Turk Neurosurg 33(5):804-810, 2023

Original Investigation

DOI: 10.5137/1019-5149.JTN.42287-22.2

Received: 08.09.2022
Accepted: 30.11.2022

Published Online: 03.03.2023

Can TRIF/TICAM-1 Dependent Pathway be Target Pathway in Lumbar Intervertebral Disc Degeneration?

Orkhan ALIZADA[1], Sibel AKYOL[2], Fatma OZLEN[3], Mehmet Yigit AKGUN[4], Semih Can CETINTAS[3], Okan TURK[5], Murat HANCI[3]

[1]Baskent University School of Medicine, Department of Neurosurgery, Istanbul, Turkey
[2]Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty, Department of Physiology, Istanbul, Turkey
[3]Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty, Department of Neurosurgery, Istanbul, Turkey
[4]Koc University, Department of Neurosurgery, Istanbul, Turkey
[5]Istanbul Education and Research Hospital, Department of Neurosurgery, Istanbul, Turkey

Corresponding author: Orkhan ALIZADA ✉ alizadaorhan@gmail.com

ABSTRACT

AIM: To elucidate the role of the TIR-domain-containing adaptor-inducing interferon-β (TRIF) dependent pathway in intervertebral disc degeneration (IVD).

MATERIAL and METHODS: A total of adult male patients with low back pain (LBP) (+/- radicular pain) were further evaluated by magnetic resonance imaging (MRI) with surgical indication for microscopic lumbar disc herniation (LDH). Preoperatively, patients were classified according to Modic Changes (MC), nonsteroidal anti-inflammatory drugs (NSAIDs) use, and the presence of radicular pain in addition to the LBP.

RESULTS: The age of the 88 patients ranged from 19 to 75 years (mean: 47.3 ± 19.6 years). Twenty eight of the patients were evaluated as MC I (31.8%), 40 as MC II (45.4%), and 20 as MC III (22.7%). The majority of patients (81.8%) had radicular LBP, while 16 patients (18.1%) had only LBP. Predominantly, 55.6% of all patients were taking NSAIDs. Levels of all adaptor molecules were highest in the MC I group and lowest in the MC III group. The levels of IRF3, TICAM1, TICAM2, NF-kB p65, TRAF6, and TLR4 were significantly increased in the MC I group compared to the MC II and MC III groups. The variations of the individual adaptor molecules showed no statistically significant difference in the use of NSAIDs and radicular LBP.

CONCLUSION: As a result of the impact assessment, the current study clearly demonstrated for the first time that the TRIF-dependent signalling pathway plays a crucial role in the degeneration process in human lumbar intervertebral disc specimens.

KEYWORDS: Degeneration, Intervertebral disc, Modic change, TICAM-1, TRIF

ABBREVIATIONS: CD4: Cluster of differentiation 4, **ELISA:** Enzyme-linked immunosorbent Assay, **hsCRP:** High sensitivity C-reactive protein, **IRF:** Interferon regulatory factors, **IVD:** Intervertebral disc, **IL 1β:** Interleukin 1β, **LBP:** Lower back pain, **LPS:** Lipopolysaccharide, **LDH:** Lumbar disc herniation, **MRI:** Magnetic resonance imaging, **MAPK:** Mitogen-activated protein kinases, **MC:** Modic change, **MyD88:** Myeloid differentiation primary response 88, **NSAIDs:** Non-steroid anti-inflammatory drugs, **NF-κB:** Nuclear factor-kappa B, **NP:** Nucleus pulposus, **NO:** Nitric oxide, **PAMP:** Pathogen-associated molecular patterns, **RIP1:** Receptor-interacting serine/threonine-protein 1, **TIR:** Toll/IL-1 receptor, **TLR4:** Toll-like receptor, **TRIF:** TIR-domain-containing adaptor-inducing interferon-β, **TICAM1:** TIR domain-containing adaptor molecule 1, **TICAM2:** TIR domain-containing adaptor molecule 2, **TBK1:** TANK-binding kinase 1, **TRAF 6:** TNF Receptor Associated Factor 6, **TNFα:** Tumor necrosis factor α

Orkhan ALIZADA : 0000-0003-0942-9906
Sibel AKYOL : 0000-0002-7720-8833
Fatma OZLEN : 0000-0003-0925-8748
Mehmet Yigit AKGUN : 0000-0003-1342-7663
Semih Can CETINTAS : 0000-0001-6036-164X
Okan TURK : 0000-0002-9514-6891
Murat HANCI : 0000-0001-9261-6686

INTRODUCTION

According to the Global Burden of Disease study, chronic low back pain (LBP) is the leading cause of disability worldwide (10,27). Conservative treatments have so far only aimed at relieving symptoms rather than preventing or inhibiting intervertebral disc degeneration (IVD) (9). Toll-like receptor (TLR) agonists can generally activate two downstream signaling pathways: myeloid differentiation primary response 88 (MyD88)-dependent and MyD88-independent (in other words TIR-domain-containing adaptor-inducing interferon-β (TRIF)-dependent) signaling pathways (29). In contrast to the MyD88-dependent pathway, the TRIF-dependent pathway, which is specific to the TLR3 and TLR4 signaling pathways, activates interferon regulatory factor 3 (IRF-3) and leads to the production of interferon (IFN) beta (3,8).

