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Connective tissue activation. XXIII. Increased plasma levels of a platelet growth factor (CTAP-III) in patients with rheumatic diseases

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Summary

Plasma levels of the CTAP-III antigen were measured by radioimmunoassay in 80 patients with rheumatic diseases. Patients with clear evidence of vasculitis usually exhibited increased plasma CTAP-III antigen. In both systemic lupus erythematosus and rheumatoid arthritis, there appeared to be a correlation between pCTAP-III values and other laboratory and clinical parameters of disease activity.

Introduction

Information concerning growth factors, long restricted to the arena of experimentalists working with *in vitro* systems, has contributed to the understanding of human disease beginning with the discovery of the family of somatomedin molecules [1]. The current literature emphasizes the possible importance of growth factors in regulating connective tissue metabolism [2], and one recent review drew attention to a possible role of platelet-derived growth factors (PDGF) in atherosclerosis, oncogenesis and myelofibrosis [3].

Definition of the clinical relevance of platelet-specific proteins in human disease should be undertaken cautiously in view of evidence that there are two or more distinct platelet growth factors [4–7]. Unlike the somatomedin group of mediators, the growth factors found in human platelets do not depend on the presence of circulating human growth hormone [8]. A role has been postulated for platelet growth factors in the pathogenesis of connective tissue diseases which exhibit a “growth” (proliferative) component, such as rheumatoid arthritis (RA), systemic

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lupus erythematosus (SLE) and less common types of vasculitis. Platelet abnormalities have been demonstrated in RA, SLE and other vascular diseases, and disease activity in RA appeared to correlate with thrombocytosis [9–11]. The destructive, proliferative pannus and accelerated formation of glycosaminoglycans (GAG) characteristic of RA clearly invite a search for a disorder of “growth factors”. Because such factors stimulate many connective tissue cell functions *in vitro*, we termed them Connective Tissue Activating Peptides (CTAP's) [5].

CTAP-III is a biologically active platelet-specific protein which has been purified to homogeneity and shown to be antigenically identical to beta-thromboglobulin (β -TG) [5,12,13]. Although CTAP-III and β -TG are antigenically identical, CTAP-III is larger by an N-terminal tetrapeptide, suggesting that the biologically inactive β -TG is a degradation product of CTAP-III [14]. Known biologic actions of CTAP-III include stimulation of prostaglandin E₂ (PGE₂) formation, increased intracellular cyclic 3',5' adenosine monophosphate (cAMP) accumulation, increased hyaluronic acid synthetase activity, increased hyaluronic acid (HA) formation, increased non-HA GAG synthesis and increased DNA synthesis [15,16].

CTAP-III is known to be released by thrombin-induced platelet aggregation. The antigenically identical degradation product, β -TG, was found to be elevated in plasma from patients with deep vein thrombosis, peripheral vascular disease, myocardial infarction, cerebral thrombosis, thrombotic thrombocytopenic purpura, diabetes mellitus and preeclampsia [17–21]. Recently, plasma CTAP-III antigen levels were shown to be elevated in most patients with rheumatoid arthritis [22]. In view of platelet abnormalities associated with the vascular disease of RA, SLE and other rheumatic disease, the present study of CTAP-III antigen was directed at these patient groups.

Materials and methods

A total of 80 patients was studied; 56 with rheumatoid arthritis, 15 with SLE, and 9 patients with other rheumatic diseases. Patients with rheumatoid arthritis and SLE fulfilled ARA criteria for these diseases. Ten RA patients were examined monthly for 6 months with assessment of 50 foot walking time, grip strength, duration of morning stiffness, Westergren erythrocyte sedimentation rate (ESR), platelet count and plasma CTAP (pCTAP). Routine laboratory studies included a complete blood count, urinalysis and measurement of serum creatinine. Patients with chronic renal failure were excluded from the study. Only 1 SLE patient had a slightly elevated serum creatinine (1.7 mg %). “Joint counts” provided a semiquantitative clinical estimate of RA disease activity based on the number of joints involved with tenderness (scored as 0 or 1) and swelling (scored as 0 or 1). Latex titer for rheumatoid factor was determined every 2 months. In selected patients, total hemolytic complement (CH₅₀) was measured by a standard method. DNA binding was measured by a Farr technique using double stranded ¹⁴C-DNA prepared from human cells.

