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## A Circulating Anticoagulant in Lepromatous Leprosy[1]

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Circulating anticoagulants have been described in collagen vascular diseases, in chronic infections, and in hemophilia ([2, 10, 19]). Although clotting disorders have been previously noted in patients with lepromatous leprosy and *erythema nodosum leprosum* (ENL) reactions, their etiology has not been defined ([1, 13, 22, 23]). This paper describes a circulating anticoagulant in a patient with lepromatous leprosy.

### CASE REPORT

A 43 year old Puerto Rican male previously in good health was referred for treatment of lepromatous leprosy. Four months prior to admission, a slightly pruritic, diffuse, nontender, erythematous rash developed over his abdomen spreading to his chest, back, and extremities over a period of three weeks. The rash progressed to crusted, pustular, plaque-like lesions with excoriation over the arms and legs.

Physical examination revealed *leonine facies*, a pigmented, serpiginous, raised rash on his abdomen, and raised pigmented plaques on both arms. Numerous areas of excoriation and skin breakdown were evident on his knees, elbows, legs, and buttocks. An erythematous rash was also noted on his cheeks and ear lobes. Neurological examination revealed anesthesia in a "stocking-glove" distribution and over the abdominal rash. Marked bilateral ulnar nerve thickening was present.

Laboratory data included a hemoglobin of 14.7 gm/100 ml and leukocyte density of 7300/mm$^3$. The platelet count was 334,000/mm$^3$. The erythrocyte sedimentation rate was 27 mm/hour. The serum SGOT, bilirubin, complement ($C_3$), and haptoglobin were normal. The albumin was 3.3 gm/100 ml, globulins 4.7 gm/100 ml, IgG 3500 mg/100 ml, IgA 500 mg/100 ml, and IgM 320 mg/100 ml. The LE prep, rheumatoid factor, antismooth muscle antibody, antimitochondrial antibody, anti-DNA antibody, and thyroid autoantibodies all were negative. Serum cryoglobulins and antinuclear antibody were both trace positive. The VDRL titer was 1:32; FTA-ABS was 2+ positive. The CSF VDRL was negative. The prothrombin

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time (PT) was 13.5 seconds with a control of 12.5 seconds, and the activated partial thromboplastin time (APTT) was 59 seconds with a control of 29.5 seconds.

Hematoxylin-eosin and Fite-Faraco stained sections of a skin biopsy confirmed the diagnosis of lepromatous leprosy, subpolar type ($LL_s$), active, according to the criteria of Ridley ([25]). Dapsone therapy (50 mg by mouth twice a week) was begun.

Parenteral vitamin K did not improve the clotting abnormality which persisted despite six weeks of dapsone therapy. The rash improved slowly; ENL reaction did not occur.

The patient was lost to follow-up after 12 weeks of treatment.

## MATERIALS AND METHODS

**Coagulation studies.** Plasma was obtained by centrifugation of blood collected on sodium citrate (3.8%). The PT and APTT were performed using rabbit brain thromboplastin and activated cephaloplastin (Dade Diagnostic, Inc., Miami, Florida) respectively. Specific coagulation factor assays were performed by one stage technics utilizing plasma deficient in the factor being tested ([3]). The PT was modified to assay factors II, V, VII, and X, and a modified APTT assayed factors VIII, IX, XI, and XII. Fibrinogen level was determined by a turbidometric technic ([9]).

**Demonstration of the circulating anticoagulant.** The APTT was performed on 0.1 ml aliquots of a mixture of equal parts of plasma from a normal subject and from the patient. Plasma mixed with equal parts of isotonic saline (0.154 M, pH 6.8) was tested in a similar fashion.

The PT was performed with thromboplastin diluted with isotonic saline. A 0.1 ml aliquot of each dilution was added to an equal volume of the patient's plasma. After five minutes of incubation at 37°C, 0.1 ml of $CaCl_2$ (0.2 M) was added, and the clotting time was measured.