This study aimed to investigate whether TLR4 expression existed in human IVD nucleus pulposus (NP) cells, and if so, whether its signaling was TRIF-dependent in other words human TIR domain-containing adaptor molecule 1 (TICAM-1)-dependent signaling pathway in a study on human intervertebral disc specimens. To our knowledge, this is the first human study to investigate whether the TRIF-dependent TLR4 signaling pathway could be a primary target for inactivating IVD degeneration. The TICAM-1/TRIF-dependent TLR4 signaling pathway, which is thought to be involved in degenerative disc disease, was investigated, and an attempt was made to associate with Modic change (MC).

MATERIAL and METHODS

This retrospective study (January 2018 to April 2020) was conducted at the Department of Neurosurgery and Physiology, Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine. Eighty-eight adult male patients who presented to our clinic with LBP and sciatica, were evaluated by magnetic resonance imaging (MRI) to detect degenerative changes. These patients were operated on in the presence of a surgical indication for microscopic lumbar disc herniation (LDH). The presence of neurological deficits and bladder, bowel, or erectile dysfunction was considered in the decision to operate. Patients admitted with LBP alone, only underwent surgery if the pain was unbearable despite medical treatment and physiotherapy. In all 88 patients, the procedures were performed through microsurgery by the same surgeon. Female patients were excluded from our study because of hormonal stress, which could cause possible differences in the immune response. In addition, patients with a history of previous LDH surgery, chronic systemic diseases, acute infectious process and endocrinological diseases were excluded. Also, patients who received preoperative steroid injections were excluded from the study.

Based on the MRI findings of the lumbar spine, patients were divided into three groups according to Modic Change (MC). The widespread use of nonsteroidal inflammatory drugs (NSAIDs) in the treatment of LBP worldwide has sparked scientific interest in their use to prevent/delay the degenerative process. We also wanted to know whether the presence of radicular pain was indicative of severe degeneration. Therefore, patients were also classified according to the use of NSAIDs and the presence of radicular LBP. The disc material was assessed from three different points of view: according to MC (I, II, and III), according to the use of NSAIDs (yes or no), and according to the radicular pain accompanying the LBP (yes or no).

The disc material was obtained intraoperatively from patients who had undergone LDH surgery. These materials were stored without delay in freezers at –80°C and transferred to nitrogen tanks at –196°C. Subsequently, the concentrations of the collected materials were analyzed using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions at Cytokines and Receptors Research Laboratory, Department of Physiology, Istanbul University-Cerrahpasa. The materials were removed from the freezers, slowly thawed, and centrifuged at 6,000 g for 2 min. Then, 350 µL of the supernatant was diluted 1:1 with a sample diluent. After the addition of 150 µL of the standard diluent, a serial dilution of 150 µL was performed from the standard tube. The sample was pipetted after each step to ensure even distribution. The 30-fold wash solution was diluted onefold with distilled water, and this solution was used for the washing steps. All materials were then subjected to the immunoassay protocol. The MultiskanTM GO microplate spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the optical density at 450 nm.

Ethical Statement

The study was approved by the Institutional Review Board of Istanbul University-Cerrahpasa (Approval no. 13/04/2017-142246). Before surgery, all patients gave written informed consent for participation in the study and publication. The privacy rights of the patients were respected at all times.

Statistical Analysis

As patients were divided into two cohorts according to the use of NSAIDs and the presence of radicular pain in addition to LBP, statistical analyzes were performed using the independent t-test in groups with normal distribution and homogeneous variance. Groups with non-homogeneous variance were compared using the Mann–Whitney U-test. Owing to the division into three groups according to the types of MC, the statistical analyzes were carried out with the one-way analysis of variance (ANOVA) test. Since ANOVA statistics do not provide reliable results at different variances, data were evaluated using the ROBUST test (Welch and Brown Forsythe). Post hoc tests were used to show which groups have differences. Tukey, HSD, Scheffe, and Bonferroni tests were used when variances were homogeneous, whereas Tamhane, Dunnett T3, Dunnett C, and Games-Howell tests when variances were not homogeneous. A difference was accepted as statistically significant if a two-sided p-value was <0.05 for the group comparison ($p<0.05$).

