Factor XIII Deficiency in BALB/c Mice with Plasmacytoma

Joseph Elpe, Vincent Yakulls, and Nicolas Costea

Hematology-Oncology Section, Department of Medicine, UCLA San Fernando Medical Program, Veterans Administration Hospital, Sepulveda, California 91343

SUMMARY

These studies examined the fate of Factor XIII (fibrin-stabilizing factor) in mice with plasmacytoma (MOPC-300, MOPC-384, MOPC-467, and J-558). Plasma Factor XIII levels in these mice decreased progressively with tumor expansion. No plasma inhibitors of Factor XIII activity could be detected. Factor XIII was found on plasmacytoma cell membranes and in the cytoplasm of the malignant cells by immunofluorescence. The inverse relationship between tumor load and plasma Factor XIII levels suggested that the fibrin-stabilizing factor was absorbed by the malignant cells.

INTRODUCTION

Factor XIII (fibrin-stabilizing factor), a plasma coagulation factor, when activated by thrombin converts loosely cross-linked fibrin into a gel that is insoluble in urea or monochloroacetic acid and is resistant to the action of proteolytic enzymes (12, 16, 17). This factor plays an important role in wound healing (1) and in the polymerization of collagen (21). Several reports have indicated that a stable clot is an important factor in the establishment and expansion of tumor metastasis (4, 5, 11, 14). It is possible that fibrin forms complexes with platelets, which enhance tumor cell adherence to the vascular endothelium. It has been shown in recent years that interference with fibrin cross-linking in experimental animals the development of tumors and their metastasis (25). In humans, acquired Factor XIII deficiency accompanies a variety of malignant tumors including multiple myeloma (20). It has been suggested that deficiency of Factor XIII activity in multiple myeloma may result from impaired production or from the presence of antibodies or paraproteins which interfere with its function (20).

The results of experiments designed to determine the fate of Factor XIII in BALB/c mice during the growth of transplanted plasmacytoma are herein reported. We present evidence that plasma Factor XIII decreases in these mice progressively with the growth of plasmacytoma and that the malignant cells appear to absorb plasma Factor XIII.

MATERIALS AND METHODS

Isolation and Assay of MFXIII.* MFXIII was isolated from

57 ml of plasma obtained from 100 BALB/c mice by a method described by Lowey et al. (18) for the isolation of human Factor XIII. Factor XIII-free fibrinogen was isolated from human plasma by DEAE chromatography as outlined by Finlayson (10). Purified MFXIII rendered human fibrin clots, obtained by addition of thrombin to Factor XIII-free fibrinogen, insoluble in 5 m urea. Its activity was expressed in units as defined by Lowey et al (18).

Preparation of Antibody to MFXIII. Isolated MFXIII incorporated into Freund's adjuvant was injected into rabbits at biweekly intervals. Serum of the immunized rabbits was absorbed with mouse plasma free of MFXIII and analyzed for specificity by immunodiffusion. Rabbit antibody to MFXIII caused fibrin clots from human and mouse plasma to be soluble in 5 m urea.

Mouse Plasmacytomas. Plasmacytomas MOPC-300, MOPC-384, MOPC-467, and J-558, obtained from Dr. Michael Potter, NIH, Bethesda, Md., were each transplanted into groups of 30 BALB/c mice. In addition, carcinoma line 26, a colon tumor, obtained from Dr. T. Corbett, Southern Research Institute, Birmingham, Ala., and a spontaneous reticulum cell sarcoma developed in our mouse colony were also used in these studies. At weekly intervals after transplantation 10 mice from each group were bled once, and the blood was collected in 3.8% sodium citrate (10:1 v/v). Plasma was obtained from groups of mice representing 1-, 2-, and 3-week samples. In addition, 10 normal BALB/c mice were bled each week, and their plasma was used as a control for the estimation of MFXIII levels. After 3 weeks, tumor-bearing mice received i.p. injections of 2 mg of cyclophosphamide. The tumors decreased in size and disappeared. These mice were bled 5 and 30 days after treatment with cyclophosphamide.

