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The Lymphocyte Phenotype in Patients with Primary Fibromyalgia

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ABSTRACT

The aim of this study was to examine the lymphocyte phenotype in fibromyalgia (FB) patients.

Nineteen patients [18 females, 1 male] who met the ACR classification criteria for FB were recruited into the study. The florescence of leukocytes was measured using Ortho Cytotron Absolute flow cytometer. Ortho-mune monoclonal antibodies [OK-Combo-Tests] were used for determination of the following antigens: CD3/CD19, CD3/HLA-DR, CD4/CD8, CD45RA/CD4. Serum IgE was established with ELISA [Merck, Germany].

There were no differences in percentage of CD3, CD19, CD4, CD8, CD3/HLA-DR and CD4/CD45RA cells as compared to healthy controls. We found a negative correlation between CD4 and CD8, CD3DR and IgE, CD8 and IgE concentration. The positive correlation between CD3 and CD3DR, CD3DR and CD8 was observed.

The inhibitory effect of CD8+ cells subpopulation on B-cells and thereby on IgE production in patients afflicting for allergy has been already observed. Moreover, the symptoms to allergy has been found in about 50% of our patients. Taken together the data thus far obtained present a coexistence of symptoms of allergy and FB.

INTRODUCTION

Primary fibromyalgia syndrome [PFS] is characterized by widespread pain and tenderness on physical examination in multiple musculoskeletal structures [tender points], frequently accompanied by fatigue, stiffness, nonrestorative sleep and other vegetative and functional symptoms (12, 30, 31, 34). The pathogenesis of PFS is unclear. Morphological, biochemical and physiological changes have been found in a large number of studies and the deviation from controls was observed with respect to: serotonin metabolism (22, 27, 32, 35), norepinephrine, epinephrine and dopamine metabolism (25), levels of substance P (33), levels of somatomedin C (2), function of autonomous nervous system (9) or the function of hypothalamus-pituitary-adrenal axis (7, 16). There are also few reports of immunological disturbances in patients with PFS. Hader et al. for example found a defect in the interleukin-2 pathway (13), Bengtsson et al. (1) and Klein et al. (15) determined the titres of different autoantibodies in serum of PFS patients. Caro has detected immunoglobulin G deposition at dermal-epidermal junction of sun-

exposed skin in 76% of PFS patients (5), Meske-Brand et al. observed a significant increase in total IgE (17). In the study of Peters and Wallace about half of 16 fibromyalgia patients had immune regulatory abnormalities characterized by elevated T-helper cell levels, Il-2 and interferon alpha (19). Russell et al. has recently identified a subset of primary PFS patients [10 of 32] falsely expressing the B-cell marker [CD19] on their CD5 positive cells (26). The aim of the present study was to analyse the lymphocyte phenotype in patients with primary fibromyalgia syndrome.

MATERIAL AND METHODS

Patients: Nineteen patients with fibromyalgia [18 females, 1 male] who fulfilled the diagnostic criteria of American College of Rheumatology (34) were randomly recruited to the study. The mean age was 41 ± 8 years [range 28-65]. None of the patients suffered from any specific rheumatic disease and other diseases associated with muscle pain. To ensure it, a blood sample from each patient was analyzed for hemoglobin, erythrocyte sedimentation rate [ESR], blood cell count, rheumatoid factor and C-reactive protein [CRP]. The patients had experienced symptoms for a mean period of 6 ± 4 years [1-11 years]. Tender points at 9 bilateral sites, as proposed by ACR were also examined with Fischer's dolorimeter with a rubber surface and a cross sectional area of approximately 0.95 cm^2 (11). The pain tolerance was measured in kg. All patients were asked about pain intensity, morning stiffness, fatigue, anxiety, vegetative and functional symptoms.

Nine examined patients showed the symptoms of atopic allergy [allergic rhinitis - 2, atopic dermatitis - 4, alimentary allergy -2, drug intolerance 1]. To confirm the diagnose of atopy, skin prick tests to a group of 16 common allergens (in the presence of a positive histamine control and a negative vehicle control) were performed. Total serum IgE concentration was determined using an ELISA technique [Merck Germany].

Immunological studies: For evaluation of the percentages of lymphocytes, whole blood was directly stained for 30 min in cold place (4°C) with two - color immunofluorescent reagents. Next, blood was treated with Ortho lysing solution for 10 min, and lymphocytes were measured using Otho Cytoron Absolute flow cytometer. Ortho-mune Monoclonal antibodies [OK-Combo-Tests] were used for detection of the following molecules: CD3/CD19 [T/B lymphocytes], CD3/HLA-DR [T lymphocytes/activated cells], CD4/CD8 [T helper/T suppressor and cytotoxic lymphocytes], CD45RA/CD4 [subpopulation of T helper lymphocytes, helper-inducer].