RESULTS

The patients were 88 men with a mean age of 47.3 ± 19.6 (range, 19–75) years at the onset of the first symptoms. The

duration of symptoms varied. If patients had a neurological deficit on admission, surgery was performed as soon as possible. In other cases, patients underwent surgery for persistent pain despite medical and physiotherapy treatment. The patients were divided into three groups based on MRI findings according to MC: 28 (31.8%) patients were classified as MC I, 40 (45.4%) as MC II, and 20 as MC III (22.7%).

Regardless of the MC classification, all patients underwent neurological examination, and two groups were formed according to their complaints: 16 (18.1%) patients had LBP alone, whereas 72 (81.8%) patients had radicular LBP. Patients were also classified according to preoperative NSAID use. Accordingly, 49 (55.6%) patients were taking NSAIDs, whereas 39 (44%) were not taking any medication.

The statistical relationship between the adaptor molecules induced by the TRIF-dependent pathway and the MC types is shown in (Figure 1). The difference between groups for CD4 in the homogeneous variance group was not statistically significant (p=0.092). The difference between MC I and MC III for IRF-3 was statistically significant (p<0.05). The difference between MC I and MC II, also MC I and MC III was statistically significant for TICAM2 (p<0.01). In the group whose variances are not homogeneous; for NF-kB p65, TLR4, TRAF6 statistically significant difference was observed among three groups. For NF-kB p65, a statistically significant difference (p<0.001) was observed between MC I and MC II, also MC I and MC III. There was also a statistically significant difference between MC I and MC III (p<0.05). Likewise for TRAF6, there was statistically significant difference (p<0.01) (p<0.001) respectivly between MC I and MC II, also MC I and MC III. As well as, the difference between MC II and MC III was statistically significant (p<0.001). The difference between MC I and MC II, also MC I and MC III for TLR4 was statistically significant (p<0.001). Also statistically significant difference was noticed between MC II and MC III (p<0.01). A statistically significant difference for TICAM1 was found between MC I and MC III (p<0.05). Finally, for TBK1, there was statistically no significance among three groups. Detailed p values for other adapter molecules are shown one by one in (Figure 1).

When patients were evaluated according to their NSAID use, the variances for each adaptor molecule were evaluated, and none of them showed a statistically significant difference (p>0.05) (Figure 2). When the patients were evaluated after their neurological examination, two groups were also formed (LBP alone and radicular LBP), and no statistically significant difference was found between these groups for any of the adaptor molecules (p>0.05) (Figure 3).

DISCUSSION

Although LBP is a worldwide problem, still no definitive treatment method has been established to prevent lumbar

