Stanično imunosni odgovor u Dupuytrenovoj bolesti

Cellular immune response in Dupuytren's disease

Márk Plander[1,2], Antal Salamon[3], Erzsébet Toldy[1,4], Gyula Kiss[3], Gábor L. Kovács[4,5]

[1]Središnji laboratorij, Sveučilišna bolnica „Markusovszky", Szombathely, Mađarska
[1]Central Laboratory, Markusovszky Teaching Hospital, Szombathely, Hungary
[2]Klinika za hematologiju, Sveučilišna bolnica „Markusovszky", Szombathely, Mađarska
[2]Department of Haematology, Markusovszky Teaching Hospital, Szombathely, Hungary
[3]Klinika za traumatologiju i rekonstruktivnu kirurgiju ruku, Sveučilišna bolnica „Markusovszky", Szombathely, Mađarska
[3]Department of Traumatology, Reconstructive and Hand Surgery, Markusovszky Teaching Hospital, Szombathely, Hungary
[4]Institut za dijagnostiku i management, Sveučilište u Pečuhu, Pečuh, Mađarska
[4]Institute of Diagnostics and Management, University of Pécs, Pécs, Hungary
[5]Institut za laboratorijsku medicinu, Sveučilište u Pečuhu, Pečuh, Mađarska
[5]Institute of Laboratory Medicine, University of Pécs, Pécs, Hungary

Sažetak

Uvod: Patogeneza Dupuytrenove bolesti je nejasna, no mogu se pretpostaviti upalni mehanizmi u njenoj pozadini.

Materijali i metode: Mjerili smo udjele različitih podvrsta monocita i limfocita u perifernoj krvi prema stadiju bolesti kod 39 bolesnika oboljelih od Dupuytrenove bolesti. Rezultate smo usporedili s rezultatima 29 zdravih kontrolnih ispitanika iz iste dobne skupine. Mjerenja su napravljena pomoću protočne citometrije.

Rezultati: U aktivnom su stadiju bolesnici imali bitno povišen udio monocita, NK-stanica sličnih T-limfocitima, dok je udio B-limfocita bio značajno snižen, uključujući CD5+ B-limfocite. Udjeli CD4+, CD8+ T-limfocita i B-limfocita nisu se znatno promijenili. U naprednom su stadiju bolesti udjeli monocita i B-limfocita ostali konstantnima, no značajno su se snizili udjeli T-limfocita. U četiri slučaja među bolesnicima koji boluju od Dupuytrenove bolesti pojavile su se limfoidne neoplazme zrelih B- ili T-limfocita.

Zaključci: Naši rezultati podupiru prijašnja mišljenja da su limfociti povezani s patogenezom Dupuytrenove bolesti te pojačavaju ulogu monocita, NK-stanica sličnih T-limfocitima i promjenu imunosnog odgovora između stadija bolesti.

Ključne riječi: Dupuytrenova bolest, aktivni i uznapredovali stadij, monociti, NK T-limfociti

Abstract

Background: The pathogenesis of Dupuytren's disease is unclear, but inflammatory mechanisms might be supposed in the background.

Materials and methods: The ratios of the subsets of monocytes and lymphocytes in the peripheral blood were measured according to the stage of the disease of 39 Dupuytren's patients. They were compared with those of 29 healthy, age-matched controls. The measurements were accomplished by flow-cytometry.

Results: In an active stage, the patients had significantly increased ratios of monocytes, NK-like T-cells, whereas significantly decreased ratio of B-lymphocytes, including CD5+ B-cells. The ratios of CD4+, CD8+ T-cells and NK-cells did not change significantly. In the advanced stage, the ratios of monocytes and B-lymphocytes remained constant, but the ratios of T-cells decreased significantly. In four cases among Dupuytren's patients, mature B- or T-cell lymphoid malignancies occurred.

Conclusions: Our results support the previous considerations that lymphocytes are involved in the pathogenesis of Dupuytren's disease and enhance the role of monocytes, NK-like T-cells and the alteration of immune response between the stages of the disease.

Key words: Dupuytren's disease, active and advanced stages, monocytes, NK-T cells

Pristiglo: 27. prosinca 2007. *Received: December 27, 2008*

Prihvaćeno: 18. travnja 2008. *Accepted: April 18, 2008*

Uvod

Dupuytrenova bolest (engl. *Dupuytren's disease*) kronični je poremećaj, koji karakterizira razvijanje kvržica i čvorića na dlanu šake i/ili tabanu stopala. Te patološke promjene nastaju zbog fibroznih procesa, no točan mehanizam nastanka skvrčenosti još je nejasan. Chiu i McFarlane (1) opisali su klinički i patološki tri stadija bolesti: rani, aktivni i uznapredovali stadij.

