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A Comparison of Lymphocyte Subpopulations Simultaneously on Local and Systemic Levels in Acute Rheumatoid Arthritis Patients

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A B S T R A C T

Rheumatoid arthritis (RA) is one of the most destructive inflammatory and autoimmune joint diseases, most frequently accompanied by extraarticular complications. The pathophysiologic mechanism and the importance of cell subpopulations in the initiation and perpetuation of synovitis are not sufficiently understood. In this study the frequency of lymphocyte subpopulations simultaneously in the synovial fluid (SF), the synovial membrane (SM) and peripheral blood (PB) of acute RA patients is determined, using flow cytometry procedures. The changes in the distribution of T lymphocyte subpopulations were significant on local levels in acute RA patients, resulting in a decreased CD4/CD8 ratio in SF, but an increased CD4/CD8 ratio in SM, compared to the ratio found in PB. The differences observed in the frequency of cells positive on natural killer (NK) cell markers suggest the role of $CD16^{-}CD56^{+}$ NK cell population in SF of RA patients. Significant differences in the observed frequency of lymphatic subpopulations suggest certain specificities of local immunological events in SM and SF in acute RA. These results confirm the T-lymphocyte hypothesis in initial pathogenic events in RA.

Key words: *T lymphocytes, NK cells, peripheral blood, synovial fluid, synovial membrane, acute rheumatoid arthritis*

Introduction

Rheumatoid arthritis (RA) is an inflammatory disease with autoimmune features affecting synovial joints. In severe cases there are extraarticular and systemic complications. The basic aetiology of the disease is not known, but aside for purely genetic predisposition, there is abundant evidence suggesting the possibility that activated lymphocytes might be central in the pathogenic mechanism of joint destruction and extraarticular complications in RA[1–4]. The pathogenetic role of different lymphocyte sets and subsets has been investigated broadly in the peripheral blood (PB), the synovial fluid (SF), and the synovial membrane (SM) of RA patients, revealing significant oscillation in their levels[5–9]. The question of which T cells contribute to the pathogenetic changes in the early phase of the disease, and which predominantly contribute to the perpetuation of synovial inflammation in RA, remain unanswered. In addition, there are differences in T cell and NK cell subsets depending on the phase of the disease and the compartment analysed (systemic vs. local).

The purpose of this study is to determine the frequency and distribution of lymphocyte subpopulations simultaneously on local (synovial fluid and synovial membrane) and systemic levels (peripheral blood) of acute RA patients. The difference in the frequency of T lymphatic subpopulations in different tissue compartments in the acute stage of the disease suggests that activated T lymphocytes play an important role in the initial pathogenic events in affected joints.

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Materials and Methods

Patient selection

This study was approved by The Ethics Committee of the Medical Faculty, University of Rijeka, and was carried out with the written consent of each subject. Peripheral blood and synovial tissue samples were obtained from patients with acute rheumatoid arthritis. All patients were hospitalized in the Clinic of Orthopaedic Surgery Lovran (Croatia). The diagnosis of RA was based on the revised criteria of the American College of Rheumatology (ACR) from 1987[10]. Patients who met a minimum of four out of seven possible criteria were studied. The selection of rheumatic patients was based solely on the availability of paired blood and synovial tissue samples (synovial membrane and synovial fluid) from patients undergoing synoviectomy or knee joint replacement surgery. Pregnant women, patients with immunological diseases or chronic diseases other than RA (chronic pancreatitis, diabetes mellitus, haematopoetic organ diseases etc.), and patients with malignant diseases were excluded from the study. A combination of clinical indicators (swollen and tender joints, skin hyperthermia) and results of laboratory testing (high erythrocyte sedimentation rate >30 mm/h, and high C-reactive protein concentration in the blood serum) was used to define the acute activity of the rheumatic articular disesase. The group of patients studied included both patients in whom RA was diagnosed for the first time and those already treated for RA, but hospitalized in the acute stage of the disease. Patients were not divided based on rheuma-factor seropositivity. Peripheral blood, synovial membrane and synovial fluid samples from 30 acute RA patients (21 female, 9 male, age range 37–60) were analysed. Synovial membrane and synovial fluid samples were routinely obtained in the Clinic of Orthopaedic Surgery Lovran, from patients undergoing diagnostic or therapeutic joint tapping, arthroscopic and open synoviectomy of the knee joint.