Estimation of MFXIII Plasma Levels. Radial immunodiffusion assays were performed as follows. Antibody to MFXIII was incorporated into agarose, and 10 μl of the plasma to be tested were placed into wells 2.5 mm in diameter. Dilutions representing 100, 50, and 25% MFXIII activity were made from a pool of plasma collected from 20 normal BALB/c mice. The immunodiffusion plates were incubated at 37° for 24 hr, and the diameter of the precipitate was measured. The plasma level of MFXIII was estimated from the linear plot of precipitin diameters obtained with dilutions of normal BALB/c plasma and reported as percentage of normal. MFXIII activity was estimated in plasma samples of normal BALB/c mice and mice with plasmacytoma according to the method of Coopland et al. (7), utilizing as...
substrate human Factor XIII-free fibrinogen. The purity of the MFXIII preparation was ascertained by polyacrylamide gel electrophoresis (5% gel in 0.03 M Tris-barbital buffer, pH 8.8). The molecular size of MFXIII was estimated by electrophoresis on sodium dodecyl sulfate-polyacrylamide gel (5.6%) in Tris-sodium acetate-EDTA buffer, pH 7.4 (9), by using as standards myoglobin, bovine serum albumin, human IgG, and fibrinogen (24). MFXIII protein isolated from pooled plasma of 100 mice was quantified by the method of Lowry et al. (19). MFXIII activity of extracts from plasmacytoma was also estimated by the method of Lowry et al. (18) with fibrinogen free of MFXIII as substrate. The tumor extract was obtained by pressing tumor fragments through wire screens and was collected in RPMI 1640. Tumor cells were washed 5 times with PBS and resuspended in 4 parts cold 0.25 M sucrose. The cells were disrupted in a Waring Blender, and the supernatant obtained by centrifugation (18,000 rpm at 4°C) was dialyzed against PBS and examined for MFXIII activity.

The possibility that plasma from BALB/c mice with plasmacytoma contains substances that may inhibit the activity of MFXIII was investigated as follows. Equal volumes of normal BALB/c mouse plasma were incubated for 1 h at 37°C with equal volumes of plasma from tumor-bearing mice or with the myeloma proteins that had been isolated by a combination of agar-gel electrophoresis and Sephadex G-200 filtration. These mixtures were assayed for MFXIII activity.

Protamine sulfate precipitation, detection of fibrin degradation products, and platelet counts were done utilizing standard methods.

**Immunofluorescent Studies.** Tumors were excised and placed in RPMI 1640, and individual cells were obtained by teasing tumor fragments with scissors. The cell suspension was filtered through gauze, placed onto Ficoll-Isopaque solution, and centrifuged at 2000 rpm for 10 min at 25°C. The cells on top of the Ficoll-Isopaque layer were removed and washed 3 times with RPMI 1640. Aliquots of these cells were dispersed into test tubes to which rabbit antiserum to MFXIII (1:5 dilution) was added. Mixtures were incubated at 25°C for 30 min and then washed 3 times in RPMI 1640. To the cell button obtained after the 3rd washing was added goat antibody to rabbit IgG conjugated with fluorescein isothiocyanate (3). The goat antibody to rabbit IgG was diluted 1:16 for optimal results. After incubation for 30 min at 25°C the cells were washed 3 times and examined with an American Optical fluorescence microscope having vertical illumination (BG 12, kV 418 exciter, and OG 515 barrier filters). Photographs of cells were taken with Kodak high-speed Ektachrome film EHB 135 (exposure time 60 to 90 sec).

For the study of intracytoplasmic MFXIII distribution, plasmacytoma cells were air dried onto microscope slides. The cells were fixed in ethanol at −20°C for 15 min, washed 3 times in PBS, and dried. The slides were first flooded with rabbit antibody to MFXIII (1:5 dilution), washed 3 times with PBS, and then overlaid with fluorescent goat antibody to rabbit IgG. The slides were washed 3 times with PBS, overlaid with phosphate-buffered glycerol (pH 7), covered, and examined. Appropriate controls were included, i.e., plasmacytoma cells incubated in normal rabbit serum or with rabbit antibody to fibrinogen overlaid with fluorescent goat antibody to rabbit IgG. In some instances plasmacytoma cells were incubated with antibody to MFXIII followed by unconjugated antibody to rabbit IgG, and finally the slides were overlaid with fluorescent antibody to rabbit IgG. Cells from tumors other than plasmacytoma and other tissues (liver, kidney, spleen, and bone marrow) were also treated as described above for detection of MFXIII.

