Natural Killer Cell Cytotoxicity in Rheumatoid Arthritis Patients Treated with TNF Inhibitors

TNF İnhibitörleri ile Tedavi Edilen Romatoid Artrit Hastalarında Doğal Öldürücü Hücre Toksisitesi

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ABSTRACT Objective: Patients with low natural killer (NK) cell activity were shown to have a higher risk of cancer development. Increased cancer risk in rheumatoid arthritis (RA) patients treated with anti-tumor necrosis factor (anti-TNF) agents is still being debated. Therefore, we think it is important to show whether anti-TNF therapy has any effects on the NK cell cytotoxicity in these patients. **Material and Methods:** We included 60 rheumatoid arthritis patients, 31 treated with anti-TNF therapy, 29 treated with other disease modifying antirheumatic drugs (DMARDs), and 51 ankylosing spondylitis (AS) patients, 35 treated with anti-TNF therapy and 16 treated with nonsteroidal anti-inflammatory drugs or conventional DMARDs as a disease control into the study. The results of two healthy control groups each consisting of 32 age- and sex- matched healthy individuals were also included. **Results:** Median values of NK cytoxicity in patients with RA who were being treated with anti-TNF and patients treated with other DMARDs were 29% and 43%, respectively (p=0.203). When we reanalyzed the results independent of clinical diagnosis, patients using anti-TNF medications and patients treated with other medicine had NK cytotoxicity median values of 32% and 43%, respectively (p=0.277). **Conclusion:** We suppose that NK cell cytotoxicity may decrease in patients with RA when they use anti-TNF agents, and this may help partly to explain why these patients are more prone to development of cancer and severe infections. We think that this study will encourage new studies with higher number of patients to better clarify effects of anti-TNF treatment on NK cell functions.

Key Words: Arthritis, rheumatoid; tumor necrosis factor-alpha; natural killer T-cells

ÖZET Amaç: Düşük doğal öldürücü (NK) hücre aktivitesi olan hastalarda kanser gelişme riskinin daha yüksek olduğu gösterilmiştir. Anti-tümör nekrozis faktör (anti-TNF) ile tedavi edilen romatoid artrit (RA) hastalarında artmış kanser riski literatürdeki farklı sonuçlarla halen bir tartışma konusudur. Bu nedenle biz RA tanılı hastalarda anti-TNF tedavinin NK hücre toksisitesi üzerine etkisi olup olmadığını göstermenin önemli olduğunu düşündük. **Gereç ve Yöntemler:** Çalışmaya 31'i anti-TNF tedavi alan, 29'u diğer hastalık modifiye edici ilaçlarla (DMARD) tedavi edilen 60 RA hastası alındı. Ayrıca hasta kontrol grubu olarak 35'i anti-TNF tedavi, 16'sı nonsteroid antiinflamatuar ilaçlar veya konvansiyonel DMARD'larla tedavi edilen 51 ankilozan spondilit (AS) hastası dâhil edildi. Hem RA hem de AS grubu için her biri yaş ve cinsiyet açısından eşleştirilmiş 32'şer hastadan oluşan iki sağlıklı kontrol grubunun sonuçları da çalışmaya dâhil edildi. **Bulgular:** Anti-TNF ilaçlarla tedavi edilen RA hastaları ile diğer DMARD'larla tedavi edilen hastaların NK sitotoksisitesi median değerleri sırasıyla, %29 ve %43'tü (p=0,203). Tanıdan bağımsız olarak sonuçlar tekrar analiz edildiğinde, anti-TNF ilaçlarla tedavi edilen hastalarla diğer ilaçlarla tedavi edilen hastaların NK sitotoksisitesi median değerleri sırasıyla %32 ve %43 olarak hesaplandı (p=0,277). **Sonuç:** Biz anti-TNF tedavi alan RA hastalarında NK hücre toksisitesinin düşebileceğini ve bunun kısmen de olsa bu hastaların malign hastalıklara ve ciddi enfeksiyonlara duyarlı olmasında etkisi olabileceğini öngörmekteyiz. Bu ilişkinin daha net ortaya konabilmesi için, bu çalışmanın daha çok sayıda hasta ile yapılacak yeni çalışmalara cesaretlendireceğini düşünmekteyiz.

