Effect of Serum Precoat on Complement Activation in Membrane Oxygenators: An In Vitro Study

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Abstract

Previous studies have shown that extracorporeal circulation devices can activate the complement cascade via the alternative pathway. Anaphylatoxins (C3a and C5a) generated by complement activation may lead to pulmonary leukocyte sequestration, pulmonary edema, granulocytopenia and microvascular lung damage. This is of particular concern in patients on cardiopulmonary bypass. The purpose of this study was to examine the impact on complement activation of precoating membrane oxygenators with serum. A pool of recalcified human serum was prepared from fresh frozen plasma stored in citrate phosphate dextrose. SciMed membrane oxygenators (N=4) were primed with 200 ml aliquots of pooled serum which were recirculated through the oxygenators for 180 minutes at 37°C. The primary recirculation serum C3a levels (mean ± SD) measured by radioimmunoassay techniques at 0, 60, 120, and 180 minutes were 122.25 ± 26.22, 430.25 ± 164.39, 456.0 ± 154.16 and 577.5 ± 163.07 ng/ml respectively. Each oxygenator was then flushed with saline and reprimed with a fresh 200 ml aliquot of pooled serum which was recirculated for 180 minutes at 37°C. The secondary recirculation serum C3a levels (mean ± SD) measured at 0, 60, 120, and 180 minutes were 130.75 ± 15.67, 213.25 ± 58.24, 283.75 ± 27.24 and 301.75 ± 19.18 ng/ml respectively. Serum C3a levels were higher in the primary serum than the secondary serum in all oxygenators. These results suggest that preconditioning the oxygenator may be a way to moderate complement activation during cardiopulmonary bypass.

Introduction

It is now widely accepted that contact between human blood and foreign surfaces such as silicon, nylon, or cellulose during extracorporeal circulation activates the alternative pathway of the complement cascade (1-7). Chenoweth and colleagues demonstrated that the complement cascade is activated by the initiation of cardiopulmonary bypass and that complement production continues through the duration of the procedure (8).

The complement cascade consists of a set of circulating serum proteins which are activated in support of both humoral and cellular defenses. During activation, the complement proteins C3 and C5 are enzymatically cleaved to yield C3a and C3b, and C5a and C5b, respectively. The C3b and C5b moieties function as intermediaries in the complement cascade, participating in the construction of the membrane attack complex which leads to lysis of the antigenic cell. The C3a and C5a moieties are called anaphylatoxins and have wide-ranging physiologic effects throughout the body (1,9).

Anaphylatoxins induce physiologic changes that facilitate the body’s host defense response to an antigenic stimulus. The anaphylatoxin C3a causes smooth muscle contraction and increased vascular permeability. A typical manifestation of these changes would be the redness and edematous swelling seen in areas surrounding a localized infection. The anaphylatoxin C5a activates neutrophils, causing them to migrate to sites of infection, adhere to the endothelial cells in the blood vessels local to the infected site, and then release bactericidal superoxide radicals. C5a also induces smooth muscle spasm, mast cell degranulation, and histamine release, which further increases local vascular permeability and swelling.

Complement activation can be stimulated via two pathways. The classical pathway is triggered by either immunoglobins bound to cell surface antigens on immune complexes, whereas the alternative pathway is activated by exposure of blood to foreign surfaces (7).

The most common natural activator of the alternative complement pathway is the lipopolysaccharide layer of gram-negative bacteria (9). It has also been shown that the molecular structure of many foreign surfaces also activate the alternative pathway (4, 6, 8,10). Because blood is exposed to foreign surface areas in extracorporeal devices, the anaphylatoxins C3a and C5a are produced by complement activation during extracorporeal circulation. The anaphylatoxins may trigger serious pathological sequelae. Pulmonary edema, increased pulmonary artery pressures, respiratory distress, leukopenia as well as other symptoms may be seen in varying degrees during and after hemodialysis (5,8), and cardiopulmonary bypass (CPB) (1,3).

The physiologic effects attributed to elevated serum anaphylatoxin levels are more easily discernable in hemodialysis patients
than in those undergoing cardiopulmonary bypass. The stable, chronic dialysis patient coming in for serial treatments can serve as his or her own control, whereas the surgery patient undergoing cardiopulmonary bypass presents with many clinical symptoms which potentially mask the manifestations of complement activation (1, 2).

