during cuprophan dialysis (11,12). Because of the potent biological activities of the anaphylatoxins and the frequently large magnitude of increase during hemodialysis that is easily detectable using radioimmunoassays, plasma C3a (and its stable des-Arginine derivative) levels have been widely used as an index of membrane biocompatibility.

During hemodialysis using cuprophan membrane, a 15- to 30-fold increase in dializer venous plasma C3a antigen concentration can be detected (11). Ivanovich et al. compared cuprophan to cellulose acetate membranes, and found that hemodialysis using the latter was associated with lower plasma C3a antigen levels (13). Chenoweth et al. and Hakim et al. showed that used cuprophan membranes reprocessed with formalin were also associated with less complement activation than unused cuprophan membranes (12, 14). Because complement activation is temperature and divalent cation dependent, dialysis using cool dialysate (15) or citrate anticoagulation (16) also induces less complement activation.

MECHANISM AND CONTROL OF COMPLEMENT ACTIVATION ON DIALYSIS MEMBRANES

Hemodialysis membranes activate complement primarily via the alternative pathway (APC) (Figure 1). The reaction is initiated by the deposition of C3b on the membrane surface. C3b, in concert with factor B and factor D, forms the APC C3 convertase (C3bBb) and C5 convertase (C3bBbC5b). Cleavage of C3 by the C3 convertase results in the generation of anaphylatoxin C3a and further C3b molecules for the amplification of APC. Cleavage of C5 by the C5 convertase results in the generation of anaphylatoxin C5a and a larger fragment C5b. Assembly of the terminal components with C5b forms the membrane attack complex (MAC). Activation of both the APC and terminal pathway have been demonstrated during clinical hemodialysis.

Figure 1. Activation of the alternative (APC) and terminal pathway of complement during hemodialysis. Activation of C3 via the alternative pathway during hemodialysis is initiated with the deposition of C3b on the membrane surface. C3b, in concert with factor B and factor D, forms the APC C3 convertase (C3bBb) and C5 convertase (C3bBbC5b). Cleavage of C3 by the C3 convertase results in the generation of anaphylatoxin C3a and further C3b molecules for the amplification of APC. Cleavage of C5 by the C5 convertase results in the generation of anaphylatoxin C5a and a larger fragment C5b. Assembly of the terminal components with C5b forms the membrane attack complex (MAC). Activation of both the APC and terminal pathway have been demonstrated during clinical hemodialysis.
There are at least three compelling lines of evidence for this argument. First, leukopenia (which is believed to be mediated by complement activation products) can be induced by dialysis membranes even in the presence of EGTA (10), a reagent that binds Ca\(^{2+}\) and prevents the activation of the classical pathway of complement. Second, the two components of the APC C3 convertase, C3b and Bb, can be found on the dialysis membrane surface (6). Third, C4a, the activation product of C4, does not increase during dialysis (11), which is expected to occur if the activation were to proceed via the classical pathway.

Because the major pathway of complement activation on dialysis membranes is APC, it would seem logical to examine the factors controlling APC activation to understand how complement activation is regulated during hemodialysis. APC activation on other biological surfaces such as zymosan appears to be initiated following covalent binding of C3b to the surface hydroxyl groups (17). Because cuprophan has an abundance of free hydroxyl groups on its glucosan rings, one may postulate that these hydroxyl groups are potential covalent binding sites for activated C3, thereby favoring C3 activation. In contrast, cellulose acetate has about 70% of the hydroxyl groups substituted by acetyl residues, one may therefore postulate that these acetyl residues block the binding sites for C3, thereby limiting C3 activation.

Our recent studies, however, did not support these hypotheses. We found that cuprophan did not bind more C3 than cellulose acetate did after 4 h of clinical hemodialysis and that the binding of C3 to cellulose membranes might not be covalent in nature (18). Instead, after exposure to serum, cuprophan membrane bound relatively more factor B (a protein that promotes APC activation) than factor H (a protein that inhibits the APC) (6). Acetylation of the cellulose membrane somehow changes the biochemical structure in a manner such that it favors the binding of factor H over that of factor B. These data suggest that the differences in complement activating potentials between cuprophan and cellulose acetate are primarily determined by their different capacities to influence the binding of the APC regulatory proteins.