Anahtar Kelimeler: Artrit, romatoid; tümör nekroze edici faktör-alfa; doğal öldürücü T- hücreleri

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Natural killer (NK) cell toxicity is an important component of innate immunity. Until now, various experimental studies were conducted regarding the role of cytokines in the activity of these cells. It was shown that human NK cells constitutively express membrane tumor necrosis factor-alpha (mTNFα), present mTNFα-dependent cytotoxic activity and stimulation with IL-2, IL-15 and IL-18 upregulates TNF-α mRNA, soluble TNF-α and mTNF-α expression in NK cells.[1] In another study, the adding interleukin-2 (IL-2) to non-target binding free cell subset of NK cells resulted in the induction of cytotoxic activity and stimulation of free cells to become binder and killer cells, and endogenous TNF-α secretion was a prerequisite for the initiation of IL-2-mediated activation of free cells into killer cells.[2] In the same study, all of these IL-2-mediated manifestations were shown to be downregulated by the adding anti-TNF-α antibody. Jewett et al. similarly demonstrated IL-12-mediated activation of natural killer cell cytotoxicity which was again dependent to endogenous TNF-alpha secretion.[3] In another study by Naume et al., NK cells were found to express TNF receptors p55 and p75 upon activation, and both receptors were involved in the generation of lymphokine-activated killer cells activity.[4] Moreover, addition of recombinant TNF-alpha increased the IL-2-induced proliferation of NK cells while mAb to p55 and p75 inhibited the IL-2-induced proliferation.[4] The role of NK cells in the pathogenesis of rheumatoid arthritis and its relevance with several cytokines, especially tumor necrosis factor is still a hot topic.[5]

It is obvious from previous studies that NK cell cytotoxicity is in a close relation with and partly dependent on to endogenous TNF-α. Currently, TNF inhibitors are widespreadly used in rheumatologic disorders. The use of these drugs have the potential to increase with their use in other inflammatory diseases. Since controversial data exists regarding increased risk of serious infections and possible cancer development with anti-TNF medications, we believe that this issue may be better clarified by data obtained from clinical studies investigating the physiology of TNF-α. Therefore, we planned to investigate the natural killer cell cytotoxicity in rheumatoid arthritis patients treated with TNF inhibitors and compate them with patients treated with non-TNF disease modifying antirheumatic drugs (DMARDs) We included ankylosing spondylitis (AS) patients as disease control and healthy individuals as control groups.

MATERIAL AND METHODS

Patients were recruited from Rheumatology outpatient clinic of Akdeniz University Hospital Department of Internal medicine. Sixty rheumatoid arthritis patients (31 treated with anti-TNF therapy, 29 treated with conventional DMARDs) and 51 ankylosing spondylitis patients (35 treated with anti-TNF therapy and 16 treated with NSAIDs or conventional DMARDs) were included in the study. The results of two healthy control groups, each consisting of 32 age- and sex- matched healthy individuals were included to compare rheumatoid arthritis and ankylosing spondylitis patients. The natural killer cytotoxicity levels of healthy patients were obtained from the data of another study in our clinic which investigated age-related immune functions. All patients with rheumatoid arthritis fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria, and patients with ankylosing spondylitis fulfilled the Assessment of SpondyloArthritis international Society classification criteria.[6,7] Patients and controls were 18 years old or older. The study procedure was consistent with Helsinki Declaration 2008. Informed consent was obtained from all patients and the study was approved by local ethic committee. Patients with a history of malignancy, active tuberculosis, symptoms and signs of acute infection, chronic renal or hepatic failure, HIV infection as well as immune compromised patients were excluded from the study. Disease activity score 28 (DAS28) and Bath ankylosing spondylitis disease activity index (BASDAI) were used to evaluate the disease activity.[8,9] Age, sex, disease duration, leukocyte and lymphocyte counts, erythrocyte sedimentation rates (ESR) and C-reactive protein (CRP) levels and ongoing therapies were recorded.

