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Elevated Salivary and Synovial Fluid β_2-Microglobulin in Sjogren's Syndrome and Rheumatoid Arthritis

Abstract. *β_2-Microglobulin is normally present in low concentrations in serum and other bodily fluids. By use of a radioimmunoassay, elevated concentrations of β_2-microglobulin were found in saliva and synovial fluid from patients with Sjogren's syndrome and rheumatoid arthritis, autoimmune inflammatory diseases that attack and destroy the salivary glands and articular tissues, respectively. Elevated β_2-microglobulin concentrations decreased in the saliva of two patients who simultaneously showed a clinical response to systemic treatment. Measurement of β_2-microglobulin in inflammatory fluids may offer a simple method of quantifying local activity in autoimmune states.*

The low molecular weight protein β_2-microglobulin (β_2m) (molecular weight 11,700) is present in low concentrations in normal serum and urine (*1*). It is increased in the serum and urine of patients with renal tubular disorders and in kidney transplant recipients, particularly during rejection crises (*2*). Amino acid sequence analysis of β_2m indicates a close homology with constant region domains of immunoglobulin polypeptide chains (*3, 4*).

β_2-Microglobulin is present on the surface membranes of peripheral blood lymphocytes (*5*). It is synthesized and secreted by lymphocytes as well as by various lymphoid and nonlymphoid tumor cell lines (*5, 6*). It is associated with both thymus-derived (T) and bone marrow–derived (B) lymphocytes since its presence has been demonstrated on thymocytes, thoracic duct lymphocytes, chronic lymphocytic leukemia cells, and cultured lymphoblastoid cell lines (*5, 6*). The latter two are generally considered to represent B cells.

Although the exact function of β_2m is unknown, it is thought to play a role in immunologic reactions since it is part of the HL-A antigen complex present on the lymphocyte cell surface (*7*). Antiserum to β_2m inhibits the response of lymphocytes to alloantigens in a mixed lymphocyte reaction (*8*). Such antiserums also induce redistribution and aggregation ("capping") of both β_2m and HL-A antigens on the lymphocyte membrane (*7–9*). The "co-capping" of β_2m and HL-A antigens is evidence of their intimate association in the living cell.

Certain autoimmune diseases such as Sjogren's syndrome and rheumatoid arthritis are characterized by intense lymphocytic and inflammatory cell infiltrations of the target tissue sites (*10, 11*). In Sjogren's syndrome, the destruction of the salivary and lacrimal glands by these infiltrates leads to the distressing symptoms of dry mouth and dry eyes (the "sicca complex"). In rheumatoid arthritis, the joint cavity is the target of attack, with cartilage destruction, bone erosion, and joint deformity occurring as a consequence. Immunoglobulins, rheumatoid factor, lymphoid cells, and inflammatory products can often be found in the biological fluids associated with these inflammatory sites, that is, the saliva and synovial fluid (*11, 12*).

A rapid simple method for quantitatively measuring the extent of inflammation in tissue sites would be extremely useful both for diagnosis and for monitoring treatment in these autoimmune and related diseases. We hoped that measurement of β_2m concentrations in inflammatory fluids might provide such a method. Accordingly, we have employed a radioimmunoassay procedure to measure β_2m in saliva and synovial fluid collected from patients with Sjogren's syndrome and rheumatoid arthritis, respectively. β_2-Microglobulin was also measured in the patients' serum and in saliva and synovial fluid collected from a variety of control subjects. Our results suggest that β_2m concentrations are increased in these inflammatory fluids, probably as a consequence of local production by infiltrating cells. Moreover, in two patients with Sjogren's syndrome a clinical response to corticosteroids or immunosuppressive drugs

