Plasma and urinary levels of $\beta 2$ microglobulin in rheumatoid arthritis

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SUMMARY Plasma and urinary levels of $\beta 2$ microglobulin have been investigated in 21 patients suffering from rheumatoid arthritis (RA). Despite a normal renal glomerular function in all patients, 50% of them had supranormal plasma $\beta 2$ microglobulin levels and 30% had a higher than normal urinary output of $\beta 2$ microglobulin generally related to the high plasma level. Plasma $\beta 2$ microglobulin levels paralleled closely the lymphocytosis and the 'joint count' both indexes of the severity of the disease. $\beta 2$ Microglobulin was normally secreted by the lymphoid tissue and it is suggested that it reflects the increase of the total mass and/or membrane turnover of the lymphoid tissue in RA. $\beta 2$ Microglobulin may be considered as a good parameter of the degree of severity of the joint and extra-articular involvement as well as a useful tool for the evaluation of drug efficacy in rheumatoid arthritis.

$\beta 2$ Microglobulin isolated from the urine of patients with tubular disorders by Berggard and Bearn in 1968 has since been considered an index of both glomerular and tubular renal function. More recently this moiety has attracted much attention following the discovery of its close relationship with the cell surface bound antigens responsible for cell recognition. Composed of 100 amino acids it has a molecular weight of 11 700 and is normally found in serum and in all biological fluids (Berggard and Bearn, 1968; Evrin and Wibell, 1972).

Sequence studies revealed a close homology of $\beta 2$ microglobulin with the constant regions of the heavy and light chains of immunoglobulins especially the so called C H₃ region (Peterson *et al.*, 1972; Cunningham *et al.*, 1973; Grey *et al.*, 1973). On the cell surface, $\beta 2$ microglobulin molecules are non covalently linked to two heavy chains constituting the histocompatibility antigen (Cresswell *et al.*, 1973; Grey *et al.*, 1973; Nakamuro *et al.*, 1973; Poulik *et al.*, 1973; Cresswell *et al.*, 1974; Peterson *et al.*, 1975). $\beta 2$ Microglobulin is found on the surface of T lymphocytes as a part of a more or less complex system (Bach *et al.*, 1973; Poulik *et al.*, 1973; McCalmon *et al.*, 1975) and is produced and secreted by T and B lymphocytes (Moore *et al.*, 1967) as well as tumoural and non tumoural

lymphoid tissue (Kithier *et al.*, 1974; Nilson *et al.*, 1974).

Some auto-immune diseases are characterised by an inflammatory process with lymphocytic infiltration responsible in the case of rheumatoid arthritis (RA) for cartilage destruction, bone erosion, and eventually articular deformation. There is a significant rise of plasma and joint fluid $\beta 2$ microglobulin values in RA patients and in the saliva of patients with Sjögren's disease (Talal *et al.*, 1975). In this last syndrome a significant positive correlation between $\beta 2$ microglobulin and the degree of lymphocytic infiltration of the salivary glands has been observed (Greenspan *et al.*, 1974; Daniels *et al.*, 1975; Michalski *et al.*, 1975) but unfortunately no information was given by these authors about the degree of severity of the disease and many of the patients reported also had an alteration of the renal function which is known by itself to increase the circulating level of $\beta 2$ microglobulin (Wibell *et al.*, 1973).

The aim of this present study was to verify the increase of circulating $\beta 2$ microglobulin in patients with RA and to correlate the levels with the degree of severity of the disease as estimated by the 'joint count' (Hollander, 1966).

Patients and methods

The plasma levels as well as 24-hour urinary excretion of $\beta 2$ microglobulin have been investigated

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in 21 patients aged from 25 to 77 years suffering from RA diagnosed according to the internationally accepted criteria (Ropes *et al.*, 1958). The mean duration of the disease was 9 years (range: 6 months to 23 years). Blood samples and 24-hour urine samples were collected at different times of the evolution of the disease for determination of $\beta 2$ microglobulin as well as other routine biological parameters. Forty-five samples were obtained in all. At the same time the joint count was evaluated by a member of the staff. Patients with RA suffering from complications such as renal insufficiency, amyloidosis, Sjögren's or infectious disease were deliberately omitted. $\beta 2$ Microglobulin was radio-immunoassayed using a commercial kit (Phadebas Pharmacia, Uppsala, Sweden).

