Interleukin-1 and its relation to biocompatibility in hemodialysis

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Since 1882 when Metchnikoff discovered phagocytosis [1], the importance of the primary response to infection, categorised as inflammatory, has been recognised. It is characterised by the rapid accumulation of poly and mononuclear phagocytes at the site of injury. Systemic symptoms may follow, which include fever, myalgia, headache, lassitude and even sleepiness. Laboratory measurements reveal increased blood neutrophils, decreased plasma zinc and iron, increased hepatic acute phase proteins and increased levels of several stress hormones. It has recently become clear that the key cell responsible for many of these actions is the monocyte rather than the granulocyte. Of the many polypeptide products («hormones») released by activated mononuclear phagocytes, interleukin-1 (IL-1) possesses a broad range of biological properties associated with both acute and chronic inflammatory changes [2].

Interleukin-1 and the acute phase response

IL-1 is a family of polypeptides with molecular weights between 17 and 18,000 daltons. In this review, the biological properties of IL-1 will be reviewed with particular attention to their relevance in patients undergoing regular maintenance hemodialysis (HD) or continuous ambulatory peritoneal dialysis (CAPD). In addition, how the HD or CAPD procedure and materials employed in both procedures can lead to monocyte/macrophage stimulation and the induction of IL-1 is considered. Evidence is now accumulating that the local inflammatory response is initiated by the dialysis membrane-monocyte interaction in HD and by the instillation of exchange fluid during CAPD. The consequence of these actions are the production of IL-1. Long-term production of IL-1 may account for the chronicity of acute phase changes observed in ESRD patients on HD and CAPD. At present, two distinct IL-1 molecules have been identified by recombinant DNA methods: one with a neutral charge (IL-1-beta) and the other with an acidic charge (IL-1-alpha) [3-6]. Since recombinant IL-1 is homogenous, doubts concerning the purity of the preparations can no longer be raised. The multiple biological activities of human recombinant IL-1-beta are listed on table I. They have been grouped into *in vivo* and *in vitro* effects.

IL-1 activates human endothelial cells *in vitro* to produce PGI_2 [7], a known vasodilator. In addition, rIL-1 stimulates endothelial cell surfaces so that neutrophils and monocytes adhere avidly [8], procoagulant activity increases [9] and smooth muscle growth factors are produced [10]. The catabolic properties of IL-1 are usually considered in terms of either local or systemic effects. For example, IL-1 produced locally acts in an autocrine fashion in destructive joint and bone disease and local tumor invasion. On the other hand, the catabolic effects of IL-1 in the systemic circulation exert their effect on liver, fat, muscle and connective tissue. IL-1 is a potent inducer of collagenase synthesis [11, 12]

Table I. Biological Activities of Recombinant Human IL-1 *

In vivo
Fever in mice, rats, rabbits, guinea pigs.
Hypozincaemia, Hypoferraemia.
Decreased cytochrome P450 enzyme activity.
Neutrophilia.
Slow wave sleep induction.
Hepatic acute phase protein synthesis.
Increased survival rate in immunosuppressed mice.
Increased bacterial clearance in immunosuppresed mice.
Increased cortisone levels in mice and rats.
Increased ACTH levels in mice and rats.
Accumulation of neutrophils in tissues.
* Human Recombinant pI 7 form (beta).
In vitro
Chemotaxis of Lymphocytes and Monocytes.
Increased IL-2 Receptors and Increased IL-2 Production.
Synergism with IL-2 Natural Killer Cell Assay (Human).
Proliferation of Dermal Fibroblasts.
Induction of Fibroblast and Endothelial GM-CSF Activity.
Induction of Endothelial Cell Procoagulant Activity.
Production of PGE-2 in Dermal and Synovial Fibroblasts.
Production of PGI-2 in Human Endothelial Cells.
Decreased Hepatocyte Albumin Synthesis.
Increased Surface Expression of HLA-A, B Antigens in Human Endothelial Cells and Dermal Fibroblasts.
Increased Neutrophil and Monocyte Thromboxane Synthesis.
Degranulation of Human Basophils (Histamine Release).
Cytotoxic for Human Melanoma Cells.
Cytotoxic for Human Beta Islet Cells (Insulin Producing).
Increased Collagenase from Human Synovial Fibroblasts.
Increased Collagenase from Chondrocytes.
Increased Bone Resorption.

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in synovial cells and chrondrocytes. IL-1 shares molecular identity with osteoclast activating factor (OAF) [13]. It is currently thought that the catabolic properties of IL-1 in cartilage and bone are primarily responsible for the tissue destruction and matrix loss of bone that is present in a variety of diseases.

In contrast to its catabolic activities, IL-1 increases fibroblast proliferation [14] and the transcription of Type I and Type III collagen [15] and Type IV (basement membrane) collagen [16].

