FACTOR VIII COMPLEX IN MYELOMATOSIS AND RELATED DISORDERS

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The levels of factor VIII/von Willebrand factor (VIII/VWF) complex and its related activities [factor VIII coagulant activity (VIII:C); factor VIII-related antigen (VIIIR:Ag); factor VIII-related ristocetin-cofactor (VIIIR:RCof)] have been frequently found to be outside the normal range in myelomatosis and related disorders[8, 13, 14, 20].

Hemorrhagic syndromes secondary to reduction of VIII/VWF are considered to be rare in monoclonal gammapathy[8, 20]. More frequently VIII:C and VIIIR:Ag are increased[13, 14], without any correlation with the thrombotic complications sometimes found in myelomatosis[10]. In general the levels of VIII:C and VIIIR:Ag are not correlated with the secretory or non-secretory type of myeloma, with abnormal protein present in the plasma, or with the presence or absence of Bence Jones proteinuria[13, 27]. The behaviour of the factor VIII ristocetin-cofactor in myelomatosis has not previously been systematically investigated. In this study we measured the levels of VIII:C and VIIIR:Ag and assessed the VIIIR:RCof. Furthermore, an attempt to explain the discrepancies between the levels of VIIIR:Ag and VIIIR:RCof was made by studying the plasma factor VIII/VWF by crossed-immunoelectrophoresis (CIE).

MATERIALS AND METHODS

Patients

Samples were obtained from 30 patients, 23 with secretory myeloma, 2 with non-secretory myeloma and 5 with macroglobulinemia. The diagnoses were based on the presence of characteristic clinical features, bone marrow

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A. *IgG myeloma (equation preferred for IgG cases)*

myeloma cell mass (cells x 10^{12})/m^2 = 0.413 + 0.256 x bone lesions*
+ 0.019 x urine M component − 0.059 x hemoglobin
+ 0.065 x serum calcium + 0.050 x serum M component

B. *All cases (useful for IgA, Bence Jones and IgG cases)*

myeloma cell mass (cells x 10^{12})/m^2 = 0.601 + 0.283 x bone lesions*
+ 0.031 x urine M component − 0.058 x hemoglobin
+ 0.051 x serum calcium + 0.028 x serum M component

* Bone lesions in the skeletal x-ray survey are scored from 0 to 3: 0 = normal bones; 1 = osteoporosis only; 2 = lytic bone lesions; 3 = extensive skeletal destruction and major fractures. Values for laboratory tests are entered directly as hemoglobin (g/100 ml), serum M component (g/100 ml), serum calcium (mg/100 ml) and urine M component (g/24 h).

Tab. 1 · Equation for calculating myeloma cell mass.

appearance and serum and urine immunochemical patterns. A complete skeletal radiological survey was also carried out and, in patients with secretory myeloma, the myeloma mass was measured indirectly, as described by SALMON and WAMPLER[23] (tab. 1). All the patients with multiple myeloma had taken or were taking specific chemotherapy for variable periods of time when studied. The patients with macroglobulinemia were off therapy.

Factor VIII/von Willebrand factor assays

After addition of 9 parts of blood to 1 part of 3.8% trisodium citrate, platelet-poor plasma was obtained by centrifugation at 5,000 *g* at 4 °C for 30 min and immediately stored at −40 °C. VIII:C was assayed in plasma stored at −40 °C by a one-stage method based on the partial thromboplastin time[3]. VIIIR:Ag was measured by rocket electroimmunodiffusion in agarose gel containing a monospecific rabbit antiserum (*Behring Institute*), by the method of ZIMMERMAN et al.[28]. Crossed-immunoelectrophoresis was performed with 10-20 µl of plasma by the method of SULTAN et al.[24]. VIIIR:RCof was measured by the method of RIVARD and DAVIAULT[22].

RESULTS

The results are shown in detail in tab. 2. The most interesting data are the generalized increase of VIIIR:Ag (21 of 25 patients with plasma cell myeloma and 2 of 5 patients with macroglobulinemia) without, usually, corresponding increases in VIII:C and VIIIR:RCof. Normal VIIIR:Ag were found in only 4 patients: of these 3 showed no osteolytic lesions radiologically.

