

## Nylon Fiber Leukapheresis: Associated Complement Component Changes and Granulocytopenia

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The possible role of complement activation in the development of transient granulocytopenia during nylon fiber filtration leukapheresis (FL) was studied in five normal donors. No changes in hemolytic complement ( $CH_{50}$ ) titers were observed in the donors' circulating plasma at any point in the procedure. However, comparison of pre- and postfilter blood samples drawn 1 min after donor blood passed the filters revealed decreases in several complement (C) components in postfilter plasma. Marked reductions in  $CH_{50}$ , hemolytic C4 and C2, and properdin protein levels were observed, as well as moderate but significant reductions in hemolytic C1, C3, and C5 titers. C1q and factor B protein levels did not fall. Partial C3 cleavage was also demonstrated in postfilter samples by electrophoretic methods. At 5 min or later, reductions in  $CH_{50}$  titers were much less marked, though significant. Maximal granulocytopenia in the donors was observed within minutes after reinfusion of the first postfilter blood. Circulating granulocytes increased to baseline or higher levels after 10–30 min, while reinfusion of postfilter blood (exhibiting only mild reductions in  $CH_{50}$  titer) continued. In a sixth donor, after the initial FL-associated granulocytopenia had cleared, a fresh set of filters was inserted in the line, and the sequence of C component reduction and granulocytopenia was repeated. The pattern of the observed C component changes was consistent with the activation of the classical C pathway, and possibly also the alternative C pathway, immediately after initial contact of blood with the nylon filters. The temporal association of these C changes with the development of the granulocytopenia in vivo within minutes after reentry of this postfilter blood into the donors' circulation suggests a causal relationship.

**F**ILTRATION LEUKAPHERESIS (FL) is a technique commonly used for collecting granulocytes for transfusion. When FL is performed on normal donors, a marked granulocytopenia occurs within minutes after blood which has passed through the nylon filters returns to the donor.<sup>1,2</sup> The granulocytopenia lasts 10–15 min and is usually followed by a significant granulocytosis, which may persist throughout the entire procedure.

The mechanism of the granulocytopenia, which occurs too rapidly and is too profound to be the result of simple cell removal, is unexplained. There is evidence that blood interacting with the nylon filters may release or activate a humoral substance(s) responsible for this phenomenon.<sup>1,2</sup> Schiffer et al.<sup>2</sup> have suggested that leukocytes reacting with nylon are responsible. However, Rubins et al.<sup>1</sup> have found that plasma alone, when passed through the nylon filters, may also produce granulocytopenia. Studies in rabbits indicate that complement (C)

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activation occurs when homologous plasma is incubated with nylon fibers *in vitro* and that a marked granulocytopenia occurs after such plasma is reinfused.<sup>3</sup> This granulocytopenia-producing activity has been attributed to a heat-stable, low molecular weight factor derived from or dependent upon C activation.<sup>3</sup> In a preliminary study, Fehr et al.<sup>4</sup> have reported that neutrophils from donors undergoing FL have reduced levels of lysosomal enzymes and impaired sensitivity to C-derived chemotactic factors.

There is, however, no direct evidence in humans demonstrating C activation or its role in inducing granulocytopenia during FL. The purpose of this report is to document marked changes in the levels of multiple C components during the earliest stages of FL in humans, and a striking temporal relationship of this phenomenon to the development of granulocytopenia *in vivo*.

## MATERIALS AND METHODS

### *Donors*

All donors were healthy males and met American National Red Cross criteria for plasmapheresis donors. Informed consent was obtained.

### *Leukapheresis*

FL was performed on five donors using the filtration leukapheresis set supplied by Fenwal Laboratories, Morton Grove, Ill. The set was filled (primed) with 0.9% NaCl solution containing heparin sulfate 1 U/ml. Donors were given a single intravenous injection of 17,500 units heparin sulfate 10 min before FL was begun. No other medications were given before or during the procedure. FL was otherwise conducted according to the manufacturer's instructions. A 50 ml/min flow rate was used in all donors.

In one additional experiment, FL was stopped after 90 min, the FL set removed and replaced with a new set primed as before, and FL was reinstated. The donor was given an additional 5000 units heparin sulfate 30 min later.

### *Sampling*

Using a three-way stopcock, blood was sampled simultaneously from the pre- and postfilter limbs of the leukapheresis set at various times during the FL procedure. Prefilter specimens (i.e., blood from the donor) were obtained at a port about 30 cm from the phlebotomy site (and 1.8 m from the filter). Postfilter specimens were obtained at a port about 1.6 m from the venipuncture site and 1.3 m from the filters. Preapheresis blood samples were obtained immediately prior to the start of the procedure, approximately 10 min after heparin had been administered. During the procedure, samples were taken from both pre- and postfilter sites at 1, 5, 10, and 30 min after donor blood first appeared in the tubing on the distal side of the filters. Plasma was separated by centrifugation within 10-15 min of sampling and promptly frozen in aliquots at  $-70^{\circ}\text{C}$  until assayed.

