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Pathogenesis of Disseminated Intravascular Coagulation*

S. A. EVENSEN**, P. F. HJORT

According to classical theory, disseminated intravascular coagulation (DIC) is the pathological process that follows extensive generation of thrombin in systemic blood. Consumption of platelets and clotting factors is produced by thrombin, and bleeding, multiple necroses and organ dysfunctions result from small vessel obstruction by platelets and fibrin. Basically, this concept is still valid, but its simplicity tends to obscure the extreme complexity of the mechanisms involved.

For this review, we have selected the two crucial questions in the pathogenesis of DIC:

First, how does disseminated clotting start? DIC is described in an ever-increasing list of apparently unrelated disorders. This, we believe, reflects the many ways clotting may be triggered, and we shall first discuss the evidence bearing on the *trigger* problem.

Secondly, which factors determine the extent of tissue injury in DIC? A healthy organism can tolerate an episode of DIC with few ill effects. In fact, slow controlled defibrination may be used in the treatment of thrombotic disease [4]. Clotting may go on in patients with normal or even increased levels of thrombin-sensitive clotting factors, and the absence of characteristic tissue lesions at autopsy does not exclude the diagnosis. Obviously, the clinical effects of DIC depend only to some extent on the potency and quantity of the trigger. The second part of this presentation, therefore, deals with the many modifying factors which determine *if* and *where* fibrin is deposited.

A. The Triggering Mechanism of DIC.

1. Definitions

In man, DIC develops as an acute or chronic complication of an underlying or preceding disorder. Fig. 1 is an attempt to group some of these according to the possible main trigger. We define a *trigger* as an agent capable of initiating clotting in vivo, and we divide triggers in two groups: direct and indirect (Fig. 2). The direct triggers are capable of activating clotting in cell-poor plasma. The most important are tissue thromboplastin and several proteolytic enzymes, including some snake venoms; Fig. 1 suggests that they directly fit the "activation lock" of the coagulation mechanism. Accordingly, DIC is a

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complication to expect when cell membranes are broken and tissue extracts are released. The indirect triggers, such as bacterial endotoxin, do not trigger clotting in vitro. Thus, endotoxin does not itself fit the "activation lock" (Fig. 1) and must act through release or activation of a *mediator* (or a sequence of mediators) which in turn acts as a direct trigger. The nature of this final mediator is still a key problem in DIC. Other indirect triggers are soluble immune complexes, various particulate agents, lipids and many others. These indirect triggers may or may not work through related mechanisms, and many sources of mediators are possible (Fig. 3).
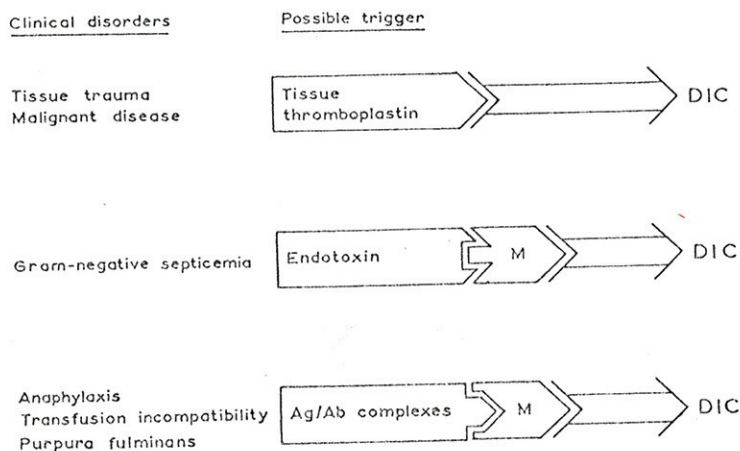
Fig. 1: DIC: Classification according to triggering mechanism. M: Mediator.

As an *accelerator* of clotting, we define a factor which accelerates the clotting process after it has been started by a direct trigger or a mediator. For example, agents which enhance polymerization of fibrin are accelerators, but not triggers.
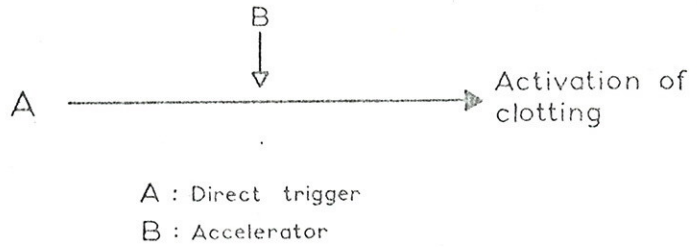
For the following discussion we have selected only a few triggers, namely bacterial endotoxin, immune complexes and a synthetic polymer, called Liquoid.

