Spontaneous and induced immunoglobulin secretion by synovial fluid B lymphocytes in rheumatoid arthritis

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SUMMARY The functional properties of B lymphocytes in synovial fluid (SF) from patients with rheumatoid arthritis (RA) were analysed by means of a reverse haemolytic plaque forming cell (PFC) assay. SF mononuclear cells spontaneously secreted IgG, but little IgM or IgA. The SF cells failed to respond to the polyclonal B cell activators pokeweed mitogen (PWM) and Epstein-Barr virus. However, SF B cells cocultured with autologous T lymphocytes from the blood and stimulated with PWM secreted IgG but little IgM or IgA. The PFC responses of blood B cells cocultured with autologous SF T cells in the presence of PWM were low; irradiation of the T cells increased the blood B lymphocyte responses, but the differences were not statistically significant. It is concluded that suppressor SF T cells may be partly responsible for the poor response of SF B cells to PWM.

In recent years evidence has been provided for the participation of both antibody-mediated and cell-mediated immune reactions in rheumatoid arthritis (RA). By the development of sensitive assays for quantitation of human B lymphocyte activation in vitro it has been shown that at least 2 cell populations regulate B cell function. Monocytes/macrophages (Mø) are mandatory for the B lymphocyte response to polyclonal B cell activators such as pokeweed mitogen (PWM) and Staphylococcus aureus. 

When present in high concentrations, Møs suppress the responses of B cells. Suppression is furthermore achieved with smaller amounts of Møs stimulated in vitro. Besides Møs, T lymphocytes subsets—that is, helper and T suppressor cells, are significant for modulation of the B cell differentiation.

In the synovial fluid (SF) of RA patients T lymphocytes and Møs are found in considerable numbers, whereas few B lymphocytes have been demonstrated. According to recent investigations synovial fluid T cells comprise more cells with suppressor phenotype as determined by monoclonal antibodies than are found in the blood. However, the functional implication of the increased proportions of T cells with the morphological characteristics of suppressor cells remains to be clarified.

The aims of the present investigation are to evaluate the ability of synovial fluid B cells from RA patients to secrete immunoglobulins (Ig) spontaneously, to investigate the responses of synovial fluid B cells to polyclonal activators, and finally to assess the influence of synovial fluid T lymphocytes on the function of autologous blood B cells.

Materials and methods

 Patients. Eight patients, mean age 48 years (range 36 to 69 years), with definite or classical RA according to the American Rheumatism Association criteria were included in the study. Four patients were receiving gold salts, 2 patients penicillamine, and 1 patient prednisone (in a dose of 10 mg daily) at the time of the study. All the patients were treated with non-steroidal anti-inflammatory drugs.

From all the patients synovial fluid free of blood was obtained by puncture of the knee. A blood specimen was drawn on the same day.

Isolation of mononuclear cells. Mononuclear cells from blood (BMC) and from synovial fluid (SMC) were isolated by centrifugation on Lymphoprep (Nyegaard, Oslo, Norway) as described previously. BMC and SMC were washed and resus-
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The content of the image appears to be a continuation of the text discussing immunoglobulin secretion, specifically focusing on the generation of PWM- and EBV-induced PFC responses. The text describes the preparation and culture of BMC and SMC, the isolation of T-depleted, MØ-depleted, and Mφ-enriched suspensions, and the quantitation of Ig-secreting cells. Additionally, it mentions the use of PWM and EBV for inducing PFC responses and the enumeration of PFC using the reverse haemolytic plaque assay. The text also briefly discusses the role of Mφ-depleted SMC in the study.

Figure 1: Spontaneous secretion of immunoglobulins by BMC and SMC. Ordinate: PFC/10^6 live cells; n=8. I: IgM-PFC. II: IgG-PFC. III: IgA-PFC.
In some experiments cells were incubated at 56°C for 15 min prior to assay for PFC activity. In additional experiments cells were incubated with 100 μg/ml of cycloheximide (Sigma) for 2 h at 37°C prior to assay for PFC. Cycloheximide was present during washings and in the agar in a similar concentration. Statistics. Data are presented as means ± 1 SEM. The results were analysed by the 2-tailed Student’s t-test. Values of p<0.05 were regarded as significant.

Results

Spontaneous Secretion of Ig
The spontaneous formation of PFC by BMC was low with regard to IgM, IgG, and IgA (Fig. 1). Thus BMC found in synovial immune complexes from the cell membranes, experiments were carried out as summarised in Table 1. SMC heated for 15 min at 56°C showed no PFC activity; hence the SMC-PFC formation requires live cells. Furthermore, the major part of PFC formation was inhibited by incubation with the protein synthesis inhibitor cycloheximide, supporting the assumption that SMC-IgG plaques are due to a protein-synthesising cell.

