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LETTER TO THE EDITOR

Effect of atorvastatin on rheumatoid arthritis–associated autoreactive T cells

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Dear Editor,

It is well known that statins exert pleiotropic effects affecting the immune system [1–4]. The intricate relationship between the endocrine and immune system is also exemplified by the hormone and cytokine prolactin [5, 6]. To further illustrate immunoendocrine interactions, we would like to report on a white man born in 1978 who presented to us in May 2017 at the age of 39 years with pain in both hands, knee joints, and feet (on a scale from 1 to 10, the pain intensity was rated as 7). The patient also complained of a reduced libido for 1 year, fatigue, and depressed mood. He did not smoke and denied symptoms of coronary artery disease. A paternal uncle had a myocardial infarction at age 55 years. Family history was negative for autoimmune disorders. His weight was 90 kg at a height of 185 cm. Blood pressure was 140/80 mm Hg. Physical examination including the thyroid and testes was normal except for painful (red color) and swollen (blue color) joints as indicated in Fig. 1.

Rheumatoid arthritis (RA) was suspected. Cyclic citrullinated peptide (CCP) antibodies in serum were elevated at 1690 U/L (normal, < 17) and rheumatoid factor was positive. Treatment was initiated with prednisone and leflunamide and after 2 months switched to prednisone 20 mg/day, methotrexate 15 mg, and folic acid 5 mg. In August 2017, Benepali (etanercept biosimilar = tumor necrosis factor alpha inhibitor) 50 mg per week was added. Six weeks later, his hemoglobin A1c was 5.5% (unchanged from before), high sensitive C reactive protein (hsCRP) elevated at 35 mg/L (normal, < 1), total cholesterol 187 mg/dl, LDL 102 mg/dl, HDL 76 mg/dl, triglycerides 69 mg/dl, and lipoprotein (a) high at 71 mg/dl. Thyroid function tests were normal. Serum testosterone was measured in the low normal range, prolactin normal at 8