Prijašnja su izvješća upućivala da bi upalni mehanizmi mogli biti povezani s patogenezom Dupuytrenove bolesti. Mnogi su autori izvijestili o kvržicama koje su sadržavale upalne stanice; uglavnom limfocite i makrofage (2). Također je biopsijom uočen povećan broj aktiviranih (HLA-DR+) T-limfocita u tkivima (3). Pronađen je i povišen udio aktiviranih T-limfocita u perifernoj krvi bolesnika s Dupuytrenovom bolesti (4). Potvrđena je i uloga citokina koje stvaraju T-limfociti. Transformacijski faktor rasta-β (engl. *Transforming Growth Factor-β,* TGFβ) izaziva transformaciju fibroblasta u miofibroblaste koji su odgovorni za skvrčavanje aponeuroze (2). Pronađena je bitna veza između Dupuytrenove bolesti, HLA-DR3 te autoantitijela i kolagena tipa I-IV (5).

Naš je cilj bio istražiti ulogu upalnih mehanizama u patogenezi Dupuytrenove bolesti i produbiti saznanja o raspodjeli podvrsta monocita i limfocita sukladno stadiju Dupuytrenove bolesti.

Materijali i metode

Bolesnici

U ovo je istraživanje bilo uključeno ukupno 39 odraslih muških bolesnika s Dupuyternovom bolesti raspona godina od 45 do 69 (57 ± 12) i skupina od 29 zdravih muških dobrovoljaca odgovarajuće dobne skupine. Iz istraživanja su isključeni oni bolesnici koji su imali bilo kakvo upalno oboljenje ili neku drugu bolest unutarnjih organa, kao npr. autoimune bolesti, šećernu bolest, cirozu jetre, epilepsiju, poznate zloćudne bolesti. Bolesnici su bili klasificirani prema Chiu i McFarlanovoj kliničkoj i patološkoj podjeli na rani, aktivni i uznapredovali stadiji (1). 23 bolesnika iz skupine s Dupuytrenovom bolesti bili su u aktivnom stadiju (stadij 2) s kvržičastim zadebljanjem na dlanu i zgrčenosti zgloba. Šesnaest je bolesnika bilo u uznapredovalom stadiju (stadij 3) s uznapredovalom zgrčenosti zgloba. Nije primijećeno da je i jedan bolesnik u ranom stadiju imao kvržicu isključivo na dlanu (stadij 1). U 36 slučajeva radilo se samo o jednoj ruci. Kod tri su bolesnika obje ruke bile zahvaćene. Lezije (oštećenja) su se uglavnom nalazile u korijenu četvrtog i petog prsta. Bolesnici su odabrani iz traumatološke ambulante bolnice Markusovszky u Szombathelyu. Uzorkovanje je trajalo 2 godine, do 2007. Istraživanje je provedeno sukladno Helsinškoj deklaraciji, a za protokol istraživanja dobiveno je odobre-

Introduction

Dupuytren's disease (DD) is a chronic disorder, characterized by development of nodules and cords in the palmar and/or plantar aponeurosis. These pathologic changes arise from a fibrotic process, but the exact mechanism of contracture is unclear. Chiu and McFarlane (1) reported a clinical-pathological description; the three stages of the disease were recognised as early, active and advanced stages.

Previous reports have indicated that inflammatory mechanisms may be involved in the pathogenesis of DD. A number of authors reported that nodules contain inflammatory cells; mainly lymphocytes and macrophages (2). An increased frequency of activated (HLA-DR+) T-lymphocytes were also detected in the biopsied tissues (3). The elevated ratio of activated T-cells has been found in the peripheral blood of patients with DD, as well (4). The role of cytokines with T-cell origin was also proved. Transforming Growth Factor-β (TGFβ) induces the transformation of fibroblasts into myofibroblasts that are responsible for the shrinkage of aponeurosis (2). A significant association was found between DD, HLA-DR3 and autoantibodies to types I-IV collagen (5).

Our aim was to investigate the participation of inflammatory mechanisms in the pathogenesis of DD and explore the distributions of monocytes and lymphocytes' subsets according to the stages of DD.

Materials and methods

Patients

A total of 39 adult male patients with DD aged between 45 and 69 (57 ± 12) years and a group of 29 age-matched, healthy male volunteers were included in this study. Those patients were excluded from the study, who suffered from any infection or from an internal disease as autoimmune disease, diabetes mellitus, cirrhosis hepatis, epilepsy, known malignancies. The patients were grouped according to the classification of Chiu and McFarlane on the basis of clinical and pathological classification, and were designated as early, active and advanced stages (1). 23 patients out of the whole Dupuytren's group were in active stage (stage 2) with nodular thickening of the palmar fascia associated with joint contracture. 16 patients were in advanced stage (stage 3) with progressive joint contracture. No patients were observed in the early group having nodule only in the palmar fascia (stage 1). In 36 cases only one hand was involved. Both hands were affected in 3 patients. The lesions were found essentially at the fourth and at the fifth ray. Patients were recruited at the Traumatology outpatient's room of Markusovszky Hospital in Szombathely. The sampling did take 2 years until 2007. The study was performed in accordance with the Helsinki

nje lokalnog etičkog odbora. Svi su bolesnici potpisali informirani pristanak za sudjelovanje u istraživanju.