Peripheral blood samples of 5-6 ml were collected from veins of forearm to heparinized test tubes, 3 cc histopaque (10771-100 ml) was added and the tubes were centrifuged at 1700 rpm for 20 minutes. Lymphocytes were collected to 15 ml falcons. We added sterile PBS until 15 ml of flacon was full, and again centrifuged at 1700 rpm for seven minutes. Supernatant was poured and 10 ml sterile RPMI was added. To achieve a $0.5x10^6$/100µl cell concentration, we diluted $5.5x10^6$ effector cell (fresh PBL) in an 1100 µl assay medium. We transferred 400 µl of the solution to a new Eppendorf tube and added 400 µl assay medium to achieve a $0.25x10^6$ cell concentration. 100 µl cells were added per well to only-effector control and assay wells. We obtained 5 ml target cell (K562 myeloid leukemia cells) from the culture and placed into 15 ml tubes and counted the alive cells by trypan blue. Cells were diluted with assay medium if the cells were in high concentration when examined by microscope. We counted target alive cells by trypan blue and diluted to achieve a concentration of $1x10^4$ cell/100 µl, and added 100 µl cells per well to only-target control, only-target plus lysis solution and assay wells. Cells were mixed by a pipette in assay wells. We added 100 µl assay medium to all wells (except only-medium and medium-lysis controls, these must be added 200µl assay medium). Plates were incubated at 37°C for four hours. Three hours after incubation, we added 30 µl lysis solution (provided with Promega Cytotox 96 kit) to target+lysis and medium+lysis controls. Wells were mixed by a pipette. We centrifuged the plate at 1400 rpm for four minutes, four hours after incubation. 50 µl supernatant was transferred to ELISA plate by a multichannel pipette. We added 50 µl substrate solution and incubated the plate at dark for 30 minutes. 50 µl stop solution was added and we observed the results by ELISA reader at 490 nm. Calculated and shown effector: target ratio was 50:1.

STATISTICAL ANALYSIS

The data were analyzed using SPSS 15.0 and MedCalc 10.0.4 software. The data analyzed by parametric tests were expressed as mean±standard deviation, and the data analyzed by non-parametric tests were expressed as median (minimum-maximum). Two- group comparisons were performed by Mann Whitney U test if the variable was not distributed normally, and by Student-t test if it was distributed normally. All comparisons for categorical data were performed by Chi-square and Fisher Exact tests. Three-group comparisons for normally distributed variables were performed by One Way ANOVA and Kruskal Wallis test for variables that were not normally distributed. The correlation between two groups was established by Spearman's correlation coefficient. Normality analysis was implemented by Kolmogorov Smirnov test. All the hypotheses were constructed two tailed, and an alpha critical value of 0.05 was accepted as significant.

RESULTS

General characteristics of the participants are shown in Table 1.

RA patients treated with anti-TNF, treated with other DMARDs and healthy control group did not differ significantly in terms of age, sex, leukocyte and lymphocyte counts. Methotrexate, leflunomide and corticosteroid treatment ratios were similar between groups. Sulfasalazine and hydroxychloroquine use were more in RA patients treated with DMARDs in comparison to anti-TNF group. Rheumatoid factor positivity, mean disease duration and CRP values were similar among groups. ESR and DAS28 values of RA patients treated with anti-TNF were slightly higher (Table 2; p values are shown on table).

AS patients treated with anti-TNF, treated with other drugs and the healthy controls were not statistically significantly different from each other in terms of age, sex, leucocyte and lymphocyte counts. Disease duration, ESR and CRP values were similar in anti-TNF group and in AS patients treated with other drugs. Methotrexate treatment ratios were similar, although sulfasalazine and nonsteroidal antiinflammatory drug therapies were more in AS patients not treated with anti-TNF. BASDAI levels of both groups were less than 3, however anti-TNF group had lower BASDAI values (Table 3; p values are shown on table).

TABLE 1: General characteristics of the participants.