Hemophan membrane provides another argument against the hypothesis that activation of complement on dialysis membranes is determined primarily by the number of surface free hydroxyl groups available for C3 binding. Hemophan is also a derivative of cellulose on which surface only ~1% of the hydroxyl groups has been substituted by DEAE groups. Yet hemodialysis using this membrane is associated with significant lower plasma C3a antigen levels than dialysis using cuprophan (no substitution) or cellulose acetate (70% substitution of hydroxyl groups) (7, 19). The mechanism(s) by which such apparently trivial modification of the cellulosic surface significantly attenuates C3 activation is intriguing.

**RELIABILITY OF FLUID PHASE C3a LEVEL AS AN INDICATOR OF COMPLEMENT ACTIVATION**

Hemodialysis using polyacrylonitrile (PAN) membranes has previously been shown to result in plasma C3a antigen concentrations far lower than that seen with cuprophan membrane (11). Both in vitro (20) and ex vivo (21) experiments, however, clearly demonstrated the high affinity of this membrane for C3a. These observations suggest that the low plasma concentration may be the result of removal of the peptide by adsorption to the membrane surface and not necessarily the result of low generation rate. More recently, we quantitated the total generation of C3a in the presence of cuprophan and AN69 (copolymer of PAN methallyl sulfonate) membranes during incubation with normal human serum (22). We found that the total generation of C3a in the presence of AN69 was in fact higher than that in the presence of cuprophan. However, because almost 90% of the generated C3a remained bound to the AN69 membrane surface, the amount of C3a in the serum was significantly less after incubation with AN69 compared with cuprophan. The high affinity of C3a (pl~9.5) to AN69 is presumably due to the surface negative charge imparted by the sulfonate groups on the membrane.

These observations raise two important points. First, the availability of free hydroxyl groups may not be essential for C3 activation on dialysis membrane surfaces. Second, because a single C3a molecule is produced when a C3 molecule is activated, total C3a (as measured by C3a + C3a\(_{act\_and\_prim\)}\) generation is an accurate indicator of C3 activation. However, the generated C3a may bind to the dialysis membrane surface (and potentially to surface of cells such as monocytes) or be removed across the dialysis membrane. Therefore, fluid phase C3a\(_{act\_and\_prim}\) level alone may not be a reliable indicator of C3 activation. The same principle applies to other proteins such as C5a and interleukin-1. C5a may also bind to dialysis membranes and cells such as neutrophils (23) and monocytes (24). Potentially, only a small fraction of the total generated amount is available for measurement in the plasma (Figure 2).

**CLINICAL EFFECTS OF COMPLEMENT ACTIVATION DURING HEMODIALYSIS**

Numerous biological effects of the anaphylatoxins C3a and C5a have been described (25–27). The precise clinical effects of these biologically active peptides on the hemodialysis patients, however, have not been clearly defined. Listed in Table 1 are some of the known properties of C5a and their potential clinical correlations. These properties fall into two general categories: anaphylactic and leukocyte-directed. Some of the anaphylactic effects of C5a are...
mediated through the stimulation of release of other biological active substances such as histamines (26) and leukotrienes (27). The release of these substances can lead to smooth muscle contraction in the airway and the pulmonary arterioles as well as interstitial edema. It may therefore account for part of the dialysis-induced hypoxemia (28) and some of the severe anaphylactoid reactions observed during clinical hemodialysis that are labeled by some as the "first use syndromes" (29, 30). Evidence supporting the roles of anaphylatoxins and membrane effects on dialysis-induced hypoxemia has been reviewed (31).

The spasmogenic properties of C3a and C5a are largely abrogated once the C-terminal arginine has been cleaved from the anaphylatoxins by serum carboxypeptidase N (32). The acute clinical effects produced by these peptides are therefore usually not drastic. They are, however, often detectable by careful observations and measurements. Animal studies have shown that infusion of cuprophan-activated plasma or sham dialysis using cuprophan membranes produces acute pulmonary hypertension (33), an effect that appears to be mediated by C3a, C5a (34), and the thromboxanes (35). Schohn et al. performed sham dialysis on uremic patients and found similar results (36). In support of the roles of anaphylatoxins in the pathogenesis of intradialytic symptoms, Dumler and colleagues have found a good correlation between these symptoms and peak plasma C3a antigen concentration during hemodialysis (37).