Preparation of synovial membrane lymphocytes (SML)

Synovial membrane tissue free of blood coagulum was rinsed several times in cold Rosswell Park Memorial Institute (RPMI) 1640 medium (Institute of Immunology, Zagreb), cut into small pieces and exposed to trypsin digestion [0.125% trypsin, (Difco, Detroit, MI) with 0.2% EDTA] at 37°C for 90 minutes with constant stirring. Trypsination was stopped by adding human AB serum to the 10% concentration of solution. Cells thus isolated were separated from the debris by passing through nylon mesh twice and by undergoing centrifugal sedimentation (600 g / 10 min). Supernatant was then aspirated and 10 ml of RPMI 1640 was added to the pellet. The cell suspension obtained was overlaid on Ficoll/Hypaque (Pharmacia, Uppsala, Sweden), and centrifuged at 800 g for 20 min. The cells accumulated at the interface were collected, rinsed twice in the RPMI 1640 medium and cultured overnight in tissue culture Petri dishes placed in a humidified CO_2 incubator. After macrophage adherence, the remaining mononuclear cell suspension was aspirated and used for immunofluorescent phenotyping. All samples were >95% viable, as assessed by Trypan Blue dye exclusion. From the synovial membrane of acute RA patients 6×10^5 to 6×10^6 MNC could be recovered, depending of the intensity of MNC infiltration of synovial membrane samples and the quantity of SM tissues obtained by diverse operative procedures and subsequently subjected to flow cytometric analysis.

Preparation of synovial fluid lymphocytes (SFL)

Synovial fluid (SF) was obtained from knee joints and selected on the basis of SF volume obtained (a minimum of 10 ml), due to mononuclear cell (MNC) requirements for flow cytometry (a minimum 1×10^6 MNC per sample). Grossly hemorrhagic SF was not used. MNC were collected after Ficoll-Hypaque density gradient centrifugation, rinsed three times in the RPMI 1640 supplemented with 10% fetal calf serum (FCS). Further steps were identical to those described for SML.

Preparation of peripheral blood lymphocytes (PBL)

Approximately 20 ml of heparinized peripheral blood was taken by cubital vein phlebotomy from patients undergoing diagnostic or therapeutic procedures before initiation of anaesthesia. Blood was overlaid onto Ficoll/Hipaque, centrifuged for 20 min at 800 g, and MNC from the interface were collected, rinsed, and cultured, as described for SML.

Phenotypic detection of cell surface antigens

The phenotypic detection of cell surface membrane antigens was performed by direct immunofluorescence and analyzed by flow cytometry immediately after cell separation. Briefly, cells were aliquoted (10^6 per aliquot) and rinsed in FACS buffer (2% fetal calf serum (FCS), 1 mmol/L EDTA, 0.1% NaN_3 in phosphate-buffered saline (PBS), pH 7.4). Double labelling method of surface antigens was used. The cells were simultaneously labeled with monoclonal antibodies directly conjugated either with FITC or PE fluoresecence dye (CD3-FITC, CD4-PE, CD8-PE, CD16-PE, CD56-PE; Becton Dickinson, Mountain View, CA) and the frequency of $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^-CD16^+$ or $CD3^-CD56^+$ cells was estimated, as well as the frequency of $CD25^+$ lymphocytes. Antibodies in the cell suspension were added in the concentration of 2 μg/ 100 μl at 4°C for 30 minutes. After three rinses in the FACS medium, the cells were resuspended in the same volume of FACS medium. In all experiments irrelevant isotype-matched murine mAbs were used as a negative control. Viable lymphocytes were gated on the basis of forward and side scatter (FSC and SSC) profile. A minimum of 10^4 cells was analysed on FACScan (Becton Dickinson), using Lysis II software. The results of this analysis were shown in dot plot graphs. The percentages of positive cells were obtained by subtracting the values of the negative controls.

