Immunobiology 231 (2026) 153176



Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio





Increased Granzyme K$^+$ CD8$^+$ T cells and senescent subset in Behçet's disease

Heera Lee [a,1], Soo-Jin Lee [b,2], Young Joon Park [b,3], Han Seul Kim [b,4], Eun-So Lee [b,*,5], Sun Park [c,*,6]

[a] Department of Dermatology, Ewha Womans University College of Medicine, Seoul, Republic of Korea
[b] Department of Dermatology, Ajou University School of Medicine, Suwon, Republic of Korea
[c] Department of Microbiology, Ajou University School of Medicine, Suwon, Republic of Korea

ARTICLE INFO

Editor: Prof. R Chakravarti

Keywords:
Behçet's disease
Granzyme K
Immunosenescence
CD8$^+$ T cells
Tumor necrosis factor-alpha (TNF-α)
Interferon-gamma (IFN-γ)

ABSTRACT

Objectives: Behçet's disease (BD) is a chronic inflammatory condition with immune system dysregulation. Recent evidence suggests proinflammatory roles of extracellular granzyme K (GzmK), particularly in aged immune cells. This study investigated GzmK-expressing CD8$^+$ T cells and their senescent subset in BD pathology.
Methods: We analysed lymphocyte subsets in blood samples from 19 active BD (aBD), 19 inactive BD (iaBD), and 19 healthy controls (HC) using flow cytometry and granzymes, as well as IL-6 using enzyme-linked immunosorbent assay.
Results: Active BD patients showed significantly higher serum GzmK levels and GzmK$^+$CD8$^+$ T cells with advanced differentiation markers. GzmK$^+$CD8$^+$ T cells positively correlated with TNF-α$^+$ and IFN-γ$^+$ lymphocytes. The senescent subset (GzmK$^+$CD27$^-$CD28$^-$CD57$^+$CD8$^+$) specifically correlated with TNF-α$^+$ lymphocytes, indicating involvement in TNF-α-centered inflammatory pathways.
Conclusion: GzmK$^+$CD8$^+$ T cells and their senescent counterparts are not merely biomarkers of disease activity but may function as extracellular proinflammatory effectors that amplify the TNF-α-mediated inflammatory loop in BD. Targeting the GzmK-senescence axis represents a promising therapeutic strategy for managing chronic inflammation in BD.

1. Introduction

Behçet's disease (BD) is a rare, chronic, and multisystem inflammatory disorder with a diverse clinical presentation that includes recurrent mucocutaneous ulceration and systemic involvements (Emmi et al., 2024). Current evidence suggests that BD is an autoimmune condition influenced by a complex interplay of genetic predisposition; environmental factors; and immune system dysregulation. Inflammation and excess immune response triggered by bacteria; viruses; and various environmental factors in susceptible individuals are implicated in BD pathogenesis (Zierhut et al., 2003). Furthermore; both innate and adaptive immune reactions are known to contribute to BD pathogenesis. Among these multifactorial etiopathogenesis; immunosenescence is thought to play one of its roles. Yang et al. have reported increased senescent CD8$^+$ T cells in active BD patients (Yang et al., 2018). In BD, this immunosenescence contributes to pathogenesis by promoting dysregulated, autoimmune-like responses. Aging CD8$^+$ T cells lose regulatory function and instead secrete excessive pro-inflammatory cytokines (e.g., tumor necrosis factor alpha (TNF-α), interleukin (IL)-6), so-called senescence-associated secretory proteins (SASP), leading to tissue damage and chronic inflammation. This process reduces the immune response to new antigens while intensifying abnormal reactions to self-

* Corresponding authors at: Postal address: 164, Worldcup-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16499, Republic of Korea.
E-mail addresses: esl@ajou.ac.kr (E.-S. Lee), sinsun@ajou.ac.kr (S. Park).
[1] https://orcid.org/0000-0002-5448-2274.
[2] https://orcid.org/0000-0002-9340-6290.
[3] https://orcid.org/0000-0003-0723-4136.
[4] https://orcid.org/0000-0001-9558-4990.
[5] https://orcid.org/0000-0003-0232-7704.
[6] https://orcid.org/0000-0003-3254-4064.

https://doi.org/10.1016/j.imbio.2026.153176
Received 7 September 2025; Received in revised form 6 March 2026; Accepted 27 March 2026
Available online 29 March 2026
0171-2985/© 2026 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

antigens, leading to inflammation.