C5a stimulates a variety of changes in neutrophils that result in adhesion and aggregation (38, 39). In addition, stimulated neutrophils release oxygen radicals (40) and intragranular proteolytic enzymes (41), which cause tissue damage. C5a is known to induce the transcription of mRNA for interleukin-1 (42). Even though the spasmogenic properties are significantly attenuated after cleavage of the arginine moiety, the leukocyte-directed effects are largely retained in C5aArg. More recently, Haeffner-

TABLE 1. Potential effects of anaphylatoxin C5a on hemodialysis patients

<table>
<thead>
<tr>
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<th>In Vitro</th>
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<tr>
<td>Anaphylactic</td>
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<td>&quot;First use syndrome&quot;</td>
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<td>Increase</td>
<td>Vascular permeability</td>
<td>Pulmonary and systemic edema</td>
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<td>Release</td>
<td>histamine</td>
<td>Allergic reactions</td>
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<td>Stimulate</td>
<td>leukotriene release and contract smooth muscles</td>
<td>Pulmonary hypertension and hypoxemia</td>
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<td>Neutrophil-directed</td>
<td>Increase CD11b/18 (CR3)* expression</td>
<td>Altered immunity and tissue damage</td>
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<td></td>
<td>Release leukotriene B4</td>
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<td>Aggregation</td>
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<td>Adherence to endothelial cells</td>
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<td>Up-regulate C3b receptors (CR1)</td>
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<td>Degranulation</td>
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<td>Stimulate release of β2MG</td>
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<tr>
<td>Monocyte-directed</td>
<td>Stimulates IL-1-TNF transcription/production</td>
<td>IL-1 and TNF effects</td>
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* CR3, complement receptor type 3; CR1, complement receptor type 1; IL-1, interleukin-1; TNF, tumor necrosis factor.
Cavaillon et al. demonstrated that C3a_desArg, which circulates in much higher concentration than C5a during hemodialysis, can also stimulate human monocytes to produce interleukin-1 (43).

ACTIVATION OF THE TERMINAL PATHWAY OF COMPLEMENT DURING HEMODIALYSIS

Activation of the alternative pathway of complement can, under favorable conditions, result in the activation of the terminal components and assembly of the membrane attack complex (MAC) (Figure 1) (44). Recent studies by Deppisch et al. demonstrated the presence of these complexes in the plasma during hemodialysis (45). In sufficient concentrations the membrane attack complex can stimulate arachidonic acid metabolism (46) and induce cell lysis (44). It is unlikely that complement-mediated lysis of erythrocytes occurs to any significant extent acutely during hemodialysis. Sublytic concentrations of MAC, however, may increase the fragility of erythrocytes and contribute to their decreased half-life in the dialysis patients.

INDICES OF COMPLEMENT ACTIVATION ASSOCIATED WITH DIALYSIS MEMBRANES

Measurement of MAC provides an additional index of complement activation. At present, a reasonable assessment of complement activation by dialysis membranes would include the measurement of total C3a_desArg, C5a_desArg, and MAC, because they reflect activation of different levels of the complement cascade with different activation potentials. Activation of C3 does not usually lead to equal molar activation of C5 or of the terminal components. Therefore, measurement of one does not substitute for the measurement of others.

There are some recent interests in the measurement of other C3 activation products as indices of C3 activation. Detection of some of these products such as IC3b and C3d is probably meaningful because they may possess their own biological activities (47). It should be cautioned, however, that unlike C3a_desArg, some of these fragments (for example, IC3b) are further degraded to various extents depending on the environment. Quantitation of a particular fragment may not accurately reflect the total number of C3 molecules that have been activated. Cross-reactivities of some of the available antibodies with various C3 fragments further complicate the interpretation of the assays.

NEUTROPHIL DEGRANULATION DURING HEMODIALYSIS

Degranulation by neutrophils with release of their intragranular proteins has been well documented during clinical hemodialysis using a variety of membranes (48,49). C5a causes neutrophils to degranulate and is a potential factor operative in the dialysis setting. HorI and colleagues have, however, observed that the changes in plasma concentrations of neutrophil granular proteins do not correlate well with changes in the plasma concentrations of C3a (49). For example, hemodialysis using polymethylmethacrylate (PMMA) membranes is associated with lower plasma concentrations of C3a but higher concentration of elastase than dialysis using cuprophan. It has therefore been suggested that neutrophil degranulation during hemodialysis may not be, at least in part, attributable to complement activation. Alternatively, it has been suggested that shearing at the cell-dialysis membrane interface contributes to the degranulation phenomenon.