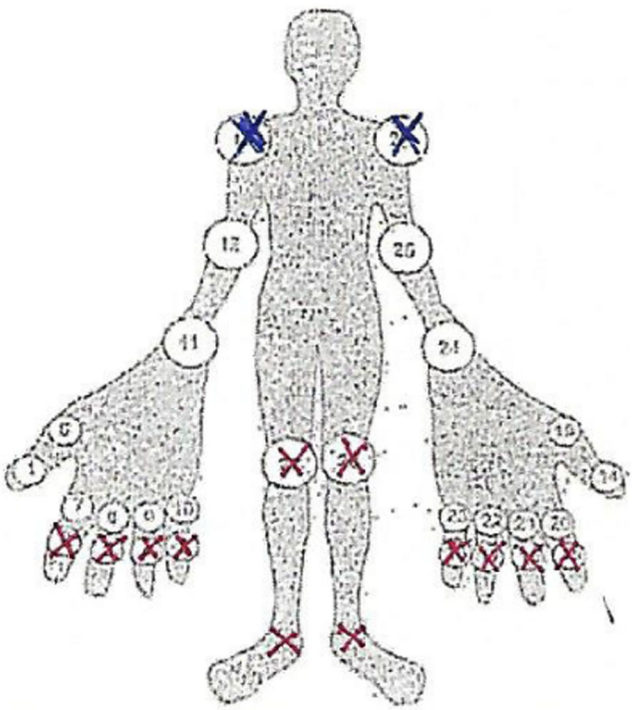

Fig. 1 Normogram to calculate the disease activity score of 28 joints

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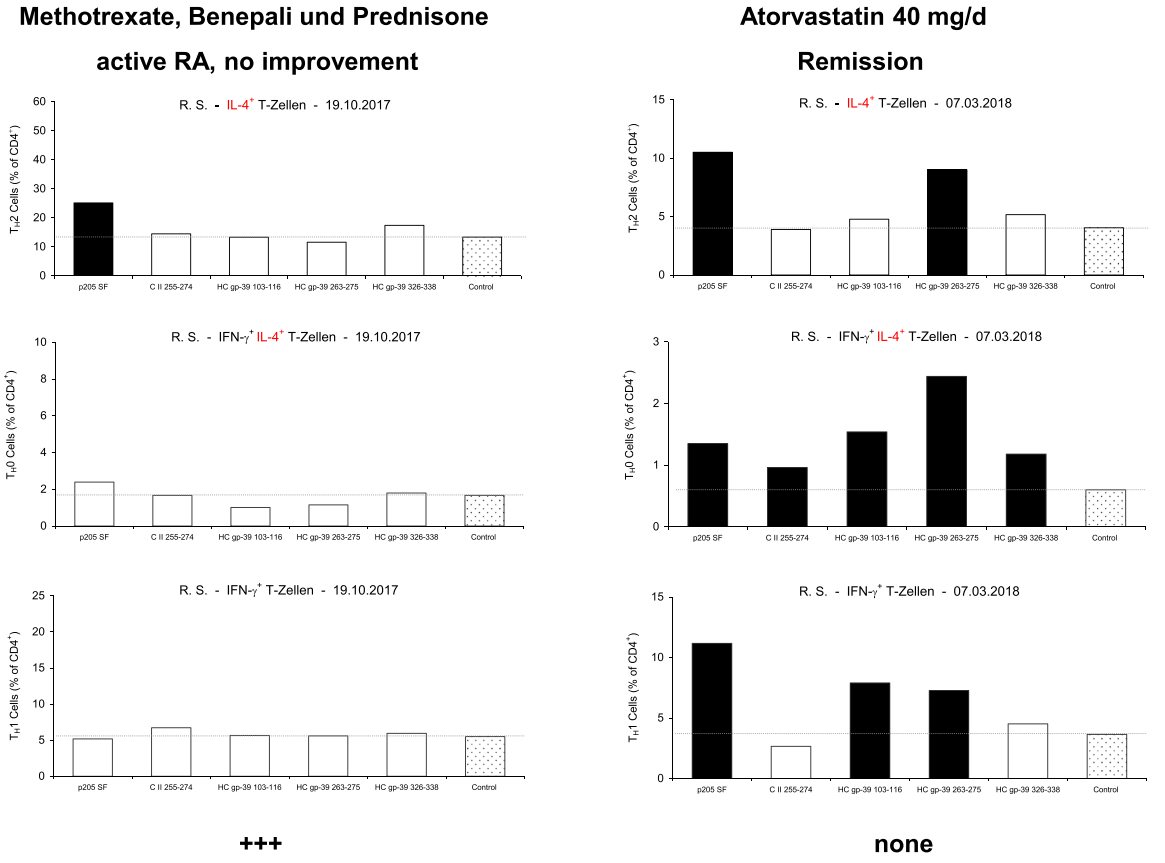

Fig. 2 Effect of atorvastatin on RA-associated autoreactive T cells, focusing on interferon gamma and IL-4 T cells. The left column depicts active RA while on treatment with methotrexate, Benepali, and prednisone. The right column shows RA in remission while on treatment with atorvastatin for approximately 4 months. Zellen = cells. A T cell response to a specific peptide was defined as positive (black columns) if the level of IL-4+, IFN-γ+IL-4+ or IFN-γ+ T cells exceeded the 150% threshold level of control culture T cells that were expanded in the presence of the peptide solvent dimethyl sulfoxide and interleukin-2 alone. The control level (right column) was calculated as mean of 6 to 9 control culture T cells seeded without peptide

ng/ml, and liver and renal function tests normal. Calcium, phosphate, and 25-hydroxyvitamin D levels were normal.

Method

Utilizing rheumatoid peptides as previously described [2], we performed an analysis of autoreactive T cells. To determine T cell activation, we used flow cytometry to measure the expression of activity markers HLA-DR and CD25 on CD4+ and CD8+ T cells. The second step comprised the analysis of the T cell reactivity to rheumatoid peptides derived from the p205 protein of synovial fluid, collagen type II (CII), and human cartilage glycoprotein 39 (HC gp-39). The third step was the Th2 provocation test with analysis of T cell reactivity in response to Th2 inducer phospholipase A2 derived from bee venom (BV-PLA2, allergen of the bee venom). The method used is identical to steps 1 and 2, clonal expansion of autoreactive T cells in the presence of autoantigen test peptide and of T cell growth factor interleukin-2 over the period of 14 days under cell culture conditions, followed by flow cytometric analysis of the intracellular cytokine production within the CD4+ T cells.