Venous blood samples were drawn in duplicate, one from each arm, using 20 gauge needles and plastic syringes. 2.5 ml of blood were obtained and immediately

transferred to plastic tubes containing 0.3 ml of EDTA and theophylline to inhibit the platelet release reaction [22]. Blood samples were immediately placed on ice and centrifuged at $1500 \times g$ for 30 min at 4°C . 0.5 ml was removed from the upper layer of plasma and frozen at -70°C . Since traumatic vena puncture may elevate pCTAP-III levels, blood from both arms was analyzed. In 128 pairs of measurements of CTAP-III antigen from both arms of subjects, values were concordant (similarly low or similarly high) in 116 instances (90%). If one arm only yields a high value, trauma has usually been recorded. Trauma to the initial arm does not result in a high value in the opposite arm. With discordant circumstances, the high value is discarded. Ideally, the measurement should be repeated at a later time. pCTAP-III antigen levels were measured by radioimmunoassay as described recently [13]. In brief, this RIA employs antisera raised in rabbits against homogeneous, biologically active CTAP-III isolated from human platelets, and ^{125}I -CTAP-III prepared by a chloramine T method. Operationally, the competition between "cold" sample CTAP-III and ^{125}I -CTAP-III for anti-CTAP-III antibody is assessed by determining the radioactivity in an immune precipitate formed by addition of a "second" antibody (goat anti-rabbit IgG).

Results

Plasma CTAP-III levels in 20 normal control subjects had a mean value of 29.4 ± 5.8 ng/ml with a median level of 28 ng/ml [22]. Patients with rheumatoid arthritis, SLE and other rheumatic diseases all showed many significantly elevated plasma CTAP-III values (Fig. 1). The mean CTAP-III value for RA patients was 81

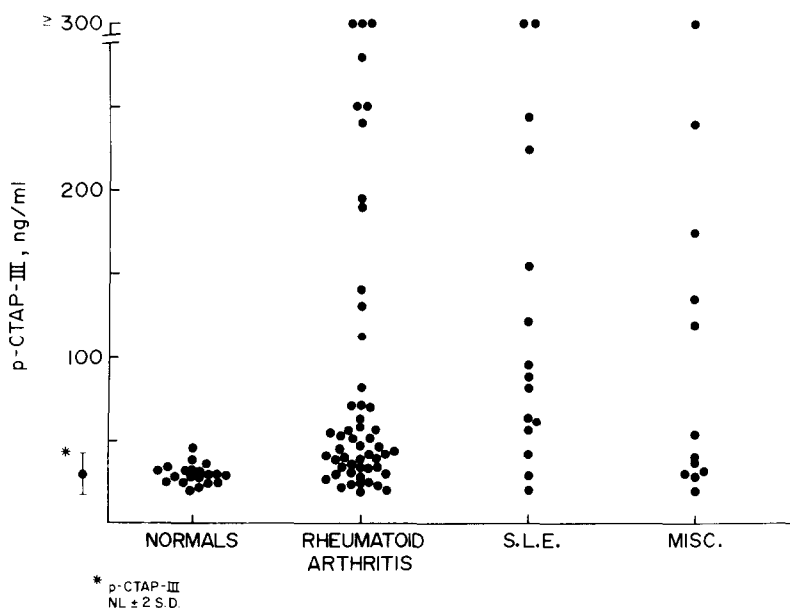


Fig. 1. A large proportion of patients with active rheumatic disease have an elevated pCTAP-III.

ng/ml, ranging from 20–2050 ng/ml. The mean pCTAP-III level for the SLE patients was 129 ng/ml with a range of 20–320 ng/ml.