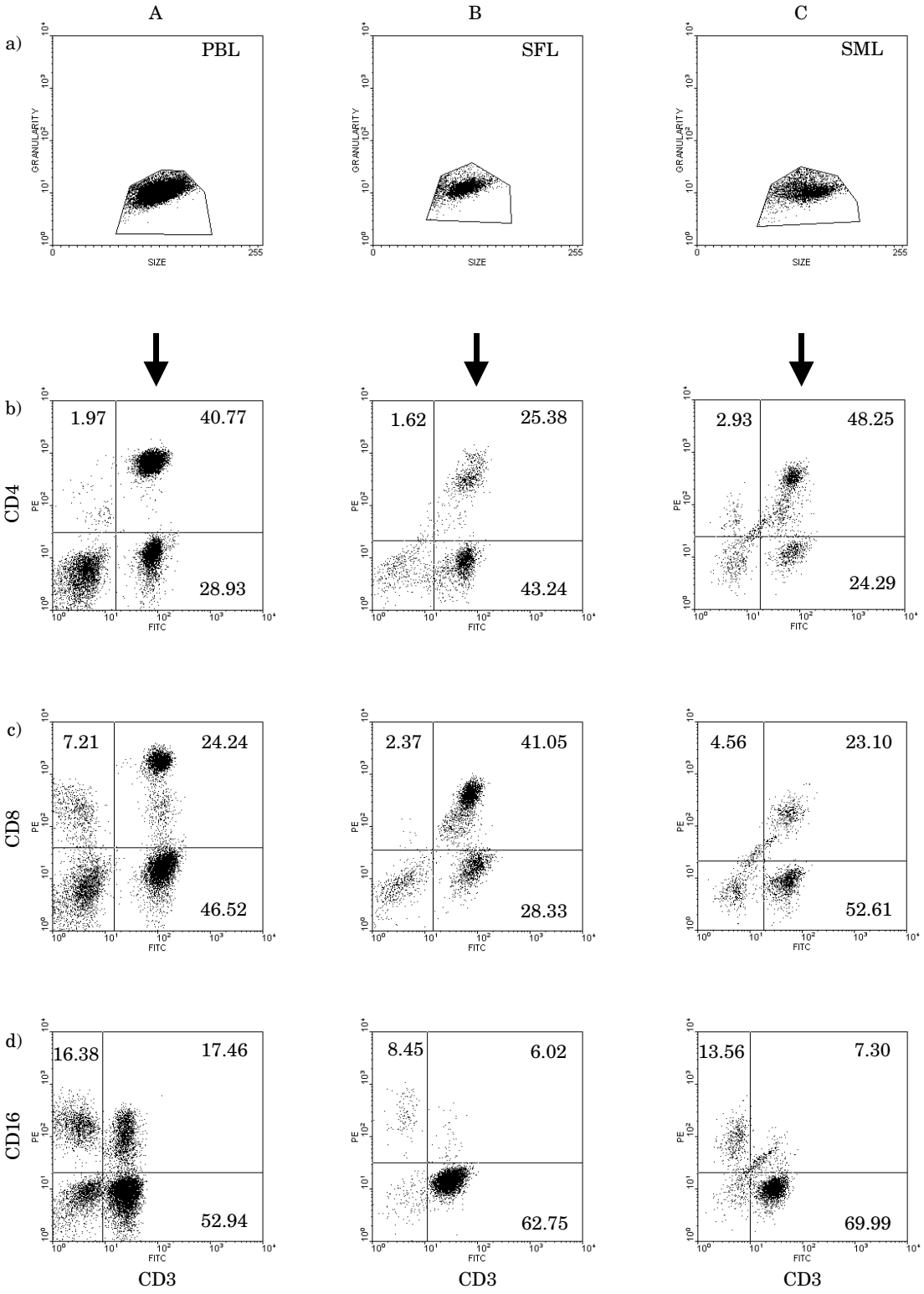

Fig. 1. Flow cytometric analysis of the expression of CD3, CD4, CD8 and CD16 antigens on cell surface of (A) peripheral blood (PBL), (B) synovial fluid (SFL), and (C) synovial membrane lymphocytes (SML) in patients with acute rheumatoid arthritis. The cells were analysed on FACScan, using two-color cytometric analysis with fluorescein isothiocyanate- (CD3-FITC) or phycoerythrin-labeled (CD4-PE, CD8-PE, CD16-PE) monoclonal antibodies. (a) The cells were first gated to include lymphocytes and a minimum of 10^4 cells were analysed for green (FITC, abscissa) and red (PE, ordinate) fluorescence. The frequency of $CD3^+CD4^+$ cells (b), $CD3^+CD8^+$ cells (c), and $CD3^-CD16^+$ (d) cells was estimated. The results shown are based on samples obtained from one representative acute rheumatoid patient.

Statistical analysis

The obtained results were analysed using Sigma Plot for Windows Version 1.02 (Jandel Scientific, Chicago, IL). Statistical analyses were performed using a standard Student's *t*-test one-way analysis for the comparison of means.

Results

Expression of cell surface antigens

In an attempt to define the immunoregulatory mechanims operating in rheumatoid arthritis we identified the frequencies of total T lymphocytes (CD3 positive cells), their subsets ($CD3^+CD4^+$ and $CD3^+CD8^+$ cells), and NK cells ($CD3^-CD16^+$ and $CD3^-CD56^+$ cells), as well as the number of activated cells bearing an IL-2 receptor ($IL\text{-}2R\alpha^+$ or $CD25^+$ cells) in acute RA patients (systemic level), and compared them to the findings of lymphocyte cell distribution in synovial membrane and synovial fluid (local level). The comparative results of statistical significance obtained from one representative acute rheumatoid patient are shown in Figure 1.

The frequency of CD3 positive cells in PBL, SFL and SML of acute RA patients

The frequencies of total T lymphocytes (CD3 positive cells) in the samples of peripheral blood lymphocytes (PBL), synovial fluid lymphocytes (SFL) and synovial membrane lymphocytes (SML) of acute RA patients were obtained by flow cytometry analysis. There was no significant difference in the percentage of total T lymphocytes found on systemic and local levels in acute RA patients (63–73%), which was presented in Figure 2.