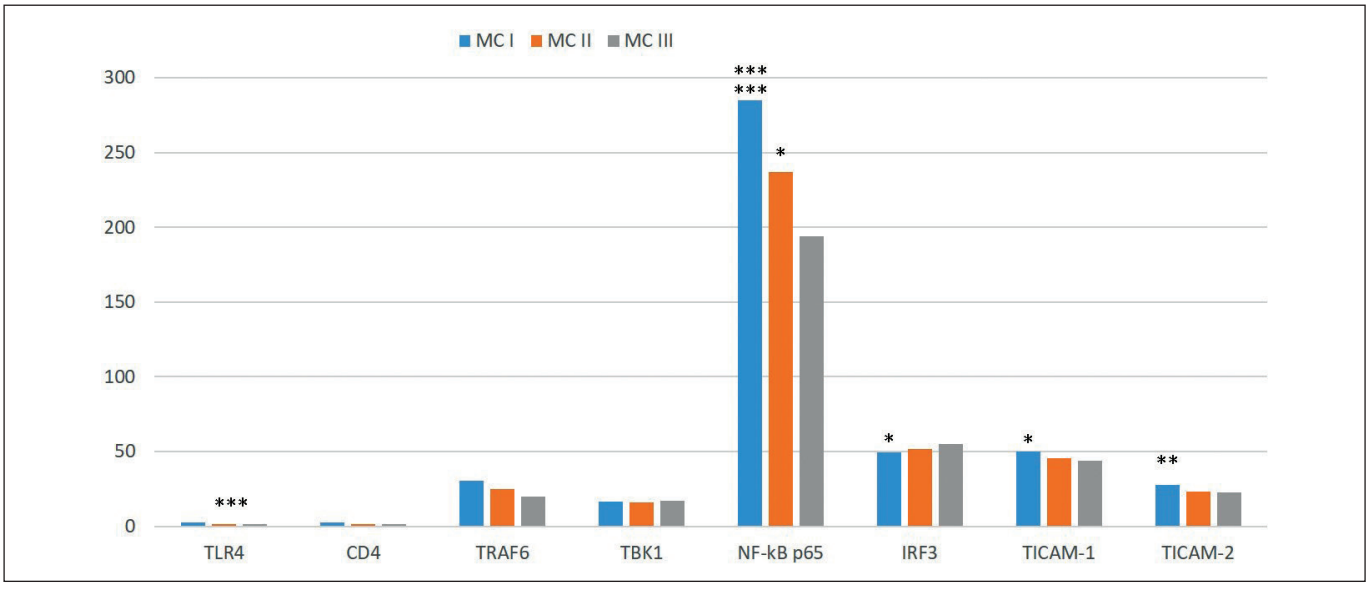

Figure 1: Statistical analysis of adapter molecules in TRIF depenent pathway according to Modic change types. **TLR4:** MCI was statistically different from both MCII and MCIII at p<0.001(***) significance level. Also MCII was statistically different from MCIII at p<0.001(***) significance level. **CD4:** No statistically significant difference was found between groups (p>0.05), while the values gradually decreased from MCI to MCIII. **TRAF6:** MCI was statistically different from both MCII and MCIII at p<0.01(**), p<0.001(***) significance level respectively. **TBK1:** There was no statistically significant difference between the groups (p>0.05). **NF-kB p65:** MC I was different from both MC II and MC III which was statistically significant p<0.001(***). Also MCII was statistically different from MCIII at p<0.05(*) significance level. **IRF-3:** MCI was statistically different from MCIII at p<0.05(*) significance level. No statistically significant difference was found between MCI and MCII, as well as MCII and MCIII (p>0.05). **TICAM-1:** Statistically significant difference was found between MC I and MC III (p<0.05). No statistically significant difference was found between MCI and MCII, as well as MCII and MCIII (p>0.05). **TICAM-2:** MCI was statistically different from both MCII and MCIII at p<0.01(**) significance level. No statistically significant difference was found between MCII and MCIII (p>0.05).

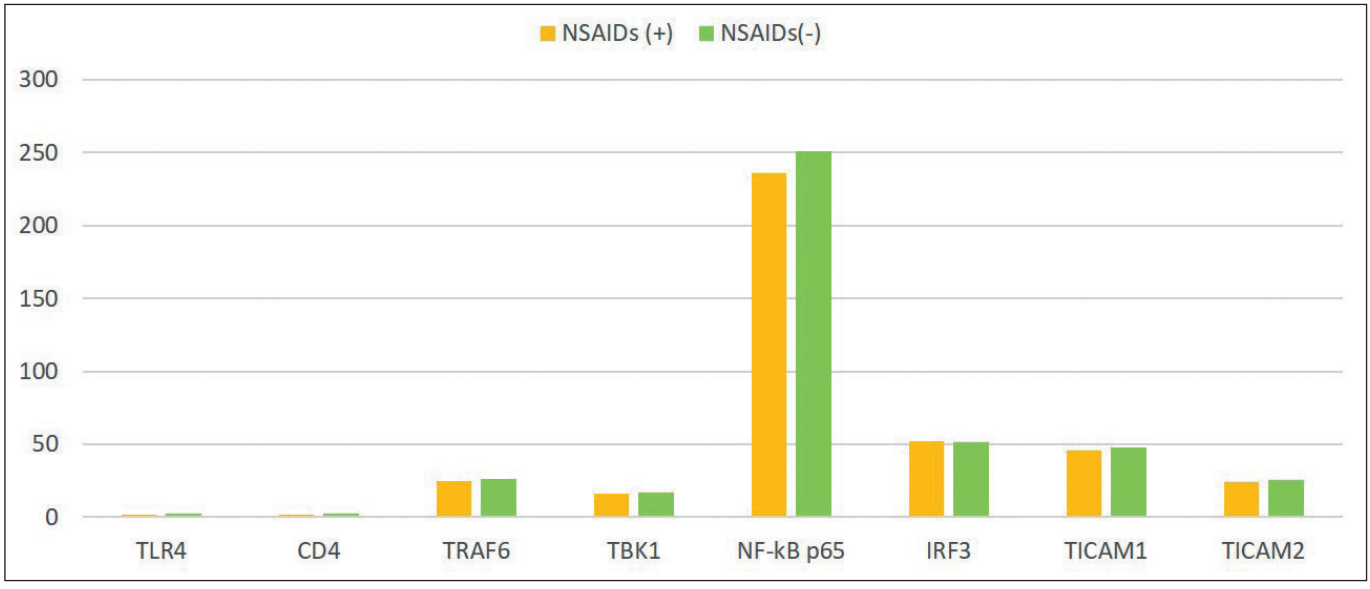

Figure 2: When the patients were evaluated according to whether they used NSAIDs or not, variances for each adapter molecules which take a part in TRIF dependent pathway were evaluated and statistically significant difference was not found between the groups (p>0.05).