There was no correlation between VIII/VWF-related activities and the paraprotein Ig type or level, the presence or absence of Bence Jones protein or renal involvement. Increased levels of VIIIR:Ag were also found in two patients with normal serum protein patterns but with extensive skeletal destruction secondary to myeloma cell infiltration. On the contrary, there was a significant correlation between cell mass and the VIIIR:Ag level ($r = 0.48$, $p < 0.01$) (fig. 1).

patients	monoclonal component		cell mass	bone lesions	VIII:C	VIIIR:Ag	VIIIR:RCof
	heavy chain	light chain					
1. T.A.	γ	λ	0.51	1	100	100	150
2. F.O.	α	κ	0.52	1	75	100	100
3. N.P.	γ	κ	0.54	1	95	110	100
4. S.F.	γ	λ	0.80	1	100	220	50
5. T.S.	γ	κ	0.81	1	150	310	100
6. C.P.	γ	λ	0.94	2	100	260	100
7. P.S.	γ	κ	1.00	2	220	300	75
8. N.L.	–	λ	1.08	2	75	150	100
9. D.L.	–	κ	1.13	2	90	250	100
10. D.D.A.	γ	κ	1.17	2	260	360	100
11. R.S.	–	κ	1.18	3	95	200	50
12. B.M.	α	κ	1.18	2	140	350	200
13. B.E.	γ	κ	1.19	2	260	360	100
14. D.A.	α	λ	1.22	1	210	420	100
15. A.V.	γ	κ	1.25	3	240	280	100
16. S.M.	γ	λ	1.26	3	220	400	200
17. M.C.	γ	κ	1.27	2	350	390	100
18. M.R.	γ	κ	1.28	2	140	200	100
19. F.F.	γ	λ	1.32	3	370	400	200
20. C.L.	γ	κ	1.35	2	150	250	200
21. F.D.	α	κ, λ	1.42	3	230	350	200
22. G.C.	γ	κ	1.43	1	350	370	100
23. P.M.	γ	κ	1.44	1	260	380	100
mean ± standard deviation		–	1.09 ± 0.28	–	188 ± 96	286 ± 97	118 ± 47
normal mean		–	–	–	94	96	90
range		–	–	–	55-135	50-150	50-150
24. E.M.	non-secretory	–	–	2	180	190	100
25. G.P.	non-secretory	–	–	2	130	320	300
26. G.A.	μ	κ	–	0	180	380	200
27. G.P.	μ	κ	–	2	200	420	200
28. H.M.	μ	κ	–	0	100	120	100
29. V.A.	μ	κ	–	0	100	110	100
30. G.G.	μ	κ	–	0	120	100	100

Tab. 2 - VIII:C, VIIIR:Ag and VIIIR:RCof levels in patients with secretory myeloma (1-23), non-secretory myeloma (24-25) and macroglobulinemia (26-30). Cell mass and bone involvement scores are also reported.