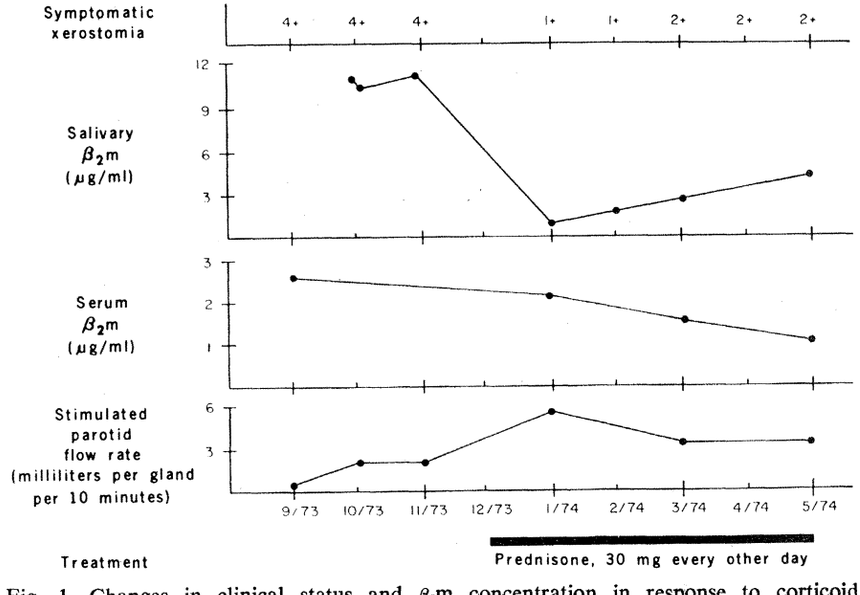

Fig. 1. Changes in clinical status and β_2m concentration in response to corticoid treatment in a patient with Sjogren's syndrome. The scale for symptomatic xerostomia is relative; there was no observation for December 1973.

Table 1. Concentration of β_2m in saliva and synovial fluid. The β_2m concentrations are given as mean ± standard error of the mean; *N*, number of patients.

Group	Diagnosis	*N*	β_2m (μg/ml)
	Saliva		
1	Sicca syndrome	17	4.94 ± 1.97
2	Sicca syndrome and connective tissue disease	7	5.16 ± 2.64
3	Sicca syndrome and rheumatoid arthritis	6	2.18 ± 0.80
4	Possible Sjogren's syndrome	11	0.96 ± 0.26
5	Connective tissue disease	6	1.02 ± 0.44
6	Other salivary gland disease	5	0.61 ± 0.17
7	Normal individuals	5	0.84 ± 0.40
1–2	All Sjogren's syndrome patients	30	4.44 ± 1.27*
5–7	All control subjects	16	0.84 ± 0.20
	Synovial fluid		
8	Rheumatoid arthritis	10	6.21 ± 0.71†
9	Inflammatory, nonrheumatoid arthritis	11	3.30 ± 0.22
10	Noninflammatory joint disease	8	2.55 ± 0.24

* Significantly greater ($P < .01$) than mean value for all control subjects (groups 5–7). † Significantly greater than the mean value for group 9 ($P < .01$) or group 10 ($P < .001$).

was accompanied by a decrease in salivary β_2m.

Parotid salivary flow rates were measured and saliva was collected following gustatory stimulation (*13*). Serum and synovial fluids were obtained by standard procedures. All specimens were stored at −20°C. Radioimmunoassay for β_2m was performed by measuring the capacity of a synovial fluid or saliva sample to inhibit the binding between [^{125}I]β_2m and antiserum to β_2m, as previously described (*7*).

Salivary specimens from 30 patients with Sjogren's syndrome were studied. Seventeen patients had an isolated sicca syndrome, seven had an associated connective tissue disease (five with systemic lupus erythematosus, one each with polymyositis and a scleroderma variant), and six had associated rheumatoid arthritis. The salivary β_2m concentration was significantly elevated ($P < .01$) in these patients (Table 1, groups 1, 2, and 3) compared to control diseased subjects (groups 4, 5, and 6) and normal individuals (group 7).

The salivary gland, like the kidney, is a tubular organ that might play a role in the normal transport of β_2m. If so, the elevated salivary β_2m might reflect a tubular abnormality rather than the inflammatory condition of the gland. For this reason, we examined another biological fluid in which tubular transport would not be a factor. Synovial fluids were obtained from 10 patients with active rheumatoid arthritis (Table 1, group 8), 11 patients with inflammatory but nonrheumatoid arthritis (group 9), and 8 patients with noninflammatory (degenerative) joint disease (group 10). β_2-Microglobulin was significantly elevated in synovial fluid from patients in the rheumatoid group compared to those in the other two groups ($P < .01$ and $P < .001$, respectively).