The control group consisted of 12 [10 females and 2 males] healthy individuals [no fibromyalgia or heavy illnesses symptoms] and was recruited from the hospital staff. The median age was 35 ± 8 years [range 25-51]. Statistical analysis was performed using the Spearman's test.

RESULTS

The mean value of tenderness for all by ACR - 1990 proposed tender points was 1.71 kg ± 0.53 [discriminatory value using Fischer's dolorimeter is 3 kg]. The typical vegetative and functional

symptoms like fatigue, sleep disturbances, headache, Raynaud's like phenomenon, paresthesia or sicca symptoms were very common in the fibromyalgia group and seldom in the control group. The total number of lymphocytes in fibromyalgia patients did not differ from the control group. We found no differences in either T-cells [CD3+] or B-cells [CD19+] percentages in the analyzed group of patients as compared to healthy controls [Table 1]. Moreover, the percentage of T-helper [CD4+] and T-suppressor/cytotoxic [CD8+] cells was found within the normal values [Table 1]. We observed slight decrease in the CD4+/CD8+ ratio as compared to the control group [1.2 ± 0.3 vs. 1.4 ± 0.3], however these changes were not statistically significant. The percentage of activated T-cells [CD3+HLA-DR+] and helper-inducer T-cells [CD4+CD45RA] were found to be unchanged in comparison with healthy donors [Table 1].

Table 1. Percentage of different types of lymphocytes in blood of 19 primary fibromyalgia patients

	Fibromyalgia patients	**Normal Range**
CD3+	72.6 ± 6.1% [62.4 - 82.4%]	75 ± 5%
CD3+HLA-DR+	3.4 ± 2.1% [1.3 - 10.1%]	5 ± 3%
CD4+	40.3 ± 6.5% [28.4 - 53.7%]	44 ± 8%
CD4+CD45RA+	12.7 ± 7.0% [5.4 - 31.1%]	16 ± 5%
CD8+	29.6 ± 7.1% [15.0 - 42.7%]	27 ± 7%
CD19+	10.5 ± 4.7% [3.1 - 19.5%]	10 ± 5%

Table 2. Correlation coefficients between percentage of lymphocyte subpopulations in blood and IgE concentration in serum

	CD3	CD3DR	CD4	CD4/45	CD8	CD19
CD3	1					
CD3DR	0.5661	1				
CD4	0.1373	-0.3888	1			
CD/45	0.3180	-0.0034	0.5916	1		
CD8	0.2738	0.5462	-0.6279	-0.3359	1	
CD19	-0.3903	-0.3228	0.0532	0.2545	-0.2994	1
IgE	-0.331	-0.6520	0.1826	-0.2762	-0.7152	0.2513

In 7 of 19 patients [37%] the total serum IgE levels were higher than 20 U/mL. The mean level of serum IgE in fibromyalgia patients was increased compared to controls [42 ± 15 vs. 19 ± 11 U/mL]. We did not find any correlation between lymphocytes phenotype as well as IgE serum level and disease duration [data not shown]. There was indeed a correlation between CD3+HLA-Dr+ and CD3 cells, CD4+CD45RA+, and a negative correlation was observed between CD4+ and CD8+ cells [Table 2]. Moreover, we found a negative correlation between IgE concentration

and the number of CD8+ cells [r=0.7152, P <0.05, Table 2] and IgE and CD3+HLA-DR+ cells [r=0.652, P <0.05, Table 2].

DISCUSSION

Since etiopathogenesis of fibromyalgia remains unknown, a number of hypotheses has been developed to understand its etiology. Fibromyalgia is a stress-related disorder. Therefore, a role of central nervous system [CNS] has been considered (3, 10). The CNS involvement may be responsible for abnormal perception of pain in the absence of recognizable peripheral tissue injury. Accordingly, a number of different neurotransmitter abnormalities have been recently identified in fibromyalgia patients. For instance, low serum level of tryptophan (23), low serum serotonin (22, 27) as well as increased cerebrospinal fluid [CSF] level of kynurenine (21) have been detected. Serotonin deficiency can cause an increase of substance P content in CSF amplifying the perception of pain (8) and in the study of Vaeroy and Russell substance P has been found increased in CSF of fibromyalgia patients (24, 33). The modulating effect of substance P is also known to influence the function of the hypothalamic-pituitary-adrenal axis (14, 18). There are published evidence indicating that substance P can stimulate lymphocytes to enhanced Il-2 production (4, 20) and it has been found that natural killer (NK) cell activity is abnormal in fibromyalgia patients (28), although they did not have fewer NK than controls. It was of interest to note that the activity of NK is regulated by monocytes which secrete NK cell cytotoxity effector in response to the concentration of serotonin. Taken together, it seems that besides CNS involvement, the immunological system may also be affected. In our study we found CD8+ cells to be slightly decreased. Similarly to allergy, IgE serum level was found to be increased. Moreover, a negative correlation between the number of CD8+ cells and IgE concentration was established in the present study.