Granzymes (Gzm) are a family of serine proteases primarily produced by cytotoxic T cells and natural killer (NK) cells, playing a crucial role against intracellular pathogens and in the induction of apoptosis in target cells (Hay and Slansky, 2022). Five types of human Gzms have been explicated so far; GzmA; B; K; M; and H. Among them; GzmB had been extensively studied in their role of target cell death. However; recent findings are expanding the understanding of GzmK beyond its intracellular cytotoxic role; demonstrating novel extracellular functions; such as endothelial activation and modulation of proinflammatory immune cytokine responses; as an important player in chronic inflammation and autoimmune diseases (Bouwman et al., 2021; Sharma et al., 2016; Wensink et al., 2014). Especially, GzmK-expressing $CD8^+$ T cells were shown to accumulate in inflamed tissues (Jonsson et al., 2022); and are associated with the secretion of pro-inflammatory cytokines; contributing to the chronic inflammatory state observed in diseases such as rheumatoid arthritis (RA) (Zheng et al., 2023). There is also growing evidence that $GzmK^+$ senescent $CD8^+$ T cells increase during normal aging process; both in human and mouse models (Mogilenko et al., 2021).

Therefore, in this study, we aimed to investigate the role of GzmK-expressing $CD8^+$ T cells and their senescent subset in the pathology of BD, as well as their association with inflammatory cytokines.

2. Methods

2.1. Subjects

Blood samples were collected from 19 patients with active BD (aBD), 19 with inactive BD (iaBD), and 19 age-matched healthy controls (HC) (Table 1). Patients who visited for the first time or were followed up at the Department of Dermatology, Ajou University Hospital, were enrolled. All patients with BD satisfied both the International Study Group criteria and Japanese criteria. The HC group consisted of age-matched individuals with no history of systemic inflammatory, autoimmune, or infectious diseases at the time of enrolment. The active BD group was defined as patients exhibiting at least one clinical symptom (e.g., oral ulcers, genital ulcers, inflammatory skin lesions, or ocular involvement) within the preceding 4 weeks, despite the continuation of maintenance therapy (Emmi et al., 2024). In contrast, the inactive group consisted of patients who had remained completely asymptomatic for a minimum of 3 months while on a stable treatment regimen. For three BD patients, blood samples were collected once during the active state of the disease and again after they improved to an inactive state to observe the changes. All informed consent was obtained prior to the study. This study was approved by the Institutional Review Board (IRB No: AJOUIRB-GEN-2022-181).

Table 1
Demographic features and major clinical findings of the enrolled patients and healthy controls.

	Active BD (n = 19)	Inactive BD (n = 19)	Healthy control (n = 19)	*P*-value
Gender, N				0.715
Men	7	5	5	
Women	12	14	14	
Age distribution, N				0.756
20–39	5	5	6	
40–59	11	13	12	
≥60	3	1	1	
Mean age, years (SD, range)	46.53 (13.43, 21–66)	46.74 (9.28, 29–61)	42 (7.18, 26–53)	0.285
Symptoms at enrollment				
Oral ulcer, N	19	NS	NS	NS
Genital ulcer, N	4	NS	NS	NS
Skin lesions, N	13	NS	NS	NS
Ocular lesion, N	2	NS	NS	NS
Arthritis, N	3	NS	NS	NS
Gastrointestinal, N	0	NS	NS	NS
Cardiovascular, N	0	NS	NS	NS
Central nervous system, N	0	NS	NS	NS
Years from diagnosis, years, mean (SD)	8.84 (5.58)	9.84 (5.51)	NS	0.582
Systemic steroid treatment at enrollment, N	7	5	NS	0.499
Biologics treatment at enrollment, N	0	3	NS	0.230
ESR, mm/h, mean (SD)	**31.53 (22.4)**	**17.8 (16.35)**	**NS**	**0.035**
CRP, mg/dL, mean (SD)	0.25 (0.34)	0.22 (0.44)	NS	0.837

BD, Behçet's disease, SD, standard deviation; ESR, Erythrocyte sedimentation rate, CRP, C-reactive protein.