To determine whether this increase reflected local production or elevated concentrations in the blood, β_2m was measured in serum samples. In the majority of patients, the β_2m concentration was higher in the saliva or synovial fluid than in the serum, which suggests that the increase in the inflammatory fluids is due to local production (Table 2).

Table 2. Concentration of β_2m in inflammatory fluids (saliva in Sjogren's syndrome and controls and synovial fluid in rheumatoid arthritis) and serum of individual patients. Of the control subjects, four had possible Sjogren's syndrome, three had connective tissue disease, and two had salivary disease (without Sjogren's syndrome). The normal concentration of β_2m in serum is taken as 1.90 ± 0.35 (13 subjects).

Patient	β_2m (μg/ml) in	
	Inflammatory fluid	Serum
	Sjogren's syndrome	
1	20.16	3.8
2	11.16	2.49
3	6.94	2.85
4	4.59	5.16
5	3.78	2.38
6	3.64	3.2
7	3.42	2.6
8	3.42	4.05
9	3.28	6.8
10	3.15	1.49
11	3.03	3.0
	Rheumatoid arthritis	
1	11.04	4.14
2	8.32	2.92
3	6.76	2.97
4	6.59	4.14
5	5.29	2.83
6	4.81	1.37
7	4.66	1.13
8	3.29	2.29
	Control subjects	
1	2.90	2.40
2	1.66	2.53
3	1.06	1.35
4	0.45	1.90
5	0.41	2.70
6	0.36	0.94
7	0.29	3.78
8	0.27	0.99
9	0.04	1.39

Since patients with Sjogren's syndrome have greatly reduced salivary flow rates compared to normal individuals, the rate dependence of the salivary β_2m concentration was tested. In two normal subjects, parotid β_2m did not change as the flow rate was varied from resting secretion to maximum stimulation.

To determine the relation between β_2m concentration and disease activity, serial determinations were performed in patients receiving systemic therapy. One patient with Sjogren's syndrome and markedly elevated salivary β_2m was started on prednisone (30 mg every other day) because of severe dry mouth (xerostomia). Ten weeks later, the salivary β_2m had fallen markedly, the symptoms of xerostomia had improved, and the parotid salivary flow rate had increased appreciably (Fig. 1). As prednisone dosage was continued over the next several months, the symptoms returned, the salivary β_2m concentration rose, and the salivary flow rate declined. The serum β_2m concentrations were at the upper limit of the normal range initially and decreased progressively during the treatment period. A second patient with Sjogren's syndrome and rheumatoid arthritis was treated with cyclophosphamide. The salivary β_2m concentration was 3.56 μg/ml initially and fell to 0.76 μg/ml after 6 months of therapy. These results indicate a correlation between elevated β_2m and disease activity, and suggest that measurement of β_2m concentrations in inflammatory fluids may permit accurate quantification of activity in autoimmune and similar disorders. Such quantification, not currently available, could serve as a guide to treatment.

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Phosphorylation of Lymphocyte Nuclear Acidic Proteins: Regulation by Cyclic Nucleotides

Abstract. *Guanosine 3′,5′-monophosphate (cyclic GMP) and cholinergic agents stimulate incorporation of phosphate into specific nuclear acidic proteins of horse peripheral blood lymphocytes. Agents that raise intracellular adenosine 3′,5′-monophosphate (cyclic AMP) inhibit nuclear acidic protein phosphorylation. The opposing effects of cyclic GMP and cyclic AMP upon nuclear protein phosphorylation parallel the effects of the cyclic nucleotides upon induction of lymphocyte proliferation.*

Chromatin protein phosphorylation is an early event preceding the extensive gene activation occurring upon induction of lymphocyte proliferation by mitogens (*1*, *2*). Certain of the effects of exogenously added mitogens upon chromatin structure and activity may be mediated by guanosine 3′,5′-monophosphate (cyclic GMP). The mitogens phytohemagglutinin and concanavalin A (Con A) have both been observed to elevate intracellular levels of cyclic GMP more than tenfold within minutes of addition to human lymphocyte cultures (*3*, *4*). On the basis of evidence obtained with a number of cell types induced to proliferate (*5*), it has been hypothesized that cyclic GMP mediates the intracellular effects of mitogens acting at the cell surface, and that, conversely, adenosine 3′,5′monophosphate (cyclic AMP) mediates actions antagonistic to the mitogenic process (*3*). We now provide evidence that opposing influences of cyclic GMP and cyclic AMP upon lymphocyte proliferation may be mediated through the effects of these agents on phosphorylation of specific nonhistone chromatin proteins.