Imunofenotipizacija

Uzorci periferne krvi stavljeni su u EDTA epruvete i obojeni u roku od 24 sata. Do početka bojenja uzorci su bili čuvani na temperaturi +4 °C. Dnevno je bila napravljena kalibracije fluorescentnih mjerenja pri čemu su se koristile standardizirane površinski fluorescentno označene mikrosfere (FITC/PE). Standardizacija stanične imunofluorescencije zasniva se na kontrolama različitih izotipova. Stanice su prvo prebrojane i nakon toga razrijeđene u PBS puferu na koncentraciju od otprilike 10^7/mL. Monoklonska antitijela konjugirana s fluorescentnim izotiocijanatom (FITC) ili fikoeritrinom (PE) dodana su u preporučenom volumenu prema uputi proizvođača. Specifičnost i kombinacije primjenjenih monoklonalnih antitijela bile su sljedeće: CD45/CD14, IgG1/IgG2 – kontrole različitih izotipova; CD3/CD19, CD3/CD16+56, CD3/CD4, CD3/CD8, CD5/CD20, CD3/HLA-DR, Kappa LC/CD19, Lambda LC/CD19. Svi su reagensi bili od proizvođača BD Bioscience (San Jose, CA, USA). 100 µL otopine razrijeđenih stanica dodana je prethodno pipetiranim antitijelima i stavljena na 15 minuta u inkubaciju na tamno mjesto na -20 °C. Zatim su eritrociti lizirani na sobnoj temperaturi. Rezultati fluorescencije dobiveni su FACSscan protočnom citometrijom, a analizirani su CellQuestovim programom (BD Bioscience). Od svakog je uzorka prikupljeno 10.000 podataka. Primjeri korištene strategije protočne citometrije prikazani su na Slici 1. Kako bi se analizirale limfocitne subpopulacije, limfociti (prikazani crnom bojom) su razvrstani na osnovi prednjeg (FSC) i bočnog (SSC) raspršenja zrake (Slika 1A). Citotoksični T-limfociti razlikovali su se prema koekspresiji CD3 i CD8 antigena (Slika 1B), dok su se pomoćnički T-limfociti razlikovali prema koekspresiji CD3 i CD4 antigena (slika 1C). NK-stanice (eng. *natural killer cells*, NK) izražavaju CD16 i CD56 antigene za razliku od CD3, NK T-limfociti CD3 i CD56 dvostruko su pozitivni (slika 1D). B-limfociti nose CD20 antigen i djelomično CD5 antigen (slika 1E).

Statistička analiza

Rezultati su izraženi u postotku od ukupnog broja leukocita i u izračunatom apsolutnom broju stanica. Rezultati su prikazani kao srednja vrijednost ± standardna pogreška (engl. *standard error*, SE). Za statističku je analizu korištena jednosmjerna analiza varijance (ANOVA) nakon koje je uslijedio *post hoc* parni test usporedbe (LSD). Razina vjerojatnosti od < 5 % prihvaćena je kao značajna. Za sve je statističke analize korišten programski paket Statistica za Windows, s intervalom pouzdanosti od 95%.

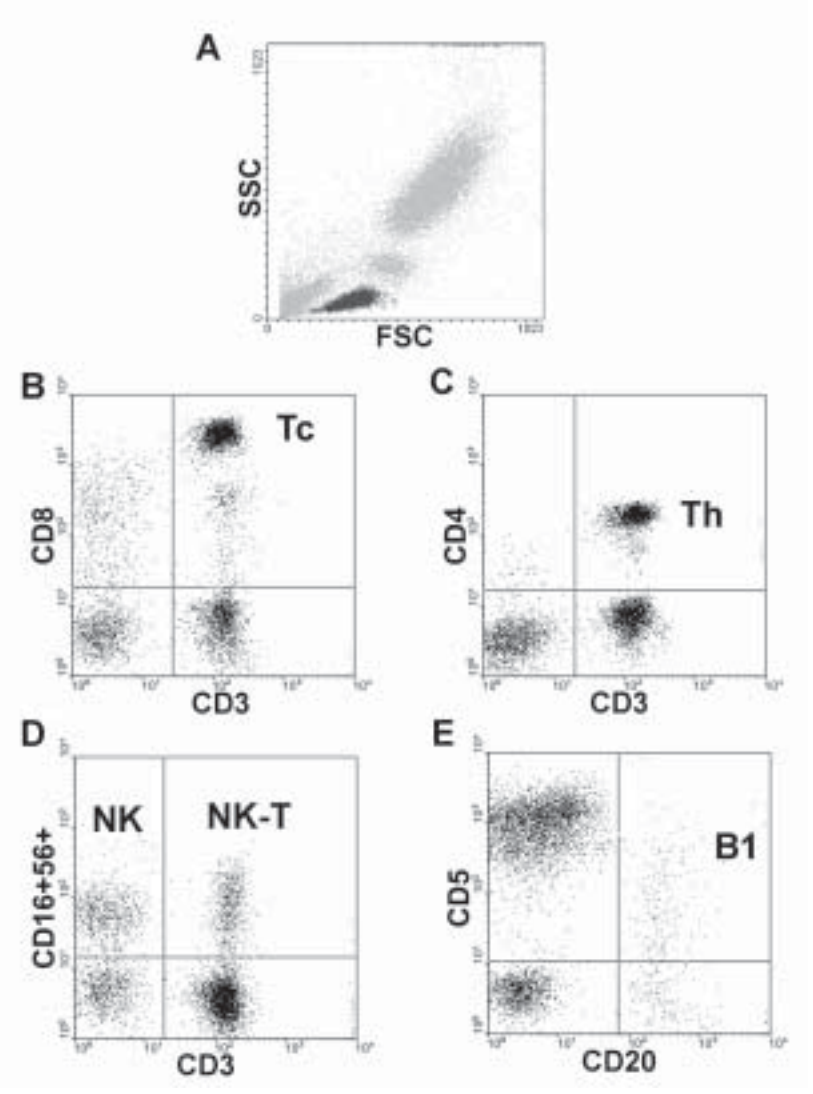
Declaration and the research protocol was approved by the local ethical committee and all patients gave their informed consent to participate.