The frequency of CD4 positive cells in PBL, SFL and SML of acute RA patients

Analyzing the population of lymphocytes positive both on CD3 and CD4 cell surface antigens (T helper cell subset) in acute RA patients (Figure 3), we found that their frequency was highest in the population of synovial membrane lymphocytes (44%), and lowest in the population of synovial fluid lymphocytes (25.8%). The resulting difference in the distribution of CD4 positive cells between the synovial fluid and peripheral blood was statistically significant at the level where $p<0.01$, and the difference in CD4 positive cell distribution in the synovial membrane and the synovial fluid was statistically significant where $p<0.001$. Despite the fact that higher values of CD4 positive cells were found in the synovial membrane, compared to those in peripheral blood, the estimated difference was not statistically significant.

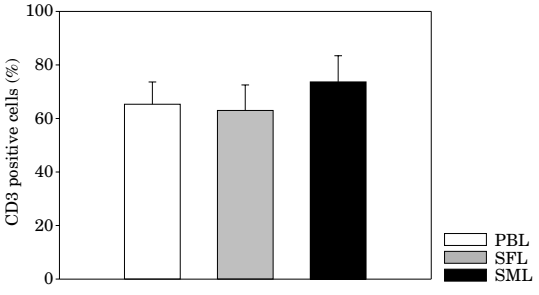

Fig. 2. The percentages of CD3 positive lymphocytes in autologous samples (PBL, SFL, SML) obtained from 30 (21 female, 9 male) acute rheumatoid arthritis patients were estimated by flow cytometry and all data sets were included in the statistical analysis (X±SE). PBL – peripheral blood lymphocytes, SFL – synovial fluid lymphocytes, SML – synovial membrane lymphocytes.

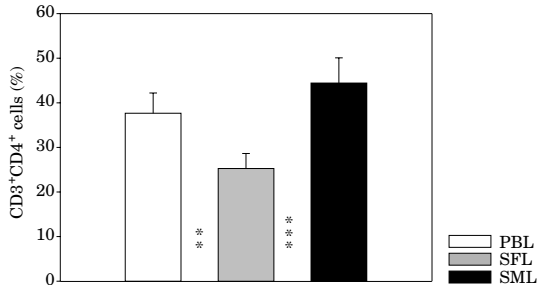

*Fig. 3. The percentages of CD4 positive lymphocytes in autologous samples (PBL, SFL, SML) obtained from 30 (21 female, 9 male) acute rheumatoid arthritis patients were estimated by flow cytometry and all data sets were included in the statistical analysis (X±SE). PBL – peripheral blood lymphocytes, SFL – synovial fluid lymphocytes, SML – synovial membrane lymphocytes. Level of significance: ** p<0.01, ***p<0.001.*

The frequency of CD8 positive cells in PBL, SFL and SML of acute RA patients

Immunophenotypic analysis of T lymphocytes positive both on CD3 and CD8 cell surface antigens (cytotoxic T lymphocytes), conducted on systemic and local level in acute RA patients, revealed significant differences in autologous tissue compartments (Figure 4). The highest percentage of these cells was found in the population of synovial fluid lymphocytes (37.1%), and statistically lower values were found in peripheral blood ($p<0.01$) and the synovial membrane ($p<0.001$).

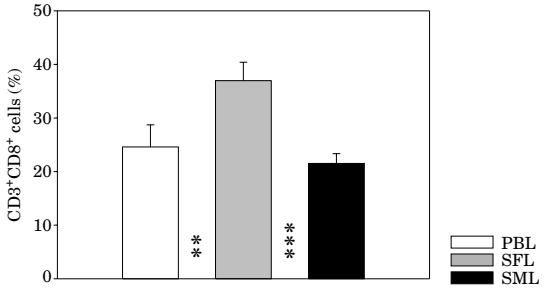

*Fig. 4. The percentages of CD8 positive lymphocytes in autologous samples (PBL, SFL, SML) obtained from 30 (21 female, 9 male) acute rheumatoid arthritis patients were estimated by flow cytometry and all data sets were included in the statistical analysis (X±SE). PBL – peripheral blood lymphocytes, SFL – synovial fluid lymphocytes, SML – synovial membrane lymphocytes. Level of significance: ** p<0.01, ***p<0.001.*

The ratio value of CD4 and CD8 positive lymphocytes in different tissue compartments of acute RA patients

In Figure 5 summarised percentages of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells and $CD3^+CD4^+/CD3^+CD8^+$ (or CD4/CD8) ratios in analysed tissue compartments (PBL, SFL, SML) of acute RA patients are shown. In peripheral blood, the ratio of helper and cytotoxic lymphocyte subpopulations was 1.5, which is in the normal range of values for adults. In comparison to the same ratio in peripheral blood, the CD4/CD8 ratio was significantly lower in synovial fluid (0.69), as a result of a simultaneous increase in CD8 and decrease in CD4 positive cells in the synovial fluid of acute RA patients. In contrast, in the synovial membrane of acute RA patients we found a higher CD4/CD8 ratio compared to that in peripheral blood (1.98).