The higher total serum IgE levels in fibromyalgia patients have been observed previously but the majority of them did not present the clinical symptoms of atopic allergy (17,29). On the other hand in the study of Cleveland, 38% of patients with chronic rhinitis fulfilled the ACR criteria of fibromyalgia (6). In conclusion the data thus far obtained may suggest a role of allergy and immunological disturbances in the pathogenesis of fibromyalgia. Future studies are required i.e. on the patients with atopic allergy or allergic symptoms.

REFERENCES

1. Bengtsson, A., Ernerudh, J., Vrethem, M. & Skogh, T.: Absence of autoantibodies in primary fibromyalgia. J Rheumatol 17:1682-1683, 1990.
2. Bennett, R.M., Clark, S.R., Campbell, S.M. & Burckhardt, C.S.: Low levels of somatomedin C in patients with the fibromyalgia syndrome. Arthritis Rheum 35:1113-1116, 1992.
3. Berg, P.A. & Klein, R.: Fibromyalgie-Syndrom. Eine neuroendokrinolo-gische Autoimmunerkrankung? Dtsch med Wschr 119:429-435, 1994.
4. Calco, C.F., Chavanel, G. & Senik, A.: Substance P enhances Il-2 expression in activated human T cells. J Immunol 148:3498-3504, 1992.

5. Caro, X.: Immunofluorescent detectionof IgG at dermal-epidermal junction in patients with apparent primary fibrositis syndrome. Arthritis Rheum 27:1174-1178, 1984.
6. Cleveland, C.H., Fisher, R.H., Brestel, E.P., Esinhart, J.D. & Metzger, W.J.: Chronic rhinitis: an underrecognized association with fibromyalgia. Allergy Proc 13:263-267, 1992.
7. Crofford, L.J.: Neuroendocrine aspects of fibromyalgia. J of Musculoskeletal Pain 3:125-133, 1994.
8. Eide, P.K. & Hole, K.: Interactions between serotonin and substance P in the spinal regulation of nociception. Brain Res 564:33-39, 1991.
9. Elam, M., Johansson, G. & Wallin, B.G.: Do patients with primary fibromyalgia have an altered muscle sympathetic nerve activity? Pain 48:371-375, 1992.
10. Ferraccioli, G., Cavalieri, F., Salaffi, F., Fontana S., Scita, F., Nolli, M. & Maestri, D.: Neuroendocrinologic findings in primary fibromyalgia (soft tissue chronic pain syndrome) and in other chronic rheumatic conditions (rheumatoid arthritis, low back pain). J Rheumatol 17:869-873, 1990.
11. Fischer, A.A.: Pressure threshold measurement of diagnosis of myofascial pain and evaluation of treatment results. Clin J Pain 2:207-214, 1987.
12. Goldenberg, D.L.: Fibromyalgia syndrome: An emerging but controversial condition. JAMA 257:2782-2787, 1987.
13. Hader, N., Rimon, D., Kinarty, A. & Lahat, N.: Altered interleukin-2 secretion in patients with primary fibromyalgia syndrome. Arthritis Rheum 34:866-872, 1991.
14. Jessop, D.S., Chowdrey, H.S., Larsen, P.J. & Lightman, S.L.: Substance P: Multifunctional eptide in the hypothalamo-pituitary system? J Endocrinol 132:331-337, 1992.
15. Klein, R., Bänsch, M. & Berg, P.: Clinical relevance of antibodies against serotonin and gangliosides in patients with primary fibromyalgia syndrome. Psychoneuroendo-crinology 17:593-598, 1992.
16. McCain, G.A. & Tilbe, K.S.: Diurnal hormone variation in fibromyalgia syndrome: A comparison with rheumatoid arthritis. J Rheumatol (suppl 19) 16:154-157, 1989.
17. Meske-Brand, S., Kapp, A. & Müller, W.: Die generalisierte Tendomyopathie, ein syndrom im rahmen der atopie? Akt Rheumatol 9:156-158, 1984.
18. Murphy, R.M. & Zemlan, F.P.: Differential effects of substance P on serotonin modulated spinal nociceptive reflexes. Psychopharmacology (Berlin) 93:118-121, 1987.
19. Peter, J.B. & Wallace, D.J.: Abnormal immune regulation in fibromyalgia. Arthritis Rheum 31:24, 1988.
20. Rameshwar, P., Gascon, P. & Ganea, D.: Immunoregulatory effects of neuropeptides. Stimulation of interleukin-2 production by substance P. J Neuroimmunol 37:65-74, 1992.
21. Russell, I.J.: Biochemical abnormalities in fibromyalgia syndrome. J Muskuloskeletal Pain 2:101-115, 1994.
22. Russell, I.J., Michalek, J.E. & Vipraio, G.A. et al: Serum serotonin and platelet 3H-imipramine binding receptor density in patients with fibromyalgia/fibrositis syndrome. J Rheumatol 19:104-109, 1992.