	RA treated with anti-TNF	RA treated with other medicine	Healthy controls for RA	AS treated with anti-TNF	AS treated with other medicine	Healthy controls for AS
	n= 31	n= 29	n= 32	n= 35	n= 16	n= 32
Age (mean ± SD)	50.55 ± 15.49	54.07 ± 10.82	49.59 ± 14.03	40.6 ± 13.73	37.38 ± 11.48	45.75 ± 14.39
Sex (F-M) (n) (%)	25-6	22-7	25-7	5-30	7-9	7-25
	80.6-19.4%	75.9-24.1%	78.1-21.9%	14.3-85.7%	43.8-56.3%	21.9-78.1%
Disease duration-year (median-min/max)	10 (2-35)	12 (1-45)		10 (2-34)	5 (1-22)	
Leukocyte (/mm^3) (mean ± SD)	7509 ± 2544	7187 ± 2127	7067 ± 1928	8183 ± 2555	8467 ± 1966	7147 ± 2053
Lymphocyte (/mm^3) (mean ± SD)	2366 ± 890	1897 ± 699	2453 ± 918	2283 ± 809	2347 ± 481	2297 ± 779
Das 28 (median-min/max)	3.75 (1.61-5.98)	2.86 (0.67-5.74)				
BASDAI (median-min/max)				1.15 (0-8.5)	2.6 (0.26-8.1)	
Infliximab (n-%)	4 (%12.9)			13 (37.1%)		
Etanercept (n-%)	16 (51.6%)			14 (40%)		
Adalimubab (n-%)	11 (35.4)			8 (22.8%)		
MTX (n) (%)	15 (48.4%)	19 (65.5%)		6 (17.1%)	6 (37.5%)	
SSZ (n) (%)	2 (6.5%)	13 (44.8%)		1 (2.9%)	12 (75%)	
HQ (n) (%)	3(9.7%)	17 (58.6%)		0	0	
Leflunomide (n) (%)	6(19.4%)	6(20.7%)		0	0	
Steroid (n) (%)	8(25.8%)	9(31%)		2 (5.7%)	0	
ESR (mm/h) (median-min/max)	34 (9-118)	21 (2-119)		20 (2-56)	27 (2-65)	
CRP (mg/dl) (median-min/max)	0.53 (0.07-7.02)	0.36 (0.05-6.56)		0.55 (0.01-8.32)	0.58 (0.09-3.23)	
RF(+) (n) (%)	20 (64.5%)	21(75%)				

Anti-TNF: Antitumor necrosis factor; AS: Ankylosing spondylitis; RA:Rheumatoid arthritis; SD:Standard deviation; F:Female; M:Male; min:Minimum; max: Maximum; mm3: Cubic milimeter; BASDAI: Bath ankylosing spondylitis disease activity index; DAS28: Disease activity score 28; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; mm: Milimeter; n: Number; MTX: Methotrexate; SSZ: Sulfasalazine; RF: Rheumatoid factor; HQ: Hydroxychloroquine.

TABLE 2: Differences among RA patients treated with anti-TNF, RA patients treated with other medicine and the control group.

	RA treated with anti-TNF	RA treated with other medicine	Control	p value
Age (mean ± SD)	50.55±15.49	54.07±10.82	49.59±14.03	0.413
Sex (F-M) (n) (%)	25-6 80.6-19.4%	22-7 75.9-24.1%	25-7 78.1-21.9%	0.711
Leukocyte (/mm^3) (mean ± SD)	7509 ± 2544	7187 ± 2127	7067±1928	0.719
Lymphocyte (/mm^3) (mean ± SD)	2366 ± 890	1897 ± 699	2453±918	0.027
MTX (n) (%)	15 (48.4%)	19 (65.5%)	-	0.181
Leflunomide (n) (%)	6 (19.4%)	6 (20.7%)	-	0.897
Steroid (n) (%)	8 (25.8%)	9 (31%)	-	0.870
SSZ (n) (%)	2 (6.5%)	13 (44.8%)	-	0.001
HQ (n) (%)	3 (9.7%)	17 (58.6%)	-	< 0.001
Disease duration-year (median-min/max)	10 (2-35)	12 (1-45)	-	0.693
Das 28 (median-min/max)	3.75 (1.61-5.98)	2.86 (0.67-5.74)	-	0.044
ESR (mm/h) (median-min/max)	34 (9-118)	21 (2-119)	-	0.047
CRP (mg/dl) (median-min/max)	0.53 (0.07-7.02)	0.36 (0.05-6.56)	-	0.528
RF(+) (n) (%)	20 (64.5%)	21 (75%)	-	0.382

Anti-TNF: Antitumor necrosis factor; RA: Rheumatoid arthritis; SD: Standard deviation; F: Female; M: Male, min: Minimum; max: Maximum; mm^3: Cubic milimeter; DAS28: Disease activity score 28; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; mm: Milimeter; n: Number; MTX: Methotrexate; SSZ: Sulfasalazine; RF: Rheumatoid factor; HQ: Hydroxychloroquine.

TABLE 3: Differences among AS patients treated with anti-TNF, AS patients treated with other medicine and the control group.