2. Endotoxin

a) *Activation of factor XII.* Endotoxin triggers clotting in man and in several animal species. In the properly prepared organism fibrin persists in the microcirculation, leading to the generalized Shwartzman reaction (GSR) or human equivalent disease [33]. We have already suggested that endotoxin initiates clotting by releasing one or several mediators (Fig. 3), mainly because it does not shorten the recalcification time of cell-poor plasma [74].

However, there is evidence suggesting activation of factor XII by endotoxin both in vitro and in vivo [43,49,50,64,75], possibly by a direct effect on the clotting mechanism. Thus, low factor XII levels were found in normal ani-

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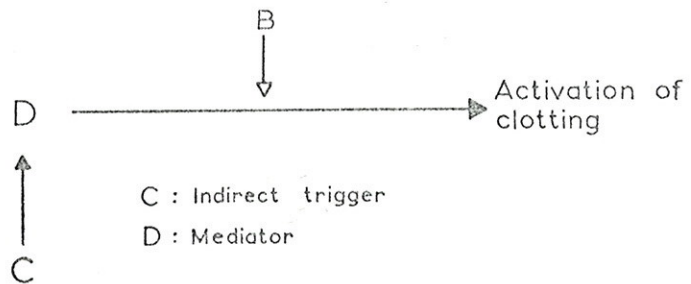a sequence
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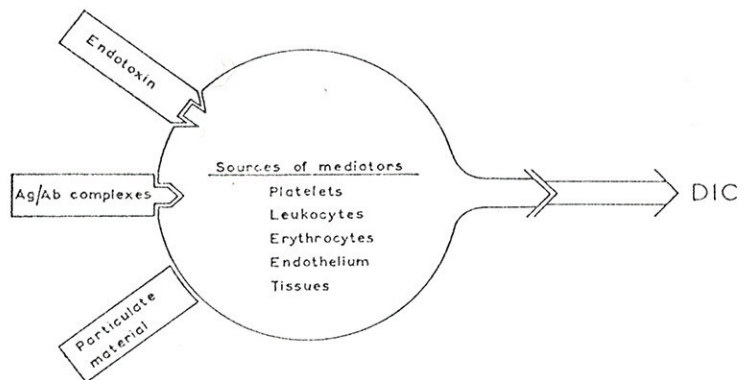Fig. 2 : Terminology of triggering mechanisms in DIC.

Fig. 3: Indirect triggers and possible sources of mediators.
(from Evensen and Jeremic: Platelets and triggering mechanism of intravascular coagulation, Brit. J. Haemat. *19*, 33 (1970))

mals injected with endotoxin, suggesting activation and subsequent removal of this factor. This effect may have been a result and not a cause of coagulation, since anticoagulation with warfarin eliminated it [43]. Furthermore, it is still uncertain whether activation of factor XII alone is sufficient stimulus for significant intravascular clotting. Kaolin, an activator of Hageman factor, does not produce the GSR when injected intravenously in prepared rabbits [85]. Whether ellagic acid, a soluble activator of factor XII, will induce the reaction in Thorotrast-prepared rabbits is disputed [7,79], but ellagic acid in combination with EACA and noradrenaline is effective [56]. These observations suggest that endotoxin activates factor XII in vivo and that activated factor XII can initiate DIC in the properly prepared animal, but it is still uncertain whether activation of factor XII is an essential step *in* or only a result *of* endotoxin-induced DIC.