2.2. Enzyme-linked immunosorbent assay (ELISA) for GzmA, GzmB, GzmK, and IL-6

Each sample's serum level was measured using an ELISA kit for GzmA (Abcam, Cambridge, UK), GzmB (Invitrogen, MA, USA), GzmK (Novus, CO, USA), and IL-6 (R&D system, MN, USA) according to the manufacturer's protocols.

2.3. Isolation and in vitro culture of peripheral blood mononuclear cells (PBMC)

PBMCs were isolated by Ficoll-Paque density gradient centrifugation (Ficoll Paque TM plus; StemCEll Technologies, Vancouver, BC, Canada) and suspended in RPMI 1640 (Hyclone; Thermo Scientific, Logan, UT, USA) containing 25 mg/mL gentamycin and 2% human serum. For the stimulation assay, two conditions were made: 1) cells activated with 1 μg/mL anti-CD3 antibody and 2.5 μg/mL anti-CD28 antibody for 1 day and 2) cells activated with 1 μg/mL anti-CD3 antibody and 2.5 μg/mL anti-CD28 antibody for 3 days at 37 °C. Also, before TNF-α and interferon gamma (IFN-γ) intracellular staining, cells were activated with 50 ng/mL phorbol myristate acetate (PMA)(Sigma Aldrich, MO, USA), 1 μg/mL ionomycin (Sigma Aldrich, MO, USA), and after 2 μM GolgiStop (BD Biosciences, NJ, USA) for 5 h at 37 °C. We then stained cells with surface markers prior to fixation and permeabilization using the Cytofix/Cytoperm kit (BD Biosciences, NJ, USA) according to the manufacturer's protocol.

2.4. Flow cytometry

We performed flow cytometry twice for different intracellular cytokine stains. First, for the granzyme intracellular cytokine stain, the surface marker PBMCs were labeled with conjugated monoclonal antibodies as follows: FITC-conjugated Fixable Viability Stain 520 (FV520), BV510-anti-CD4, BV650-anti-CD8, APC-H7-anti-CD27, BV421-anti-CD28, and PerCP-Cy5.5-anti-CD57. Additionally, for the intracellular staining, conjugated monoclonal antibodies of PE-Cy7-anti-GzmA, PE-Cy7-anti-GzmB, and PE-anti-GzmK were used. For TNF-α and IFN-γ intracellular staining, the surface marker PBMCs were labeled with conjugated monoclonal antibodies as follows; FITC-FV520, BV510-anti-CD4, BV650-anti-CD8, PE-Cy7-anti-CD27, BV421-anti-CD28, and PerCP-Cy5.5-anti-CD57. Conjugated monoclonal antibodies of PE-anti-TNF-α and APC-anti-IFN-γ were used for the intracellular staining. The gating strategy is demonstrated in Supplementary Fig. 1. All antibodies were titrated to optimal concentrations according to the manufacturer's protocol. All stained samples were analysed using an LSR Fortessa cell analyzer (BD Biosciences, NJ, USA). Because of the number of laser color limitations, we separately stained each sample replicated in three bottles when performing granzyme intracellular cytokine staining. All flow cytometry data were analysed using FlowJo software (Tree Star, OR,

USA).

2.5. Statistical analysis

In the demographic characteristics presented in Table 1, Pearson's chi-square test and Fisher's exact test were applied for categorical data, while an independent samples *t*-test was conducted to examine differences in the continuous variables. Next, to compare Gzms and IL-6 serum levels measured by ELISA, the mean fluorescent intensity (MFI) of Gzm in $CD8^+$ T cells, in $CD4^+$ T cells, and in senescent $CD8^+$ T cells across the active BD group, HC group, and inactive BD groups, a one-way analysis of variance (ANOVA) was used if data transformation achieved a normal distribution; otherwise, the Kruskal-Wallis test was employed. When a significant difference was observed, Tukey's honestly significant difference (HSD) test was applied as a post-hoc analysis to determine specific differences between each pair of groups. Pearson correlation analysis was conducted to examine the associations between $GzmK^+$ $CD8^+$ T cells or $GzmK^+$ senescent $CD8^+$ T cells and TNF-α^+ or IFN-γ^+ lymphocytes. All analyses were performed using SPSS (version 22.0, IBM, Armonk, NY, USA). For all analyses, *p*-values of less than 0.05 with a 95% confidence interval were accepted as significant. GraphPad Prism (version 10.0.0 for Windows, GraphPad Software, MA, USA) was used for visualization.