**RESULTS**

Plasma (57 ml) obtained from 100 BALB/c mice yielded 654 µg of MFXIII. Clot-stabilizing activity was estimated to be 970 units per mg of the MFXIII preparation. Polyacrylamide gel electrophoresis of MFXIII revealed a single band migrating slightly faster than human FXIII. The estimated molecular weight of MFXIII was approximately 230,000, whereas human FXIII had a molecular weight of approximately 300,000. Rabbit antibody to MFXIII after absorption with plasma free of MFXIII developed precipitin reactions only with isolated MFXIII or mouse plasma but not with plasma free of MFXIII as demonstrated by immunodiffusion (Fig. 1). Plasma MFXIII antigen decreased to 48, 36, and 28%, respectively, 7, 14, and 21 days after implantation of plasmacytoma MOPC-467 (Chart 1). A similar phenomenon was observed with plasma from BALB/c mice in which plasmacytoma J-558 had been implanted. The fall in plasma MFXIII antigen in plasmacytoma-bearing mice was statistically significant when compared with pretransplantation levels (p < 0.001).

Plasma MFXIII antigen levels of mice transplanted with plasmacytoma MOPC-384 decreased to 41.2% 7 days after implant; however, as the tumor grew there was a slight elevation of the antigen (59.7% after 3 weeks). A progressive decrease of plasma MFXIII antigen was also observed in groups of 10 BALB/c mice bearing plasmacytoma MOPC-300. Cyclophosphamide was injected i.p. 3 weeks after implantation when the level of MFXIII antigen reached 37% of the preimplantation value. Five days after treatment, MFXIII antigen returned to normal values (91.6%) at a time when monoclonal IgG was still present in the serum (Chart 2). Plasma MFXIII activity assayed by fibrin clot stability in 5 µl urea decreased progressively in parallel with the level of MFXIII plasma antigen (Table 1).

MFXIII inhibitors were not detected in the plasma of tumor-bearing mice since mixtures of normal mouse plasma and plasma from plasmacytoma-bearing mice had normal MFXIII activity. Furthermore, increments of myeloma protein isolated from the serum by electrophoresis and Sephadex G-200 filtration from mice with tumor when added to normal mouse plasma did not reduce the activity of MFXIII.

Since the protamine sulfate test and platelet counts were within normal limits and the search for fibrin degradation products yielded negative results, disseminated intravascular coagulation was not present in the tumor-bearing mice. Plasma cells isolated from different plasmacytomas were incubated with antibody to MFXIII followed by goat anti-rabbit IgG conjugated with fluorescein isothiocyanate.
Factor XII in Mouse Plasmacytoma

Plasma cells from plasmacytomas J-558, MOPC-467, and MOPC-384 demonstrated surface and cytoplasmic staining (Fig. 2). Most of the cells had a faint circumferential fluorescent ring with superimposed bright spots. Plasma cells of plasmacytoma MOPC-300 exhibited only cytoplasmic fluorescence. In some tumors numerous plasmablasts contained coarse intracytoplasmic fluorescent staining (Fig. 2). Plasmablasts incubated with normal rabbit serum or rabbit antibody to fibrinogen before the addition of fluorescent goat antibody to rabbit IgG did not fluoresce. Incubation of plasmablasts with antibody to MFXIII followed by addition of unconjugated goat antibody to rabbit IgG decreased appreciably the immune fluorescence after fluorescent antibody to rabbit IgG had been added.

Cell suspensions from liver, spleen, and kidney failed to demonstrate fluorescence with antibody to MFXIII; however, megakaryocytes and platelets contained MFXIII when treated in a similar fashion.