There is also a direct effect of IL-1 on hepatocyte transcription of acute phase proteins, including serum amyloid A (SAA) protein which is the precursor of amyloid fibril [17]. Therefore, fibrosis and deposition of abnormal proteins in tissues appears, in part, to be mediated by IL-1. In rheumatoid joint disease, IL-1 is thought to contribute to the pannus formation [12]. The liver's response to IL-1 also includes the synthesis of metalloproteins which bind serum iron and zinc and account for the hypozincemia and hypoferremia induced by IL-1 [18].

IL-1 and hemodialysis

Fever is a frequent sign associated with hemodialysis therapy, particularly when the dialysate fluid is heavily contaminated [19, 20]. However, most patients undergoing uncomplicated hemodialysis exhibit a slight but consistent rise in body temperature during and after the procedure [21, 22]. Since injecting as little as 100 ng/kg. of rIL-1 into experimental animals results in the rapid onset of fever, it is clear that IL-1 is potent endogenous pyrogen [23]. One might indeed speculate that IL-1 induced PGI_2 production by endothelial cells leads to hypotension during hemodialysis [24, 25], especially as we have unpublished data demonstrating that rIL-1 drops the blood pressure of the rabbit in a spectacular fashion.

There is a direct relationship between the magnitude of the febrile response and the level of contamination in the dialysate [19, 20]. The amount of endotoxin which can be detected using the Limulus test in the blood side of heavily contaminated dialysate has been consistently low or absent as reported in several studies [26, 27]. Human monocytes produce IL-1 *in vitro* in response to concentrations of endotoxin as low as 50-100 pg/ml. Therefore, stimulation of human monocyte IL-1 production may offer a more specific and relevant method for detecting dialyser membrane integrity to endotoxins in the dialysate. In most experimental designs, investigators used endotoxin which had been extracted from Gram-negative bacteria with hot phenol. This results in the formation of large molecular aggregates (1,000 kD) which are excluded by the dialyser membrane. However, dialysate contains live bacteria and enzymatic degradation of endotoxins into small molecular weight fragments. Degraded endotoxin is comprised of lipid A with the unique R core structure attached to a single O-polysaccharide side chain [28].

These endotoxin fragments are reactive in the Limulus assay; however, when further degradation takes place, and the O-polysaccharide is removed, the solubility of the remaining Lipid A markedly decreases and this is associated with decreases in Limulus reactivity. Protein may help solubilise these fragments.

In addition, only the LAL-non-reactive carbohydrate moiety of leads to complement activation [28]. Thus, negative LAL reactivity in the blood side could still be associated with stimulation of IL-1 *via* a microbial mediated mechanism. Endotoxin sub-units isolated from the blood of human subjects are less than 1 kD, but the majority of endotoxin fragments are about 20-30 kD [29]. We have recently reported a model of *in vitro* closed loop hemodialysis in which human blood was dialysed in the absence or presence of purified *E. coli* endotoxin (1 µg/ml). The spontaneous production of IL-1 from monocytes was then measured after 4 hours of recirculation of the blood in the closed loop [30].

Regenerated cellulosic capillary dialysers were used and the 24 hour monocyte supernate was used to assess IL-1 production. Under the conditions where there was endotoxin-free dialysate, no significant release of IL-1 during a subsequent 24 hour incubation took place; in contrast, IL-1 production increased markedly when endotoxin was present in the dialysate. In a similar study, IL-1 inducing substances have been isolated from the blood compartment containing only culture medium and human plasma when endotoxin or bacterial filtrate was present in the dialysate [31]. Recently support for these observations have been presented from *in vivo* dialysis [32]. Non-endotoxin molecules can also induce IL-1; these include polypeptides exotoxins (20-30 kD) from *Staphylococcus aureus* [33] and muramyl dipeptides (fig. 1) [34]. Toxic shock toxin induces IL-1 at 20 pg/ml and muramyl dipeptides can induce IL-1 at concentrations as low as 12 pg/ml (fig. 2) [34]. Muramyl dipeptides are small molecular fragments of the glycoprotein cell wall common to all bacteria and fungi. Neither staphylococcal toxins nor the muramyl dipeptides are reactive in the Limulus test. During hemodialysis, muramyl dipeptides generated in the dialysate could pass through the membrane and stimulate IL-1 production. In general, however, the intact glycopeptide cell wall requires a specific enzymatic cleavage step and this is accomplished by human lysozyme and related enzymes found in plasma. These enzymes are the major secretion products of granulocytes and are released as a result of degranulation of the neutrophil on activation by the dialysis membrane [35]. In addition, muramyl dipeptides have been found in sterile CAPD drain fluid [34], which has yielded IL-1 after chromatographic separation of circulating protein inhibitors of IL-1. The maximal IL-1 activity corresponded with maximal stimulation of