Of the 10 RA patients followed serially for 6 months, 6 had pCTAP-III levels that correlated with clinical estimates of disease activity, and in general, with ESR and joint count. Two patients had pCTAP-III values which correlated with disease activity assessed subjectively, but not with ESR and joint count. In the other 2 RA patients, pCTAP-III levels showed no obvious correlation with disease activity. Data from a patient with active rheumatoid arthritis and a flare of pericarditis is shown in Fig. 2. A patient with mild, stable rheumatoid arthritis had persistently normal pCTAP-III levels (Fig. 3).

Twelve of 15 patients with SLE had elevated pCTAP-III levels, and 9 of the 12 had high DNA binding values (Fig. 4). Ten of 13 patients with abnormal pCTAP-III values had a low CH_{50} , and of the 13 patients, 10 had very active to moderate disease activity (Fig. 4). Of the 3 patients with normal pCTAP-III values, 1 had a normal CH_{50} and 2 had low CH_{50} values. Fig. 5 is of interest in that it demonstrates that the concordance between DNA binding and CH_{50} was of the same order as that between CTAP-III and DNA binding or CH_{50} .

CTAP-III values in patients with miscellaneous rheumatic diseases are shown in Table I. Two patients with early scleroderma had normal pCTAP-III levels, while another with digital ulcers had an elevated value. One of the 2 patients with

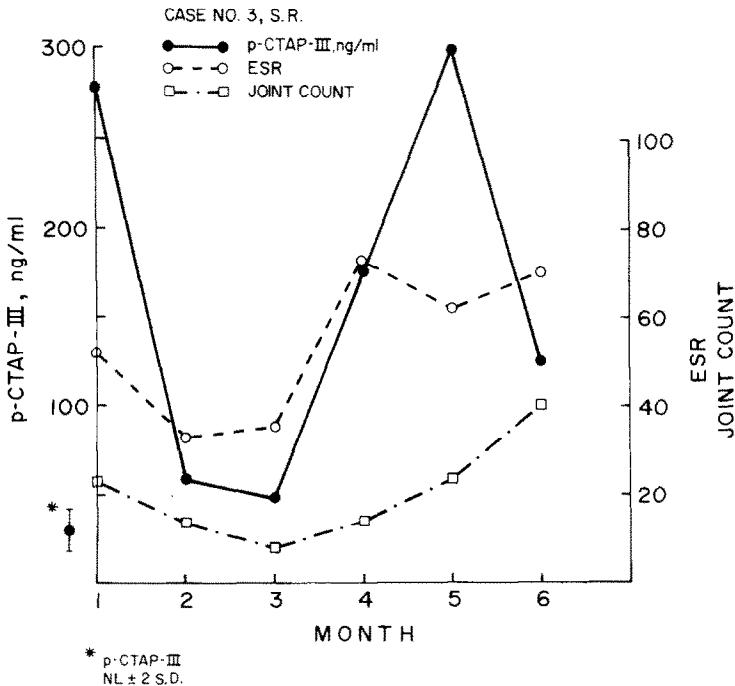


Fig. 2. Patient S.R. developed pericarditis believed to be an extraarticular manifestation of active rheumatoid arthritis; this was accompanied by increased articular and laboratory abnormalities.