Because of the likelihood of hemodilution with the priming solution in the 1-min postfilter specimens, hematocrits were performed on these samples and compared with simultaneously drawn prefilter specimens where hemodilution was not present. The amount of hemodilution is shown in Table 1, and all postfilter complement values for the 1-min specimens were corrected accordingly. Dilution in samples obtained later than 1 min was negligible. The priming solution itself did not interfere with C titrations.

White blood cell counts (Coulter ZBI apparatus) and differential counts (200 cells) were obtained for each time interval on the prefilter sample.

### *Complement Assays*

Functional, hemolytic titrations for total complement, C1, C4, C2, C3, and C5 were performed as previously described.<sup>5</sup> Single radial immunodiffusion assays for C4, C3, and C5 utilized commercial kits (C4 and C5; Meloy Laboratories, Springfield, Va.; C3; Hyland Division, Travenol Labora-

**Table 1. Hemolytic Complement Assays on Samples Obtained Simultaneously on Both Sides of the Nylon Filters 1 min After Donor Blood Became Visible Exiting From Filters**

Donor	CH <sub>50</sub> *		C1†		C4†		C2†		C3†		C5†		Dilution‡ (%)
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
1702	101	37	85.3	42.5	77.5	33.3	9.4	1.2	38.6	30.1	206	186	7
1699	114	44	71.3	38.3	81.6	35.1	8.2	1.7	34.9	24.6	236	207	7.7
1694	108	54	68.9	61.2	91.9	69.1	6.5	1.3	37.3	26.7	214	185	16.7
1693	119	40	57.9	40.8	132.6	75.4	7.8	0.8	33.0	23.7	216	186	10
1691	155	72	51.5	49.2	118.7	83.2	8.7	2.8	33.0	23.8	217	213	4.9
Normal pool§	149		75		100		8.5		37		250		
Mean	119.4	49.4	66.9	46.4	100.5	59.2	8.1	1.6	35.4	25.8	217.9	195.4	
SEM	8.40	5.66	5.21	3.68	9.64	9.36	0.43	0.31	1.01	1.08	4.42	5.29	
Paired <i>t</i>	13.4466		2.702		7.157		12.899		24.953		4.654		
<i>p</i>	< 0.0005		< 0.05		< 0.005		< 0.0005		< 0.0005		< 0.005		

\* U/ml (normal range 80–160). Postfilter values are corrected for dilution.

† U/ml ( $\times 10^{-3}$ ). All postfilter values are corrected for dilution.

‡ Calculated from hematocrit changes.

§ Pool of 30 normal sera assayed concurrently with donor samples.

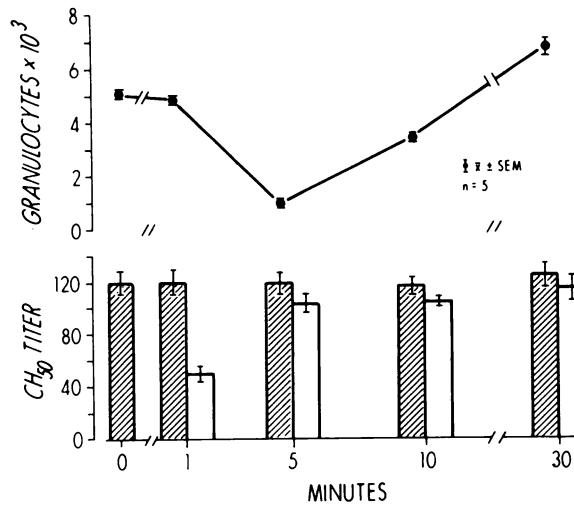
atories, Costa Mesa, Calif.). Similar radial immunodiffusion assays were performed for properdin factor B and C1q utilizing monospecific antisera obtained from Atlantic Antibodies (Westbrook, Me.). Monospecific antiproperdin for radial immunodiffusion was prepared by immunizing a rabbit with highly purified human properdin. Immunoelectrophoretic analysis for conversion products of C3 and properdin factor B was performed as reported.<sup>5</sup>

**Statistics**

Statistical analyses of the differences between pre- and postfilter C values utilized the single-tailed paired *t* test for hemolytic values and the double-tailed paired *t* test for immunodiffusion data.