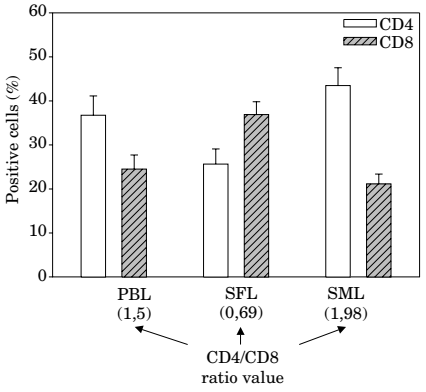

Fig. 5. The ratios of CD4 and CD8 positive lymphocytes in the population of peripheral blood lymphocytes (PBL), synovial fluid lymphocytes (SFL) and synovial membrane lymphocytes (SML) obtained from 30 (21 female, 9 male) acute rheumatoid arthritis patients.

The frequency of CD16 and CD56 positive cells in PBL, SFL and SML of acute RA patients

Aiming to analyse NK cell involvement in acute RA pathogenic processes, we estimated the expression of cell surface antigens characteristic for this lymphocyte population, CD16 (Figure 6) and CD56 (Figure 7), on the $CD3^-$ lymphocyte subpopulation. In autologous samples of peripheral blood, synovial fluid and synovial membrane lymphocytes of acute RA patients, significant differences were found. The highest percentage of $CD16^+$ cells was obtained in the peripheral blood lymphocyte population (16.3%). A statistically lower percentage of these cells were found on a local level (synovial membrane = 13%, $p<0.01$, synovial fluid 8.5%, $p<0.001$). In contrast to the CD16 surface antigen, the CD56 surface molecule was not expressed differently in autologous tissue samples of acute RA patients. The resulting higher frequency of $CD56^+$ cells in peripheral blood was not statistically significant in comparison to that in the other two examined compartments.

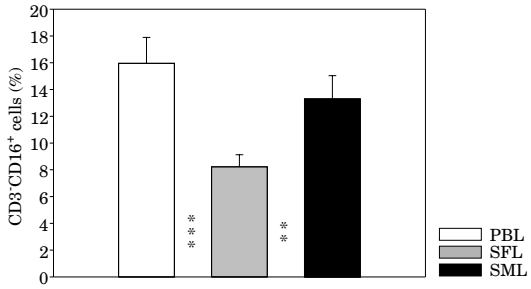

*Fig. 6. The percentages of CD16 positive lymphocytes in autologous samples (PBL, SFL, SML) obtained from 30 (21 female, 9 male) acute rheumatoid arthritis patients were estimated by flow cytometry and all data sets were included in the statistical analysis (X±SE). PBL – peripheral blood lymphocytes, SFL – synovial fluid lymphocytes, SML – synovial membrane lymphocytes. Level of significance: ** p<0.01, ***p<0.001.*

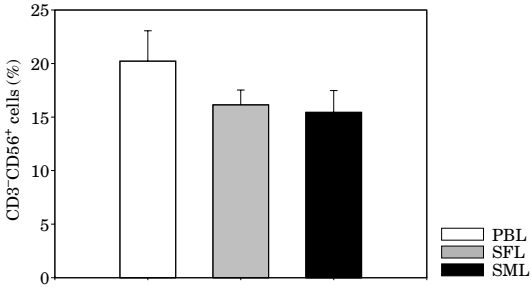

Fig. 7. The percentages of CD56 positive lymphocytes in autologous samples (PBL, SFL, SML) obtained from 30 (21 female, 9 male) acute rheumatoid arthritis patients were estimated by flow cytometry and all data sets were included in the statistical analysis (X±SE). PBL – peripheral blood lymphocytes, SFL – synovial fluid lymphocytes, SML – synovial membrane lymphocytes.

The ratio value of CD16 and CD56 positive lymphocytes in different tissue compartments of acute RA patients

The percentage of $CD3^-CD16^+$ and $CD3^-CD56^+$ lymphocytes in different tissue compartments (PBL, SFL, SML) in acute RA patients and their ratios are shown in Figure 8. The $CD3^-CD16^+$ / $CD3^-CD56^+$ ratio (or CD16/CD56 ratio) was identical (0.8) in peripheral blood and the synovial membrane, but it was lower (0.5) in the synovial fluid resulting from a significantly diminished frequency of $CD16^+$ cells in this joint compartment.

The frequency of CD25 positive cells in PBL, SFL and SML of acute RA patients

The CD25 antigen or high affinity IL-2 receptor (IL-2Rα) is prominent on activated T lymphocytes. The distribution of this antigen among different tissue compartments in acute RA patients is shown in Figure 9. A statistically significant difference in the level of expression of this antigen was found in different compartments, with a higher level of manifestation found in peripheral blood lymphocyte populations than in the synovial membrane populations ($p<0.05$).