Results

The results of plasma and urinary $\beta 2$ microglobulin

are presented in Table 1 together with the joint count evaluation, creatinine clearance, and other useful information. More than 50% of the plasma $\beta 2$ microglobulin levels fell above the upper limits of our normal values for a mixed population of men and women closely matched for their age with the group of patients reported here (normal range 0.8–2.9 mg/l, $n = 134$). Our normal range does not differ from that reported in the literature using radio-immunoassay techniques (Wibell *et al.*, 1973). The mean value of plasma $\beta 2$ microglobulin of our RA patients resembles that observed in patients suffering from Sjögren's disease with or without associated RA (Talak *et al.*, 1975). The urinary values show an unusual frequency of elevated $\beta 2$ microglobulin compared to the range observed in normal individuals (less than 0.03 to 0.3 mg [1.78 to 17.8 mmol]/24 hours, $n = 133$). More than 30% of the patients excrete amounts above the upper limit of normal.

Table 1

Case	Sex	Age (years)	Duration of disease (years)	Plasma $\beta 2$ microglobulin (mg/l)	Urinary $\beta 2$ microglobulin (mg/24 h)	Creatinine clearance (ml/min)	Joint count	Lymphocytes (mm^{-3})	ESR (mm/h)
1	M	72	7	4.8 4.0 5.0 6.0 7.2 16.8 14.4 8.8 7.2	3.36 2.48	93	7 4 8 11 14 14 14 13	2673	85
2	F	30	3	1.5	Traces	120	1	1632	40
3	F	40	5	4.3	0.22	120	2	1739	20
4	M	25	4	1.7	Traces	125	0	946	8
5	M	65	12	3.6	0.16	98	4	1003	108
6	F	76	5	4.2	0.17	93	6	2673	113
7	F	76	11	4.1	0.68	79	5	1300	45
8	F	71	10	3.3	Traces	92	2	1975	40
9	F	49	7	1.3	Traces	86	0	1650	35
10	M	64	4	3.6	3.12	90	3	1575	130
11	F	77	20	4.7 2.2 2.2	0.85 Traces Traces	92	9 3 0	3450	115 76 33
12	M	71	13	2.2	Traces	94	0	1512	77
13	F	34	17	3.2	0.08	110	2	2006	97
14	F	40	23	2.0	0.05	90	0	1083	16
15	F	3.2	0.5	2.7	0.43	103	1	1050	74
16	F	63	12	2.1	Traces	92	0	1575	120
17	F	65	12	4.4 1.8 3.2	0.71 0.88 0.05	73	14 3 3	2205 1330 1525	120 120 120
18	M	72	3	7.0 3.7 4.2 6.6 4.8 4.9 4.9 5.4 6.4	Traces 0.22 Traces Traces Traces 0.04 0.04 0.04	88	14 4 6 17 7 7 7 11 15	1525 1932	54 90
19	F	60	4	4.0 4.1	1.92 1.28	116	6 6 4	2125	97 73 10
20	M	56	2	1.8	Traces	100	4	884	62
21	F	48	15	1.3–16.8	0.3–3.6	73–125	0–17		
Range		25–76	0.5–17	0.8–2.9	0–0.3	80–120	0		
Normal range		20–80							

The correlation between different parameters shown in Table 1 disclosed a significant positive linear correlation between plasma $\beta 2$ microglobulin and joint count (Fig. 1) and between plasma $\beta 2$ microglobulin and lymphocyte count (Fig. 2) but no significant correlation could be established between $\beta 2$ microglobulin and other parameters such as total white blood count, polymorphonuclear count, red blood cell count, or sedimentation rate.