The granules of neutrophils contain a variety of proteins, some of which are potent proteolytic enzymes involved in host defense (48). Release of these enzymes into the plasma can therefore damage circulating proteins. Indeed, protein catabolic state has been demonstrated during hemodialysis (50,51). The extent to which neutrophil degranulation contributes to this catabolic state is unclear at present.

MONOCYTE STIMULATION AND PRODUCTION OF INTERLEUKIN-1 (IL-1)

Among many other cell types, circulating monocytes are known to produce IL-1, a class of potent mediators of inflammatory and immunologic responses with M r 17,000 (52). The “interleukin hypothesis” was first put forward by Henderson et al. to offer a potential explanation for the phenomena of dialysis-induced hypotension and dialysis-induced fever (53). Because the activated monocytes also produce other proinflammatory molecules such as tumor necrosis factor (TNF), the hypothesis has been renamed by some as the “monokine hypothesis.” Further, some of the biological effects of IL-1 appear to be mediated by interleukin-6 (54). Because IL-6 is also produced by lymphocytes in addition to monocytes, the hypothesis has to be extended to include the lymphocytes. Hence the term “cytokine hypothesis” has evolved.

Several substances relevant to the hemodialysis setting has been shown to induce IL-1 production in vitro (Figure 2). One of the best known stimuli of IL-1 is endotoxin (52), which may be present in the contaminated dialysate. Bingel et al. demonstrated that when endotoxin was placed into the dialysate compartment of cellulose membrane dialyzers in vitro, the monocytes on the blood side somehow became activated to produce IL-1 (55). This is rather surprising because the endotoxin aggregate is approximately 1 x 10⁹ daltons in molecular weight and
is therefore not expected to cross the intact dialysis membrane. Indeed, several groups of investigators
have failed to find the passage of endotoxins through membranes of intact dialyzers (56,57).

Alternatively, it has been postulated that muramyl peptides, which are fragments of the bacterial cell wall,
may cross the dialysis membrane because of their relatively small sizes (~1,000 daltons). These peptides are not
detectable by the conventional limulus lysate assay for endotoxins, but are potent stimulants of IL-1 production (58).
The magnitude and mechanism(s) by which endotoxins and their fragments traverse hemodialysis membranes is
controversial at present, and may be dependent on the type of membrane employed and the species of bacteria
from which the endotoxins are isolated. If the transfer is primarily by convection, then back-filtration of
fluid through high permeability membranes would be an important issue. An argument has been made
that dialysates should be sterilized and free of endotoxins to minimize IL-1 production during hemodi-
alysis.

Schindler and colleagues recently presented evidence that recombinant C5a was capable of inducing
transcription of IL-1 (and TNF) in human mononuclear cells, but that lipopolysaccharide from Esche-
richia coli was subsequently required to stimulate its release (42). In contrast, Haeffner-Cavaillon et al.
showed that C3a(desArg), in the absence of endotoxins, can stimulate IL-1 production by human monocytes
(43). The effective concentration of C3a(desArg) was only 10^{-8} M (~100 ng/ml), which can be easily
achieved in the plasma during hemodialysis (11). The implication of this study is that complement activa-
tion products generated during hemodialysis per se can contribute to the production of cytokines, regard-
less of whether bacterial fragments can traverse the dialyzer membranes.

Interestingly, Bingel’s group demonstrated that IL-1 production by mononuclear blood cells in vitro
could also be stimulated by acetate buffer at 20 mM, an effect that was reportedly synergistic with that of
endotoxin (59). Although the circulating plasma acetate concentration is much lower than 20 mM, mono-
cytes adherent to the dialysis membrane surface may be exposed to such high concentration. Incubation of
monocytes with cuprophan membrane, in the absence of serum, has also been shown to induce IL-1 prod-
cution (60,61), indicating a direct membrane ef-

**PRODUCTION OF IL-1 DURING CLINICAL HE-
MODIALYSIS**

Clinical studies confirmed that monocytes can be stimulated to produce IL-1 during hemodialysis (62–64).
Intracellular IL-1 levels may increase within 2 h of hemodialysis using cuprophan membranes and
appear to correlate with the increase in plasma C3a(desArg) levels (63). At least part of the cell-associa-
ted IL-1 can be subsequently released (63), resulting in an elevation of plasma IL-1 concentrations (62,64).