Results

Analysis of the fresh blood sample showed a slight elevation of activated CD4+ T cells with expression of the late activation marker HLA-DR and a strong elevation of activated CD8 + HLA-DR+ T cells. There was an aberrant, overproportional strong activation of CD8+ T cells, characteristic for destructive autoimmune disorders such as multiple sclerosis, RA, type 1 diabetes mellitus, and

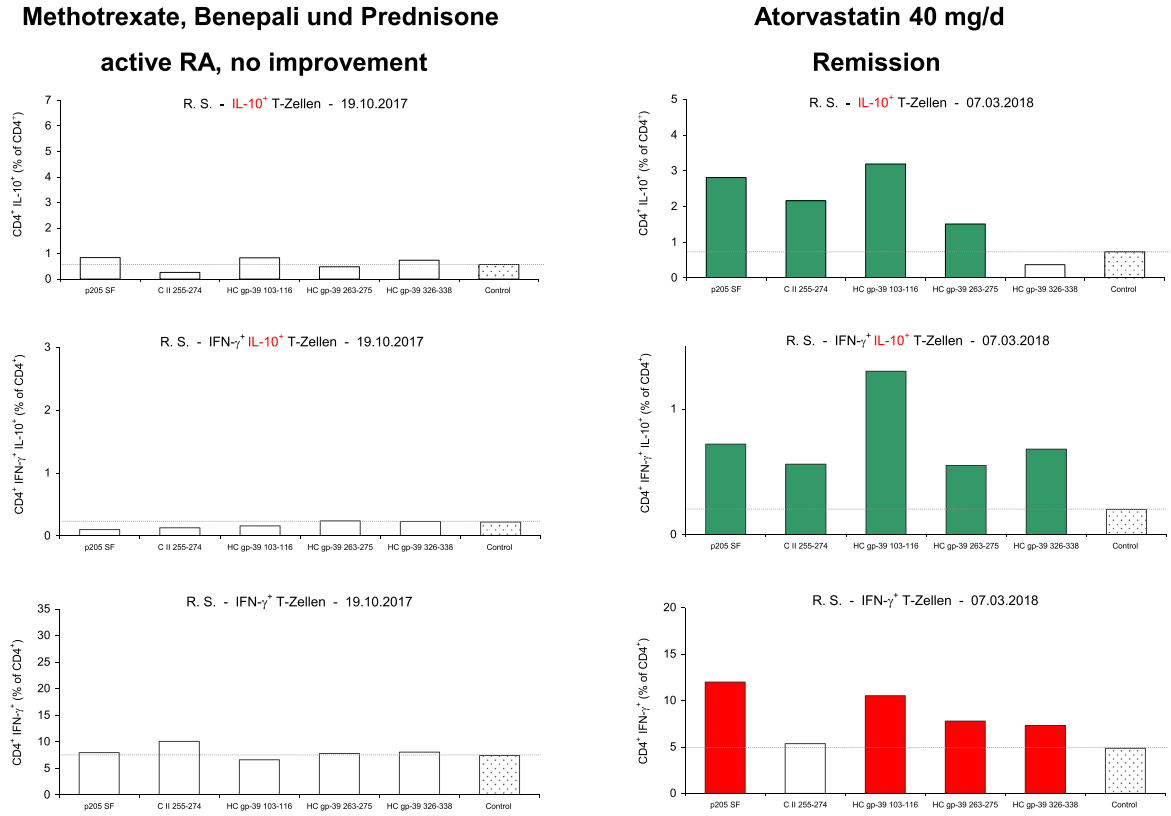

Fig. 3 Effect of atorvastatin on RA-associated autoreactive T cells, focusing on interferon gamma and IL-10 T cells. The left column depicts active RA while on treatment with methotrexate, Benepali, and prednisone. The right column shows RA in remission while on treatment with atorvastatin for approximately 4 months. Zellen = cells. A T cell response to a specific peptide was defined as positive (green and red columns) if the level of IL-10+, IFN-γ+IL10 or IFN-γ+ T cells exceeded the 150% threshold level of control culture T cells that were expanded in the presence of the peptide solvent dimethyl sulfoxide and interleukin-2 alone. The control level (right column) was calculated as mean of 6 to 9 control culture T cells seeded without peptide

Hashimoto thyroiditis, usually occurring years prior to the clinical manifestation of the respective autoimmune disorder.