b) *Release of clot-promoting platelet factors*. Platelets are generally held to be the primary target of bacterial endotoxin in vivo. Endotoxin reduces the electrokinetic charge of rabbit platelets [30], thereby facilitating massive aggregation both in vitro and in vivo, with subsequent release of platelet material that might serve as mediator of coagulation (Fig. 3). The most potent agent is platelet factor 3, a lipid factor masked in intact platelets, which accelerates clotting at least in vitro [15,35]. Platelets may also aquire tissue thromboplastin-like activity when incubated at 37° for 16 to 20 hrs in the presence of factor XII [5]. The chemical nature and in vivo significance of this activity are not yet known. Finally, endotoxin releases platelet factor 4 or antiheparin factor from rabbit platelets [45], a factor which may precipitate fibrin from soluble fibrin monomer complexes in a non-enzymatic reaction. In our opinion platelet factor 4 may serve as accelerator (Fig. 2), but not as mediator of coagulation. These data clearly show that platelets contain clot-promoting activity, but can platelet material *initiate* intravascular coagulation? This critical question is still open.

Several investigators have failed to induce intravascular coagulation by infusing large amounts of homologous disintegrated platelets [17,32]. Experiments with heterologous material have given confusing results. Rodríguez-Erdmann reported in 1965 [76] and confirmed in 1969 [77] that an extract of bovine platelets produced the GSR in Thorotrast-prepared rabbits. However, Müller-Berghaus et al. [60] were unable to reproduce this finding with a similar preparation of platelet factor 3, and workers from the same group also failed to produce fibrin deposits in the glomerular capillaries in rabbits and monkeys with soybean phospholipids representing up to 420 per cent of the platelet factor 3 activity normally available in an animal [56].

Recent work from our laboratory may explain some of these conflicting results [20]. Normal and Thorotrast-prepared rabbits received an amount of frozen-thawn homologous platelet material at least equivalent to the number of circulating platelets in a normal animal of the same weight. Fig. 4 shows that in normal rabbits fibrinogen was not consumed. In Thorotrast-prepared animals, however, the disappearance of labelled fibrinogen was increased, and the thrombin-sensitive clotting factors decreased significantly. None of the

animals developed the GSR. Thus, platelet injury obviously can initiate mild clotting in properly prepared animals, but the normal animal clears injured platelets and their weak clot-promoting activity without significant clotting. Attempts have also been made to test the role of the platelet directly by injecting endotoxin in antibody-induced thrombocytopenic animals [29,44,47]. Margaretten and McKay [47] injected the antiserum 16 hrs after the first injection of endotoxin and prevented clotting at this time with heparin. Four
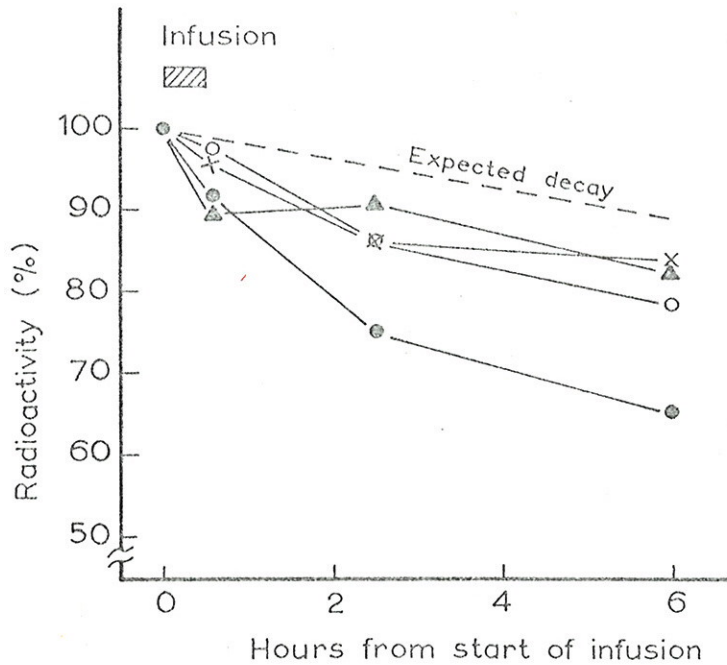