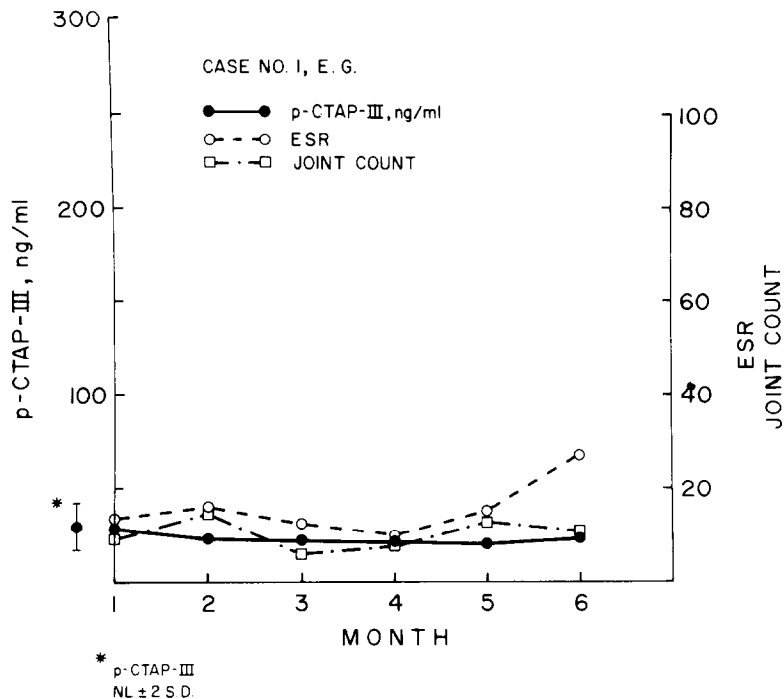


Fig. 3. Patient E.G. had clinically mild, stable rheumatoid arthritis.

Wegener's granulomatosis had elevated pCTAP-III associated with severe disease and digital ulcers; the other had minimal disease activity and was in remission on prednisone and cyclophosphamide. One patient with polyarteritis nodosa had a very high platelet factor level concurrent with very active clinical disease. A patient with temporal arteritis and an ESR of 120 mm/h had a pCTAP-III of 175 ng/ml; values which returned to normal after 2 months of prednisone therapy. A patient with polymyalgia rheumatica had a normal pCTAP-III value in the face of moderate to severe disease activity.

Six additional patients with rheumatoid arthritis, most with very active disease, a high ESR and high latex titers are reviewed in Table II. One patient with Felty's syndrome, rheumatoid nodules and lower extremity ulcers had a pCTAP-III value of 190 ng/ml. Two other patients with high latex titers had pCTAP-III values of 250 ng/ml. One of these patients had mild disease, the other had moderate disease activity. A patient with low latex titer, Raynaud's phenomenon, Sjögren's syndrome and RA had a pCTAP-III value of 140 ng/ml.

Discussion

In the present study, pCTAP-III values were found to be elevated in patients with rheumatic diseases who had clear evidence of vasculitis. Particularly in the patients