Activated regulatory CD4+ T helper cells with expression of early activation marker CD25 (IL-2 receptor) were also elevated.

Cell population	19.10.2017	7.3.2018	
Activated CD4+HLA-DR+ T cells:	2.98%	2.48%	(Normal, < 2%)
Activated CD8+HLA-DR+ T cells:	11.25%	8.08%	(Normal, < 2%)
Activated + reg. CD4+CD25+ T cells:	22.22%	19.39%	(Normal, < 20%)

Figures 2 and 3 illustrate the results of the second step of our method, namely on interferon-γ/IL-4/IL-10 producing RA-associated autoreactive T cells.

In October 2017, there was a weak anti-inflammatory Th2 reaction in response to the peptide of the p205 protein from synovial fluid, otherwise negative test results. There were no regulatory T cells with production of the anti-inflammatory, immunoregulatory, and immunosuppressive acting IL-10 and there were no measurable proinflammatory Th1 cells. The Th2 provocation test showed a good reaction in the setting of remarkably elevated basal values of anti-inflammatory Th2 cells.

In conclusion, there was a total lack of regulatory T cell reactivity and a lack of proinflammatory Th1 cells in blood because of their migration into the inflammatory tissues around the affected joints.

These findings along with a strong elevation of hsCRP indicated a high disease activity of the RA and failure of the current therapy. After informed consent, the patient was started on atorvastatin 40 mg daily on November 28, 2017, with continuation of low-dose ("adrenal reserve") prednisone 5 mg daily. Methotrexate and Benepali were discontinued. Total cholesterol and LDL cholesterol dropped by 20% and hsCRP declined to 1.4 mg/L by March 2018. Creatine kinase levels and aspartate transaminase remained within normal limits.

On atorvastatin therapy since November 2017, follow-up in March 2018 showed a positive reaction in response to all 5 RA-associated peptides with regard to interferon-γ/IL-4/IL-10 producing RA-associated T cells. There were now undifferentiated Th0 cells. For 2 peptides, there were now anti-inflammatory Th2 cells rising and for 3 peptides there was retraction of proinflammatory Th1 cells in the peripheral blood stream. In all 5 RA-associated peptides, we could now measure regulatory T cells with production of anti-inflammatory IL-10. Proinflammatory Th1 cells were withheld in peripheral blood with 4 RA-associated peptides and were counterregulated by IL-10 producing T regulatory cells. The Th2 provocation test showed now a normal reaction with a reduction of the Th1/Th2 ratio from initially (Oct 2017) 0.9 to 0.1.

In conclusion, atorvastatin 40 mg daily combined with prednisone 5 mg caused an upregulation of anti-inflammatory Th2 cells, especially of IL-10 producing T regulatory cells, and mobilization of proinflammatory Th1 cells into the peripheral blood from the initially inflamed tissue regions/joints, and overall clinical improvement of the RA. This indicated that prednisone therapy at present/that time was no longer necessary, especially when considering that prior higher dose prednisone therapy had no measurable positive effect on the immune system.

In January 2019, rheumatoid factor in serum was 64 IU/ml (normal, < 14) and the ratio of CCP antibodies was 5.98 (normal, < 1). The erythrocyte sedimentation rate was 3 mm/h compared with 57 mm/h in October 2017. Using the disease activity score of 28 joints, the patient reported now no pain and had no swelling at any joint.

In patients with RA, inflammation is a consistent and independent predictor of coronary atherosclerosis progression [7]. A recent randomized, double-blind, placebo-controlled trial in patients with RA age 50 years and older or with a disease duration of > 10 years who were not known to have clinical atherosclerosis, diabetes mellitus, or myopathy found that those taking atorvastatin 40 mg daily had a 34% risk reduction in cardiovascular events with the primary end point being a composite of cardiovascular death, myocardial infarction, stroke, transient ischemic attack, or any arterial revascularization [8, 9]. This trial also showed that atorvastatin 40 mg is safe in RA patients. Apparently, statins exert anti-inflammatory effects in RA patients with atorvastatin being superior to simvastatin [10]. As demonstrated here in a patient with RA, our immunodiagnostics method can uniquely assist in measuring and monitoring immune disease activity of various autoimmune disorders [2].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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