3. Results

3.1. Demographics and clinical characteristics

19 patients with active BD, 19 with inactive BD, and 19 HC were enrolled in this study. The baseline clinical characteristics of patients with BD are summarized in Table 1. There was no significant difference between the three groups in terms of sex and age. In the active group, 19 patients showed mucocutaneous symptoms, with ocular and arthritic symptoms being the most and least common, respectively. In the inactive BD group, 19 patients had their symptoms under control by maintenance therapy, such as colchicine, mycophenolate mofetil, sulfasalazine, adalimumab, and systemic steroid if needed. More patients in the active BD group received systemic steroid treatment at enrolment. On the other hand, only three patients in the inactive BD group received biologics, all adalimumab, at enrolment. A significantly higher ESR level was noted in the active BD group than in the inactive BD group.

3.2. Increased GzmK serum level in patients with active BD

In Fig. 1, only the GzmK level was significantly higher in the active BD group than in the HC and inactive BD group, as measured with ELISA using serum samples. The GzmA levels revealed no meaningful differences between the three groups. GzmB level was higher in patients with BD than in HC; however, there was no statistical significance when comparing the active group to the HC or inactive BD group. IL-6, a known severity marker in BD, was significantly elevated in the active BD group compared to the HC group, distinguishing active BD from inactive BD. Further, patients with uveitis showed especially high IL-6 levels. The consistency of IL-6 levels and trends with previous findings further supports our classification of active and inactive BD, consistent with existing reports.

3.3. Identification of major secreting cells for each Gzm

As shown in Fig. 2, the expression levels and cellular sources of GzmA, GzmB, and GzmK were analysed in $CD4^+$ T cells, $CD8^+$ T cells, and other cell types across the aBD, iaBD, and HC groups. Total GzmA and GzmB expression levels did not show significant differences among the groups. However, $CD8^+$ T cells and other cell types were the main source of GzmA and $CD4^+$ T cells and others were identified as the primary source of GzmB. In contrast, GzmK expression levels were significantly elevated in the aBD group compared to the iaBD and HC groups. $GzmK^+$ $CD8^+$ T cells were the dominant source of GzmK, showing significantly higher proportions in the aBD group. These findings highlight $CD8^+$ T cells as the major producers of GzmK specifically upregulated in aBD group.

3.4. MFI of GzmK in $CD8^+$ T cells were significantly higher in active BD patients' group under ex vivo condition

In the flow cytometry analysis, the MFI of GzmK in $CD8^+$ T cells was significantly higher in the active BD group than in the HC group. However, the MFI of GzmK in $CD4^+$ T cells demonstrated no significant difference between the three groups. (Fig. 3) Neither the MFI of GzmA in $CD8^+$ T cells nor in $CD4^+$ T cells had any significant differences in counts between the three groups. Similarly, both MFI of GzmB in $CD8^+$ T cells and in $CD4^+$ T cells didn't show any meaningful difference in counts between the three groups. (Supplementary Fig. 2).