Sucrose extracts from plasmacytoma cells contained MFXIII activity as demonstrated by their ability to render insoluble in 5 M urea Factor XIII-free fibrin clots. Furthermore, the activity of these extracts was inhibited by prior incubation with antiserum to MFXIII. Cells isolated from tumors other than plasmacytoma (carcinoma line 26 and reticulum cell sarcoma) did not carry MFXIII. In addition, their plasma MFXIII levels and activity were unchanged during tumor growth.

**DISCUSSION**

The results of our experiments indicate that in mice with plasmacytoma the level of plasma MFXIII decreases with expansion of the tumor. This phenomenon was observed in all mice bearing plasmacytoma regardless of the type of paraprotein synthesized by the tumors. After eradication of the plasmacytoma by i.p. injection of cyclophosphamide, MFXIII plasma levels returned to normal values.

In patients with multiple myeloma, the decreased MFXIII level was considered to be due to the inhibitory effect of paraprotein which may have interfered with the functions of this coagulation factor (22). Results of our experiments indicate that in mice with plasmacytoma there was no interaction between myeloma proteins and the activity of MFXIII, since both MFXIII activity (as determined by clot stability in 5 M urea) and antigen levels returned to normal values after these animals were treated with cyclophosphamide at a time when monoclonal Ig was still present in their sera. Results of mixing experiments in which normal mouse plasma was incubated with monoclonal Ig isolated from the...
that the paraproteins synthesized by tumors MOPC-300, demonstrated by immunofluorescence, and extracts of all variety of conditions (8). In congenital Factor XIII deficiency, tentative changes revealed a progressive decline of both MFXIII activity and antigen.

Reduction of MFXIII in mice with plasmacytoma is due to cellular molecules is unlikely since assay for quantitative and qualitative changes revealed a progressive decline of both MFXIII activity

The accumulation of MFXIII on or in plasmablasts was demonstrated by immunofluorescence, and extracts of all plasmacytomas investigated yielded active MFXIII.

Reduction of plasma Factor XIII has been observed in a variety of conditions (8). In congenital Factor XIII deficiency, although a quantitatively near-normal amount of this factor has been detected in the plasma, its function was abnormal, probably because of synthesis of a defective molecule (23). In mice with plasmacytoma, synthesis of defective MFXIII molecules is unlikely since assay for quantitative and qualitative changes revealed a progressive decline of both MFXIII activity and antigen.

We have also investigated the possibility that disseminated or localized intravascular coagulation may have been responsible for the reduction of this consumable coagulation factor. We did not detect in the plasma of tumor-bearing animals by-products of intravascular coagulation, nor could we demonstrate fibrin on the surface of malignant cells. Deposition of fibrin in and around tumors appears to be a transient phenomenon (4). We conclude that reduction of MFXIII in mice with plasmacytoma is due to cellular uptake of this factor and in some instances by ingestion (endocytosis) of this coagulation factor by the malignant plasma cells. It is also conceivable that MFXIII might have been incorporated as part of the transamidation of polymerized fibrin deposited during the establishment of the tumor. We have not detected MFXIII in plasma cells of splenic or bone marrow origin of tumor-bearing mice. Clot-forming and -stabilizing activity was also found in a fraction from plasma cell tumor YPC-1 (13).

Several reports have indicated that deposition of polymerized fibrin plays an important role in the growth of malignant tumors and the establishment of their metastasis (4, 6). Studies in both humans and experimental animals indicate that interference with fibrin deposition retards growth and metastasis (2, 15, 25). Interference with the formation of a fibrin network and stabilization by Factor XIII may represent a rational approach for the treatment of certain tumors. Factor XIII absorbed by malignant plasma cells may also represent a propitious target for active or passive immunotherapy.

**REFERENCES**

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Fig. 2. Immunofluorescent staining of tumor cells with rabbit antibody to MFXIII and goat antibody to rabbit IgG labeled with fluorescein isothiocyanate. Staining of viable cells (A, B) and fixed cells (C, D).
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