**RESULTS**

Figure 1 illustrates the temporal relationship between the donors' circulating granulocyte counts and whole hemolytic C (CH<sub>50</sub>) titers in blood samples ob-



**Fig. 1. Changes in hemolytic complement (CH<sub>50</sub>) titers and granulocyte counts in five donors during FL. Shaded bars, CH<sub>50</sub> titers in prefilter blood samples; open bars, postfilter samples. Differences in CH<sub>50</sub> titers were significant for all time intervals (*p* = 0.025 or less). Zero time, prefilter specimen obtained 10 min after heparin administration.**

**Table 2. Radial Immunodiffusion Assays on Samples Obtained Simultaneously on Both Sides of the Nylon Filters 1 min After Donor Blood Became Visible Exiting From Filters**

Donor	C1q ( $\mu\text{g/ml}$ )		C4 (mg/dl)		C3 (mg/dl)		C5 ( $\mu\text{g/dl}$ )		Factor B ( $\mu\text{g/ml}$ )		Properdin ( $\mu\text{g/ml}$ )	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1702	187	188	18	14	112	124	105	104	254	261	15.5	2.2
1699	152	150	20	14	115	114	97	98	224	243	14.6	2.2
1694	156	166	27	16	130	126	105	108	231	262	16.3	4.1
1693	197	208	37	27	105	110	97	100	250	270	15.5	2.8
1691	157	152	31	23	99	94	114	110	238	250	9.4	1.2
Normal range	126–190		14–51		70–220		67–137		157–293		11–27	
Mean	169.8	172.8	26.6	18.8	112.2	113.6	103.6	104	239.4	257.2	14.3	2.5
SEM	8.26	9.94	3.13	2.35	4.69	5.13	2.82	2.04	5.04	4.26	1.11	0.42
Paired <i>t</i>	0.935		6.091		0.441		0.302		4.377		12.934	
<i>p</i>	NS		<0.01		NS		NS		<0.02		<0.001	

Postfilter values are corrected for dilution.

tained simultaneously pre- and postfilter. Prefilter  $\text{CH}_{50}$  values were normal throughout the procedure. In samples collected 1 min after donor blood first exited the nylon filters, marked reductions in  $\text{CH}_{50}$  titers were evident in the postfilter samples. By 5 min, postfilter  $\text{CH}_{50}$  titers had returned to near normal levels and remained so throughout the rest of the observation period. Nevertheless, the falls in postfilter  $\text{CH}_{50}$  titers at 5, 10, and 30 min, although small, were consistent and statistically significant ( $p < 0.001$ ). Granulocyte counts were unchanged at 1 min but fell to a mean level of 18% of baseline by 5 min (Fig. 1). Recovery of the peripheral circulating granulocyte count was rapid and by 30 min a modest granulocytosis ensued (Fig. 1), which usually persisted throughout the procedures (not shown).

For more detailed analysis of the C changes, attention was focused on the 1-min samples, which had shown the greatest reduction in postfilter  $\text{CH}_{50}$  titers. By hemolytic assays (Table 1), reductions in  $\text{CH}_{50}$ , C4, and C2 were most striking; the postfilter falls in C1, C3, and C5 were smaller but statistically significant (Table 1). All prefilter C determinations were within normal limits.

Concentrations of C proteins in the 1-min samples, measured by radial immunodiffusion, are shown in Table 2. There were no significant differences between pre- and postfilter levels for C1q, C3, and C5. C4 levels were moderately decreased, properdin was markedly reduced, and factor B levels were slightly increased in the postfilter samples.\*

Immunoelectrophoresis performed on the 1-min pre- and postfilter samples from each experiment revealed partial electrophoretic conversion of C3 in all postfilter samples. Conversion of factor B was not detectable by this method, but a more sensitive electroimmunodiffusion method (kindly performed by Dr. Martin Klemperer) revealed modest conversion of factor B in the postfilter samples.

\*Measurements of C proteins by immunodiffusion methods may be falsely elevated by the presence of cleavage products, leading to underestimation of the degree of consumption or even to an apparent increase in concentration.

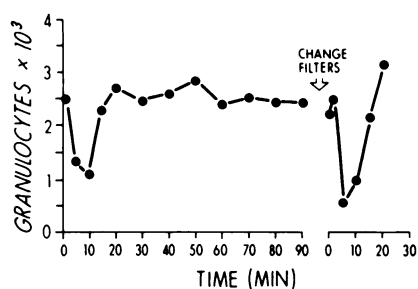


Fig. 2. Changes in granulocyte count during FL and after second set of filters was inserted.

Figure 2 demonstrates the reoccurrence of granulocytopenia when a new set of nylon filters was inserted during an FL procedure. CH<sub>50</sub>, C1, C4, C2, and C3 hemolytic titers fell in both postfilter specimens obtained at 1 min, while C5 remained unchanged after the second filtration (Table 3). Protein levels of C4 and properdin were also reduced in both postfilter samples (Table 3).