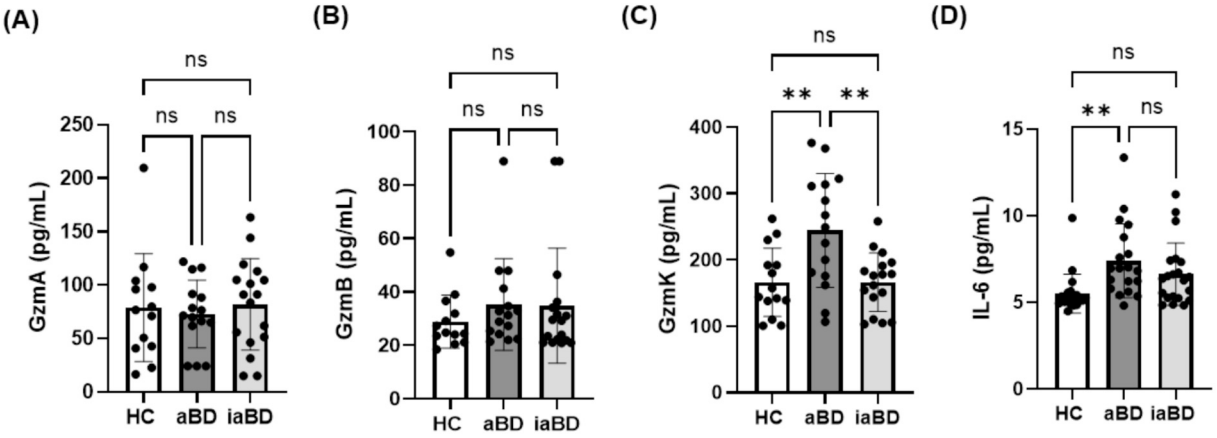


Fig. 1. Serum levels of GzmA, GzmB, GzmK, and interleukin (IL)-6 in Behçet's disease (BD) patients and healthy controls (HC) by using Enzyme-linked Immunosorbent Assay (ELISA). (A, B) Serum levels of GzmA and GzmB in aBD, iaBD, and HC group, showing no significant differences between the groups. (C) Serum levels of GzmK were significantly higher in the aBD group compared to the HC ($p = 0.0048$) and iaBD groups ($p = 0.0032$). (D) Serum IL-6 levels also showed significantly higher levels in the aBD group compared to other groups ($p = 0.0026$), consistent with the tendency observed for GzmK.
Each dot represents an individual sample. Data are presented as mean ± standard deviation (SD). $^{**}p < 0.01$, ns: not significant.
Abbreviations: BD, Behçet's disease; aBD, active BD group; iaBD, inactive BD group; HC, healthy control; Gzm, granzyme.

