Synovial fluid $\beta_2$ microglobulin and hydroxyproline fractions in rheumatoid arthritis and nonautoimmune arthropathies

D. MANICOURT, H. BRAUMAN, AND S. ORLOFF

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SUMMARY

Fourteen patients with classical and definite seropositive rheumatoid arthritis (RA), 5 patients with microcrystalline arthritis, and 7 patients with osteoarthritis were studied with respect to markers of newly synthesised collagen (synovial NDOH pro levels); markers of connective tissue resorption (synovial DOH pro and NBH levels); markers of lymphoid tissue activity (synovial and plasma $\beta_2m$ levels). Higher amounts of NDOH pro in RA synovial fluid are compatible with the hypothesis of a local connective tissue production as suggested by Utito et al. on basis of a higher protocollagen proline hydroxylase activity in RA synovial tissue. DOH pro and NBH do not differ significantly in synovial fluid from RA or gouty patients, but the correlations between these forms of OH pro and, respectively, synovial lymphocytes and polymorphonuclear leucocytes are indicative of different processes of connective tissue remodelling in the 2 conditions. Synovial $\beta_2m$ levels are a direct function of total synovial lymphocyte counts independently of the type of arthropathy being explored. The ratio of synovial to plasma $\beta_2m$ is systematically above unity in RA patients only.

Recent work on $\beta_2$ microglobulin ($\beta_2m$) led to the discovery of its immunogenetic aspect, though its exact function in this respect is still unknown (Marx, 1974). This 11 700 molecular weight (MW) protein has a close homology with the constant domains of the heavy and light chains of immunoglobulins, especially with the CH3 domain (Berggard and Bearn, 1968; Peterson et al., 1972; Smithies and Poulik, 1972; Cunningham et al., 1973).

$\beta_2m$ is normally present in low concentrations in serum and generally speaking in all biological fluids (Evrin et al., 1971; Evrin and Wibell, 1972). The circulating $\beta_2m$, however, does not derive from a splitting of immunoglobulins, and its production does not depend only on B cell function (Cejka et al., 1973; Hutteroth et al., 1973; Nilson et al., 1973). $\beta_2m$ is a part of the major histocompatibility cell-membrane-bound antigenic complexes (Cresswell et al., 1973; Grey et al., 1973; Nakamura et al., 1973; Cresswell et al., 1974; Peterson et al., 1974; Tanigaki and Pressman, 1974) and is produced by lymphocytes (T and B) and cell lines of tumoral or nontumoral lymphoid tissues (Bernier and Fanger, 1972; Hutteroth et al., 1973; Nilson et al., 1973; Poulik and Bloom, 1973; Kithier et al., 1974; Nilson et al., 1974).

Except for cases of renal insufficiency or pregnancy, increased serum levels of $\beta_2m$ were reported in patients with advanced malignant disease and in some inflammatory autoimmune disorders such as ulcerative colitis, regional enteritis, systemic lupus erythematosus (SLE) dysglobulinaemic purpura, and rheumatoid arthritis (RA) (Evrin and Wibell, 1973; Brauman et al., 1977). Talal et al. (1975) reported elevated concentrations of $\beta_2m$ in saliva and synovial fluid from patients with Sjögren's syndrome. In patients with RA we described increased circulating $\beta_2m$ parrelling the lymphocytosis and reflecting the severity and extension of the joint involvement as well as the onset of extra-articular involvement (Manicourt et al., 1978). An intense subsynovial lymphocytic and inflammatory cell infiltration characterises the RA joint cavity (Talal, 1972), and an increased collagenase production by this hypercellular rheumatoid synovial tissue may be an
important cause of joint destruction (Evanson et al., 1968; Harris et al., 1969; Wegelius et al., 1970).

Hydroxyproline (OH pro) was described as a metabolic marker of the collagen whose biosynthesis is increased in RA synovial tissue (Uitto et al., 1972).

