Comment on "Disease activity in patients with long-lasting rheumatoid arthritis is associated with changes in peripheral blood lymphocyte subpopulations"

To the Editor   I read with keen interest the article by Smoleńska et al.[1] indicating alterations in the main subpopulations of peripheral blood lymphocytes in patients with long-lasting rheumatoid arthritis (RA). Interestingly, they found a higher proportion of CD4+CD28− in patients with more active disease and demonstrated a trend towards an increase in the number of natural killer T cells. All these findings are new and stimulating. However, the study raises a number of questions.

First of all, the authors did not find any significant associations between the duration of the disease and alterations in the percentage of lymphocyte subpopulations. They performed the study in patients with a relatively short duration of the disease (mean 3, 5, and 2.5 years of disease duration in the study groups). It would be definitely more valuable to compare lymphocyte subpopulations in patients with preclinical RA, early RA (<1 year of disease duration),[2] and long-lasting RA (>5 or even >10 years). Because circulating autoantibodies (immunoglobulin M rheumatoid factor and anticitrullinated protein antibody) may precede the clinical onset of the disease for years,[3] and the earliest phases of RA are likely to represent the crucial therapeutic windows,[4] the differences in T-cell subpopulations (if detectable at these stages) could help identify these patients.

Another important question is whether any of these peripheral blood T-cell subpopulations (especially CD4+CD28−) are directly involved in the initiation of the disease, maintain the activity of RA, or reflect the increased concentrations of tumor necrosis factor α in these patients, as reported previously by the same authors.[5] Based on their findings, the authors could possibly indicate a new potential target for the future therapeutic agent in RA.

Regarding treatment, I agree that the lower doses of methotrexate than the currently recommended 25 mg per week did not control active disease in patients with the disease activity score 28 exceeding 5.1; therefore, the effect of treatment on lymphocyte subpopulations could have been missed.

Finally, there are contradictory statements in the abstract, namely, "A higher proportion of CD4+CD28− was associated with more active disease" and "The proportion of CD4+CD28− was not associated with disease activity". Considering the data from figure 1A and 1C,[1] the latter statement seems to be related to CD4+CD28− T cells. This issue deserves clarification to improve data interpretation in the future.

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REFERENCES

1 Smoleńska Ż, Pawłowska J, Daca A, et al. Disease activity in patients with long-lasting rheumatoid arthritis is associated with changes in peripheral blood lymphocyte subpopulations. Pol Arch Med Wewn. 2012; 122: 591-598.

2 Gerlag DM, Raza K, van Baarsen LG, et al. EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid arthritis. Ann Rheum Dis. 2012; 71: 638-641.

3 Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum. 2004; 50: 380-386.

4 van de Sande MG, de Hair MJ, van der Leij C, et al. Different stages of rheumatoid arthritis: features of the synovium in the preclinical phase. Ann Rheum Dis. 2011; 70: 772-777.

5 Bryl E, Vallejo AN, Matteson EL, et al. Modulation of CD28 expression with anti-tumor necrosis factor alpha therapy in rheumatoid arthritis. Arthritis Rheum. 2005; 52: 2996-3003.

Authors' reply   We would like to thank for the interest in our recent paper.[1] In this paper, we indeed presented data on the disease of a relatively short duration, but in a previously published

paper,[2] we reported that the percentages of activated CD4^{+} T cells are not different in patients with rheumatoid arthritis (RA) with the duration of the disease ranging from 1.5 to 22.0 years. Similar data (unpublished so far) were obtained regarding the CD28 expression on CD4^{+} and CD8^{+} T cells; there was no correlation between the duration of the disease and percentages of CD4^{+}CD28^{−} and CD8^{+}CD28^{−} T lymphocytes in the peripheral blood.

We agree that it would be valuable to compare lymphocyte subpopulations between various phases of the disease, including preclinical or early RA. In fact, our next paper, in which we make such comparison, is currently under review.

The preclinical stage of RA is an interesting issue in itself. The presence of circulating immunoglobulin M rheumatoid factor (IgM-RF) and/or anticitrullinated protein antibodies (ACPA) may precede the clinical onset of the disease for years; however, according to the published papers, only about 25% of the patients with arthritis who are positive for those antibodies would eventually develop RA.[3-5]

A study on the changes of lymphocyte subpopulations in preclinical RA should include many more patients than is possible for one clinical and research center. In our opinion, it would be feasible only as a multicenter cooperation and would require long prospective studies, in which we would love to participate.