**Immunoadsorption of the anticoagulant.** Aliquots of 0.1 ml of rabbit antisera to human IgG, IgA, or IgM (Behring Diagnostics, Subs. Hoechst Corp., Somerville, NJ) were incubated with 0.4 ml aliquots of patient's plasma for one hour at 37°C. After centrifugation for 30 minutes, 0.1 ml of this mixture was diluted 1:1 with normal untreated plasma, and 0.1 ml of activated cephaloplastin added. After three minutes of incubation at 37°C, 0.1 ml of $CaCl_2$ (0.02 M) was added, and the clotting time recorded.

**Gel filtration.** Globulin fractions were prepared from control and patient's plasma by precipitation with cold ammonium sulfate to a final 50% saturation. The precipitated fractions were dissolved in barbital-buffered saline (pH 7.4) and fractionated by chromatography on 2.5 × 45 cm columns of Sephadex G-200 in barbital-buffered saline. The optical density of each effluent fraction was read at 280 $\mu$m. The fraction corresponding to each protein peak was concentrated fivefold and assayed for anticoagulant activity by performing the APTT on a mixture of equal aliquots (0.1 ml) of normal plasma and each fraction. The immunoglobulin type of the concentrated fractions was determined by immunodiffusion analysis with specific antisera to IgM, IgA, and IgG.

TABLE 1. *Coagulation studies.*

| | PT (sec) | APTT (sec) | TT[a] (sec) | PT with diluted thromboplastin 1:10 | 1:100 | 1:1000 |
|---|---|---|---|---|---|---|
| Normal | 12.5 | 29.5 | 19 | 13.5 | 22.0 | 37.5 |
| Patient | 13.5 | 59 | 19 | 26.0 | 51.0 | 88.0 |
| Normal + patient | | 47 | | | | |
| Normal + saline | | 35 | | | | |
| Patient + saline | | 36.5 | | | | |

[a] Thrombin time.

## RESULTS

Concentrations of all coagulation factors were normal. The PT was minimally prolonged (Table 1). Marked prolongation of the APTT was noted which persisted when the patient's plasma was diluted 1:1 with normal plasma. Dilution of the patient's plasma with isotonic saline (1:1), however, corrected the abnormality. Marked prolongation of the PT was observed when diluted thromboplastin was used.

TABLE 2. *Immunoadsorption of the anticoagulant.*

| | APTT (sec) |
|---|---|
| Normal plasma adsorbed with anti-IgG | 30.0 |
| Normal plasma adsorbed with anti-IgA | 31.0 |
| Normal plasma absorbed with anti-IgM | 31.0 |
| Patient's plasma adsorbed with anti-IgG | 47.0 |
| Patient's plasma adsorbed with anti-IgA | 46.5 |
| Patient's plasma adsorbed with anti-IgM | 38.5 |

Greatest shortening of the APTT was achieved when the patient's plasma was adsorbed with anti-IgM antiserum (Table 2). When larger amounts of anti-IgM antiserum were added to the patient's plasma, the decrease of the anticoagulant activity could not be differentiated from the correction due to dilution of the inhibitor. However, fractionation of the patient's globulin with a Sephadex G-200 column revealed that the anticoagulant activity was associated with the early (high molecular weight) protein fractions (Table 3). On immunodiffusion with specific antisera, the fraction reacted only with the anti-IgM antiserum. No anticoagulant activity was detected in corresponding fractions from normal plasma.

TABLE 3. *Separation of the anticoagulant by Sephadex G-200 fractionation.*

| Mixture | APTT (sec) |
|---|---|
| Normal plasma + buffered saline | 38 |
| Normal plasma + early protein peak | 52 |
| Normal plasma + middle protein peak | 35 |
| Normal plasma + late protein peak | 33 |

## DISCUSSION

Spontaneously occurring circulating anticoagulants in nonhemophiliac patients have been shown to be immunoglobulins, primarily IgG, but occasionally IgM or mixed IgG-IgA ([2, 11, 17, 19, 28, 30]). In this case, specific antiserum adsorption and Sephadex G-200 fractionation suggested that the inhibitor was an IgM immunoglobulin. It is impossible to know the antigenic stimulus at which this immunoglobulin was directed. It is interesting to speculate, however, that this protein was not an autoantibody. Rather, it may have been directed at the mannophosphoinositides of *Mycobacterium leprae* that cross react with the phospholipid used as the platelet substitute in coagulation reactions ([14]). Unfortunately, testing of this hypothesis by absorption of the patient's serum with phospholipid in an attempt to reverse the anticoagulant effect was not possible because of the lack of availability of the mycobacterial mannophosphoinositides.