	AS treated with anti-TNF	AS treated with other medicine	Control group	p value
Age (mean ± SD)	40.6±13.73	37.38±11.48	45.75±14.39	0.104
Sex (F-M) (n) (%)	5-30 14.3-85.7%	7-9 43.8-56.3%	7-25 21.9-78.1%	0.066
Leukocyte (/mm^3) (mean ± SD)	8183±2555	8467±1966	7147±2053	0.088
Lymphocyte (/mm^3) (mean ± SD)	2283±809	2347±481	2297±779	0.960
MTX (n) (%)	6 (17.1%)	6 (37.5%)	-	0.157
SSZ (n) (%)	1 (2.9%)	12 (75%)	-	<0.001
Disease duration-year (median-min/max)	10 (2-34)	5 (1-22)	-	0.064
BASDAI (median-min/max)	1.15 (0-8.5)	2.6 (0.26-8.1)	-	0.006
ESR(mm/h) (median-min/max)	20 (2-56)	27 (2-65)	-	0.388
CRP(mg/dl) (median-min/max)	0.55 (0.01-8.32)	0.58 (0.09-3.23)	-	0.795

Anti-TNF: Antitumor necrosis factor; AS: Ankylosing spondylitidis; SD: Standard deviation; F: Female; M: Male; min: Minimum; max: Maximum; mm^3: Cubic milimeter; DAS28: Disease activity score 28 ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; mm: Milimeter; n: Number; MTX: Methotrexate; SSZ: Sulfasalazine.

Median (min-max) NK cytotoxicity values were 29% (9-89); 43% (8-99) and 49.57% (6-99) in RA patients treated with anti-TNF, in RA patients treated with other drugs and in healthy controls, respectively (p=0.203). AS patients treated with anti-TNF, AS patients treated with other drugs and healthy controls had median (min-max) NK cytotoxicity values of 35% (6-85); 41.5% (11-99) and 39.77% (6-80), respectively (p=0.892). Median (min-max) NK cytotoxicity values of patients treated with anti-TNF and patients treated with other drugs regardless of their diagnoses were 32% (6-89) and 43% (8-99), respectively (p=0.277). RA patients and AS patients had median (min-max) NK cytotoxicity values of 32.5% (8-99) and 37% (6-99), respectively, regardless of their treatment modality (p=0.277) (Table 4). There was no correlation between NK cytotoxicity values and anti-TNF

TABLE 4: NK cytotoxicity and P values of (1) RA patients regarding treatment with anti-TNF or other medicine, compared to healthy controls; (2) AS patients regarding treatment with anti-TNF or other medicine, compared to healthy controls; (3) RA and AS patients together, treated with anti-TNF or treated with other medicine; (4) RA patients and AS patients independent of treatment.

	NK cytotoxicity% median-(min/max)	p value
RA treated with anti-TNF	29-(9/89)	
RA treated with other medicine	43-(8/99)	0.203
Control group for RA	49.57-(6-99)	
AS treated with anti-TNF	35-(6/85)	
AS treated with other medicine	41.5-(11/99)	0.892
Control group for AS	39.77-(6/80)	
RA and AS patients treated with anti-TNF	32-(6/89)	0.277
RA and AS patients treated with other medicine	43-(8/99)	
All RA patients	32.5(8/99)	0.277
All AS patients	37-(6/99)	

Anti-TNF: Antitumor necrosis factor; NKC: Natural killer cell; AS: Ankylosing spondylitis; RA: Rheumatoid arthritis.

treatment durations (p= 0.506). Effects of three anti-TNF drugs on NK cytotoxicity were similar (p=0.710). Again, there was no difference for monoclonal antibodies and receptor fusion protein (p=0.602).

DISCUSSION

RA is a chronic inflammatory and autoimmune disorder characterized by extensive synovitis leading to cartilage and bone erosions and hence joint destruction.[10] RA is typically considered a Th1-driven disease, although recent findings suggest a prominent role for Th17 cells.[11,12] RA synovial membrane is rich in different immune cells, such as macrophages, T cells, B cells, dendritic cells, neutrophils, and plasma cells. In addition to these cells, a subset of NK cells has been detected in the synovial fluid of RA patients.[13] NK cells are large granular lymphocytes derived from pluripotent hematopoietic stem cells, and their development occurs primarily extrathymically. NK cells mainly contribute to innate immunity and adaptive immune responses by killing target cells directly, or they may participate indirectly by prompting the production of a variety of cytokines and chemokines. Natural killer (NK) cells are large, granular and short-lived cells of the innate immune system that represent approximately 10-15% of circulating lymphocytes and 5% of lymphocytes in lymphoid tissue. NK cells carry cytotoxic proteins placed in secretory lysosomes. When they recognize and connect to foreign or abnormal cells, they produce a lytic immunologic synapsis at the contact region. Finally, they emerge with plasma membrane whereby they secrete cytotoxic gradients into the target cell.[14]