The effect of membrane materials on IL-1 production during hemodialysis has not been clearly deline-
ated at present. Haeffner-Cavaillon et al. found that clinical dialysis using AN69 membranes did not lead
to an increase in intracellular IL-1 in most patients (although there were significant variations among
patients), in contrast to dialysis using cuprophan membranes (63). The predialysis intracellular IL-1
levels were, however, substantially higher in the AN69 group. Conceivably, different factors were oper-
ative in stimulating the leukocytes during hemodi-
alysis using the two different types of membranes. For example, large quantities of C3a(desArg) in the
plasma might have stimulated the cells during cupro-
phan dialysis; whereas during dialysis with AN69,
endotoxin fragments in the dialysate might have slowly traversed the more permeable membrane to
stimulate the monocytes, which subsequently release
the IL-1 during the interdialytic period. Difficult to
reconcile with this theory were the observations
of Bingel and colleagues, which showed that clinical
dialysis with either cuprophan or Hemophan (a
weaker complement activator) resulted in significant increases in plasma IL-1 levels (64).

More recently, Herbelin and colleagues showed that the
duration for which the patients had been on
hemodialysis could influence the plasma IL-1 levels
during the treatment (62). An increase was observed
in patients who had been on dialysis for a mean
duration of 46 months but not in patients who were
undergoing hemodialysis for the first time. Whether
this susceptibility to leukocyte stimulation in the
long-term dialysis patients is due to the more pro-
longed uremic state, chronic exposure to dialysis
membranes, or other factors is unclear. In apparent
contrast to the earlier studies which showed no acute
intradialytic increase in intracellular IL-1 levels (63),
these investigators also found an increase in plasma
IL-1 during hemodialysis using the AN69 membrane
(62).

It is apparent from the above discussion that, IL-1 production cannot be assessed by plasma IL-1 levels
alone. The generated IL-1 may stay intracellularly,
exist freely in the plasma after being released, bind
to the hemodialysis membrane (65), or it may even
be lost to the dialysate compartment (Figure 2). All
these pools should be accounted for to assess IL-1
generation accurately. Complicating matters further
are the assays available for IL-1. The thymocyte co-
proliferation assay has been the standard measure-
ment of this cytokine and has been used in most of
the hemodialysis studies. Some of the effects of IL-1, however, are shared by interleukin-6, including its ability to augment T cell proliferation and to induce the production of hepatic acute phase proteins (54). Interleukin-6 is produced not only by monocytes, but also by lymphocytes and other cell types. Thus, measurement of "IL-1" by the coproliferation assay is neither specific for IL-1 nor necessarily an accurate reflection of the state of monocyte activation.

Tumor necrosis factor (TNF) is another cytokine produced by monocytes. Despite distinct amino acid sequence and separate receptor, TNF shares a number of features with IL-1 (66). One of the most potent stimuli of TNF production is again endotoxin. TNF and IL-1 share the same spectrum of and act synergistically in a variety of biologic responses. In contrast to IL-1, however, TNF does not have a direct effect on lymphocyte activation. Studies so far have failed to demonstrate the release of significant amounts of TNF into the circulation during hemodialysis (62).

**EFFECTS OF IL-1 ON HEMODIALYSIS PATIENTS**

The spectrum of biological activities of IL-1 is wide. Although the precise clinical consequences of dialysis-induced IL-1 production are at present unknown, the fact that IL-1 functional activity (assuming that the coproliferation assays are measuring primarily IL-1) can be detected in plasma obtained during hemodialysis indicates that these molecules are biological active and are likely to be clinically relevant.

Dinarello and colleagues published an updated version of the "interleukin hypothesis" in which they have speculated upon the effects of IL-1 on the hemodialysis patients (67). Some of these are depicted in Figure 3. IL-1 induces fever through the production of prostaglandin E2 and may account for part of the intradialytic as well as postdialytic elevation in core temperature. It had been debated whether IL-1 production during hemodialysis is sufficiently rapid to account for the intradialytic fever. Recent data showed that IL-1 production and its release from monocytes could occur within only 2 h (63). Therefore, the temporal relationship does not argue against the role of IL-1 in dialysis-associated fever. The magnitude of the temperature elevation may be relatively mild. Profound "pyrogenic reactions" with temperature exceeding 101°F are the exception rather than the rule during routine hemodialysis unless there are coexisting infections. This does not imply that intradialytic IL-1 production is clinically unimportant. Besides raising the immediate concerns of the medical staff caring for the patients, fever induction by IL-1 suggests that this multifunctional cytokine may also produce other acute and chronic effects on the hemodialyzed patients.