Among the different OH pro fractions dialysable OH pro (DOH pro) and mainly nonprotein bound OH pro (NBH) appear to be relevant markers of bone collagen disorders and especially of bone resorption (Kivirikko, 1970; Krane et al., 1970; Bishop and

Table 1  Group A patients with rheumatoid arthritis

<table>
<thead>
<tr>
<th>Plasma β2m mg/l</th>
<th>Synovial β2m mg/l</th>
<th>Synovial OH pro (µg/ml)</th>
<th>Synovial leucocytes count</th>
<th>TOH pro</th>
<th>DOH pro</th>
<th>NDOH pro</th>
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<th>Neutrophil polymorphs</th>
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Table 2  Patients with microcrystalline osteoarthrosis (Group B) and osteoarthritis

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<th>Synovial β2m mg/l</th>
<th>Synovial OH pro (µg/ml)</th>
<th>Synovial leucocytes count</th>
<th>TOH pro</th>
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<th>Neutrophil polymorphs</th>
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<td>0.6</td>
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Group C: Osteoarthrosis

|                 |                   |                         |                           |         |         |         |     |                      |            |
|                 |                   |                         |                           |         |         |         |     |                      |            |
| 1 1.92          | 1.57              | 9.0                     | -                         | -       | -       | -       | -   | 0.67                 | 0.31       |
| 2 1.20          | 0.83              | 10.0                    | -                         | -       | -       | -       | 0.82| 0.18                 |            |
| 3 1.63          | 1.07              | 7.5                     | -                         | -       | -       | -       | 1.10| 0.63                 |            |
| 4 1.90          | 1.30              | 8.5                     | -                         | -       | -       | -       | 0.76| 0.57                 |            |
| 5 1.40          | 1.00              | 9.2                     | -                         | -       | -       | -       | 0.75| 0.12                 |            |
| 6 1.70          | 0.93              | 8.0                     | -                         | -       | -       | -       | 1.00| 0.02                 |            |
| 7 2.53          | 1.66              | 8.7                     | -                         | -       | -       | -       | 0.8  |                      |            |
| M 1.7           | 1.2               | 8.7                     | -                         | -       | -       | -       | 0.2  |                      |            |
| ± SD 0.4        | 0.3               | 0.8                     | -                         | -       | -       | -       | 0.2  |                      |            |
Synovial fluid $\beta_2$ microglobulin and hydroxyproline fractions in rheumatoid arthritis

Smith, 1971; Kaye, 1971; Sagar et al., 1971; Goulding et al., 1974; Varghese et al., 1973), whereas the nondialysable OH pro (NDOH pro) are derived either from collagen newly synthesised and rapidly degraded or from fragments recently synthesised but not incorporated into tropocollagen (Krane et al., 1967, 1970; Haddad et al., 1970).

As $\beta_2$m production is associated with lymphocytes it was of interest to study the levels of $\beta_2$m in the synovial fluid of patients suffering from RA and to correlate these data with the synovial cytology, the synovial total and different OH pro fractions, and the degree of disease activity as expressed by the joint count (Hollander and McCarty, 1972), the erythrocyte sedimentation rate (ESR), the serum latex fixation test (LF), and the plasma $\beta_2$m levels (Manicourt et al., 1978). Furthermore it was interesting to compare the data observed in RA with the values observed in degenerative or inflammatory nonautoimmune conditions.

**Patients and methods**

Fourteen patients with classical and definite seropositive RA according to the criteria of the American Rheumatism Association were admitted in this study (group A), and patients with renal insufficiency amyloidosis or Sjögren's syndrome were excluded. The control group consisted of 5 patients presenting an inflammatory nonrheumatoid arthritis (gout or pseudogout, group B) and 7 patients with osteoarthritis (group C).
Blood and synovial fluid samples were collected simultaneously for $\beta_2 m$ and OH pro determinations as well as for other routine biological parameters (ESR, LF). On the same day a physical examination by a member of the staff evaluated the joint count (JC) according to standard directions (Hollander and McCarty, 1972).