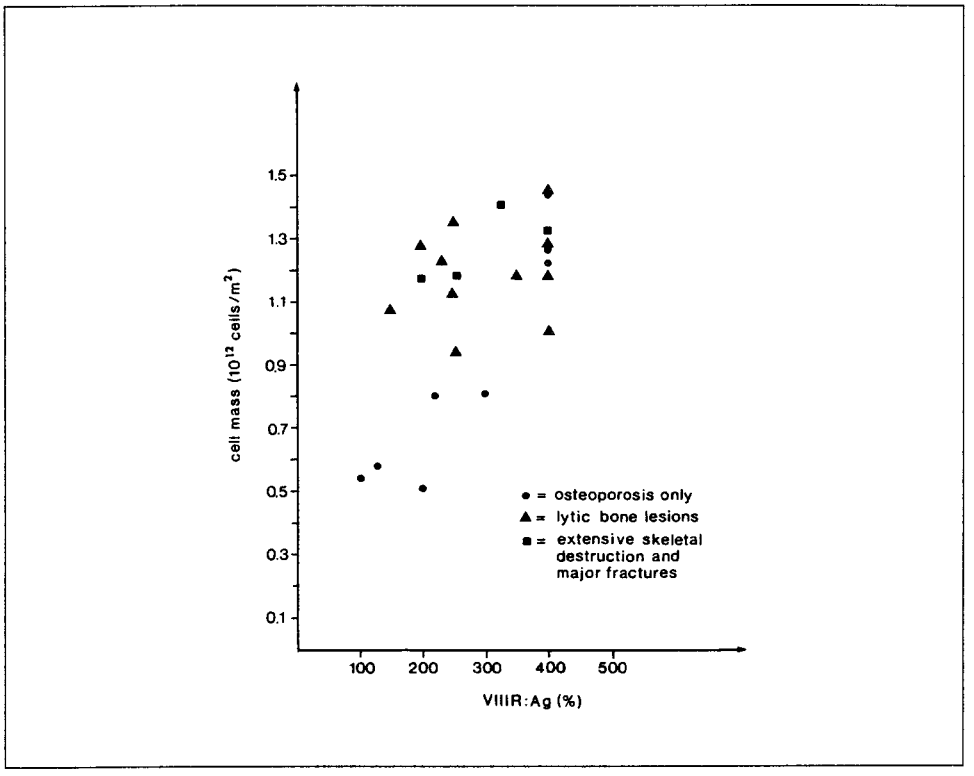

Fig. 1 - Correlation between cell mass and VIIIR:Ag level ($r = 0.48$, $p < 0.01$).

Factor VIII:C was increased in 11 of the 25 patients with myelomatosis (9 of them had lytic bone lesions). Although the VIII:C was increased less than the VIIIR:Ag, there was good correlation between these two parameters ($r = 0.68$, $p < 0.01$).

Factor VIIIR:RCof was increased in only six patients, all with extensive bone involvement. There was no correlation between the VIIIR:RCof and the VIIIR:Ag.

Crossed-immunoelectrophoresis was performed in 2 patients showing simultaneous increases in VIIIR:Ag and VIIIR:RCof and in 2 patients showing high VIIIR:Ag only. The CIE pattern of the plasmas, not discrepant for VIIIR:Ag and VIIIR:RCof, showed no deviation from normal. On the contrary, the VIIIR:Ag immunoprecipitation peaks of the plasmas with an increase of only VIIIR:Ag were asymmetric, with their peaks shifted slightly towards the cathode, as compared with the symmetric VIIIR:Ag peak present in the normal plasma (fig. 2).

DISCUSSION

A temporary increase in the factor VIII/VWF complex is known to occur in various physiological and pathological conditions: after stress or strenuous muscular exercise or following the infusion of adrenaline, 1-deamino-8-D-ar-

ginine vasopressin (DDAVP), factors VIII:C and VIIIR:Ag rise and this rise is likely to be due to release from storage deposits[5,19]. A disproportionally greater rise in VIIIR:Ag than in VIII:C has been found in some pathological conditions, such as ischemic limb disease[7], glomerulonephritis[12], diabetes mellitus[9], renal failure[25] and malignancy[14], and in clinical conditions typically associated with increased plasma proteolysis [acute pancreatitis, disseminated intravascular coagulation (DIC) and thrombolytic therapy][17]. The behaviour of VIIIR:RCof has been less frequently reported. In pathological conditions, both a strict correlation with VIIIR:Ag[25] and a discrepancy[18] have been found.

In myelomatosis and related disorders, VIIIR:Ag and, less frequently, VIII:C are generally increased[13,27], although some cases of hemorrhagic diathesis due to reduced VIII/VWF complex have been reported[8,20]. Our data confirm these previous reports and further show that VIIIR:RCof does not increase in parallel with VIIIR:Ag. VIIIR:Ag increases independently of the type of myeloma protein present in the plasma and of the presence or absence of Bence Jones protein or renal involvement. On the contrary, there is a strict correlation between VIIIR:Ag and cell mass. Moreover, high levels of VIIIR:Ag were found in patients with bone lesions, even without monoclonal protein in the serum.