Immunophenotyping

Peripheral blood samples were taken into EDTA tubes and stained within 24 hours. Until staining procedure, specimens were stored at 4 °C. Fluorescence measurements were calibrated daily using standardized fluorescent surface labelled microspheres (FITC/PE). Standardization of cellular immunfluorescence relied on isotype control.
Cells were first counted and then diluted in PBS buffer to a concentration of approx. 10^7/mL. Monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) were added in the recommended volume on the manufacturer's data sheet. The specificity and combinations of applied monoclonal antibodies were as follows: CD45/CD14, IgG1/IgG2 - isotype controls; CD3/CD19, CD3/CD16+56, CD3/CD4, CD3/CD8, CD5/CD20, CD3/HLA-DR, Kappa LC/CD19, Lambda LC/CD19. All reagents were purchased from BD Bioscience (San Jose, CA, USA). 100 µL of diluted cell suspension was added to the prepipetted antibodies and incubated for 15 minutes on ice in the dark. Then, the erythrocytes were lysed at room temperature. Fluorescence data were acquired on a FACScan flow-cytometer and analyzed with CellQuest software (BD Bioscience). 10,000 events were collected from each sample.
Examples of the applied gating strategy are shown on Figure 1. To analyze the lymphocyte subpopulations, the lymphocytes (displayed in black) were gated on the basis of forward (FSC) and side scatter (SSC) (Figure 1A). Citotoxic T-lymphocytes were discriminated by the coexpression of CD3 and CD8 antigens (Figure 1B), while helper T-lymphocytes by the coexpression of CD3 and CD4 antigens (Figure 1C). Natural Killer cells (NK) express CD16 and CD56 antigens in contrast to CD3, Natural Killer (NK)-type T-cells are CD3 and CD56 double positive (Figure 1D). B-lymphocytes bear CD20 antigen and partly CD5 antigen (Figure 1E).

Statistical analysis

Results are expressed in the percent of the total leukocyte number and in the calculated absolute cell-counts. Results are presented as mean ± standard error (SE). One-way analysis of variance (ANOVA) was used for statistical analysis followed by a *post-hoc* paired comparison (LSD) test. A probability level of <5 % was accepted as significance. Statistica for Windows program package was used for all statistical analysis, by 95% confidential interval.



Slika 1. Postupak odabira stanica.
Limfociti su razvrstani na osnovi prednjeg (FSC) i bočnog (SSC) raspršenja zrake (Slika 1A). Citotoksični T-limfociti CD3 i CD8 dvostruko su pozitivni (gornji desni kvadrant, Slika 1B). Pomoćnički T-limfociti izražavaju zajedno CD3 i CD4 (gornji desni kvadrant, Slika 1C). NK-stanice nose antigene CD56 i CD16 (gornji lijevi kvadrant, Slika 1D), NK T-limfociti su pozitivni na CD3 i CD56 antigene (gornji desni kvadrant, Slika 1D). B-limfociti izražavaju CD20 i B1 populaciju, dakle antigen CD5 (gornji desni kvadrant, Slika 1E).

Figure 1. The gating procedure.
Lymphocytes were gated on the basis of forward (FSC) and side scatter (SSC) (Figure 1A). Citotoxic T-lymphocytes are CD3 and CD8 double positive (upper right quadrant, Figure 1B). Helper T-cells express CD3 and CD4 together (upper right quadrant, Figure 1C). NK-cells bear CD56 and CD16 antigens (upper left quadrant, Figure 1D), NK-T lymphocytes are positive for CD3 and CD56 antigens (upper right quadrant, Figure 1D). B-lymphocytes express CD20 and the B1 population also the CD5 antigen (upper right quadrant, Figure 1E).

Rezultati

Kod 4 bolesnika iz skupine s Dupuytrenovom bolesti su restrikcijom lakog lanca imunoglobulina i/ili patološkim imunofenotipom dokazane su zrele limfoidne neoplazme u tihom obliku, kao npr. kronična limfocitna leukemija B-stanica (engl. *B-cell chronic lymphocytic leukaemia*, CLL) i leukemija granuliranih T-limfocita (T-LGL), dok kod ispitanika iz zdrave skupine nije pronađena takva bolest. Lim-

Results

In 4 patients of the Dupuytren's group mature, indolent lymphoid malignancies, such as B-cell chronic lymphocytic leukaemia (CLL) and T cell granular lymphocytic leukaemia (T-LGL) were proved by immunoglobulin light chain restriction and/or abnormal immunophenotype, whilst such a disease was not found in the healthy group.

fomi su se nalazili u ranom stadiju bez ikakvih kliničkih znakova.