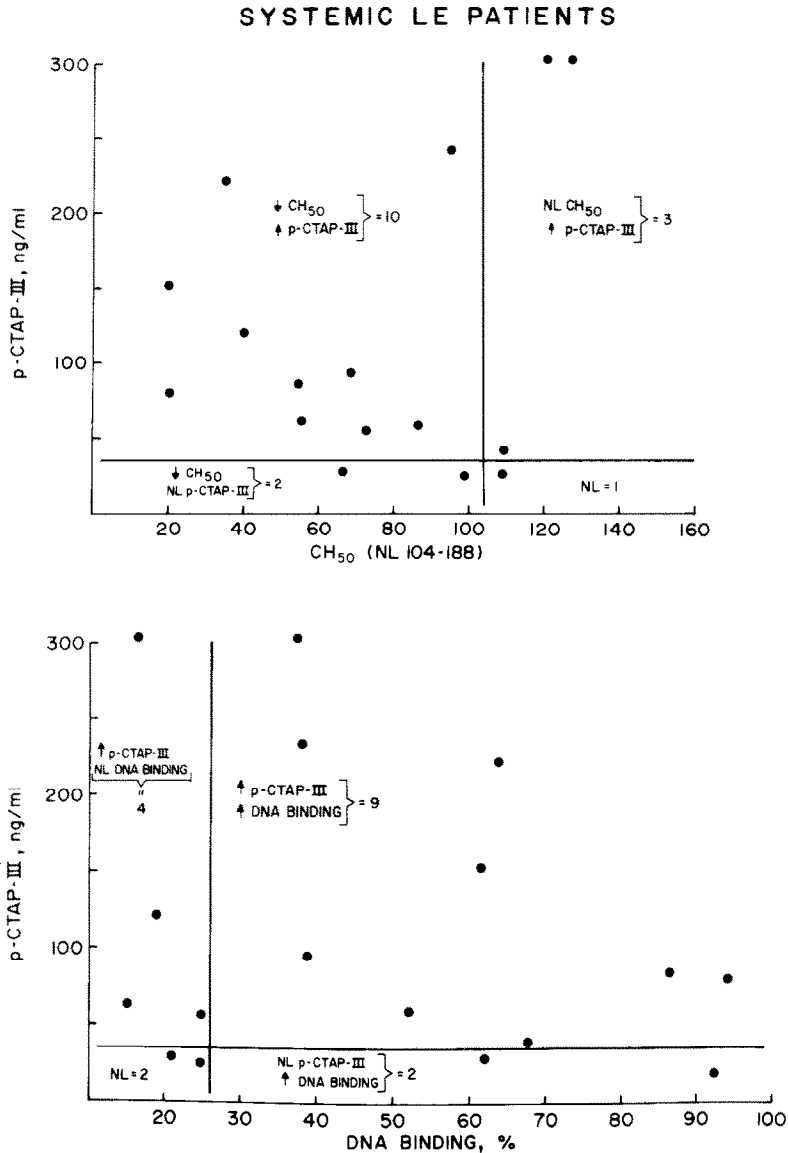


Fig. 4. The upper panel shows that in systemic lupus erythematosus patients, an elevated pCTAP-III usually occurs in the setting of a reduced CH₅₀, while the lower panel suggests that an elevated pCTAP-III was usually found with an abnormal DNA-binding value.

with RA and SLE, elevated pCTAP-III values occurred very frequently in association with the laboratory and clinical parameters of "clinical disease activity". The limited study population renders elaborate statistical analysis no more helpful than inspection of the data. It is important to note that the data basically reflect platelet activation and secretion of specific protein mediators, presumably within a few

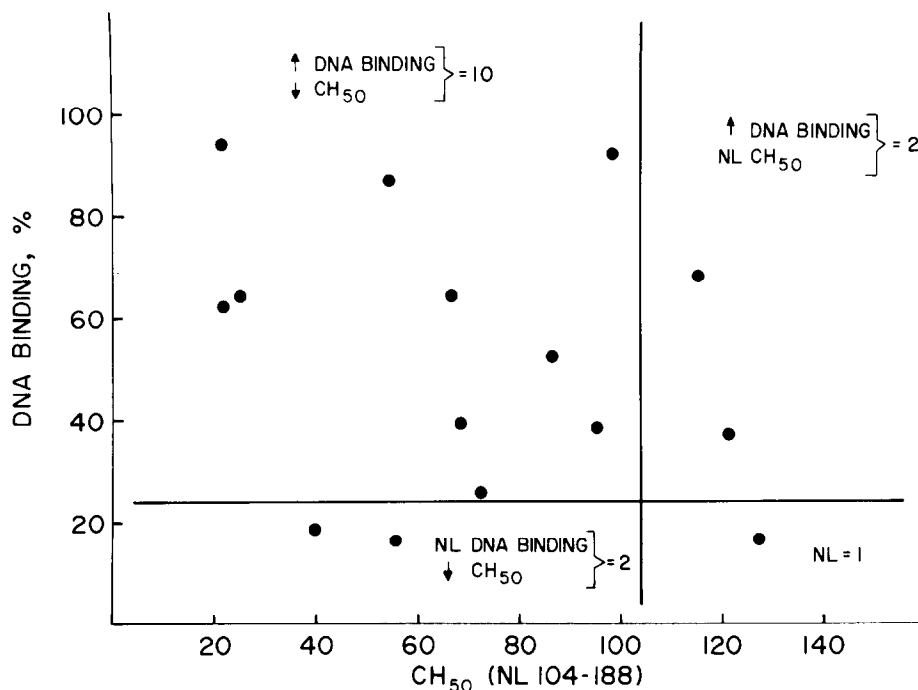


Fig. 5. Increased DNA binding and reduced CH₅₀ values usually occur together.

hours of sampling in view of data indicating that the half-life of this molecular species is of the order of 100 min [23].