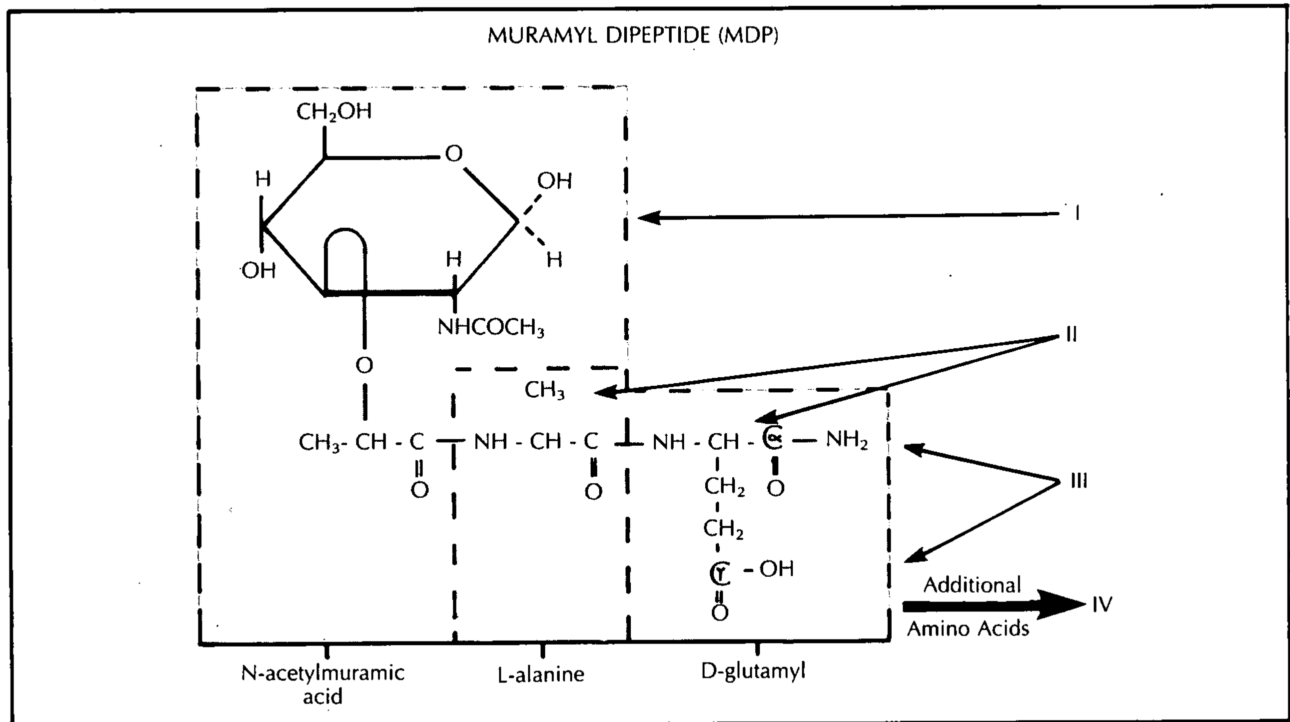

Fig. 1.—The chemical structure of muramyl dipeptide derived from bacterial glycoprotein. Lyzozymes cleave the peptide chain at site III.

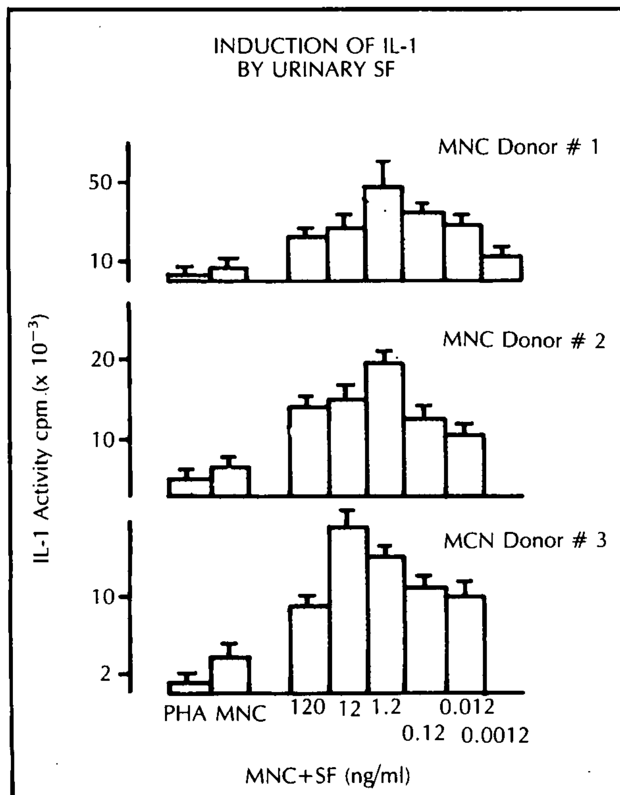

Fig. 2.—The potency of muramyl dipeptide (SF) (otherwise known as the sleep factor) in stimulating human blood monocytes (MNC) at 12 pg/ml (0.012 ng/ml) (derived from ref. 34).

fibroblasts on an *in vitro* assay [36]. This data suggests that peritoneal fibrosis may be related to muramyl dipeptide activation of macrophages. The origin of the muramyl dipeptides in the CAPD drain fluid still remains speculative.