Ovi su hematološki slučajevi isključeni iz daljnje analize. Statistička razlika među ispitanika kontrolne skupine (N = 29), bolesnika koji boluju od Dupuytrenove bolesti stadija 2 (N = 21) i stadija 3 (N = 14) prikazana je u Tablicama 1 i 2.

U oba stadija Dupuytrenove bolesti udio monocita znatno se povisio u usporedbi s kontrolnom skupinom (5,75 ± 0,21 prema 7,49 ± 0,35, P = 0,002 za skupinu s Dupuytrenovom bolesti, i 8,08 ± 0,78, P < 0,001 za kontrolnu skupinu) i ostao gotovo nepromijenjen u naprednom stadiju (Tablica 1). Udio NK T-limfocita također je znatno porasao u stadiju 2 (0,66 ± 0,09 prema 1,07 ± 0,16, P = 0,021), i zatim se snizio u stadiju 3 (0,47 ± 0,1 prema 1,06 ± 0,16, P = 0,012) (Tablica 2.). Tablica 1 prikazuje znatno sniženje udjela B-limfocita (2,88 ± 0,16 prema 2,13 ± 0,21, P = 0,013) uključujući i B1 (koekspresiju CD 20+5+) populaciju (1,44 ± 0,14 prema 0,57 ± 0,09, P = 0,009) kod bolesnika s Dupuytrenovom bolesti u usporedbi sa kontrolnom skupinom. Udio B-limfocita nije se promijenio tijekom bolesti. Nije primijećeno statistički značajno povišenje udjela T-limfocita uključujući populacije pomoćničkih (CD4+) i citotoksičnih (CD8+) između bolesnika s Dupuytrenovom bolesti stadija 2 i zdravih kontrolnih ispitanika (Tablica 2). Udio CD4+/CD8+ T-limfocita snizio se u aktivnom stadiju (Tablica 2). Međutim postotak čitave populacije T-limfocita i populacije pomoćničkih T-limfocita razlikovao se značajno između stadija 2 i 3 kod bolesnika s Dupuytrenovom bolesti (19,02 ± 0,82 prema 15,9 ± 1,38, P = 0,043 za stadij 2 i 13,04 ± 0,85 prema 10,27 ± 1,09, P = 0,023, za stadij 3) (Tablica 2). Udjeli citotoksičnih i aktiviranih (HLA-DR+) T-limfocita i NK-stanica slijedili su promjene u populaciji pomoćničkih T-limfocita, i iako se niti jedan od tih udjela nije znatno promijenio, povećanje HLA-DR+T-limfocita pokazuje jaku tendenciju (P = 0,07) među bolesnicima oba stadija Dupuytrenove bolesti (Tablica 1 i 2).

The lymphomas were in an early stage without any clinical sign.

These haematological cases were excluded from the further analyses. The statistical differences among controls (N = 29), and patients in Dupuytren's stage 2 (N = 21) and stage 3 (N = 14) are shown in the Tables 1 and 2.

In both stages of Dupuytren's disease, the ratio of monocytes increased significantly as compared with the control group (5.75 ± 0.21 *vs*. 7.49 ± 0.35, P = 0.002, and 8.08 ± 0.78, P < 0.001, respectively) and remained almost unchanged in the advanced stage (Table 1.). Natural Killer (NK)-type T-cells rose also significantly in stage 2 (0.66 ± 0.09 *vs*. 1.07 ± 0.16, P = 0.021), then fell in stage 3 (0.47 ± 0.1 *vs*. 1.06 ± 0.16, P = 0.012) (Table 2.). Table 1 depicts the significant reduction in B-lymphocytes (2.88 ± 0.16 *vs*. 2.13 ± 0.21 respectively, P = 0.013), including the B1 (CD 20+5+ coexpression) population (1.44 ± 0.14 *vs*. 0.57 ± 0.09, P = 0.009) in Dupuytren's patients compared with the healthy group. The ratio of B-lymphocytes did not change during the course of the disease. No statistically significant elevation were found in T-lymphocytes, including helper (CD4+), cytotoxic (CD8+) populations between stage 2 of Dupuytren's disease and healthy controls (Table 2). The ratio of CD4+/CD8+ T-cells fell in active stage (Table 2). Nevertheless, the percentage of entire T-lymphocyte population and that of helper T-cells was found to be significantly different between stages 2 and 3 of Dupuytren's patients (19.02 ± 0.82 *vs*. 15.9 ± 1.38, P = 0.043 and 13.04 ± 0.85 *vs*. 10.27 ± 1.09, P = 0.023, respectively) (Table 2). The ratios of cytotoxic T-, activated (HLA-DR+) T-cells and NK cells followed the alterations of helper T-lymphocyte populations, although neither of them changed significantly, the increase in HLA-DR+T lymphocytes show a strong tendency (P = 0.07) between the two DD groups (Table 1 and 2).

Tablica 1. Usporedba broja monocita, B-limfocita i NK-stanica između kontrolne skupine i bolesnika s Dupuytrenovom bolesti.