NK cells have the ability to destroy tumor cells by two main cytotoxic pathways; the first one being perforin/granzyme-mediated secretory/necrotic killing and the second one being TNF family ligand-mediated apoptotic killing. The former mechanism is operative mainly against a few cultured leukemia cell targets, while the latter mediates substantial activity against most tumor cell targets. It also appears that the apoptotic mechanism is the main antitumor pathway.[15] IL-2 has been shown to be a potent stimulatory factor for NK cells. In addition, IL-7 and IL-12 have also been shown to possess direct and potent stimulatory effects on NK cells. Furthermore, endogenous production of TNF seems to play an important role in mediating effects induced by several stimulatory cytokines.[16] CD56bright NK cell subset, found in RA synovial membrane, has a great capacity to secrete a large amount of cytokines including TNF, a critical mediator in RA. IL-12, IL-15 and IL-18,

Th1 cytokines detected in RA, can in turn induce CD56bright NK cells to produce pro-inflammatory cytokines.[17] In these experiments, NK cells could stimulate monocytes to produce TNF in a cell contact-dependent manner and conversely, monocytes activated NK cells.[18]

Studies of NK cell function in various rheumatic diseases revealed reduced cytotoxicity in rheumatoid arthritis; in contrast normal NK function was found in patients with ankylosing spondylitis.[19-21] Recently, Park et al. reported a significantly reduced percentages and absolute numbers of NK cells, cytotoxicities, and lymphokine-activated killer (LAK) activity against K562 cells in the peripheral blood of systemic lupus erythematosus (SLE) and RA patients compared to healthy controls.[19] In the same study, these changes were not observed in patients with Behcet's Disease or AS.[19]

Since NK cytotoxicity depends partly on endogenous TNF cytokine and could easily be blocked either with anti-TNF antibodies or anti-TNF receptor antibodies, we hypothesized that in vivo NK cytotoxicity could be affected in patients who were being treated with anti-TNF medications. In our study, NK cytoxicity tended to decrease in patients with RA who were being treated with anti–TNF when compared to patients treated with other DMARDs. When we reanalyzed the results independent of clinical diagnosis, patients who used anti-TNF medications also had a tendency to demonstrate lower NK cytotoxicity when compared to patients who did not use anti-TNF. However, we were not able to detect any statistical significance between the groups probably due to small number of patients in the groups. Based on the supportive data from literature indicating NK cytotoxicity can be influenced by TNF cytokine, we believe that a larger study on more patients could have given more reliable data.

There are several investigations suggesting that anti-TNF treatments may increase the risk of tuberculosis.[22-28] Furthermore, post-marketing surveillance and observational studies have shown that there is a positive correlation between anti-TNF use and the risk of severe infections.[29-32] There are some conflicting results regarding lymphoma and solid malignancy development during anti-TNF use.Some studies have suggested an increased risk for lymphoma whereas others showed the opposite.[33-36] Particularly, the risk of solid tumor development is further increased in patients who concurrently use cyclophosphamide and anti-TNF agents.[37] Imai et al. has shown that patients with low NK activity have a higher risk of cancer development during a follow-up period of 11 years.[38] The interactions between proliferating tumor cells, human leukocyte antigen system and NK-receptors have also been postulated to be responsible for tumor survival and progression.[39] Therefore, it is reasonable to think that, NK activity changes may contribute to cancer development and progression in patients with rheumatic diseases, especially those treated with immunosuppressive drugs and anti-TNF.

Even though our study has some limitations, such as small number of patients in each group, and lack of assessing NK function in the same patients before and after anti-TNF treatment in order to show the relationship between anti-TNF treatment and NK cytotoxicity,we think that it is still important to see the effect of anti-TNF medications on NK cytotoxicity in limited number of patients. Our data suggest that further studies should be perfomed in this field. We believe that our data suggest anti-TNF related NK cell dysfunction, and it may help to explainincreased systemic infections and secondary malignancies, especially lymphoma, in patients who are treated with anti-TNF agents.

CONCLUSION

We suppose that NK cell function and cytotoxicity may decrease in RA patients using anti-TNF agents, and this may partly explain the reason why these patients are more prone to development of cancer and severe infections.

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