Non microbial mechanisms of IL-1 induction during hemodialysis

There is no demonstrable defect in the ability of human blood monocytes from uremic patients on chronic hemodialysis or chronic ambulatory peritoneal dialysis to produce IL-1 [37]. In the absence of endotoxin or any other detectable stimulant, human blood monocytes which settle and spread onto plastic or glass surfaces demonstrate transcription of mRNA for IL-1, whereas non-adherent mononuclear cells circulating in the blood have no detectable IL-1 mRNA. IL-1 mRNA has been detected within 15 minutes of attachment. However, of considerable importance for hemodialysis and bioincompatibility in general is the fact that the IL-1 synthesised by monocytes activated by adherence remains intracellular. The function of IL-1 inducers seems to be involved with the mechanisms of processing the precursor IL-1 and secreting it into the medium. During hemodialysis, although it remains to be established, attachment of blood monocytes to the membrane would be a sufficient enough signal to de-repress the IL-1 gene and initiate transcription of mRNA. Indeed, preliminary results have surprisingly

shown that healthy donor blood monocytes incubated in a siliconised Petri dish show more activation for IL-1 mRNA transcription when PAN membranes were used compared to regenerated cellulose [38]. The role of substances such as endotoxin or other microbial toxins or products is to accelerate the processing of translated IL-1 mRNA. Adherent monocytes transcribe as much as 5 % of their total polyadenylated pool into IL-1 mRNA. We know of no other inducible molecule with this magnitude of response. Indeed, it suggests that for short periods of time, IL-1 is the major synthetic product of the activated monocyte.

In terms of IL-1 production during hemodialysis, these findings on the molecular biology of IL-1 require a new assessment. For example, monocytes adhering to the dialyser membrane become activated and small amounts of microbial toxins or products diffusing through the membrane could signal the processing of intracellular IL-1. C_5a, a known IL-1 inducer [39], and one which is produced as a result of serum contact with regenerated cellulosic membranes when the 5th component of complement is activated by contact with OH radicles on the surface of the membrane, would be in a high concentration in the vicinity of adhering monocytes. A recent study supports a nonmicrobial activation of monocyte IL-1 production by complement generating dialysis membranes during sham dialysis with human volunteers, where blood was recirculated through cuprophan membranes without the presence of dialysate [40]. Other factors such as the ionic components of the dialysis fluid may also contribute to the processing of intracellular IL-1. We have recently shown that sodium acetate has a pronounced effect on the normal human blood monocyte IL-1 production *in vitro* at concentrations likely to be encountered by adherent monocytes on the dialysis membrane [41]. In addition, other factors related to dialysis may be involved and will require experimental models. Recruitment of monocytes from the bone marrow during dialysis will permit large number of cells to interact with the membrane and allow for a greater magnitude of the response in patients than that which can be evaluated *in vitro*. Precisely how many monocytes and what type of monocyte interacts with the membrane remains to be established. The importance of these observations of IL-1 at the molecular level, however, re-inforce the need for biocompatibility of dialysis membranes.

In summary IL-1 is a family of polypeptides with molecular weights between 4 and 17,000 daltons which are produced by a variety of cell types in response to injury, infection, inflammation or immunological challenge. Two separate gene products coding for human IL-1 precursors (mw 31,000 daltons) have been cloned and the entire amino acid sequences are known. These precursor polypeptides are processed into smaller biologically active IL-1 peptides. IL-1 has diverse biological properties; for example, it causes fever, hypozincemia, neutrophilia and IL-2 production. In addition, it is a potent catabolic agent causing bone, muscle and cartilage breakdown whilst stimulating endothelial cell and fibroblast proliferation. It also enhances sleep and acute phase protein synthesis at the expense of albumin synthesis.

Many of these and other biological properties of IL-1 can be observed in patients on longterm hemodialysis. The human blood monocyte appears to be a major source of IL-1 and exposure to pg/ml. concentrations of endotoxin or even adherence to foreign surfaces results in the transcription and translation of large amounts of mRNA coding for IL-1. In this paper, the multiple biological activities of human monocyte and recombinant IL-1 has been discussed with particular emphasis on those activities relevant to hemodialysis. In addition, the various mechanisms (endotoxin passage through the dialyser membrane, the membrane itself, C_5a and acetate), which activate blood monocytes to produce IL-1 during hemodialysis has been reviewed.

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