Fig. 1 Linear relationship between plasma $\beta 2$ microglobulin and the joint count, expressed in arbitrary units. $n = 37$. $y = 0.31 (\pm 0.2)$ $x + 2.17 (\pm 0.19)$. $r = 0.898$. $P < 0.0001$.

Fig. 2 Linear relationship between plasma $\beta 2$ -m and plasma lymphocyte count. $n = 23$. $y = 1.34 (\pm 0.43)$ $x + 1.09 (\pm 0.78)$. $r = 0.564$. $P < 0.001$.

There was no correlation with age or sex and it is interesting that there was no correlation of $\beta 2$ microglobulin levels with $\alpha 2$ or gammaglobulin. There was a positive, but non-linear correlation, between joint count and sedimentation rate (Fig. 3).

As far as the levels of urinary $\beta 2$ microglobulin are concerned there was no correlation between the urinary excretion and plasma $\beta 2$ microglobulin levels although patients showed a tendency to excrete more $\beta 2$ microglobulin when the plasma level was well above the upper limit of normal. Some of them occasionally excreted high amounts of $\beta 2$ microglobulin (see case 17, Table 1) despite normal values of plasma $\beta 2$ microglobulin and creatinine clearance suggesting some tubular involvement of the kidney.

Figure 4 was chosen to illustrate a typical parallelism between the evolution of plasma $\beta 2$ microglobulin level and the simultaneous joint count evaluation. It should be stressed that in this particular case acute manifestations of polyneuritis and angitis accompanied a supplementary rise of $\beta 2$ microglobulin. The joint count was stabilised at that time. $\beta 2$ Microglobulin returned to normal values after corticotherapy. Unfortunately the patient died shortly after the last determination.

Discussion

It appears from this study that plasma $\beta 2$ microglobulin is frequently increased in RA and that the increase closely parallels the joint count which is a reasonable estimate of the degree of severity of the

Fig. 3 Non-linear relationship between red blood corpuscles sedimentation rate (SR) and joint count (arbitrary units). $r = 0.511$. $P < 0.001$.

Fig. 4 Evolution of joint count in arbitrary units and plasma $\beta 2$ microglobulin in a patient with RA. Note the dramatic and supplementary increase of $\beta 2$ microglobulin (arrows A and B), despite a stabilisation of the joint count, coinciding with the appearance of extra articular manifestation of RA (polyneuritis and angitis) and the rapid fall of $\beta 2$ microglobulin after corticotherapy (arrow C).

disease. Plasma $\beta 2$ microglobulin is also closely related to lymphocytosis and a supplementary increase of $\beta 2$ microglobulin can be observed when inflammatory manifestations of other organs than the joints appear during the evolution of the disease (Fig. 4). Our values of plasma $\beta 2$ microglobulin levels are comparable with those reported (Michalski *et al.*, 1975) in Sjögren's disease with or without RA.

As the renal function of our patients was within the normal limits and there was no other possible source to affect plasma $\beta 2$ microglobulin levels, the elevation observed must be related to the degree of RA involvement. The linear relationship between $\beta 2$ microglobulin and the joint count supports this. The joint involvement being due to an inflammatory process in which the lymphocyte infiltration is predominant, it is not surprising that we also found a good correlation between plasma $\beta 2$ microglobulin levels and the lymphocyte count. This correlation

has probably the same meaning as that observed between the salivary $\beta 2$ microglobulin and the degree of lymphocytic involvement of the salivary glands in cases of Sjögren's disease (Michalski *et al.*, 1975). The increase in $\beta 2$ microglobulin seems to reflect very well the increase of the total mass of lymphoid tissue and/or the increase of membrane turnover of this tissue, the lymphoid tissue being well known as a major source of $\beta 2$ microglobulin.

The apparent discrepancy between the presence of a linear correlation between $\beta 2$ microglobulin and joint or lymphocyte count and the absence of a linear correlation between joint and lymphocyte counts cannot be explained satisfactorily in the light of our results. It should, however, be remembered that when an additional manifestation occurs as in patient 1 (Fig. 4) the plasma $\beta 2$ microglobulin increases independently of the degree of joint involvement.