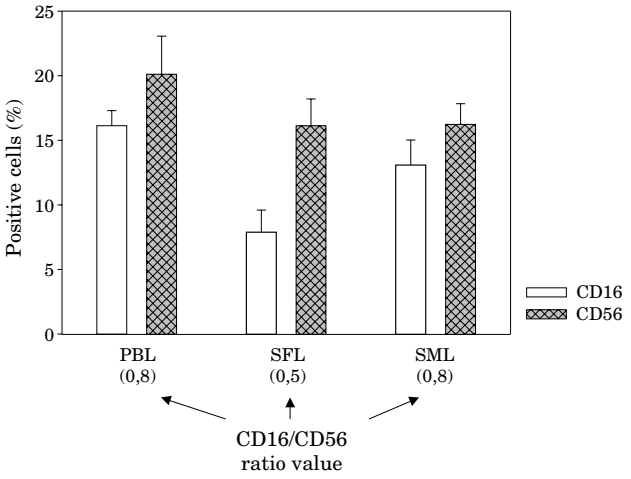

Fig. 8. The ratios of CD16 and CD56 positive lymphocytes in the population of peripheral blood lymphocytes (PBL), synovial fluid lymphocytes (SFL) and synovial membrane lymphocytes (SML) obtained from 30 (21 female, 9 male) acute rheumatoid arthritis patients.

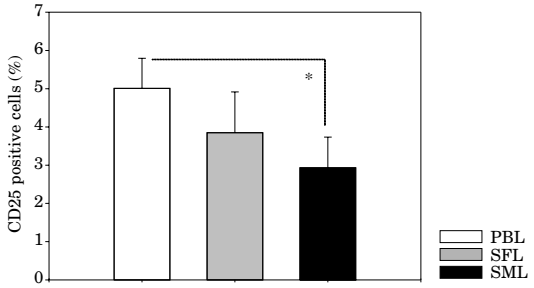

*Fig. 9. The percentages of CD25 positive lymphocytes in autologous samples (PBL, SFL, SML) obtained from 30 (21 female, 9 male) acute rheumatoid arthritis patients were estimated by flow cytometry and included in the statistical analysis (X±SE). PBL – peripheral blood lymphocytes, SFL – synovial fluid lymphocytes, SML – synovial membrane lymphocytes. Level of significance: * p<0.05.*

Discussion

Rheumatoid arthritis (RA) is a chronic, systemic imflammatory disease that affects approximately 1% of the world population. Despite many years of intensive study, neither the etiology, nor the pathogenic regulatory mechanisms of RA have been defined. Several allelic variants of HLA-DRB1 genes (HLA DRB1*0401 and *0404) have been associated with RA, suggesting that T-cell receptor HLA-antigen interactions play an important role in the pathologic process in RA[1–4]. However, although a large body of data suggests that T lymphocytes play a crucial role in the pathogenic process, there is much controversy regarding their role in and contribution to the initiation and perpetuation of RA, giving rise to both T-dependent and T-independent hypotheses regarding the pathogenesis of RA[11–16]. The first hypothesis describes T lymphocytes as the main initiative cell population which can be activated by a certain, as yet undefined, specific exogenous antigen, or that the autoreactive T lymphocytes in the joint can be triggered by certain tissue antigens. The second hypothesis proposes that the perpetuation of RA is not necessarily an antigen-driven process and that T cells are only one of several important factors in the perpetuation of RA. Considerable uncertainty also remains regarding the frequency and distribution of distinct lymphocyte subpopulations in different tissue compartments in acute RA patients, as well as regarding their role in different stages of the disease[11].

The importance of the T cell subpopulations in RA was emphasized in our investigation as well. The results of the present study confirm certain previous findings where the frequency of total T lymphocytes was similar on both local and systemic levels in the acute stage of RA. However, the percentages measured (70%) in our study were close to the upper range of values found in other investigations (30–70%).

To determine the potential role of T lymphocytes in the initiation of RA, we analysed the distribution of different T lymphocyte subpopulations both on systemic level (peripheral blood) and the local level (synovial membrane and synovial fluid), where terminal joint destruction occurs. In spite of the fact that no difference was detected in the level of $CD3^+$ cells, significant changes were found in the $CD4^+$ subpopulation of $CD3^+$ cells which, during the acute phase of RA, were the highest in SM, where the pathologic process occurs. The association of RA with a class II MHC epitope implies that antigen-specific responses of $CD4^+$ T lymphocytes are required for the development of the disease[16]. Most studies identify this lymphocyte subpopulation as the most important one in recognizing a potential arthritogenic antigen present on specialized antigen presenting cells (such as dendritic cells in SM). However, some longitudinal studies suggest a negative corelation between the number of CD4 synovial membrane lymphocytes and the clinical intensity of the disease, suggesting in turn the protective role of $CD4^+$ lymphocytes[17]. Our results, indicating an increased number of $CD4^+$ T cells in the synovial membrane in the initial stage of RA patients, do not uphold this hypothesis. However, significantly lower values of $CD3^+CD4^+$ cells were detected in the synovial fluid, suggesting either that the CD4 positive cells play a different role in these two joint compartments, or that there is a difference in the time dynamics of their arrival. $CD3^+CD4^+$ cells are generally regarded as Th1 or Th2 cells producing either inflammatory or noninflammatory network of cytokines. The investigation of Th1/Th2 cytokine profiles dominating in RA did not confirm the classical theory of the division of cytokine production. Rather, this investigation confirmed the existence of a sequence of pathologic cell-cell interactions in RA that eventually lead to the transformation of the synovial tissue into a component of the systemic lymphoid system[17–20]. There is also direct evidence that cytolytic $CD4^+$ cells containing intracellular protein per-