The generation of PGE2 in cerebral tissues induced by IL-1 may produce lassitude and headache. Its ability to increase slow-wave sleep may correlate with the sleep disturbances seen in hemodialysis patients. IL-1 reduces food intake in animals (68) and may contribute to anorexia. It increases the rate of muscle proteolysis, an effect which is also mediated by PGE2. Protein catabolism has been well described during hemodialysis and has been attributed to neutrophil degranulation as described above. In addition, Bergstrom et al. performed sham hemodialysis on normal human volunteers and found the release of free amino acids suggestive of protein catabolism (51). This phenomenon could be prevented by the administration of the prostaglandin synthetase inhibitor indomethacin, lending support to the hypothesis that IL-1 contributes to intradialytic protein catabolism. IL-1 stimulates the production of prostacyclines (PGI2) from endothelial cells and may theoretically induce hypotension (67).

IL-1 has several effects on the skeletal system that may have relevance to the dialysis patients (67). As an osteoclast-activating factor, it induces bone resorption. It induces synthesis of collagenase in synovial cells and metalloproteinases in chondrocytes. In addition, as part of its ability to mediate acute-phase responses, IL-1 stimulates the release of amyloid A from the liver. These effects may contribute to the articular and periarticular diseases in the uremic patients. IL-1 inhibits lipoprotein lipase activity and therefore affects lipid metabolism. It promotes procoagulant activities and adherence of neutrophils to endothelial cells. Together with its ability to stimu-
late the proliferation of smooth muscle cells. IL-1 may predispose the patients to premature atherosclerosis. Finally, chronic preactivation of T cells by IL-1 can lead to subnormal T cell responses to subsequent stimuli. This may be one of the mechanisms causing the immunodeficiency state in dialysis patients.

LYMPHOCYTE STIMULATION AND INTERLEUKIN-2 (IL-2) DURING HEMODIALYSIS

Information on the effects of hemodialysis on lymphocytes is sparse, but is gradually accumulating. Only a brief description of lymphocyte activation and IL-2 will be given here. Stimulation of T lymphocytes by antigens in the presence of IL-1 induces two events: the production of IL-2 and the expression of its receptor (IL-2R) on the cell surface (69). After released from the cell, IL-2 binds to the surface IL-2R as part of an autocrine system. This ligand-receptor interaction then leads to the proliferation of the T cell itself. An activated helper T cell in turn serves an important function of mediating B lymphocyte maturation as well as activation of cytotoxic T cells, monocytes, natural killer cells, and hematopoietic precursors.

The receptor IL-2R consists of an α and β chain. A portion of the cell membrane-associated IL-2R can be released into the fluid phase under physiologic conditions. The released molecule (~45,000 daltons) corresponds to a truncated form of the β chain and is known as soluble IL-2R (70). The presence of soluble IL-2R in the plasma reflects a state of T lymphocyte activation. In addition, soluble IL-2R retains the ability to bind IL-2 and may therefore decrease the availability of IL-2 to activate the target cell. It has therefore been suggested that an increased amount of soluble IL-2R in the plasma, as can be seen in patients with active systemic lupus or rheumatoid arthritis (71,72), represents a state of immune down-modulation.

Recent studies of Beaurain et al. demonstrated that the expression of IL-2R on T lymphocyte surface was abnormally enhanced in hemodialysis patients (73). Soluble IL-2R levels in the plasma were correspondingly increased. Probably as a result of this state of preactivation, T cells from hemodialysis patients exhibited abnormal responses to mitogen stimulation. The effect of the hemodialysis procedure on T lymphocyte activation is unclear. Beaurain reported no change in plasma soluble IL-2R levels after hemodialysis treatments. Preliminary results from Hakim and colleagues, however, showed that the expression of IL-2R on lymphocytes during hemodialysis was influenced by the types of membranes employed (74). These data suggest that dialysis membrane biocompatibility may have an effect on the competence of lymphocytes in host-defense.

Several investigators have also reported alterations in lymphocyte subsets during clinical hemodialysis using cellulosic membranes (75,76). Information on other peripheral mononuclear cells are scanty. Kay and Raij studied the effect of membrane materials on natural killer cell function during perfusion of whole blood through intact dialyzers in vitro (77). More decrease in cell function was associated with cuprophan than with polycarbonate membranes.