Fig. 4: The increased disappearance of ^{125}I-fibrinogen in Thorotrast-pretreated rabbits receiving platelet material.
O — O : Saline-pretreated animals infused with platelet material.
X — X : Saline-pretreated animals infused with saline.
⊙ — ⊙ : Thorotrast-pretreated animals infused with platelet material.
▲ — ▲ : Thorotrast-pretreated animals infused with saline.
(from Evensen and Jeremic: Platelets and triggering mechanism of intravascular coagulation. Brit. J. Haemat.: *19*: 33 [1970])

hours later, when heparin was no longer present, the animals received the second injection of endotoxin. Thrombocytopenia prevented the development of early deposits of fibrin in glomerular capillaries, suggesting an essential role of platelets in the pathogenesis of the GSR. Further support came from Evans and Mustard [18], who reported significant reduction in the incidence of endotoxin-induced GSR in rabbits pretreated with phenylbutazone, which prevents platelet aggregation in vitro. In the baboon, however, the production of severe thrombocytopenia with platelet antiserum did not prevent the late decreases in clotting factors induced by endotoxin [84]. Endotoxin added to human

platelet-rich plasma only occasionally induces clumping [72], produces no change in the electrophoretic mobility of platelets [30], and releases little or no platelet factor 3, facts which illustrate the limitations of the animal models. We conclude that platelets appear to be essential in the production of the GSR by endotoxin in the rabbit, and rabbit platelets contain clot-promoting activity which may serve as the yet unidentified mediator of endotoxin. In man and other animals the role of platelets is still unresolved.

c) *The need for granulocytes.* Granulocytes apparently are required for endotoxin to trigger DIC. Prepared rabbits made granulocytopenic with nitrogen mustard are protected not only against the GSR when challenged with endotoxin [85], but also against the thrombin-induced decreases in factors V and VIII [43]. The observation that infusion of neutropenic rabbits with suspensions of viable granulocytes reinduced marked decreases in fibrinogen and factor VIII and the GSR following endotoxin administration, further indicates that granulocytopenia diminishes the generation of thrombin after endotoxin [24,34]. Severe granulocytopenia due to acute leukemia likewise protected against DIC in patients with Gramnegative septicemia [40].

These observations strongly suggest a mediator released from granulocytes, but do granulocytes contain enough clot-promoting activity to provoke DIC? In vitro observations of Rapaport and Hjort [71] and the failure of McKay et al. [54] to induce glomerular thrombi by infusing peritoneal leukocyte lysate in prepared animals suggest that the answer to this question probably is no. Furthermore, antibody-induced neutropenia did not prevent the classical GSR [46]. In fact, the antigen-antibody reaction itself prepared for the GSR, probably by stimulating α-adrenergic receptor sites (see below).

Thus, the role of the granulocyte in the triggering mechanism of endotoxin is still controversial. The profound granulocytopenia following endotoxin injection shows that these cells are involved in some way; *how*, is still a mystery. At present, it is difficult to explain the effect of endotoxin by the release of a mediator from the granulocytes, but they may supply a yet undetected conditioning factor for DIC. For example, it would be interesting to investigate whether nitrogen mustard pretreatment interferes with the production of glomerular thrombi following a single dose of endotoxin and infusion of noradrenaline [56].

d) *Haemolysis.* The haemolysis induced by endotoxin is secondary to intravascular coagulation [8], and incubation of erythrocytes with very high concentrations of endotoxin does not produce haemolysis. Thus, red cells probably supply accelerators of clotting, but not mediators.

e) *Endothelium and tissue injury* as the missing link in the triggering mechanism of endotoxin has so far received little attention. Tissues could be damaged, either directly by endotoxin or secondary to the obstruction of vessels by platelet aggregates, with subsequent release of tissue thromboplastin. Endotoxin induces many biochemical and ultrastructural changes, but, unfortunately, most of these injuries may be secondary to the obstruction of the microcirculation by fibrin. Thus, McKay et al. [53] gave endotoxin to pregnant rats and found that endothelial damage occurred secondary to platelet and fibrin

deposits in the capillary lumina, with the exception of hepatic sinusoids, where early swelling of the Kupffer cells was observed. In contrast, Müller-Berghaus and Lasch [61] demonstrated that thrombin generation in recalcified normal rabbit plasma was more rapid when the plasma was incubated in aorta preparations isolated after a second injection of endotoxin than in normal aortas. In agreement with this finding, circulating endothelial cells were found in rabbits after a single intravenous dose of endotoxin [27], and examination of arterial endothelium after endotoxin administration has revealed extensive injuries [27,52].