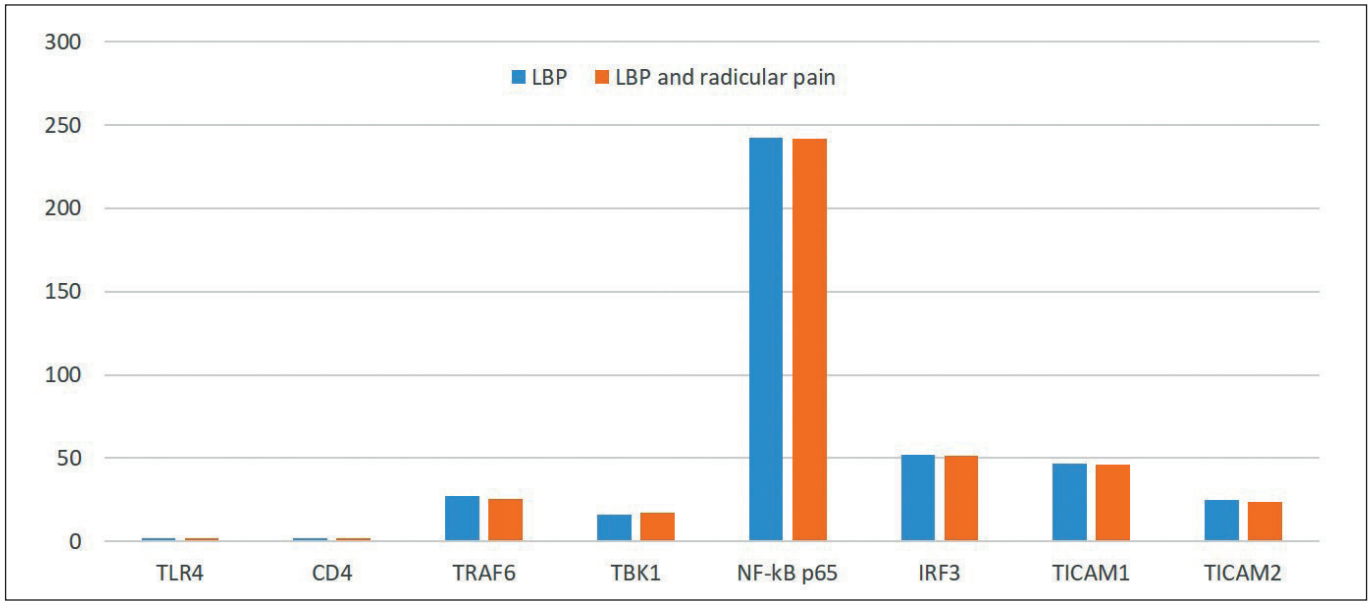

Figure 3: When the patients were evaluated in terms of their neurological examination (back pain and accompanying radicular pain), statistically significant difference was not found between the groups (p>0.05).

degeneration. Thus far, in vitro studies have confirmed the expression of the *TLR4* gene in IVD NP cells. According to these studies, TLR4 plays a significant role in the molecular mechanism of IVD degeneration (20,21). TLR4 can affect signaling in two ways via both the MyD88-dependent and TRIF-dependent pathways, whereas TLR3 can only act through TRIF (1). Qin et al. demonstrated the MyD88-dependent nature of the signaling transduction pathway that activates the TLR4 in NP cells in IVD (20). However, whether the TRIF-dependent pathway induces TLR4 activation in NP cells in IVD remains unknown.

Studies to date have confirmed that the *TLR4* gene is expressed in IVD NP cells and plays an important role in the molecular mechanism of IVD (21). Recent results regarding the activity of the TRIF-dependent TLR4 signaling pathway have not been reported. In the present study, the effect of TLR4 on degeneration in the TRIF-dependent pathway was investigated in materials obtained from human degenerated disc tissues.

Modic et al. reported that MC I generally progressed to MC II over time, but it can also return to normal (15). The results

of a cohort study of 40-year-old Danish men and women showed that MC I was more common than MC II, whereas a Finnish cohort study by Kuisma et al. who analyzed male train engineers and a control group of local people confirmed the predominance of MC II changes (12,14). Similarly, we found a 45.4% MC II predominance in our study group of 88 male patients.

Although most of the previous studies have found a closer association between LBP and MC I (2,14,16,17,24), others have not found an association between MC and LBP (5,13). Although the pathobiology behind MC-related pain is unknown, it may occur following an increase in the protein gene product-9.5 (PGP-9.5) nerve fiber count and the appearance of tumor necrosis factor-α-positive cells in damaged vertebral endplates adjacent to MC sites (6,17). Dudli et al. found no significant difference in pain between MC I and MC II (4). The correlation between MC and accompanying LPB, which has been shown in various publications, was not found in our study. When the protein levels in the TRIF-dependent pathway were compared with the patient's admission clinic (LBP alone or accompanying radicular pain), no statistically significant difference was found ($p>0.05$). These results clarify that radicular pain develops secondary to the pressure of the disc herniation to the root and the resulting edema rather than degeneration.