DISCUSSION

Significant destruction or consumption of multiple C components has been documented during FL. The most marked changes in C occur very early in the procedure, apparently as a result of first contact of the heparinized blood with the saline-primed filters. The maximum fall in circulating granulocytes occurs within minutes after this postfilter blood reenters the donor, as described previously.<sup>1,2</sup> Although low-grade C consumption may continue throughout the procedure (Fig. 1), the leukocyte counts recover rapidly and surpass pretreatment levels. It is not clear why this recovery should take place. One possibility is that the donor rapidly develops a refractoriness to a granulocytopenia-producing factor. If so, the effect is transient; later in the procedure the donor promptly exhibits a second cycle of granulocytopenia after reinfusion of blood which has just passed through a pair of fresh filters (Fig. 2). Alternatively, it can be postulated that the granulocytopenia-producing activity is a product

Table 3. Complement Component Assays on Samples Obtained Simultaneously on Both Sides of the Nylon Filters 1 min After Donor Blood Became Visible Exiting From the Filters

Filter	Hemolytic Assays*											
	CH <sub>50</sub>		C1		C4		C2		C3		C5	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
First	102	55	91.7	40.0	55.1	44.9	8.4	3.3	42.3	31.3	230	214
Second	101	34	103.7	39.3	64.2	28.6	8.4	1.2	35.6	28.0	215	214

Filter	Immunodiffusion Assays									
	C1q (μg/ml)		C4 (mg/dl)		C3 (mg/dl)		Factor B (μg/ml)		Properdin (μg/ml)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
First	128	131	28	18	121	116	254	220	18.2	2.6
Second	134	139	27	19	121	116	254	253	17.0	1.1

Postfilter values are corrected for dilution.

\*CH<sub>50</sub> titers in U/ml. All other hemolytic values in U/ml (x 10<sup>-3</sup>). See Tables 1 and 2 for normal values.

only of initial contact between blood and a "fresh" filter. It is of interest that the destruction or consumption of C components, as measured by CH<sub>50</sub> titers, also diminishes rapidly after the marked initial falls at 1 min (Fig. 1).

Closer analysis of the C changes provides evidence that components of both the classical and alternative pathways are affected (Tables 1-3). The pattern of classical C component reductions, i.e., moderate fall in C1 with more marked reductions in C4 and C2, is consistent with activation of C1 esterase. The finding of reduced C1 function (Table 1) without a fall in Clq protein (Table 2) raises the possibility that C1 is being activated without being bound and removed from solution. The moderate reductions in functional C3 and C5 would also be consistent with classical pathway activation, although a contribution from alternative pathway activation is possible.

Our analysis of the alternative C pathway changes is incomplete. The available data are somewhat puzzling in that properdin levels are so profoundly altered out of proportion to relatively small changes in factor B. It is possible that at least a portion of the properdin loss may result from a nonspecific affinity for nylon fibers. On the other hand, it is known that C3b is the major binding site for properdin on the immune complex.<sup>6</sup> Although we do not have evidence for large amounts of C3b binding on the fibers, the quantitative relationships are such that a small proportion of the plasma C3, binding as C3b, might bind a large percentage of the available properdin.

The mechanism by which C activation may occur during FL remains to be clarified. Aggregation of immunoglobulins on nylon fibers or generation of plasmin, which has C1-cleaving activity,<sup>7</sup> could initiate classical pathway activation. Alternative pathway activation could be a secondary consequence of a classical pathway-initiated feedback via C3b;<sup>8</sup> or it might result from direct contact of plasma with the foreign surfaces or, in part, from leukocytic enzyme release.<sup>9,10</sup> Direct cleavage of C components by leukocytic enzymes is also conceivable.<sup>9,11,12</sup> However, passage of plasma alone through the filters can induce granulocytopenia,<sup>1</sup> an observation which we have confirmed in additional studies, together with finding preliminary evidence for C consumption. This finding suggests that leukocyte-derived factors are not essential for the granulocytopenic effect, although they might be contributory when whole blood is being processed. These questions are a subject of continuing investigation.

The present studies do not establish a causal relationship between complement activation and granulocytopenia, but the temporal association is very suggestive. Administration of known C-activating substances (cobra venom factor, inulin, or aggregated  $\gamma$ -globulin) to rabbits produces an immediate neutropenia, followed by neutrophilia, similar to that seen in FL donors.<sup>3,13</sup> Furthermore, an analogy can be made with humans undergoing hemodialysis, during which alternative C pathway activation and granulocytopenia have been related to plasma contact with cellophane membranes.<sup>14</sup>

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