Thus, low concentrations of tissue thromboplastin from the endothelium may serve as the mediator of several indirect triggers. We have attempted to demonstrate circulating clot-promoting activity after addition of large doses of endotoxin to the isolated perfused rabbit liver. So far, however, these experiments have been negative.

3. Antigen-Antibody Complexes

The intravenous injection of preformed antigen-antibody complexes in mice or rabbits [42,51] or antigen into specifically immunized rabbits [6,42], produced the characteristic depletion of platelets and clotting factors and kidney lesions indicative of the GSR. Immune complexes formed in the presence of antigen excess make platelet factor 3 available and accelerate clotting of platelet-rich, but not that of platelet-poor plasma [35,37,74]. In this respect, antigen-antibody complexes mimic the in vitro effect of endotoxin, but heparin protects against acute allergic reactions in man [38] and anaphylaxis in animals [37], suggesting that platelet injury may be secondary to clotting. However, these observations could also be explained by the anticomplementary effect of heparin.

Other cells may also be involved in the reaction since antigen or antibody can be prefixed to cell membranes, especially of red cells. Haemolysates contain platelet-like clot-promoting activity [69] and large amounts of ADP which releases platelet factor 3 [58]. The amount of phospholipids in the total erythrocyte mass far exceeds that of the platelets, and rapid infusions of autologous haemolysate initiate moderate intravascular clotting [70]. In spite of these findings, most patients with severe haemolysis do not develop DIC; haemolysis in victims of DIC is usually secondary to clotting, since the red cells are injured during the passage through small vessels obstructed by fibrin threads [8]. However, immune haemolysis is more likely to cause DIC than simple haemolysis [13,41,87], suggesting that antigen-antibody reactions add to the weak effect of simple haemolysis.

Recently, the finding of fibrin deposits in glomerular capillaries in hyperacutely rejected kidney allografts has been taken as an equivalent of the GSR [39,82]. However, thrombi have not been found in other organs [9], and Rosenberg et al. [80] found low levels of the thrombin-sensitive clotting factors only in the venous blood flowing from the kidney, not in the arterial blood. Thus, clotting is probably local, triggered by the interaction of preformed humoral

antibodies in the recipient with antigen in the donor kidney, and does not represent the GSR.

4. Liquoid

Liquoid is a synthetic polymer with anticoagulant effect. Nevertheless, it consistently produces severe DIC and the GSR in rabbits, and anticoagulation with warfarin gives complete protection [22,78], indicating that Liquoid must serve as an extremely potent trigger of coagulation, breaking through its own short-lasting anticoagulant effect, but not through that of warfarin.

In vitro, Liquoid prolongs the clotting time of blood, and its in vivo effect, therefore, appears to be that of an indirect trigger. Platelets are a possible source of a mediator, since profound and immediate thrombocytopenia develops before the thrombin-sensitive clotting factors start to fall. However, extreme thrombocytopenia prior to the injection of Liquoid does not prevent the progressive consumption of clotting factors and the GSR [19]. Recent findings of Müller-Berghaus and Lasch [59] suggest, that Liquoid may trigger DIC directly through the activation of the contact phase of coagulation. Factor XII activity decreased precipitously after intravenous injection, and animals infused with lysozyme, an inhibitor of factor XII activation [66], were protected against the GSR after a single injection of Liquoid. However, Liquoid is far more potent than other contact activators, such as kaolin or ellagic acid, and additional mechanisms are therefore probably involved.

B. Modifying factors

1. *Release of accelerators.* Thrombin aggregates platelets, makes platelet factor 3 available [11], induces granulocytopenia [65], and haemolysis [8]. In theory, all of these effects contribute to a vicious circle by enhancing clotting, but in practice the effect may be weak. Thus, we found that DIC following a moderate dose of tissue thromboplastin was equally severe in extremely thrombocytopenic rabbits as in normal rabbits, suggesting that the platelet injury in normal animals did not enhance clotting [21].
2. *Reticuloendothelial system.* In the normal organism, RES protects against DIC by effectively removing clotting intermediates, fibrin macromolecules and various triggers, such as endotoxin [12]. The clearing mechanism is powerful and specific, attacking clotting factors only in the activated form [14,26].
We do not know exactly how RES functions are impaired during DIC. Probably many factors are involved, including fibrin, endotoxin, endotoxin-induced hyperlipemia, steroids and stress [81].
3. *Fibrinolysis* breaks down circulating and locally deposited fibrin, thus maintaining the microcirculation. Increased amounts of circulating fibrin degradation products are formed which may produce bleeding through interference with fibrin polymerization and platelet aggregation. It is not surprising that EACA enhances intravascular coagulation in man [63], that the combined