In the present study, when patients were evaluated according to NSAID use, no statistically significant difference was found between the drugs and protein levels in the TLR4/TRIF-dependent pathway ($p>0.05$). Although NSAIDs is widely used for patients with LBP, our results clearly showed that they only alleviate symptoms and cannot prevent degeneration.

As a result of the examination of the disc materials by the ELISA method, which were taken from patients classified in three groups according to Modic degeneration, data obtained for TLR4 were statistically significantly higher in the stage with the highest inflammation, i.e., MC I, than in MC II and III stages ($p<0.001$).

Considering the importance of TLR4 in the inflammatory process, our results showed that inflammation peaks in MC I. Assuming that MC I represent an acute inflammatory stage, the finding that TLR4 values are the highest in MC I (different from both MC II and MC III at the statistical significance level of $p<0.001$) proves the undeniable contribution of TLR4 in IVD. Likewise, the finding that TLR4 values in MC II were different from those in MC III (at $p<0.001$ significance level) supports the hypothesis that the inflammatory process continues in some cases with MC II.

Although the MyD88- and TRIF-dependent pathways use different adapter molecules, both pathways involve activation and nuclear translocation of NF-kB, leading to the expression of multiple proinflammatory cytokines (26). Interestingly, Dudli et al. found that NF-kB, a proinflammatory mediator, was downregulated in MC II (4). In our study, the proinflammatory mediator NF-kB p65 was increased ($p<0.001$) in MC I compared with the other two groups. This suggests that inflammation is significantly severe in MC I. The finding that the values of this adapter molecule are the lowest in MC III, that is, in the sclerosis stage, indicates that the inflammation has ended in this group. The variable results of studies suggest that there may be pathobiological subphenotypes in every MC type that cannot be evaluated using standard T1- and T2-weighted MRI. These results suggest that the TRIF-dependent TLR4 signaling pathway is a target pathway in IVD.

TICAM-1 induces the activations of NF-kB and IRF-3. TICAM-1 triggers the robust activation of the IFN-β, whereas the activation via MyD88 is negligible. Mutations of TRAF6-binding motifs in TICAM-1 N+ TIR give rise to complete abrogation induction of IFN-β. These mutations also reduce the activation of transcription factors that are critical for IFN-β expression, primarily including IRF-3 and NF-kB (22). TRAF6 is indispensable in LPS-mediated IP-10 induction and NF-kB activation (11). In the TRIF-dependent pathway, TRAF6 uptakes the receptor-interacting serine/threonine protein 1 molecule that interacts with TAK1, causing the release of NF-kB and inflammatory cytokines. TRAF6 mediates NF-kB activation via TLR4 by interacting with TICAM-1. Recently, Sato et al. reported that TRAF6 binds to TICAM-1 and plays an important role in NF-kB activation in the TRIF-dependent pathway (23). Sasai et al. also showed that the polyubiquitination of TICAM-1 by TRAF-6 is essential for the TRIF-dependent pathway, and through TRAF6, the induction of IRF-3 and NF-kB activation by TICAM-1 is possible (22). Contrary to other studies, we revealed statistically significant differences between MC types in terms of TRAF6 (4). Our results indicated a significant positive correlation between MC I and TRAF6. In MC I, TRAF6 was higher than that in MC III ($p<0.001$) and more than that in MC II ($p<0.01$). Considering that TRAF6 induces the release of inflammatory cytokines, as shown in the literature, inflammation is most common at MC I, and inflammation is least observed at MC III. On the contrary, IRF3 is one of the major efectors of TLR4/TICAM1-dependent signaling pathway. Recent studies have shown that IRF3 activation upregulates the expression of IFNs and several inflammatory genes (18,28). Likewise, IRF-3 was statistically significant in MC I compared with MC III ($p<0.05$). Thus, we suggest that the present study has revealed the incontrovertible role of TRAF6 and IRF3 in the degeneration process through the TRIF-dependent pathway.

TICAM-1 strongly bound TLR3; however, a direct association was not recognized between TICAM-1 and TLR4. Oshiumi et al. found and named this new adapter molecule TIR-containing adapter molecule-2 (TICAM-2), in other words TRAM, which physically connects TLR4 and TICAM-1 and functionally transmits the LPS-TLR4 signal to TICAM-1, eventually activating IRF-3. This study reported that TLR4 could only interact with TICAM-1 in the presence of TICAM-2 (19). Significantly increased TICAM-1-mediated IFN-β activation was observed in the existence of a perpetual volume of TICAM-2 expressions. The presence of TICAM-2 also brought about IRF-3 phosphorylation induced by TICAM-1 transfection (19). Although TICAM-1-dependent NF-kB activation is weak compared with that by MyD88, both MyD88 and TRIF mediate NF-kB and IRF-3 activations (25). In our study, the values of TICAM-1 were the highest in MC I, and statistically significant

differences were found between MC I and MC III. These results corroborate our hypothesis and clearly show that when activated, the TICAM-1-dependent signaling pathway led to the release of inflammatory factors that participated in IVD.