The absence of correlation between the sedimentation rate and $\beta 2$ microglobulin is probably due to the fact that $\beta 2$ microglobulin is related in a more specific way to RA than sedimentation rate. The same holds true of the relation between $\beta 2$ microglobulin and lymphocytosis as compared to the relation between $\beta 2$ microglobulin and polymorphonuclear count. For instance case 3 had a slightly elevated sedimentation rate but a high plasma $\beta 2$ microglobulin level together with a severe destruction of the wrist. It could be assumed that there is some correlation between $\beta 2$ microglobulin and immunoglobulin levels due to the structural relationship between $\beta 2$ microglobulins and some parts of the heavy or light chains of these proteins. It has, however, been shown that $\beta 2$ microglobulin does not originate from these immunoglobulins (Cejka *et al.*, 1973; Nilson *et al.*, 1973).

Finally, the presence of high values of urinary $\beta 2$ microglobulins in some of our patients can be explained in most of the cases by the increased plasma $\beta 2$ microglobulin. It has been well demonstrated that tubular reabsorption of $\beta 2$ microglobulin is a saturable process (Wibell and Evrin, 1974). On these grounds a high urinary level of $\beta 2$ microglobulin in conjunction with a moderately increased plasma level may be suggestive of tubular impairment. Despite the absence of amyloidosis on rectal biopsy case 1 might have renal amyloidosis.

$\beta 2$ microglobulin plasma levels appear thus to reflect the severity and extension of joint involvement in RA as well as the presence of extra-articular involvement. This parameter may be considered an early warning of abarticular complication and may be proposed as a useful tool for the evaluation of drug efficacy and for the adjustment of dosage schedules.