β2-MICROGLOBULIN (β2MG)

Deposition of β2MG in tissues is now recognized as a form of amyloidosis (AH) in dialysis patients (78). Although there is no good correlation between the plasma level of β2MG and clinical AH disease (79), it is generally believed that significantly elevated serum β2MG level is undesirable. Various membranes and modes of treatment have been compared for their efficacy in removing β2MG (2,3). Transport of this protein to the dialysate/ultrafiltrate compartment, may it be diffusive or convective, is considered to be an expected function of the artificial kidney membrane and would not be discussed in the context of biocompatibility. Instead, we shall address the issues of generation of β2MG during the treatment and the binding of the protein to the artificial membrane.

The net production of β2MG during hemodialysis using cuprophan membranes has been controversial. β2MG (Mr ~11,000) is the light chain of the HLA antigen and is normally shed from the peripheral blood cell surfaces and removed by the kidneys (80). There is little reason to suspect that its cellular production and release should cease during hemodialysis. The more pertinent questions are (a) whether the release of β2MG is enhanced during cuprophan hemodialysis, and (b) whether the rate of release exceeds that of elimination so that there is a net increase in plasma β2MG concentration during cuprophan hemodialysis.

In a study by Ritz and Bonmer, hemodialysis using cuprophan membranes was associated with ~35% increase in plasma β2MG levels (81). Even after correcting for hemoconcentration as a result of fluid removal, an increase of ~15% (~7 μg/ml) could still be demonstrated. Is this a biocompatibility issue? An increase of 2-3 μg/ml from a starting plasma concentration of 50 μg/ml may superficially appear to be trivial. It is, nonetheless, rather significant in biological terms, considering that the normal plasma concentration is only ~1 μg/ml. A sudden burst of release of β2MG in a relatively short duration probably indicates substantial activation of certain cells. What is the tissue origin of this protein and the stimuli during this abrupt release?

Bjerrum and colleagues demonstrated the presence of β2MG in neutrophils, presumably residing inside
their specific granules (82). It can be co-released in vitro along with other proteins during degranulation induced by certain stimuli such as phorbol and calcium ionophore. Because degranulation of neutrophils occurs during cuprophan hemodialysis (48,49), it is conceivable that $\beta_2$MG is released into the circulation by this mechanism. The neutrophil intragranular content of $\beta_2$MG is, however, relatively small (82). Even assuming total discharge of this content, the elevation in plasma $\beta_2$MG concentration would only be $\sim 0.02 \mu g/ml$ by calculation, and would be below the detection limit of commercially available radioimmunoassay. $\beta_2$MG is also located on the plasma membrane of neutrophils, which can potentially be shed during passage of the cells through the dialyzer. We have detected $\beta_2$MG on cell surfaces using fluorescein-conjugated antibody and flow cytometry. In some patients we found a decrease in $\beta_2$MG expression on neutrophil surface with time on dialysis using cuprophan membranes (personal observation). In addition, a difference between the dialyzer afferent and efferent line could be seen. The results among different patients were, however, inconsistent.

An alternative cellular source are the monocytes. Recently, Knudsen et al. demonstrated that a decrease of 35 mmol/kg in osmolarity (manipulated with mannitol) could induce the release of $\beta_2$MG from peripheral blood leukocytes in vitro (83). The magnitude of this release was very small, extrapolated to only 0.04 $\mu g/ml$ in whole blood. When isolated monocytes were subjected to stimulation using various agents, $\gamma$-interferon [a lymphokine from T cells] was found to be most effective. releasing an amount of $\beta_2$MG corresponding to $\sim 1 \mu g/ml$ in whole blood. Lipo polysaccharide from E. coli or TNF was half as effective. Because monocytes are activated during hemodialysis, it is conceivable that blood-membrane interactions and endotoxin-like substances from contaminated dialysate may contribute to the increase in plasma $\beta_2$MG during hemodialysis (Figure 4).

In contrast, hemodialysis using AN69 (3) or polysulfone (2) membranes causes a decrease in plasma $\beta_2$MG levels. This decrease may be due to either transfer to the dialysate compartment or adsorption onto the dialysis membranes. In vitro studies by Goldman and colleagues confirmed that AN69 membrane adsorbed a significant amount of this protein (3). Does the adsorption of $\beta_2$MG mean that the membrane is biocompatible? If one defines biocompatibility as lack of any perturbation in the blood constituents, then adsorption of $\beta_2$MG is not biocompatible. Is it beneficial? To the extent that lowering of plasma $\beta_2$MG levels may prevent (84) or ameliorate AH amyloid, its adsorption by the dialysis membrane is beneficial.