Although recent studies have confirmed TICAM-2 as a bridging adapter, just as Mal/TIRAP, functions other than bridging should be further explored (7,19). The ability of TICAM-2 to activate NK-kB and IFN-β promoters is very limited. Although recent data revealed just the bridging ability of TICAM-2, our study demonstrated that TICAM-2 can do much more. In our study, levels of TICAM-2 expression were statistically significantly different between MC I and MC II–III. Accordingly, TICAM-2 not only acts as a bridge between TLR-4 and TICAM-1 but also contributes significantly to the inflammation process as much as TICAM-1. The major limitation of the current study was that this pathway was evaluated only in the small subgroup of patients with IVD who underwent discectomy.

CONCLUSION

As a result of our impact assessment, this study can provide direct evidence for the hypothesis that the TRIF-dependent TLR4 signaling pathway plays an important role in IVD. Treatments that prevent the activation of adaptor molecules that have been shown to contribute to degeneration along this pathway could represent a breakthrough in the treatment of IVD. If the activation of these adaptor molecules can be prevented with new pharmacological agents, we can at least slow down the process and treat intractable LBP. Consequently, this study may provide a theoretical basis for exploring molecular mechanisms that will lead to the identification of new therapeutic targets and approaches.

ACKNOWLEDGEMENTS

This study was funded by Scientific Research Projects Coordination Unit of Istanbul University Cerrahpasa; Project number: 25405.

AUTHORSHIP CONTRIBUTION

Study conception and design: OA, MH, FO
Data collection: OA, SA, YA, SC
Analysis and interpretation of results: OA, SA, YA, SC
Draft manuscript preparation: MH, OT, FO
All authors (OA, SA, FO, MYA, SCC, OT, MH) reviewed the results and approved the final version of the manuscript.

REFERENCES

1. Akira S, Takeda K, Kaisho T: Toll-like receptors: Critical proteins linking innate and acquired immunity. Nature Immunol 2:675-80, 2001
2. Braithwaite I, White J, Saifuddin A, Renton P, Taylor BA: Vertebral end-plate (Modic) changes on lumbar spine MRI: Correlation with pain reproduction at lumbar discography. Eur Spine J 7:363-368, 1988
3. Doyle S, Vaidya S, O'Connell R, Dadgostar H, Dempsey P, Wu T, Rao G, Sun R, Haberland M, Modlin R, Cheng G: IRF3 mediates a TLR3/TLR4-specific antiviral gene program. Immunity 17:251-263, 2002
4. Dudli S, Sing DC, Hu SS, Berven SH, Burch S, Deviren V, Cheng I, Tay BKB, Alamin TF, Ith MAM, Pietras EM, Lotz JC: ISSLS Prize in Basic Science 2017: Intervertebral disc/bone marrow cross-talk with Modic changes. Eur Spine J 26:1362-1373, 2017
5. el Barzouhi A, Vleggeert-Lankamp CLAM, van der Kallen BF, Lycklama a Nijeholt GJ, van den Hout WB, Koes BW, Peul WC; Leiden–The Hague Spine Intervention Prognostic Study Group: Back pain's association with vertebral end-plate signal changes in sciatica. Spine J 14:225-233, 2014
6. Fields AJ, Liebenberg EC, Lotz JC: Innervation of pathologies in the lumbar vertebral end plate and intervertebral disc. Spine J 14:513-521, 2014
7. Funami K, Matsumoto M, Oshiumi H, Inagaki F, Seya T: Functional interfaces between TICAM-2/TRAM and TICAM-1/TRIF in TLR4 signaling. Biochem Soc Trans 45:929-935, 2017
8. Hoshino K, Kaisho T, Iwabe T, Takeuchi O, Akira S: Differential involvement of IFN-beta in Toll-like receptor-stimulated dendritic cell activation. Int Immunol 14:1225-1231, 2002
9. Ireland D: Molecular mechanisms involved in intervertebral disc degeneration and potential new treatment strategies. Biosci Horiz 2:83-89, 2009
10. Kassebaum NJ, Arora M, Barber RM, Bhutta ZA, Brown J, Carter A et al. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990-2015: A systematic analysis for the Global Burden of Disease Study 2015. Lancet 388:1603-1658, 2016
11. Kawai T, Takeuchi O, Fujita T, J Inoue, Mühlradt PF, Sato S, Hoshino K, Akira S: Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. J Immunol 167:5887-5894, 2001
12. Kjaer P, Leboeuf-Yde C, Korsholm L, Sorensen JS, Bendix T: Magnetic resonance imaging and low back pain in adults: A diagnostic imaging study of 40-year-old men and women. Spine 30:1173-1180, 2005
13. Kovacs FM, Arana E, Royuela A, Estremera A, Amengual G, Asenjo B, Sarasíbar H, Galarraga I, Alonso A, Casillas C, Muriel A, Martínez C, Abraira V; Spanish Back Pain Research Network: Spanish Back Pain Research Network. Vertebral endplate changes are not associated with chronic low back pain among Southern European subjects: A case control study. AJNR Am J Neuroradiol 33(8):1519-1524, 2012
14. Kuisma M, Karppinen J, Niinimäki J, Ojala R, Haapea M, Heliövaara M, Korpelainen R, Taimela S, Natri A, Tervonen O: Modic changes in endplates of lumbar vertebral bodies: Prevalence and association with low back and sciatic pain among middle-aged male workers. Spine (Phila Pa 1976) 32:1116-1122, 2007
15. Modic MT, Masaryk TJ, Ross JS, Carter JR: Imaging of degenerative disk disease. Radiology 168:177-186, 1988