Table 1. Comparison of monocytes, B-lymphocytes and NK-cell counts between controls and Dupuytren patients.

	Monocytes (CD14+)	B-Ly (CD19+)	B1-Ly (CD20+5+)	NK-cells (CD16+56+)
	Mean ± S.D. G/l (in % of the total WBC count)			
Control	0.37 ± 0.10 (5.8 ± 1.20)	0.20 ± 0.06 (2.88 ± 0.94)	0.09 ± 0.05 (1.44 ± 0.80)	0.20 ± 0.10 (2.80 ± 1.5)
Stage2	0.51 ± 0.14** (7.5 ± 1.7)	0.16 ± 0.03* (2.13 ± 1.01)	0.04 ± 0.02*** (0.57 ± 0.44)	0.22 ± 0.14 (3.10 ±1.60)
Stage3	0.55 ± 0.13** (8.10 ± 2.90)	0.14 ± 0.03** (1.93 ± 1.15)	0.04 ± 0.03*** (0.38 ± 0.33)	0.23 ± 0.15 (3.40 ±1.70)
ANOVA (P)	< 0.001	0.21	< 0.001	0.27

Significantly different from the control group by the LSD test: ***P < 0.001; **P < 0.01; *P < 0.05.

Tablica 2. Usporedba broja T-limfocita između kontrolne skupine i bolesnika s Dupuytrenovom bolesti.

Table 2. Comparison of T-lymphocyte counts between controls and Dupuytren patients.

	T-Ly (CD3+)	NK T-Ly (CD3+56+)	Activated T-Ly (CD3+DR+)	T-helper (CD3+4+)	T-cytotoxic (CD3+8+)	CD3+4+/ CD3+8+ ratio
	Mean ± S.D. G/l (in % of the total WBC count)					
Control	1.18 ± 0.33 (18.80 ± 4.20)	0.04 ± 0.03 (0.66 ± 0.54)	0.25 ± 0.13 (3.80 ± 1.80)	0.76 ± 0.20 (12.10 ± 2.50)	0.41 ± 0.18 (6.40 ± 2.70)	2.40 ± 1.40
Stage2	1.38 ± 0.37 (19.20 ± 4.00)	0.08 ± 0.05**b (1.07 ± 0.80)	0.32 ± 0.18 (4.70 ± 3.40)	0.85 ± 0.22 (13.00 ± 4.10)	0.49 ± 0.30 (7.10 ± 4.90)	2.00 ± 1.20
Stage3	1.06 ± 0.42*a (16,10 ± 5.20)	0.04 ± 0.03*a (0.48 ± 0.38)	0.23 ± 0.08 (3.20 ± 1.10)	0.68 ± 0.33 (10.30 ± 4.10)	0.38 ± 0.17*a (5.80 ± 2.30)	2.10 ± 1.50
ANOVA (P)	0.21 (NS)	0.0016	0.07 (NS)	0.14 (NS)	0.24 (NS)	0.27 (NS)

a – Significantly different from stage 2; b – Significantly different from both the control and from stage 3 groups by the LSD test; **P < 0.01; *P < 0.05.

Rasprava

Promatrali smo različite i karakteristične promjene u broju limfocita i monocita u perifernoj krvi sukladno kliničkim stadijima Dupuytrenove bolesti. U aktivnom se stadiju udio monocita i NK T-limfocita značajno povisio. Slično se povisio i udio CD4+, CD8+, aktiviranih T-limfocita i NK-stanica, no te promjene nisu bile značajne. Ovi nalazi potvrđuju prethodna mišljenja da su T-limfociti povezani s patogenezom Dupuytrenove bolesti (3,4). Ovdje nismo istraživali monocite, NK T-limfocite niti stanično imunosni odgovor sukladno stadiju Dupuytrenove bolesti.

Prikazano je i značajno smanjenje udjela B-limfocita, uključujući tzv. B1-stanice (CD5+) što je djelomično povezano s rezultatima Gudmundssona i sur. (4). Oni su izvijestili o snižavanju udjela CD5+ B-limfocita. Naša prethodna zapažanja o prisustvu antinuklearnih antitijela odnose se također na ulogu B-limfocita u Dupuytrenovoj bolesti (6). Fibrozne bolesti, npr. plućna i jetrena fibroza, sistemska skleroza povezane su s protuupalnim T pomoćničkim 2 (T_H2) staničnim odgovorom (7-10). TGFβ je jedan od najvažnijih citokina od T_H2 posrednim imunosnim odgovorom. Značaj TGFβ u Dupuytrenovoj bolesti dokazali su mnogi autori (10-12). Makrofagi su jedan od glavnih izvora TGFβ i za TGFβ takvog porijekla vjeruje se da je profibrozan (13). Povišen broj monocita u Dupuytrenovoj bolesti može osigurati trajan dotok energije makrofagima u zahvaćenom tkivu.