23. Russell, I.J., Michalek, J.E., Vipraio, G.A., Fletcher, E.M. & Wall, K.: Serum aminoacids in fibrositis/fibromyalgia syndrome. J Rheumatol 19:158-163, 1989.
24. Russell, I.J., Orr, M.D., Littman, B., Vipraio, G.A., Alboukrek, D., Michalek, J.E., Lopez, Y. & McKillip, F.: Elevated cerebrospinal fluid levels of substance P in patients with fibromyalgia syndrome. Arthritis Rheum 37:1593-1601, 1994.
25. Russell, I.J., Vaeroy, H., Javors, M. & Nyberg, F.: Cerebrospinal fluid biogenic amine metabolites in fibromyalgia/fibrositis syndrome and rheumatoid arthritis. Arthritis Rheum 35:550-556, 1992.
26. Russell, I.J., Vipraio, G.A., Craig, F.E., King, D.E. & Monteil, M.M.: False CD19 positive-CD5 cells in fibromyalgia. Arthritis Rheum 37 (suppl):350, 1994.
27. Russell, I.J., Vipraio, G.A. & Lopez, Y.M.: Serum serotonin in fibromyalgia syndrome, rheumatoid arthritis, osteoarthritis and healthy normal controls. Arthritis Rheum 36:223, 1993.
28. Russell, I.J., Vipraio, G.A., Tovar, Z., Michalek, J. & Fletcher, E.: Abnormal natural killer cell activity in fibrositis syndrome in responsive in vitro to Il-2. Arthritis Rheum 31:24, 1988.
29. Samborski, W., Pawlik, I., Konys, J. & Mackiewicz, U.: The assesments of atopia symptoms in fibromyalgia patients. Reumatologia 4:45-52, 1994.
30. Samborski, W., Stratz, T., Kretzman, W., Mennet, P. & Muller, W.: Vergleichende undersuchugen uber das vorkommen vegetativer und funktioneller beschwerden bei lumbalgien und generalisierten tendomyopathien (fibromyalgia). Z Rheumatol 50:378-381, 1991.
31. Samborski, W., Stratz, T., Kretzman, W., Mennet, P. & Muller, W.: The comparison of incidence of vegetative and functional symptoms in patients with fibromyalgia and chronic low back pain syndrome. Scand J Rheumatol 94 (suppl):16, 1992.
32. Stratz, T., Samborski, W., Hrycaj, P., Pap, T., Mackiewicz, S., Mennet, P. & Müller, W.: Die serotoninkonzentration im serum bei patienten mit generalisierter tendomyopathie (fibromyalgie) und chronischer polyarthritis. Med Klin 88:458-462, 1993.
33. Vaeroy, H., Helle, R., Forre, O., Kass, E. & Terenius, L.: Elevated CSF levels of substance P and high incidence of Raynaud's phenomenon in patients with fibromyalgia: New features for diagnosis. Pain 32:21-26, 1988.
34. Wolfe, F., Smythe, H.A. & Yunus, M.B. et al.: The American college of rheumatology 1990 criteria for the classification of fibromyalgia. Arthritis Rheum 33:160-172, 1990.
35. Yunus, M.B., Dailey, J.C. & Aldag, J.C. et al.: Plasma tryptophan and other amino acids in primary fibromyalgia: A controlled study. J Rheumatol 19:90-94, 1992.

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