It is also important to recall that secretion may not necessarily require aggregation and release such as might occur at a site of endothelial injury. For instance, IgG, IgM and fibrinogen are known to enhance platelet aggregation *in vitro* [24]; interaction of RA platelets with insoluble IgG-rheumatoid factor complexes results

TABLE I

PLASMA CTAP-III LEVELS IN DIFFERENT RHEUMATIC DISEASES

Rheumatic disease	pCTAP-III (ng/ml)	Disease activity
Wegener's (1)	28	remission
Wegener's (2)	135	severe
Scleroderma (1)	120	severe
Scleroderma (2)	31	mild
Scleroderma (3)	20	moderate
Polymyalgia rheumatica	34	moderate
Temporal arteritis	175	acute, severe
Polyarteritis	430	moderate
Dermatomyositis	37	acute, severe

Normal pCTAP levels = 17-41 ng/ml (mean \pm 2 S.D.).

TABLE II
PLASMA CTAP-III AND OTHER PARAMETERS IN RHEUMATOID ARTHRITIS

Patient	pCTAP-III (mg/ml)	ESR (mm/h)	Platelets $\times 10^3$	Latex titre	Joint count	Disease activity	Comments
1 C.M.	190	105	194	1:20480	6	severe	Felty's syndrome, rheumatoid nodules, lower extremity ulcers, aspirin
2 A.L.	54	26	327	1:5120	33	moderate	Bowel vasculitis, penicillamine aspirin, rheumatoid nodules
3 G.S.	43	6	219	1:5120	10	mild	Moderate MCP synovitis, aspirin, solganol
4 L.M.	250	80	338	1:163840	24	moderate	Solganol discontinued 1 month prior, Indocin, aspirin
5 L.S.	140	40	269	1:320	29	moderate	Myochrysin, prednisone 8 mg/day, sulindac, Raynaud's, Sjögren's
6 V.N.	250	10	267	1:2560	36	mild	Solganol q 4 weeks, subcutaneous nodules

Normal ESR (Westergren) is less than 20 mm/hr. Platelet counts normally range from 200,000-400,000/mm³, and latex titers for rheumatoid factors are abnormal in titers greater than 1:80.

in release of platelet products such as serotonin [25]. Elevated pCTAP-III levels in SLE might also be explained by immune complex induced endothelial cell injury with subsequent platelet aggregation and release of platelet growth factors. In addition to thrombocytosis, rheumatoid patients may exhibit an increased rate of platelet turnover and decreased platelet half-life [9]. There is evidence that thrombocytosis often correlates with RA disease activity and extra-articular manifestations [10], and recent studies show that RA platelets contain decreased acid phosphatase, saline-soluble protein and CTAP-III [26]. In the present study, no relationship between pCTAP-III levels and circulating platelet count was noted for any of the disease entities studied.

It is important to not over-interpret the current evidence, and in this vein, it should be noted that the radioimmunoassays available do not distinguish between biologically active CTAP-III and biologically inactive β -TG. The assay does in fact, primarily reflect recent platelet secretion of the antigenic material. In this context, it is also important to note that the levels of circulating antigen measured in disease states, even though elevated, would not be expected to exert a significant effect on sensitive peripheral connective tissue cells. The concentrations measured, even if entirely composed of biologically active CTAP-III are believed, on the basis of in vitro experiments, to be too low to exert biological effects. It seems likely that these materials are primarily active in the micro-environment at the site of platelet secretion.

It is attractive to postulate a hypothetical role for this platelet-derived growth factor based on its known capacity to stimulate connective tissue cell DNA synthesis and formation of connective tissue matrix components. One might visualize CTAP-III as an important signal mechanism in the inflammatory process. The platelet growth factor (CTAP-III) might have a role in the pathogenesis of inflammation, chronic vascular disease and the growth and repair of connective tissue by virtue of its ability to "activate" connective tissue cells at the site of injury with consequent increase in connective tissue cell replication and formation of extracellular matrix materials. Further studies are needed to determine whether the findings in patients are of a primary pathogenetic significance or are a secondary phenomenon.

Acknowledgement

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