As B lymphocytes are few in suspensions of SMC, it was of interest to ensure the B cell origin of spontaneous plaques. Synovial fluid T-depleted cells generated no PFC, whereas the capacity for spontaneous PFC formation was preserved in synovial fluid T-depleted cell suspensions (Table 1). In additional experiments IgG-PFC were found to a similar extent in suspensions of Mφ-depleted SMC, whereas less than 100 IgG-PFC/10⁶ cells were recorded among from patients with rheumatoid arthritis were not spontaneously activated into Ig-secreting cells, which is similar to the findings in normal persons. By contrast SMC contained a considerable number of PFC, restricted to the IgG class (Fig. 1). Thus SMC generated more IgG-PFC than did BMC (p<0.001), whereas IgM-PFC were found in similar numbers. SMC IgA-PFC were discovered to a lower degree as compared to BMC (Fig. 1) (p<0.05).

To exclude the possibility that the spontaneous SMC-PFC were due to release of IgG-containing immune complexes from the cell membranes, experiments were carried out as summarised in Table 1. SMC heated for 15 min at 56°C showed no PFC activity; hence the SMC-PFC formation requires live cells. Furthermore, the major part of PFC formation was inhibited by incubation with the protein synthesis inhibitor cycloheximide, supporting the assumption that SMC-IgG plaques are due to a protein-synthesising cell.

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Table 1  Spontaneous PFC formation: influence of heating (56°C for 15 min) and of treatment with cycloheximide (100 μg/ml for 2 hours)

<table>
<thead>
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<th>Expt. 1</th>
<th>Expt. 2</th>
<th>Expt. 3</th>
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<td>1869</td>
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<tr>
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<td>2</td>
<td>0</td>
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<tr>
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<td>2413</td>
<td>1208</td>
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<tr>
<td>SF T-depleted cells</td>
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<td>2604</td>
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<td>SF T-enriched cells</td>
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1 Results are given as IgG-PFC/10⁶ live cells.

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Fig. 2  PWM-induced PFC responses of 50% blood T-depleted cells cocultured with 50% autologous T-enriched cells; n=8. Ordinate: PFC/10⁶ originally cultured cells.

Tblood: T-enriched cells from blood.
TSF: T-enriched cells from SF.
\( \# \): denotes irradiation (2500 rad).
I: IgM-PFC. II: IgG-PFC. III: IgA-PFC.
Mø-enriched SMC. Thus SMC-IgG plaques are caused by B lymphocytes.

POKEWEED-MITOGEN-INDUCED IG SECRETION

In a previous communication it was shown that BMC from patients with rheumatoid arthritis generated considerable numbers of Ig-secreting cells in response to PWM, whereas SMC failed to do so.\(^{19}\) Preliminary experiments revealed that optimal PWM-PFC responses in RA patients were obtained with cocultures of 50% T-depleted and 50% T-enriched cells from the blood, in agreement with findings in normal adults\(^{20}\) and in neonates.\(^{21}\) T-depleted or T-enriched cells cultured alone failed to respond to PWM (data not shown).

First, we investigated the balance between helper and suppressor T cells from the blood using irradiation of T cells to eliminate suppressor activity.\(^{22-23}\) As outlined in Fig. 2, the PFC responses of blood T-depleted cells cocultured with blood T cells were similar to those obtained with irradiated T cells, indicating that T cells in the blood of a patient with RA do not include a significant number of T lymphocytes with suppressor activities, which is in agreement with earlier findings.\(^{24}\) Secondly, similar experiments were performed with SF T cells. Fig. 2 shows that T-enriched cells from synovial fluid supported PFC responses to a lesser degree than autologous blood T cells (p<0.01). Irradiated SF T-enriched cells yielded PWM-induced PFC responses which were higher than those obtained with unirradiated cells, though the differences did not reach statistical significance.

It was of interest to investigate whether SF B cells could respond to PWM after removal of T lymphocytes. These experiments are summarised in Fig. 3. SF T-depleted cells cultured alone failed to respond to PWM (data not shown). The same cells cocultured with T-enriched cells from the blood generated a considerable number of PFC in the presence of PWM. As in the case of spontaneous SMC-PFC formation, the PWM-induced SF B cell response was restricted to IgG-secreting cells, whereas few IgM- or IgA-PFC were found (p<0.01). With irradiated blood T cells the SF B cells generated more IgG-PFC, but the difference did not reach statistical significance.

EBV-INDUCED IG SECRETION

Because SMC B cells were able to respond to pokeweed mitogen in the presence of blood T cells, it was of interest to evaluate the SMC responses to the direct B cell activator EBV.\(^{25}\) BMC and T-depleted blood cells responded well to EBV (Table 2). By contrast, neither SMC nor SF T-depleted cells secreted Ig when challenged with EBV.