![Figure 4. Potential mechanisms of $\beta_2$ microglobulin ($\beta_2$MG) production during hemodialysis. Complement C5a is a known stimulant of neutrophils (PMN) and monocytes. Both cell types have been shown to release $\beta_2$MG. TNF is a potent stimulant for $\beta_2$MG release by monocytes. Increases in plasma TNF levels during hemodialysis appear to be insignificant. TNF, however, may potentially serve as an autoantigens to stimulate monocytes without achieving high concentration in the plasma. Large osmolar changes also induce the release of $\beta_2$MG from leukocytes, but the magnitude of the release is small.](image)

**ADSorption BY DIALYSIS MEMBRanes AS AN INDEX OF BIoCOMPATIBILITY**

We have mentioned that adsorption of albumin onto artificial surfaces decreases platelet adhesion and that adsorption of $\beta_2$MG decreases plasma concentration of the peptide. Both represent situations which are biocompatible (based on the "inert surface" definition) but beneficial. Adsorption of proteins, however, is not necessarily beneficial. For example, adsorption of fibrinogen to artificial surfaces favors platelet adhesion and promotes coagulation (1). This phenomenon is therefore bioincompatible and also nonbeneficial. Another scenario is the adsorption of beneficial substances onto the dialysis membrane. For example, we have recently demonstrated that AN69 membranes adsorb far more erythropoietin in vitro than do cuprophan membranes (85). Although the clinical impact of this phenomenon has not been determined, removal of erythropoietin from the plasma may be considered as nonbeneficial.

**CONCLUSION**

Numerous alterations occur in the blood as a result of contact with the hemodialysis membrane. Many of these changes have not been covered in this brief review or have not yet been realized. At times, these changes can produce clinical pictures that are acute and drastic as exemplified by anaphylactoid reactions. At other times, the results may not be apparent and can only be detected by laboratory examinations. Analogous situations in which the clinical outcome is highly variable can be seen in many areas of renal disease and general medicine. Penicillins do not usually cause interstitial nephritis and administration of gentamycin does not invariably lead to oliguric acute renal failure.
The precise clinical impacts of each of these bloincompatible events are difficult to determine for several reasons. First, the uremic state per se is associated with numerous physiologic and metabolic disorders that can be confused with the effects of membrane biocompatibility. Second, the clinical status and underlying illness of the patient population are often highly heterogeneous and fluctuating, making controlled trials aiming at clinical outcome difficult to conduct. Third, many different cellular and noncellular elements are affected upon exposure to the dialysis membranes. These elements often interact in a complex manner to produce biological effects; individual effects are difficult to dissect out.

Even the subtle bloincompatible reactions, however, should not be ignored, as emphasized by Hakim and colleagues (14). The dialysis patients are exposed to the extracorporeal circuit two or three times a week for many years. Effects of the membranes can accumulate over these periods of time. Catabolism of a few grams of plasma proteins [attributed to neutrophil degranulation or IL-1 release, for example] during a single dialysis session may be inconsequential, but repetitious loss can be deleterious. These chronic effects are particularly important in the uremic patients who are already subjected to many other factors predisposing them to diseases.

Finally, it should be reiterated that biocompatibility and beneficial effects of dialysis membranes vary depending on the criteria employed. In this article, distinction has been made between a "biocompatible" phenomenon and a "beneficial" phenomenon. We should first examine if a biocompatible event is beneficial or deleterious, which by itself may not be an easy task. Ten years ago, the adsorption of β2MG by dialysis membranes might have been considered an epiphenomenon. After we understand the effects of a particular phenomenon, we may wish to choose a particular membrane in order to fulfill a particular biocompatibility purpose. The choice of the membrane is of course also influenced by other factors such as its transport characteristics and economic constraints. At the same time we should recognize that this particular membrane may be associated with other effects that are undesirable. The long-term clinical outcome is influenced by the combined beneficial and deleterious effects of the membrane. Continued and concerted efforts should be made by the biomedical scientists and bioengineers to understand the many aspects of blood-membrane interactions and to design circuits in which the positive features can be exploited and the negative side-effects minimized.

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