infusion of EACA and thrombin results in the GSR [48], and that activation of the rabbit's fibrinolytic system with streptokinase completely prevents the GSR [10]. Further, endotoxin activates fibrinolysis in man and in the dog, but not in the rabbit [57]. In fact, urokinase excretion decreased considerably in endotoxin-infused rabbits which developed glomerular fibrin deposits [28], and much smaller quantities of tissue plasminogen activator could be extracted from kidneys of endotoxin-treated rabbits than from normal controls [45]. These observations may explain the unusual sensitivity of the rabbit to the GSR.

4. *Pregnancy* predisposes for DIC. A single injection of endotoxin elicits GSR in pregnant animals [91] and the majority of patients with fulminant DIC are pregnant women. One reason may be the decrease in fibrinolytic activity during pregnancy. The level of plasminogen activator in the kidney cortex was significantly lower in pregnant animals of different species as compared to non-pregnant controls [16]. The phagocytic capacity of the RES is normal or increased during pregnancy [89].

5. *Localizing factors*. The distribution of fibrin between various organs in DIC differs from the distribution of circulating fibrinogen, indicating that vascular factors are involved in the deposition of fibrin. For example, quantitative measurements after thrombin infusion in rabbits showed that the kidney accumulated, as fibrin, 15 times the amount of fibrinogen present in the renal blood volume [73].

Many experiments point to the role of the adrenergic system. Adrenergic blocking agents [23,62] and sympathetic denervation [68] have a protective effect in GSR. In agreement with these findings McKay et al. [56] produced glomerular fibrin by a combination of a single injection of endotoxin and an infusion of noradrenaline, while a single injection of endotoxin failed to produce fibrin deposits. Noradrenaline probably produced sufficient capillary stagnation for microcoagulation to occur. This experiment, therefore, resembles the stasis experiments in veins by Wessler [88].

Thus, stimulation of the α-adrenergic receptor sites is essential for the localization of fibrin in the microcirculation. The larger the role of this factor in a patient, the more difficult it is to differentiate between DIC and thrombosis. It should be noted, however, that endotoxin also induces the release of other vasomotor substances, like kinins, histamine and serotonin. Thrombin [67,90] and fibrinopeptides [3] also have vasomotor effects.

6. *Plasma lipids* are elevated following the administration of endotoxin in rabbits [1,31], during infection by Gram-negative bacilli in patients [25] and after trauma [86]. Hypercoagulability, inhibition of fibrinolysis [2] and depression of the RES [55,83] are possible results, and these effects undoubtedly modify the response to intravascular clotting. Indeed, Huth et al. [36] showed that the combination of fat infusion and endotoxin injection led to severe DIC, while administration of either agent alone had only moderate effects on the clotting system.

C. Conclusions and Summary

DIC continues to delight the experimentator and to frustrate the clinician. New observations constantly add to the complexity of the syndrome, and a series of complicated mechanisms contribute to the final result. For this reason, we believe that no experimental models and no patients are exactly alike. However, the following ideas are now well documented:

First, intravascular coagulation must be triggered, either directly, or indirectly through mediators. Many different triggering mechanisms exist, such as release of tissue factors, disintegration of platelets, and activation of factor XII. Most indirect triggers probably have two things in common: they are weak, but continue to act over several hours. For this reason, it is so difficult to demonstrate and isolate the factors involved.

Secondly, clotting is accelerated by many mechanisms, aggregation of platelets with release of platelet factors, release of factors from damaged tissues, enhancement of fibrin polymerization, and interference with normal neutralizing mechanisms.

Thirdly, the response of the organism is modified by systemic (fibrinolysis, RES, lipids) and localizing factors. Generally, these factors determine the clinical effects.

In further research, it is necessary to explore each of these mechanisms and their interactions, not to find *the* factor or *the* mechanism, but to achieve a deeper understanding which finally may lead to more efficient therapy.

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