NK T-limfociti mogu izlučiti tipične T_H2 citokine, npr. IL-13 (14). Aktivacija TGFβ1 ovisi o metaloproteinazi matriksa 9 (MMP-9), pospješenoj s IL-13 koji cijepa peptide povezane s latencijom (15). U eksperimentalnim modelima autoimunih bolesti, NK T-limfociti su štitili od šećerne bolesti

Discussion

We observed different and characteristic changes in numbers of peripheral blood lymphocytes and monocytes according to clinical stages of DD. In active stage, monocytes and NK-type T-cells increased significantly. Similarly, CD4+, CD8+, activated T-cells and NK-cells rose also, but these alterations were not significant. These findings support the previous observations that T-cells are involved in the pathogenesis of DD (3, 4). Monocytes, NK-T-cells and cellular immune response according to stages of DD were not investigated by this time.

A significant reduction of B-lymphocytes, including the so-called B1-cells (CD5+) was also here shown, which correlates partly with the results of Gudmundsson et al (4). They found a decrease in the ratio of CD5+ B-cells. Our previous observations about the presence of antinuclear antibodies refer also to the involvement of B-lymphocytes in DD (6). Fibrotic diseases, e.g. pulmonary fibrosis, hepatic fibrosis, systemic sclerosis are associated with anti-inflammatory T helper 2 (T_H2)-cell responses (7-10). TGFβ is one of the most important cytokines of T_H2 mediated immune response. The significance of TGFβ in Dupuytren's disease was proved by more authors (10-12). Macrophages are one of the main sources of TGFβ and macrophage-derived TGFβ is thought to be pro-fibrotic (13). Increased ratio of monocytes in DD may ensure permanent supply for macrophages in the affected tissue.

NKT-cells are able to secret typical T_H2 cytokines, e.g. IL-13 (14). The activation of TGFβ1 depends on the matrix metalloproteinase-9 (MMP-9), upregulated by IL-13 that cleave the latency-associated peptide (15). In experimental models of autoimmune diseases, NKT cells protected

ili eksperimentalnog autoimunog encefalomijelitisa premještanjem ravnoteže s T_H1 prema T_H2 imunom odgovoru (16,17). Bolesnici koji boluju od drugih fibroznih bolesti (npr. sistemska skleroza) pokazuju promijenjenu homeostazu B-limfocita sličnu našim rezultatima (18).

U uznapredovalom su se stadiju udjeli CD4+ T-limfocita i NK T-limfocita snizili značajno, dok su se udjeli CD8+ i HLA-DR+ T-limfocita snizili, ali ne značajno. Udjeli monocita i B-limfocita ostali su konstantni u usporedbi s onima u aktivom stadiju. Tendencija snižavanja udjela T-limfocita navodi na razmatranje da je ona dio protuupalne reakcije koja može biti krajnji rezultat T_H2 posredovanog imunosnog odgovora. Uzimajući sve u obzir, naši se podaci mogu odnositi na T_H2 posredovani imunosni odgovor povezan s masivnom fibrozom u patogenezi Dupuytrenove bolesti. Međutim treba naglasiti da do sada nismo direktno istražili citokinski profil T-limfocita kod Dupuytrenove bolesti. Česta pojava zrelih limfoidnih neoplazmi u tihom obliku među našim bolesnicima s Dupuytrenovom bolesti može podržati hipotezu o autoimunoj pozadini Dupuytrenove bolesti, zato što različite autoimune bolesti, npr. autoimuna hemolitička anemija često kompliciraju CLL. Naravno da je potrebno napraviti velika istraživanja kako bi se opravdala veza između Dupuytrenove bolesti i hematoloških zloćudnih bolesti.

Zaključujemo da su monociti, T-limfociti (a posebno NK T-limfociti) i B-limfociti vjerojatno povezani s patogenezom Dupuytrenove bolesti, te da promijene u njihovom broju mogu ukazivati na upalni mehanizam u tijeku. Međutim, potrebno je dalje istražiti T-limfocite koji prodiru u aponeurozu, raspodijelu i profil citokina u T-limfocitima u perifernoj krvi kod bolesnika s Dupuytrenovom bolesti u ranom stadiju.

Zahvale

Uz dužno poštovanje zahvaljujemo Riti Jáger M.D. iz Mađarske banke krvi za kontrolne uzorke krvi i gđi J. Gál te gđi. M. Horváth za tehničku pomoć.

from diabetes or experimental autoimmune encephalomyelitis by shifting the balance from T_H1 toward T_H2 response (16,17).

Patients with other fibrotic disease (e.g. systemic sclerosis) show similarly altered B-cell homeostasis to our results (18).

In the advanced stage, the ratios of CD4+ T-cells and NK-T-cells fell significantly, while that of CD8+ and HLA-DR+ T-cells decreased not significantly. The ratios of monocytes, B-lymphocytes remained constant compared with those in the active stage. The lowering tendency of T-cells is tempting to be speculated as part of an anti-inflammatory reaction that can be the final result of the T_H2 mediated immune response. Taken together, our data may refer to a T_H2 mediated immune response associated with massive fibrosis in the pathogenesis of DD. It should, however, be emphasized that by this time we have not investigated directly the cytokine profiles of T-cells in DD.

The frequent occurrence of mature, indolent lymphoid malignancies among our Dupuytren patients may support also the autoimmune hypothesis, because different autoimmune diseases, e.g. autoimmune haemolytic anaemia often complicate CLL Of course, larger series are needed to justify an association between DD and haematological malignancies.