forin ($CD4^+P^+$) are generated *in vivo* in the joints of RA patients, which suggest their involvement in the cell--mediated cytotoxicity operating in RA joints[21].

Contrary to the results regarding the $CD4^+$ T cell population, the frequency of $CD8^+$ cytotoxic T lymphocytes – known to constitutively express perforin molecule in their cytoplasm – was higher in the synovial fluid of acute RA patients, compared to their frequency in peripheral blood and synovial membrane. The pathogenic role of cytotoxic T lymphocytes in RA was emphasized, and previous studies revealed that this cytolytic cell subpopulation is involved in pathological cellular interaction[21], leading to the activation of T and B lymphocytes, and the degradation of collagen, mediated by synoviocytes [22,23]. Their role in cartilage and bone degradation could be direct or indirect. Activated cytotoxic lymphocytes T and NK cells, were shown to secrete serine proteases (grenzimes) and perforin, cytolytic molecules involved in cell membrane degradation[24]. They could degrade type IV collagen $\alpha 2$ chain in basal membrane, stongly inducing transendotelial migration of circulating T lymphocytes and mononuclear cells of monocytic/macrophage lineage, thus contributing to synovial hyperplasia[25,26]. These results suggest that intense cell infiltration in synovial tissue is a result of pronounced migration of both $CD4^+$ and $CD8^+$ lymphocytes, but that the chemotactic signals from the synovial fluid mainly attract $CD8^+$ cells. This chemoattractive role is mainly attributed to IL-6, a well-known proliferative stimulus for $CD8^+$ cells, predominantly detected in SF of RA patients[27]. These results were followed by changes in the CD4/CD8 ratio, which was decreased in SF, but increased in SM, compared to PB. The predilection of $CD4^+$ cells for the synovial membrane, and of $CD8^+$ cells for the synovial fluid is not yet understood.

Despite many controversial results regarding the frequency and the role of T lymphocytes in RA, there is considerable agreement regarding the activation state of inflamed joint tissue lymphocytes. A number of phenotypic and functional studies putatively demonstrated a substantial expression of the early activation antigen CD69, the late activation antigen HLA-DR, the transferrin receptor, but without an augmentation in the IL-2 receptor (CD25)[28–32]. These phenotypic changes are characteristic neither for naive, nor for the cells activated by classical pathways. Our results regarding the activation status of lymphatic cells confirmed the low level of IL-2R expression on peripheral blood cells, as well as on the synovial membrane and the synovial fluid cells in acute RA patients. Since it is known that IL-2R can disappear from the surface of activated cells[33], it was proposed that lymphocytes in RA are in the state of late activation. The low expression of IL-2R on RA lymphocytes could also be the result of a long-lasting *in vivo* stimulation of lymphocytes in RA. However, in spite of a certain degree of activation, only T lymphocytes in close contact with the dendritic cells in the transient zone of subintima showed characteristics of blastic transformation, and the lymphocytes isolated from other synovial layers had minimal cytoplasmic changes and resembled unstimulated cells[34]. Moreover, the suboptimal proliferative response of the synovial membrane lymphocytes revealed upon mitogen-, anti-CD3- or cytokine-stimulation, could be explained by the abundance of suppressive factors in the joints of RA patients (for example TGF?, transforming factor beta)[35–38]. A preliminary immunophenotypic study in RA patients, using double staining methods, has revealed a decreased frequency of the $CD4^+CD25^+$ cell population, known to be T regulatory cells (not shown). In addition to their important role in clonal deletion and anergy, T regulatory cells are also essential for the down regulation of T cell responses to both foreign and self antigens, and for the prevention of autoimmunity[39–41].

Furthermore, with the intention of explaining the role of NK cells in the pathogenic mechanism in RA, we analysed the frequency of cells that display NK cell antigens CD16 and CD56. The results were interesting, as the percentage of $CD3^-CD56^+$ cells was not significantly different on local and systemic levels, and the significantly lower percentage of $CD3^-CD16^+$ cells in the synovial fluid implied the presence of a $CD16^-CD56^+$ NK cell subpopulation.