$\beta_2 m$ was radioimmunoassayed by the Phadebas $\beta_2 m$ microtest (Phadebas Pharmacia, Uppsala, Sweden). The total and free OH pro (FOH pro) were measured in hydrolysed and unhydrolysed samples (Delfosse et al., 1975); the nondialysable fraction (NDOH pro) was isolated (Haddad et al., 1970) and the OH pro content was determined as

![Graphs of linear correlation between synovial fluid $\beta_2 m$ microglobulin and the joint count (JC) and between synovial fluid $\beta_2 m$ and plasma $\beta_2 m$ in RA patients (group A) (mg/l).](http://ard.bmj.com/)

Fig. 3 Linear correlation between synovial fluid $\beta_2 m$ microglobulin and the joint count (JC) and between synovial fluid $\beta_2 m$ and plasma $\beta_2 m$ in RA patients (group A) (mg/l).
mentioned above. The dialysable fraction (DOH pro) was assessed by subtracting NDOH pro from total OH pro and the NBH by subtracting FOH pro from DOH pro. When several values for synovial fluid appear for the same patient, as in Tables 1 and 2, they were obtained from various joint cavities.

**Results**

The relevant biological data are presented in Table 1 and 2.

Synovial $\beta_2$m levels are highest in RA patients (group A) and differ significantly between the 3 groups ($P<0.001$) (Fig. 1).

Total OH pro levels for groups A and B (Fig. 1) are significantly higher when compared to group C ($P<0.001$); no statistical difference is found between groups A and B ($P<0.1$). The NDOH pro values (Fig. 1) in group A are significantly higher than those observed in group B ($P<0.001$) whereas there is no statistical difference between the DOH pro and the NBH values in these 2 different groups (Fig. 1).

Irrespective of the type of arthropathy a signi-

![Graph](https://example.com/image1.png)

![Graph](https://example.com/image2.png)

**Fig. 4** (Upper) Linear correlation between synovial fluid $\beta_2$m microglobulin ($\beta_2$m) and the erythrocyte sedimentation rate (ESR) in the RA patients (Lower) Linear correlation between synovial fluid $\beta_2$m microglobulin ($\beta_2$m) and the logarithm of the inverse of the latex fixation test (1/dil. latex) in RA patients (group A)
Fig. 5 Correlation between synovial fluid $\beta_2$ microglobulin ($\beta_2$m) and synovial fluid total hydroxyproline (OH pro), dialysable hydroxyproline ($D$ OH pro), and nonprotein-bound hydroxyproline (NBH) in the RA patients (group A).

Fig. 6 Correlation between synovial fluid lymphocyte count and synovial fluid total hydroxyproline (OH pro), dialysable hydroxyproline ($D$ OH pro) and nonprotein-bound hydroxyproline (NBH) in RA patients (group A).
Significant positive linear correlation is found between the synovial $\beta_2$m levels and the synovial lymphocytosis (Fig. 2). In RA patients (group A) measurements of overall disease activity (JC, plasma $\beta_2$m levels, ESR, and LF) show a positive correlation with synovial $\beta_2$m levels (Figs. 3 and 4). Moreover, in the same patients (group A) the synovial $\beta_2$m values correlate significantly with the synovial total OH pro ($r=0.70$; $P<0.001$), with the synovial DOH pro ($r=0.83$; $P<0.001$) and with the synovial NBH ($r=0.81$; $P<0.001$) (Fig. 5). As might be expected from the data in Fig. 2, RA patients further show a positive correlation between synovial lymphocyte counts and synovial OH pro values, namely $r=0.70$, $P<0.01$, for synovial total OH pro; $r=0.75$, $P<0.01$ for synovial DOH pro; and $r=0.77$, $P<0.001$ for synovial NBH (Fig. 6). No such correlations were found in control groups.

Interestingly enough, in gouty patients (group B) the synovial OH pro fractions are correlated with the synovial polymorphonuclear neutrophil (PMN) count (Fig. 7) and not with the synovial lymphocyte count.

In none of the groups studied is there any correlation between synovial NDOH pro levels and synovial cells count (PMN or lymphocytes).

It is further noted that in each RA patient synovial $\beta_2$m levels are systematically higher than plasma $\beta_2$m levels in contrast to the 2 other groups (Tables 1 and 2).