16. Nguyen C, Bendeddouche I, Sanchez K, Jousse M, Papelard A, Feydy A, Revel M, Poiraudeau S, Rannou F: Assessment of ankylosing spondylitis criteria in patients with chronic low back pain and vertebral endplate Modic I signal changes. J Rheumatol 37:2334-2339, 2010
17. Ohtori S, Inoue G, Ito T, Koshi T, Ozawa T, Doya H, Saito T, Moriya H, Takahashi K: Tumor necrosis factor-immunoreactive cells and PGP 9.5-immunoreactive nerve fibers in vertebral endplates of patients with discogenic low back Pain and Modic Type 1 or Type 2 changes on MRI. Spine 31:1026-1031, 2006
18. Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T: TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. Nat Immunol 4:161-167, 2003
19. Oshiumi H, Sasai M, Shida K, Fujita T, Matsumoto M, Seya T: TIR-containing adapter molecule (TICAM)-2, a bridging adapter recruiting to toll-like receptor 4 TICAM-1 that induces interferon-beta. J Biol Chem 278:49751-49762, 2003
20. Qin C, Zhang B, Zhang L, Zhang Z, Wang L, Tang L, Li S, Yang Y, Yang F, Zhang P, Yang B: MyD88-dependent Toll-like receptor 4 signal pathway in intervertebral disc degeneration. Exp Ther Med 12:611-618, 2016
21. Rajan NE, Bloom O, Maidhof R, Stetson N, Sherry B, Levine M, Chahine NO: Toll-Like Receptor 4 (TLR4) expression and stimulation in a model of intervertebral disc inflammation and degeneration. Spine 38:1343-1351, 2013
22. Sasai M, Tatematsu M, Oshiumi H, Funami K, Matsumoto M, Hatakeyama S, Seya T: Direct binding of TRAF2 and TRAF6 to TICAM-1/TRIF adaptor participates in activation of the Toll-like receptor 3/4 pathway. Mol Immunol 47:1283-1291, 2010
23. Sato S, Sugiyama M, Yamamoto M, Watanabe Y, Kawai T, Takeda K, Akira S: Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF) associates with TNF receptor-associated factor 6 and TANK-binding kinase 1, and activates two distinct transcription factors, NF-kappa B and IFN-regulatory factor-3, in the Toll-like receptor signaling. J Immunol 171:4304-4310, 2003
24. Schistad EI, Espeland A, Rygh LJ, Roe C, Gjerstad J: The association between Modic changes and pain during 1-year follow-up in patients with lumbar radicular pain. Skeletal Radiol 43(9):1271-1279, 2014
25. Seya T, Oshiumi H, Sasai M, Akazawa T, Matsumoto M: TICAM-1 and TICAM-2: Toll-like receptor adapters that participate in induction of type 1 interferons. Int J Biochem Cell Biol 37:524-529, 2005
26. Shamji MF, Setton LA, Jarvis W, So S, Chen J, Jing L, Bullock R, Isaacs RE, Brown C, Richardson WJ: Proinflammatory cytokine expression profile in degenerated and herniated human intervertebral disc tissues. Arthritis Rheum 62:1974-1982, 2010
27. Vos T, Allen C, Arora M, Barber RM, Bhutta ZA, Brown A. et al. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: A systematic analysis for the Global Burden of Disease Study 2015. Lancet 388(10053):1545-1602, 2016
28. Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Hideki Sanjo, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S: Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science 301:640-643, 2003
29. Youn HS, Lee JY, Fitzgerald KA, Young HA, Akira S, Hwang DH: Specific inhibition of MyD88-independent signaling pathways of TLR3 and TLR4 by resveratrol: Molecular targets are TBK1 and RIP1 in TRIF complex. J Immunol 175:3339-3346, 2005