The results of previous studies regarding cytotoxic cells in peripheral blood, synovial fluid and membrane of RA patients are controversial. Studies of morphologic and functional characteristics of NK cells tested for both types of characteristics revealed a difference in either the number or the cytotoxic ability of NK cells[16,42]. This can be explained not only by the differences in therapeutic procedures used on RA patients, but also by the functional sensitivity of cytotoxic tests used in different groups of patients[43]. In addition, there is substantional heterogeneity in the expression of surface NK cell antigens. Furthermore, it is known that certain conventional surface NK cell markers can disappear from the cell surface upon activation. Combe et al. observed a decrease in NK activity in peripheral blood, synovial fluid and synovial membrane in RA patients, disproportional to disease intensity[42]. Reinitz et al., however, observed an elevation in NK cell cytotoxic activity in the synovial tissue, in comparison to that in autologous peripheral blood[43]. Hendrich et al. also observed a decrease in the frequency and density of CD16 marker on the surface of synovial fluid lymphocytes, as well as a decrease in cytotoxicity and ADCC (antibody dependent cell cytotoxicity). This can be explained by the presence of the rheuma factor (RF), since it is known that the RF of IgG isotype in a population of peripheral blood lymphocytes from healthy donors can modulate the expression of the CD16 molecule on the cell surface, decrease NK activity and ADCC ability, and induce transcription and production of IFNγ and TNFα[44].

A diminished number of NK cells, even lower than 1% of mononuclear cells in synovial tissue of rheumatic patients, were reported by many authors[42–46]. These results concord with our results regarding NK cells on the local level, especially in the synovial fluid, suggesting

that chronic lymphoid stimulation is potentially present in rheumatic joints as a result of the functional disability of NK cells. However, it is known that NK cells can decrease immune responses, such as immunoglobulin production or autologous mixed reaction (AMLR), hence, it is possible that the lower frequency of NK cells is responsible for the pathogenic immune processes involved in RA[45].

Moller et al. divided NK cells into two groups, based on the expression of CD16 and CD56 surface molecules. In decidua, the prevalence of $CD16^{-/dim+}$ $CD56^{+}CD3^{-}$ phenotype was detected, such as in the normal colon and some malignancies[46]. $CD16^{-/dim+}$ NK cells have shown various spectra of cytotoxic abilities when examined on K562 and JAR target cells, compared to bright CD16 positive cells. The observed higher frequency of CD56 positive cells compared to the frequency of CD16 positive cells in all examined groups suggests the presence of $CD16^{-}CD56^{+}$ cells in RA, and in OA (not shown). We observed that the frequency of $CD16^{-}CD56^{+}$ cells was lowest in peripheral blood, similar to the findings of Moller et al.[46], who demonstrated that more than 90% of NK cells in peripheral blood have the $CD16^{+}CD56^{+}CD3^{-}$ phenotype. A possible explanation for the diminuition in the CD16 expression can be found in specific cytokine network in RA joints, or in the fact that this type of cells selectively infiltrates the joints of RA patients, and because their inadequate cytotoxic abilities contribute to the progression of the disease.

In conclusion, a significantly different lymphocyte distribution was observed on local and systemic levels in the same group of acute RA patients. This observed specificity of local immune responses occurring in the synovial membrane and the synovial fluid suggests that lymphocytes play a crucial pathogenic role in the initial stages of rheumatoid arthritis, and confirms the hypothesis of T-lymphocyte involvement in RA pathogenesis.

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USPOREDBA ZASTUPLJENOSTI LIMFOCITNIH SUBPOPULACIJA ISTODOBNO NA LOKALNOJ I SISTEMSKOJ RAZINI U BOLESNIKA S AKUTNIM REUMATOIDNIM ARTRITISOM

S A Ž E T A K

Reumatoidni artritis (RA) je jedna od najtežih upalnih i autoimunih destruktivnih bolesti zglobova, koja je često praćena nizom vanzglobnih poremećaja. Patofiziološki mehanizam i važnost pojedinih staničnih subpopulacija u pokretanju i održavanju sinovitisa još nisu posve razjašnjeni. U ovom smo radu, metodom protočne citometrije, istovremeno određivali zastupljenost pojedinih limfocitnih subpopulacija u sinovijalnoj tekućini (ST), sinovijalnoj membrani (SM) i perifernoj krvi (PK) bolesnika u akutnom stadiju reumatoidnog artritisa. Promjene zastupljenosti T limfocitnih subpopulacija bile su značajne na lokalnoj razini u akutnih RA bolesnika, što je imalo za posljedicu smanjenje CD4/CD8 omjera u ST, a povećanje u SM u usporedbi sa vrijednostima omjera u PK. Opažene razlike u zastupljenosti stanica koje nose NK stanične biljege ukazale su na moguću ulogu $CD16^{-}CD56^{+}$ NK stanične populacije u ST. Značajne razlike u zastupljenosti pojedinih limfocitnih subpopulacija ukazuju na važnost lokalnih imunoloških zbivanja u SM i ST u akutnih RA bolesnika te potvrđuju značenje T-limfocitne hipoteze u početnim patogenetskim događajima u RA.