**Discussion**

In the present investigation the reverse haemolytic PFC assay was used to quantify both spontaneous and in-vitro-induced Ig secretion. The spontaneous Ig secretion by RA blood lymphocytes was low. By contrast, SMC secreted Ig spontaneously, but with restriction to IgG, in accordance with the recent findings of Al-Balaghi et al.\(^{26}\) IgG-containing immune

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**Table 2. EBV-induced PFC responses of BMC and SMC, and of T-depleted cells from blood or synovial fluid**

<table>
<thead>
<tr>
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<tr>
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</tr>
<tr>
<td>T-depleted cells</td>
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<td></td>
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</tr>
<tr>
<td><strong>SF</strong></td>
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</table>

1 Data are expressed as PFC/10\(^6\) originally cultured cells. Cells from 3 RA patients were investigated.
complexes are abundant in the synovial fluid of patients with RA, and these immune complexes bind to cells with Fc receptors. Experiments were performed to exclude the possibility that SMC-PFC complexes were due to passive release of such immune complexes. First, heating of cells to 56°C completely abolished PFC formation. Secondly, on the addition of a protein synthesis inhibitor, cycloheximide, the majority of PFC disappeared. Thirdly, SMC-PFC formation occurred only in suspensions of SF B cells. Thus the spontaneous IgG-PFC of synovial fluid seem to be live, protein-synthesising B lymphocytes.

In an earlier report we found that SMC did not generate PFC in response to pokeweed mitogen. According to recent experiments the possibility that the low SMC responses were due to Mc- mediated suppression of Ig-secreted cells has been ruled out (Petersen, unpublished data). As the low SMC-PFC formation might be due to the occurrence of synovial fluid T suppressor cells, SF SMC were depleted of T lymphocytes, and the remaining cells—that is, SF B cells plus Msps, were cocultured with autologous T lymphocytes from the blood. Under these conditions synovial fluid B cells secreted Ig. Again, the secreted Ig was restricted to the IgG class. The experiments thus show that, although SF B cells spontaneously secrete IgG, probably due to activation in vivo these cells also secrete IgG in response to PWM when SF T cells are replaced with blood T cells. As SF B lymphocytes could respond to PWM under certain conditions, it was of interest to assess the SF B cell response on direct stimulation with EBV. In normal adults EBV induces a PFC response which is mainly of the IgM class. In the present study we could not generate SMC-PFC responses by means of EBV. These results disagree with earlier findings, indicating that SMC from patients with rheumatoid arthritis may transform into cell lines once they are infected with EBV.

The recent development of hybridoma techniques has provided important tools for the characterisation of T lymphocyte subpopulations. Thus SMC from RA patients have recently been reported to contain more T cells with the putative suppressor phenotype than cells from blood. In this report we analysed the ability of SF T cells to co-operate in the PWM-induced activation of autologous B cells obtained from the blood. It was clearly shown that employment of SF T cells gave a lower B cell response than did blood T cells from the same individual. As irradiation of T lymphocytes abolishes their suppressive effect, this procedure was applied to SF T cells to investigate the balance between helper and suppressor T functions. Irradiation of SF T cells before addition to blood B cells increased the PFC responses, although the increase was not demonstrated statistically. Thus, in vivo-activated SF T suppressor cells may be of importance for the low SMC-PFC response to pokeweed mitogen. Experiments with SF suppressor T cells purified by means of monoclonal antibodies are in progress in our laboratory, and these experiments may clarify the role of SF T cell subsets with regard to Ig secretion of SF B cells. Furthermore, such experiments may elucidate whether synovial fluid B cells from patients with RA are amenable to suppression to the same degree as blood B cells.

This work was supported by the National Danish Association against the Rheumatic Diseases, Fonden til Lægevidenskabens Fremme, and the Ferdinand and Ellen Hindsdaugs Fund. EBV was a gift from Dr Jette Hesse. The excellent technical assistance of Mrs Anne Ambjørnsen and Mrs Vita Weibull is gratefully acknowledged.

References
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Book review


Apart from the clinical examination no skill is more necessary for the rheumatologist than that of local injection. The proposers of the motion at the final meeting of the Heberden Society that steroid injections did more harm than good to patients had an impossible task despite their powerful advocacy.

The production of a pocket size manual on the subject is to be warmly welcomed. The second edition of this monograph has been revised and enlarged. After a general introduction it deals with each area of the body. The indications for and the techniques of injection into each region are succinctly discussed. Excellent illustrations in the form of clinical photographs, radiographs, and diagrams clarify the procedures. No junior doctor on the rheumatological or orthopaedic service can afford to be without this book, and most consultants will be grateful for it too. We have ordered a copy for each ward in the Regional Rheumatology Centre at Harrogate.

V. WRIGHT
Spontaneous and induced immunoglobulin secretion by synovial fluid B lymphocytes in rheumatoid arthritis.

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