While abnormal serum proteins have been noted in lepromatous leprosy, none has been associated with anticoagulant activity ([5, 7, 20, 24]). The abnormal APTT previously observed with lepromatous leprosy is corrected by addition of normal plasma ([23]). Such coagulation defects are presumably caused by interference with coagulation factor synthesis secondary to hepatic dysfunction or low-grade consumptive coagulopathy ([1, 22]). No evidence of hepatic dysfunction or low-grade coagulopathy was found in this patient.

The ENL reaction has been associated with spontaneously reversible clotting abnormalities ([22, 23]). In this patient, persistence of the clotting abnormality for six weeks suggests a different mechanism.

This patient's anticoagulant resembles those described in association with systemic lupus erythematosus (SLE) ([8, 18, 19, 21, 27]). The mechanism of action of these anticoagulants is poorly understood. Interference with the activation of a specific coagulation factor has been reported ([6, 15]), but interference with the phospholipid component of the prothrombin activator occurs more commonly ([16, 19]). The interaction between the inhibitor and phospholipid may alter the lipid surface of the prothrombin activator interfering with clotting factor adsorption ([29]).

This patient's anticoagulant was much less

active when diluted with saline than with normal plasma. This finding suggests that normal plasma supplies a co-factor which sustains the anticoagulant effect of the inhibitor. Saline dilution may decrease co-factor concentration below the optimal range for inhibitory activity. A plasma co-factor is similarly necessary for the activity of the lupus anticoagulant ([26]).

No specific clotting factor inhibition or bleeding diathesis was caused by this patient's anticoagulant. Although rare, bleeding manifestations may occur with this type of anticoagulant, usually in association with thrombocytopenia or other hemostatic defects ([10, 12]). Thrombotic manifestations, which have also been reported in association with a circulating anticoagulant, were absent in this patient ([4, 10]).

The finding of a circulating anticoagulant in our patient extends the variety of conditions in which anticoagulants can occur.


## SUMMARY

We observed a patient with lepromatous leprosy and a circulating anticoagulant. Intrinsic pathway inhibition was demonstrated by prolongation of the activated partial thromboplastin time. Extrinsic pathway inhibition was demonstrated by prolongation of the prothrombin time when performed with diluted thromboplastin. A plasma co-factor was required for inhibition. Immunoadsorption with specific antisera and Sephadex G-200 fractionation suggested that the anticoagulant was an IgM immunoglobulin. The similarities between this patient's anticoagulant and those associated with other disease states are discussed.

## RESUMEN

Observamos un paciente con lepra lepromatosa y un anticoagulante en circulación. La inhibición del mecanismo intrínseco de la coagulación se demostró por una prolongación del tiempo de tromboplastina parcial activada. La inhibición del mecanismo extrínseco se demostró por una prolongación del tiempo de protrombina en presencia de tromboplastina diluída. Para la inhibición se requirió un co-factor plasmático. La inmunoadsorción con antisueros especificos y el fraccionamiento con Sephadex G-200, sugirío que el anticoagulante fue una inmunoglobulina IgM. Se discuten las similitudes entre el anticoagulante de este paciente y aquellos asociados con otras enfermedades.

## RÉSUMÉ

On a observé un malade atteint de lèpre lépromateuse et présentant un anticoagulant circulant.

L'inhibition du système intrinsèque a été mis en évidence par l'allongement du temps de céphaline activé. L'inhibition du système intrinsèque a été mis en évidence par l'allongement du temps de prothrombine, déterminé avec de la thromboplastine diluée. Un cofacteur plasmatique était nécessaire pour l'inhibition. L'immunoadsorption avec un antiserum spécifique et le fractionnement sur colonne Sephadex G-200, suggère que l'anticoagulant est une immunoglobuline IgM. On discute les ressemblances entre l'anticoagulant observé chez ce malade et ceux retrouvés dans d'autres maladies.


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