There is no data available for direct CD4^{+}CD28^{−} involvement in the initiation of the disease, because there is no knowledge on when and how RA starts in humans. According to Nielen et al.,[4] IgM-RF and ACPA are risk factors but there is no evidence that they initiate the disease. There is the second hit theory but there is no definitive answer what factor(s) are responsible for the initiation of the disease. According to our findings, the CD8^{+}CD28^{−} subpopulation could maintain the activity of RA because the number of these cells is higher in RA patients with a higher disease activity score DAS28. Higher numbers of these cells were found in the synovium of patients with RA.[6] The cells contain granzyme B and perforin, and they definitely behave differently from classic CD4^{+}CD28^{+} cells. CD4^{+}CD28^{−} T cells were shown to be cytotoxic against own tissues (including the endothelium of blood vessels in acute coronary syndromes)[7] and were associated with the recurrence of those syndromes.[8] They are not solely responsible for endothelial damage; inflammation and oxidative stress in patients with RA and high disease activity could also contribute to accelerated atherosclerosis as suggested by Kwaśny-Krochin et al.[9] The increased percentage of CD4^{+}CD28^{−} T cells could also reflect increased concentration of tumor necrosis factor (TNF) because TNF directly works on the promoter region of the *CD28* gene.[10] Thus, the proportion of CD4^{+}CD28^{−} lymphocytes can change in response to anti-TNF treatment as shown by a number of

studies.[11] However, other factors can also be involved in the process of losing CD28, for example, vigorous T-cell proliferation.[12]

Based only on our recent paper,[1] we could not indicate any new potential target for the future therapeutic agent. However, based on our previously published papers, we would rather suggest certain molecular targets in the CD4^{+} cells, for example, Klotho[13] or ZNF334,[14] since they were found to be decreased in CD4^{+} T cells of patients with RA. Based on our findings, we showed that even at a clinically early stage of undifferentiated arthritis, the kinetics of the cell cycle of CD4^{+} T cells allows to distinguish patients with the pre-RA stage in the cohort of patients with undifferentiated arthritis.[15] In our opinion, this is where future therapeutic agents could be sought.

Finally, regarding the apparently contradictory statements in the abstract; unfortunately, the English version of the abstract contains a mistake. The statement should be "The proportions of CD8^{+}CD28^{−} was not associated with disease activity". The Polish abstract is correct and consistent with the presented data. We hope that despite that error, the results included in the figures are clear enough and can be easily interpreted.

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REFERENCES

1 Smoleńska Ż, Pawłowska J, Daca A, et al. Disease activity in patients with long-lasting rheumatoid arthritis is associated with changes in peripheral blood lymphocyte subpopulations. Pol Arch Int Med. 2012; 122: 591-598.

2 Pawłowska J, Smoleńska Z, Daca A, et al. Older age of rheumatoid arthritis onset is associated with higher activation status of peripheral blood CD4+ T cells and disease activity. Clin Exp Immunol. 2011; 163: 157-164.

3 Gerlag DM, Raza K, van Baarsen LG, et al. EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid arthritis. Ann Rheum Dis. 2012; 71: 638-641.

4 Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum. 2004; 50: 380-386.

5 van de Sande MG, de Hair MJ, van der Leij C, et al. Different stages of rheumatoid arthritis: features of the synovium in the preclinical phase. Ann Rheum Dis. 2011; 70: 772-777.

6 Pawłowska J, Mikosik A, Soroczynska-Cybula M, et al. Different distribution of CD4 and CD8 T cells in synovial membrane and peripheral blood of rheumatoid arthritis and osteoarthritis patients. Folia Histochem Cytobiol. 2009; 47: 627-632.

7 Nakajima T, Schulte S, Warrington KJ, et al. T-cell mediated lysis of endothelial cells in acute coronary syndromes. Circulation. 2002; 105: 570-575.

8 Liuzzo G, Biasucci LM, Trotta G, et al. Unusual CD4+CD28null T lymphocytes and recurrence of acute coronary events. J Am Coll Cardiol. 2007; 50: 1450-1458.

9 Kwaśny-Krocin B, Głuszko P, Undas A. Plasma asymmetric dimethylarginine in active rheumatoid arthritis: links with oxidative stress and inflammation. Pol Arch Int Med. 2012; 122: 270-276.

10 Bryl E, Vallejo AN, Weyand CM, Goronzy JJ. Down-regulation of CD28 expression by TNF-alpha. J Immunol. 2001; 167: 3231-3238.

11 Bryl E, Vallejo AN, Matteson EL, et al. Modulation of CD28 expression with anti-tumor necrosis factor alpha therapy in rheumatoid arthritis. Arthritis Rheum. 2005;52: 2996-3003.

12 Bryl E, Witkowski JM. Decreased proliferative capability of CD4(+) cells of elderly people is associated with faster loss of activation-related antigens and accumulation of regulatory T cells. Exp Gerontol. 2004; 39: 587-595.

13 Witkowski JM, Soroczyńska-Cybula M, Bryl E, et al. Klotho – a common link in physiological and rheumatoid arthritis-related aging of human CD4+ lymphocytes. J Immunol. 2007; 178: 771-777.

14 Soroczyńska-Cybula M, Bryl E, Smoleńska Z, Witkowski JM. Varying expression of four genes sharing a common regulatory sequence may differentiate rheumatoid arthritis from ageing effects on the CD4(+) lymphocytes. Immunology. 2011; 132: 78-86.

15 Pawłowska J, Smoleńska Ż, Zdrojewski Z, et al. Changes in proliferation kinetics of T cells: a new predictive cellular biomarkers for early rheumatoid arthritis? J Clin Immunol. 2012; 32: 991-999.