These data probably rule out any interference of M component in the VIIIR:Ag assay and stress the importance of the tumor mass. Yet the pathogenesis of this uneven increase in VIII/VWF activities is not clear. VIIIR:Ag is synthesized by endothelial cells[6] and megakaryocytes[21]: an increased level of VIIIR:Ag without a correspondent increase of VIII:C has been suggested[7-11] to indicate the endothelial damage. Recent studies, both *in vivo* and *in vitro*, suggest that the discrepancies between VIII/VWF-related activities

Fig. 2 - VIIIR:Ag crossed-immunoelectrophoresis of a normal subject (bottom) and of a patient with increased VIIIR:Ag but normal VIIIR:RCof (top). The anode is on the left.

could be attributed to the proteolytic activation[1,2,17]. The exposure of purified VIII/VWF to trypsin or plasmin induces, after a rapid and brief increase, a destruction of the procoagulant activity (VIII:C), while the Laurell 'rocket' reaction (VIIIR:Ag) increases and the VIIIR:RCof remains unchanged or slightly reduced[2,3]. Thus, an increase of VIIIR:Ag could be found without any real increase in VWF protein synthesis or release. Of course this possibility must be taken into consideration, particularly when it is not possible to demonstrate corresponding increases of the other VIII/VWF-related activities. In fact, in clinical conditions characterized by increased plasma proteolysis, such as acute pancreatitis, decompensated DIC or during thrombolytic therapy, the VIIIR:Ag is disproportionally increased with respect to VIII:C[17]. In this situation an additional fast-moving precipitin peak appears in immunoelectrophoresis in agar gel. The additional VIIIR:Ag peak is probably the result of *in vivo* fragmentation of factor VIII/von Willebrand factor by proteolytic enzymes[17].

Although in myelomatosis the behaviour of VIII/VWF-related activities appears to be similar to that reported in pancreatitis or in malignancy with decompensated DIC, it is difficult to suppose they have the same pathogenesis: proteolytic enzymes are released from damaged tissues and leukocytes during acute-phase reactions, while myelomatosis is a typical chronic disease. Moreover, decompensated DIC could not be demonstrated in any of our patients. Furthermore, although the patterns in crossed-immunoelectrophoresis for some patients with myelomatosis were different from normal, suggesting an altered distribution in size of the various multimers forming VWF[15], additional peaks were never demonstrated.

At present it is not possible to rule out a real increase of VIIIR:Ag released by endothelial cells, which might be stimulated in the bone marrow by plasma cell infiltration. Discrepancy between the high level of VIIIR:Ag and the normal or low levels of VIIIR:RCof could result from additional factors: human plasma contains variable amounts of proteins that can inhibit ristocetin-induced agglutination[14]. High concentration of an inhibitor of the ristocetin-mediated agglutination has been found in patients with sickle cell hemoglobinopathies[16]. In those patients, high levels of VIIIR:Ag have been found without any corresponding increase in VIIIR:RCof[18]. The possibility that an inhibitor of ristocetin agglutination is present in plasmas of patients with gammapathy should also be considered.

SUMMARY

The behaviour of the factor VIII/von Willebrand factor complex (VIII:C, VIIIR:Ag and VIIIR:RCof) was investigated in 23 patients with secretory myeloma, in 2 patients with non-secretory myeloma and in 5 patients with macroglobulinemia. In most patients (21 of 25 patients with plasma cell myeloma and 2 of 5 patients with macroglobulinemia) VIIIR:Ag was increased usually without corresponding increases in VIII:C and VIIIR:RCof. There was no correlation between the VIIIR:Ag levels and paraprotein Ig type or level nor with the presence or the absence of Bence Jones proteins in serum and urine. Furthermore, increased levels of VIIIR:Ag were found in patients with non-secretory myeloma. In general, VIIIR:Ag was higher in patients with extensive bone lesions and there was a significant correlation between cell mass and the VIIIR:Ag level. The crossed-immunoelectrophoresis of plasmas with discrepant VIIIR:Ag and VIIIR:RCof showed variation from the normal pattern.

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