Discussion

From our results it emerges that synovial fluid NDOH pro is significantly higher in RA than in gouty patients and that no correlation exists between this fraction and the cellular content of the synovial fluid. Since NDOH pro is said to originate from fragments recently synthesised but not incorporated into tropocollagen (Krane et al., 1967; Haddad et al., 1970), a higher content of this fraction could be evidence of a higher rate of collagen biosynthesis, which agrees with a protocollagen proline hydroxylase hyperactivity in RA synovial tissue (Uitto et al., 1972). In this respect RA joint connective tissue remodelling would appear to differ from microcrystalline arthritis on the grounds of the presence of an active repair process that we cannot demonstrate in microcrystalline arthritis. Nevertheless the high OH pro content of Clq (MW 40 9000; 45 residues per 1000; Reid et al., 1972) may also reflect on the synovial NDOH pro levels and account for the difference seen between RA and gouty patients. We were not in a position to measure systematically the synovial complement levels in our patients, and it is thus impossible to assert to what extent synovial NDOH pro reflects connective tissue activation or increased complement levels or both. However, the usually low Clq synovial values in seropositive RA and the generally normal Clq synovial levels in gout (Hedberg, 1973; Versey et al.,
1973; Bunch et al., 1974; Williams, 1976) suggest that NDOH pro in RA reflects rather connective tissue activation. This conclusion would hold unless it can be shown that the low levels described in synovial fluid are not only due to a high consumption rate but to a turnover that is fast enough to produce more NDOH pro in RA than in gout but also specific enough to stop at this stage, since total OH pro appears to be identical in synovial fluid of both conditions.

The amounts of synovial total OH pro, DOH pro, and NBH do not significantly differ in fluids from patients with RA or gout, but the correlation between these OH pro fractions and the 2 types of synovial fluid cells (Figs. 6 and 7) indicates that their production is related to different mechanisms involved in these different inflammatory processes rather than to the different rates of connective tissue remodelling. Indeed synovial fluid PMN cells have a key role in the pathogenesis of gout or pseudogout, where it has been shown that, when leucocytes take up urate or calcium phosphate crystals, the latter interact with the lysosomal membrane, causing its rupture, with the liberation of collagenase and various proteolytic enzymes responsible for articular destruction (McCarty et al., 1962; Weissmann, 1966, 1967, 1972; McCarty, 1971; Wright and Malawista, 1973).

In contrast the positive correlation between lymphocyte count and DOH pro or NBH levels in synovial fluid from RA patients (Fig. 6) stresses the primordial contribution of lymphocytes in RA joint injury and destruction. Indeed PMN do not seem to play a key role in RA joint lesions. PMN are not characteristically seen both at the invasion zones and in the synovium itself, where the infiltrate mainly comprises mononuclear cells. Collagenase not originating from PMN has been detected at the cartilage/pannus junction (Woolley et al., 1977). Moreover, macrophages incubated with lymphokines can produce collagenase (Wahl et al., 1975), and Dayer et al. (1977) have recently reported that lymphocytes produce a low molecular weight factor (MW 11 500) stimulating collagenase production by rheumatoid synovial cells in culture.

Our observation that synovial \( \beta_2 \)m levels are a direct function of synovial lymphocyte count irrespective of the joint pathology (Fig. 2) is not surprising since the lymphoid tissue is well known to be a major source of \( \beta_2 \)m (Bernier and Fanger, 1972). Synovial \( \beta_2 \)m overproduction (Tables 1 and 2) and the correlation between markers of bone collagen resorption (namely, DOH pro and NBH) and synovial \( \beta_2 \)m levels on the one hand (Fig. 5) and synovial lymphocyte count on the other (Fig. 6) are observed only in RA patients whose overall disease activity is already shown to correlate with synovial \( \beta_2 \)m levels (Figs. 3 and 4). These data appear as an in-vivo confirmation of Dayer et al.'s in-vitro observation of the stimulating effect of lymphocytes on collagenase production (Dayer et al., 1977).

Synovial \( \beta_2 \)m can be thus considered as a fair index of synovial lymphoid tissue overactivity in RA and of the severity of RA joint involvement and destructive potential. Since \( \beta_2 \)m has no known function per se (either as an enzyme or as a stimulator), and in absence of further characterisation of the low molecular weight (MW 11 500) factor described by Dayer et al. (1977), it is at best to consider them as special evidence of lymphoid cells activity in RA. This is further substantiated by the observation that the synovial to plasma \( \beta_2 \)m ratio is systematically raised above unity in only RA in contrast to non-autoimmune arthropathies (microcrystalline arthritis or osteoarthrosis), where it is systematically lower than unity, an observation that could eventually be useful in differentiating both conditions.

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References


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