Some of the symptoms attributable to the activation of complement by a dialysis membrane often appear as a complex of symptoms referred to as the "first-use syndrome" (10). The name "first-use syndrome" came from the observation that symptoms tend to manifest themselves when new dialyzer membranes are used - especially membrane made of a cellulose. When dialysis membranes that have been used (exposed to plasma) are stored in formalin and reused, these symptoms are not seen (10). Upon investigation, it was found that serum anaphylatoxins levels during the initial use of a dialysis membrane were much higher than those in subsequent sessions (5, 11).

It has been speculated that during serial dialyzer use, the dialyzer membrane becomes saturated with serum proteins, which mask the sites on the membrane surface which activate the complement alternative pathway, thereby blocking any further complement activation (7). It has also been noted that plasma C3a levels peak within two hours of initiation of neonatal ECMO and return to normal after 24 hours, although the procedure may last for days or weeks (12). This finding also supports the notion that complement activation subsides when potential membrane activating sites are masked by serum proteins.

Although several manufacturers are currently investigating techniques to produce biomaterials that will not activate complement, complement activation remains a potential problem in many clinical settings. One solution to control complement activation would be to precoat the blood interface of the extracorporeal device with serum protein, some fraction thereof or even a totally different substance which would mask activating sites thereby precluding alternate pathway complement activation.

In order to investigate the notion that complement activation by a membrane oxygenator may be limited through its previous exposure to serum, we performed a series of in-vitro experiments comparing the levels of the anaphylatoxin C3a generated by recirculating human serum through new, unused oxygenators versus those that had been previously exposed to serum. To a large degree, our methods are based upon those devised by Henderson and Chenoweth for the laboratory analysis of hemodialyzers (13).

**Laboratory Extracorporeal Oxygenation Circuit**

The test circuit shown in Figure 1 consisted of a small closed loop of 1/4" Cobe polynylchloride tubing leading from a 300ml transfer bag through a rollerhead pump into a 0.4 m² silicon Sci-Med membrane oxygenator and then back into the transfer bag which served as a reservoir. The reservoir was partially clamped in order to insure that the serum flowing through it would follow a circuitous path and be well mixed.

The circuit was primed with 350ml of 0.9% NaCl. The reservoir bag was placed in a water bath and the prime recirculated by the pump until a temperature of 37°C was reached.

**FIGURE 1: Test Circuit**

![Test Circuit Diagram]

**Materials and Methods**

**Preparation of Pooled Human Serum**

Serum complement activity is maintained in fresh frozen plasma stored using normal blood banking procedures (13,14). Fresh frozen plasma (all from type O-positive donors) stored in citrate phosphate dextrose (CPD) was obtained from a local blood bank (Red Cross Northeast Regional Blood Bank, Dedham, MA). Plasma was thawed and mixed in a large beaker to insure a homogenous pool. Plasma was then decanted into 500 ml polyethylene centrifuge bottles and warmed in a water bath to 37°C. Once warmed, 30 ml of 0.1M CaCl₂ and 1000 units of bovine thrombin were added to each of the bottles, which were incubated at 37°C for 30 minutes to allow clot formation. Serum was expressed via centrifugation from the fibrin clot in each bottle, divided into 200 ml aliquots and then stored at -70°C.

Chenoweth and Henderson demonstrated that sufficient quantities of Ca++ and Mg++ must be present in order for recalcified serum to be used as a source of functionally active complement components (13). Therefore, serum Ca++ and Mg++ concentration were adjusted to 22 mg% and 5 mg% respectively with CaCl₂ and MgCl₂ just prior to the beginning of each procedure.
prime. The serum was then recirculated at 37°C at a flowrate of 0.35 LPM through the oxygenator for 180 minutes. The gas inlet ports of the oxygenator were sealed throughout the procedure.

Samples from the serum prime were taken just prior to its introduction into the circuit (time zero minutes), and then at 60, 120 and 180 minutes thereafter. Each sample was placed in a Vacutainer tube containing 5.5mg of disodium ethylenediamine tetraacetate (EDTA) to prevent any further activation of complement. Samples were frozen immediately and maintained at -70°C until they were analyzed.

In order to measure complement activation which occurred independently of the effects of the oxygenator circuit, 10ml of serum from the same aliquot was incubated undisturbed in a test tube at 37°C throughout the procedure. This was used as our control and samples were collected for analysis at the same time interval as the circuit serum.

After 180 minutes of recirculation, the serum was flushed out the circuit with 2L of 0.9% NaCl. The circuit remained completely primed during the flush procedure. At no time was air, that might form bubbles, allowed to enter the circuit.