We conclude that monocytes, T- (especially NK-T) and B -lymphocytes are likely involved in the pathogenesis of Dupuytren's disease and alteration in their numbers may refer to an ongoing inflammatory mechanism. Further studies, however, should be launched to investigate the aponeurosis infiltrating T-lymphocytes, the distribution and cytokine profile of the peripheral blood T-lymphocytes in early stage DD patients.

Acknowledgments

We are indebted to Rita Jáger M.D. at the Hungarian Blood Bank for control blood samples and to Mrs. J. Gál and Ms. M. Horváth for technical assistance.

Adresa za dopisivanje:

Institute of Laboratory Medicine, University of Pécs
Ifjúság St. 13
H-7624 Pécs
Hungary
e-pošta: gabor.l.kovacs@aok.pte.hu
tel: +3672 536-120
faks: +3672 536-121

Corresponding author:

Univ. Prof. Gábor L. Kovács MD, PhD, D.Sc.
Institute of Laboratory Medicine, University of Pécs
Ifjúság St. 13
H-7624 Pécs
Hungary
e-mail: gabor.l.kovacs@aok.pte.hu
phone: +36 72 536-120
fax: +3672 536-121

Literatura/References

1. Chiu HF, McFarlane RM. Pathogenesis of Dupuytren's contracture: A correlative clinical-pathological study. J Hand Surg 1978;3:1-10.
2. Thurston AJ. Dupuytren's disease. J Bone Joint Surg 2003;85:469-77.
3. Baird KS, Alwan HW, Crossan JF, Wojciak B. T-cell mediated response in Dupuytren's disease. Lancet 1993;341:1622-3.
4. Gudmundsson KG, Arngrimsson R, Arinbjarnarson S, Olafsson A, Jonsson T. T- and B-lymphocyte subsets in patients with Dupuytren's disease. J Hand Surg 1998; 23:724-7.
5. Neumüller J, Menzel J, Millesi H. Prevalence of HLA-DR3 and autoantibodies to connective tissue components in Dupuytren's contracture. Clin Immunol Immunpathol 1994;71:142-8.
6. Salamon A, Józsa L, Toldy E. The role of different factors in the pathogenesis of Dupuytren's disease. Proceedings of the 8th Congress of the IFSSH. 2001;363-9.
7. Dlugovitzky D, Bay ML, Rateni L, Urízar L, Rondelli CF, Largacha C, et al. In vitro synthesis of interferon-gamma, interleukin-4, transforming growth factor-beta and interleukin-1 beta by peripheral blood mononuclear cells from tuberculosis patients: relationship with the severity of pulmonary involvement. Scand J Immunol 1999;49:210-7.
8. Wallace WA, Ramage EA, Lamb D, Howie SE. A type 2 (Th2-like) pattern of immune response predominates in the pulmonary interstitium of patients with cryptogenic fibrosing alveolitis (CFA). Clin Exp Immunol 1995;101:436-41.
9. Vaillant B, Chiaramonte MG, Cheever AW, Soloway PD, Wynn TA. Regulation of hepatic fibrosis and extracellular matrix genes by the TH response: new insight into the role of tissue inhibitors of matrix metalloproteinases. J Immunol 2001;157:7017-26.
10. Hasegawa M, Fujimoto M, Kikuchi K, Takehara K. Elevated serum levels of interleukin 4 (IL-4), IL-10 and IL-13 in patients with systemic sclerosis. J Rheumatol 1997;24:328-32.
11. Bayat A, Stanley JK, Watson JS, Ferguson MW, Ollier WE. Genetic susceptibility to Dupuytren's disease: transforming growth factor beta receptor (TGFbetaR) gene polymorphisms and Dupuytren's disease. Br J Plast Surg 2003;56:328-33.
12. Kloen P, Jennings CL, Gebhardt MC, Springfield DS, Mankin HJ. Transforming growth factor-beta: possible roles in Dupuytren's contracture. J Hand Surg 1995;20:101-08.
13. Wynn AT. Fibrotic disease and the T_H1/T_H2 paradigm. Nat Rev Immunol 2004; 4:583-94.
14. Godfrey DI, Kronenberg M. Going both ways: immune regulation via CD1d-dependent NKT cells. J Clin Invest 2004;114:1379-88.
15. Lee CG, Homer RJ, Zhu Z, Lanone S, Wang X, Koteliansky V, et al. Interleukin-13 induces tissue fibrosis by selectively stimulating and activating the transforming growth factor β1. J Exp Med 2001;194:809-21.
16. Hong S, Wilson MT, Serizawa I, Wu L, Singh N, Naidenko OV, et al. Natural killer T-cell ligand alpha-galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. Nat Med 2001;7:1052-6.
17. Jahng AW, Maricic I, Pedersen B, Burdin N, Naidenko O, Kronenberg M, et al. Activation of natural killer T cells potentiates or prevents experimental autoimmune encephalomyelitis. J Exp Med 2001;194:1789-99.
18. Fujimoto M, Sato S. B lymphocytes and systemic sclerosis. Curr Opin Rheumatol 2005;17:746-51.