Lymphocytes were purified from horse peripheral blood by centrifugation over a cushion of Ficoll-Isopaque (*2*). Cells prepared as described were >90 percent lymphocytes, the remaining cells consisting primarily of monocytes, which were removed by glass adherence. Lymphocytes, cultured for 24 hours prior to use in experiments, were treated with [^{32}P]-phosphate as described in the legend to Fig. 1. After cell activity was stopped by rapidly freezing at −70°C, nuclei were isolated and nuclear acidic proteins were extracted (*2*, *6*, *7*). The protein fraction contained only 10 to 20 percent of the total lymphocyte nuclear protein (*2*). These proteins constitute a class of phosphoproteins that are species specific, tissue specific, and that have been observed to stimulate transcription from isolated DNA in vitro (*6*). These phenol-extracted acidic phosphoproteins were subjected to sodium dodecyl sulfate disc-gel electrophoresis, and [^{32}P]phosphate was assayed in individual electrophoretic protein bands (*7*).

The differing effects of cyclic GMP and cyclic AMP on the amount of [^{32}P]phosphate incorporated into lymphocyte nuclear acidic proteins are shown in Fig. 1A. At a concentration of $10^{-6}M$ cyclic GMP stimulates phosphorylation of the total phenol-extracted protein fraction about twofold 10 minutes after addition to the culture medium. In contrast to this stimulatory effect of cyclic GMP, cyclic AMP, at any concentration tested, does not increase phosphate incorporation into the total nonhistone chromatin protein fraction. Both cyclic GMP and cyclic AMP inhibit nuclear acidic protein phosphorylation at concentrations $>10^{-5}M$. The 8-bromo derivative of cyclic GMP stimulates nuclear acidic protein phosphorylation at lower concentrations than does cyclic GMP. One-and-a-half– to twofold stimulation of phosphate incorporation into total nuclear acidic proteins was obtained at concentrations of 8-bromo cyclic GMP ranging from $10^{-7}M$ to $10^{-9}M$ in different experiments. 8-Bromo cyclic GMP does not inhibit nuclear acidic protein phosphorylation at concentrations as high as $10^{-5}M$. Monobutyryl derivatives of both cyclic GMP and cyclic AMP have no effect when used at low concentrations and are strongly inhibitory to phosphate incorporation at concentrations $>10^{-5}M$.

Agents known to elevate cyclic GMP concentrations in lymphocytes have effects on nuclear acidic protein phosphorylation opposite to those of agents known to elevate cyclic AMP. Cholinergic agents selectively elevate cyclic GMP in several tissues including lymphocytes (*8*). The data in Fig. 1B show that acetylcholine ($10^{-6}M$) stimulates the rate of phosphate incorporation into lymphocyte nuclear acidic proteins. The rate of protein phosphorylation is stimulated maximally within 10 minutes of acetylcholine addition and returns to control values within 90 minutes. Prostaglandin E_1 (PGE_1), an agent observed to raise cyclic AMP in lymphocytes (*9*), has effects on nuclear protein phosphorylation opposite to those exerted by the cholinergic agents (Fig. 1B). At $10^{-4}M$, PGE_1 inhibits nonhistone chromatin protein phosphorylation 60 percent within 45 minutes of addition to lymphocyte cultures. At the concentrations used, neither acetylcholine nor PGE_1 affects the viability of lymphocytes up to 24 hours after addition. Acetylcholine stimulated phosphorylation maximally at concentrations ranging from $10^{-6}M$ to $10^{-8}M$ in different experiments, while maximal inhibition by PGE_1 was observed from $10^{-4}M$ to $10^{-6}M$. The cyclic nucleotide phosphodiesterase inhibitors theophylline and papaverine were also examined for