References

- Bach, M. L., Huang, S. W., and Hong, R. (1973). B2-microglobulin association with lymphocytes receptors. *Science*, **182**, 1350-1352.
- Berggard, I., and Bearn, A. G. (1968). Isolation and properties of a low molecular weight B2-globulin occurring in human biological fluids. *Journal of Biological Chemistry*, **243**, 4095-4099.
- Cejka, J., Cohen, F., and Kithier, K. (1973). Serum B2-microglobulin levels in normal children and sex linked agamma globulinemia patients. *Clinica Chimica Acta*, **47**, 59-61.
- Cresswell, P., Springer, T., and Strominger, J. L. (1974). Immunological identity of the small subunit of HLA antigens and B2-microglobulins and its turnover on the cell membrane. *Proceedings of the National Academy of Sciences*, **71**, 3123-3127.
- Cresswell, P., Turner, M. J., and Strominger, J. L. (1973). Papain solubilized HLA antigens from cultured human lymphocytes contain two peptide fragments. *Proceedings of the National Academy of Sciences*, **70**, 1603-1606.
- Cunningham, B. A., Wang, J. L., Berggard, I., and Peterson, P. A. (1973). The complete amino acid sequence of B2-microglobulin. *Biochemistry*, **12**, 24-27.
- Daniels, T. E., Silverman, S., and Michalski, J. P. (1975). The oral component of Sjögren's syndrome. *Oral Surgery*, **39**, 875-885.
- Evrin, P. E., and Wibell, L. (1972). The serum level and urinary excretion of B2-microglobulin in apparently healthy subjects. *Scandinavian Journal of Clinical and Laboratory Investigation*, **29**, 69-79.
- Greenspan, S. S., Daniels, T. E., and Talal, N. (1974). The histopathology of Sjögren's syndrome in labial salivary gland biopsies. *Oral Surgery*, **37**, 217-229.
- Grey, H. M., Kubo, R. T., Colon, S. M., Poulik, M. D., Cresswell, P., Springer, T., Turner, M. J., and Strominger, J. L. (1973). The small subunit of HLA antigens is B2-microglobulin. *Journal of Experimental Medicine*, **138**, 1608-1612.
- Hollander, J. L. (1966). *Arthritis and Allied Conditions*, pp. 269-290. Lea and Febiger, Philadelphia.
- Kithier, K., Cejka, J., and Belamarie, M. (1974). B2-microglobulin: occurrence in fetal life and malignancy. *Clinica Chimica Acta*, **52**, 293-299.
- McCalmon, R. T., Kubo, R. T., and Grey, H. M. (1975). Effect of anti B2-microglobulin on antigen and of human lymphocytes. *Journal of Immunology*, **114**, 1766-1770.
- Michalski, J. P., Daniels, T. E., Talal, N., and Grey, H. M. (1975). B2-microglobulin and lymphocyte infiltration in Sjögren's syndrome. *New England Journal of Medicine*, **293**, 1228-1231.
- Moore, G. E., Gerner, R. E., and Franklin, H. A. (1967). Culture of normal human leucocytes. *Journal of American Medical Association*, **199**, 519-521.
- Nakamuro, K., Tanigaki, N., and Pressman, D. (1974). Multiple common properties of human B2-microglobulin and the common portion fragment derived from HLA antigen molecules. *Proceedings of the National Academy of Sciences*, **70**, 2863-2865.
- Nilson, K., Evrin, P. E., Berggard, I., and Ponten, J. (1973). Involvement of lymphoid cells in the production of B2-microglobulin: a homologue of the constant domain of IgG. *Nature*, **244**, 44-45.
- Nilson, K., Evrin, P. E., and Welsh, K. I. (1974). Production of B2-microglobulin by normal and malignant human cell lines and peripheral lymphocytes. *Transplantation Review*, **21**, 53-84.
- Peterson, P. A., Cunningham, B. A., Berggard, I., and Edelman, G. M. (1972). B2-microglobulin. A free immunoglobulin domain. *Proceedings of the National Academy of Sciences*, **69**, 1697-2001.
- Peterson, P. A., Rask, L., and Lindblom, J. B. (1974). Highly purified papain-solubilized HLA antigens contain B2-microglobulin. *Proceedings of the National Academy of Sciences*, **71**, 35-39.
- Peterson, P. A., Rask, L., Sege, K., Klareskog, L., Anundt, H., and Ostberg, L. (1975). Evolutionary relationship between immunoglobulins and transplantation antigens. *Proceedings of the National Academy of Sciences*, **72**, 1612-1616.
- Poulik, M. D., Bernoco, M., Bernoco, D., and Creppell, J. (1973). Aggregation of HLA antigens at the lymphocyte surface induced by antiserum to B2-microglobulin. *Science*, **182**, 1352-1355.
- Poulik, M. D., and Bloom, A. D. (1973). B2-microglobulin production and secretion by lymphocytes in culture. *Journal of Immunology*, **110**, 1430-1433.
- Ropes, M. W., Burnett, C. A., and Cobb, S. (1958). Diagnostic criteria for rheumatoid arthritis. *Bulletin of Rheumatoid Diseases*, **9**, 175-176.
- Silver, J., and Hood, L. (1975). An approach to amino acid sequence analysis of transplantations antigens. *Nature*, **256**, 63-64.
- Talal, N., Grey, H. M., Zivafier, N., Michalski, J. P., and Daniels, T. E. (1975). Elevated salivary and synovial fluid B2-microglobulin in Sjögren's syndrome and rheumatoid arthritis. *Science*, **187**, 1196-1198.
- Wibell, L., and Bjorsell-Ostling, E. (1973). Endogenous creatinine clearance in apparently healthy individuals determined by 24 hour ambulatory urine collection. *Uppsala Journal of Medical Sciences*, **78**, 43-47.
- Wibell, L., Evrin, P. E., and Berggard, I. (1973). Serum B2-microglobulin in renal diseases. *Nephron*, **10**, 320-331.
- Wibell, L., and Evrin, P. E. (1974). *Urinary B2-microglobulin in Patients with Renal Diseases; a Study during Augmented Diuresis*. Thesis, Uppsala University.