Secondary Recirculation

In order to assess the impact of membrane preexposure to serum on complement activation, 200ml of the saline prime was displaced from the circuit in which the primary recirculation had taken place. In its place 200ml of pooled serum were added to the circuit. The serum was then recirculated for 180 minutes at a temperature of 37°C and at a flowrate of 0.35 LPM. Control serum incubation and sampling was performed in a manner identical to the primary recirculation.

The entire procedure consisting of a primary and secondary recirculation and their respective controls was carried out on four oxygenators.

Anaphylatoxin Radioimmunoassays

Serum levels of C3a antigen were quantitated by the radioimmunoassay (RIA) methods described by Hugli (17) using commercially available assay kits. Results were converted to ng/ml of serum.

Results

The mean concentrations of C3a in control, primary and secondary serum samples are shown in Tables 1 and 2. The changes in mean C3a levels from baseline were compared in both the Primary and Secondary recirculation serum and shown in Figures 2 and 3. Time-dependent production of C3a is demonstrated by these data. The serum C3a level increases from baseline in all systems with the Primary recirculation serum values showing the largest overall increases. For example, the mean secondary recirculation serum C3a levels reach only 52% of the primary mean recirculation serum C3a levels (301.75 v 577.5 ng/ml respectively).

The impact of prior membrane exposure to serum on complement activation is reflected in the relationship of the recirculation serum and the control serum C3a values. The

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primary serum C3a levels increased 429 ng/ml above the control value at 180 minutes while the secondary serum C3a levels increase only 11 ng/ml above the control value at 180 minutes. The relationship among all values is shown in Figure 4.

**FIGURE 4: Serum C3a Levels (ng/dl) During Primary and Secondary Recirculation**

![Graph showing C3a levels during primary and secondary recirculation](image)

**Discussion**

The biomaterial membranes used in extracorporeal devices are assumed to be the primary source of the complement cascade activation commonly seen during hemodialysis and cardiopulmonary bypass. It is believed that this process occurs via the alternative pathway and begins when a metastable C3b molecule binds to a reactive site on the membrane surface. This C3b molecule is then transformed into C3 convertase by factor B. The surfacebound C3 convertase enzymatically cleaves any nearby C3 molecules into C3a and C3b moieties. The C3 convertase binds to the newly formed C3b molecule, and the whole complex is transformed into surface-bound C5 convertase. The C5 convertase then splits local C5 molecules into C5a and C5b moieties. The liberated C3a and C5a molecules are then free to circulate in the blood as anaphylatoxins (1,5-7). It is suspected that these anaphylatoxins are the source of various unexplained adverse effects (i.e., pulmonary edema, respiratory distress, leukopenia and others) sometimes associated with extracorporeal devices such as hemodialyzers (4,7,10) or cardiopulmonary bypass oxygenators (1-4).

The increase in the levels of the anaphylatoxin C3a in the primary recirculation serum was consistent with those seen in other studies of complement activation in new, unused membrane oxygenators (3,12, 15). However, the C3a production in the secondary recirculation serum was markedly decreased. This finding supports the concept that the previous exposure to human serum will limit complement activation in silicone membrane oxygenators when new serum is introduced. Analogous results have been seen in vivo and in vitro studies of re-used hemodialyzers (10,11).

It has been suggested that the saturation of all possible reactive binding sites for C3b by complement and other serum proteins is a rate limiting factor for complement activation on biomaterial membrane surfaces (7). Our findings tend to support this hypothesis.

The notion that some form of preconditioning solution may render an oxygenator less complement-activating may have clinical value. However, due to epidemiological factors (hepatitis, HIV, etc.), human serum would not be a well suited choice for such a preconditioning solution. Even so, one can envision the development of a "preconditioning cocktail" priming solution (perhaps based on albumin, hetastarch, or some other substance) that could be used to prepare the membrane prior to use.

In conclusion, we have demonstrated that exposure to human serum limits complement activation in silicone membrane oxygenators. The masking of complement reactive binding sites is thought to be the reason for the reduction of C3a levels seen in our study. Analogous studies are required in other types of oxygenators (such as hollow fiber membranes) because of design differences that may affect the mode of complement activation in those devices. Further investigation to determine if a similar reduction in oxygenator-mediated complement activation may be produced by other substances that might be included in the circuit prime is also indicated.

**References**