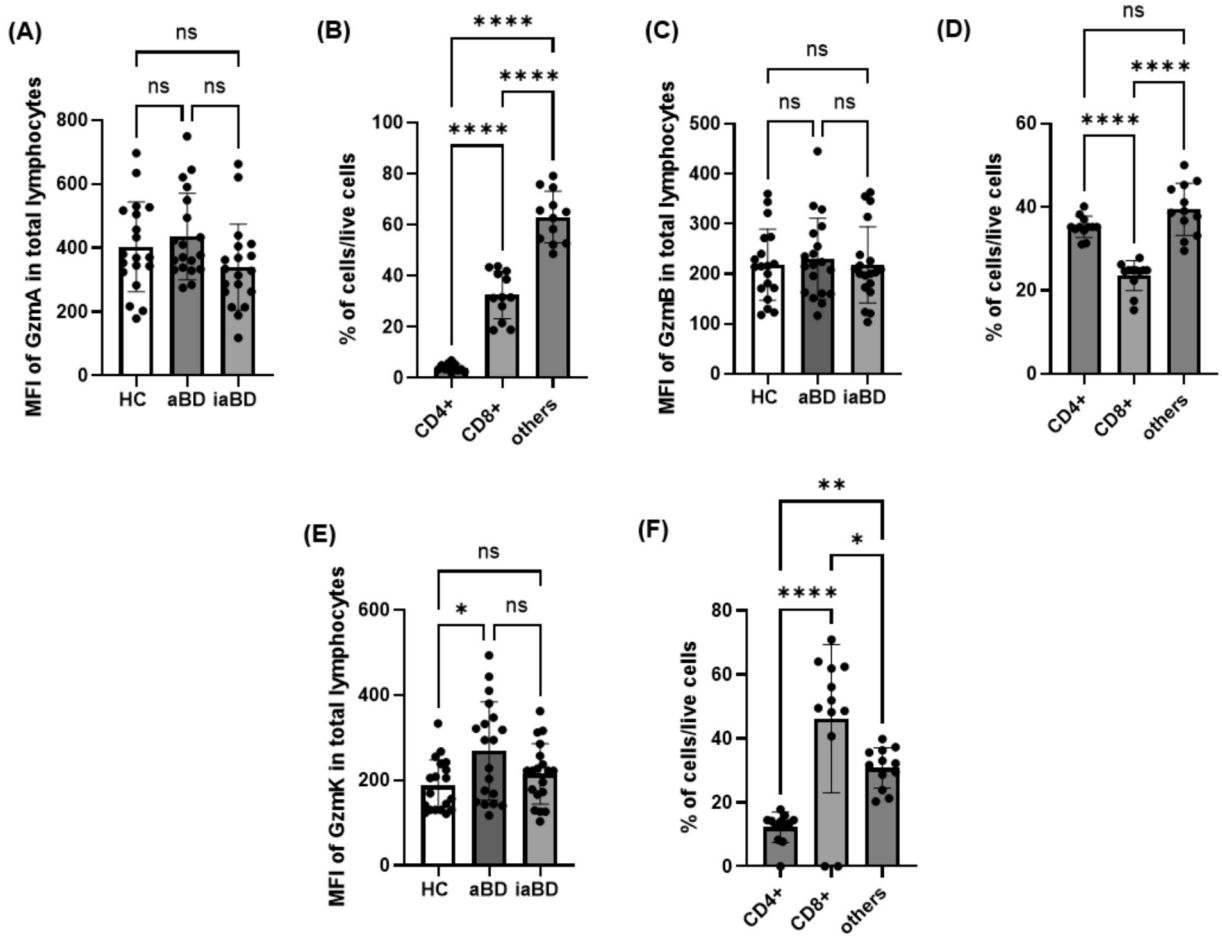


Fig. 2. Expression and Cellular Sources of granzyme (Gzm) A, B, and K. (A, B) GzmA expression. (A) Comparison of MFI of GzmA in the total lymphocyte population among HC, active BD (aBD), and inactive BD (iaBD) group. No significant differences were observed between groups. (B) Distribution of GzmA$^+$ cells among CD4$^+$ T cells, CD8$^+$ T cells, and other cell types. The "others" population and CD8$^+$ T cells were identified as the primary sources of GzmA, showing significantly higher proportions compared to CD4$^+$ T cells (all pairwise comparisons, $p < 0.0001$). (C, D) GzmB expression. (C) MFI of GzmB in lymphocytes across HC, aBD, and iaBD groups, showing no statistical significance. (D) Analysis of cellular sources for GzmB. CD4$^+$ T cells and other lymphocytes were the dominant producers of GzmB, with significantly lower expression in the CD8$^+$ T cell compartment ($p < 0.0001$). (E, F) GzmK expression. (E) MFI of GzmK was significantly elevated in the aBD group compared to both the HC ($p = 0.0136$). (F) Identification of GzmK-secreting cells. CD8$^+$ T cells were the predominant source of GzmK, exhibiting a significantly higher percentage compared to CD4$^+$ T cells ($p < 0.0001$) and other cell types ($p = 0.0306$). Data are presented as mean ± standard deviation (SD). Statistical significance across the three groups was determined using one-way ANOVA followed by Tukey's multiple comparisons test (A-D) or Kruskal-Wallis test followed by Dunn's post-hoc test (E, F). *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, and ns: not significant. Error bars represent the mean ± SD. Abbreviations: MFI, mean fluorescence intensity; BD, Behçet's disease; aBD, active BD group; iaBD, inactive BD group; HC, healthy control; Gzm, granzyme.

3.5. Increased MFI of GzmK in senescent CD8$^+$ T cells in the active BD group and their association with clinical symptoms

In Fig. 3, the MFI of GzmK in senescent CD8$^+$ T cells were significantly higher in the active BD group than in the HC and inactive BD groups. These levels decreased as the symptoms of three patient with active BD improved. (Fig. 3(E)) While formal statistical significance was not reached due to the limited sample size ($n = 3$), the uniformity of this downward trajectory across all monitored cases provides preliminary evidence linking these cells to clinical recovery.

3.6. Stimulation assay reveals GzmK upregulation in BD may not derive from abnormal T cell activation

To clarify whether an increased expression of GzmK in patients with active BD is the result of an abnormal response of T cells to the activating stimuli, Gzm expressions were analysed after in vitro stimulation of T cells with anti-CD3 and anti-CD28 antibodies. We observed similar patterns of each Gzm expression change among the three experimental groups according to the stimulation. The MFIs of GzmA or GzmB were increased after 3-day stimulation except for GzmA in CD8$^+$ T cells and their senescent subset, whereas the MFIs of GzmK in CD4$^+$ T cells and in CD8$^+$ T cells were all decreased. (Fig. 4) These results suggest that GzmK upregulation in CD8$^+$ T cells might not be secondary to their abnormal response to T cell receptor stimulation.

3.7. Correlation between TNF-α$^+$ or IFN-γ$^+$ lymphocytes and MFI of GzmK in CD8$^+$ T cells

In Fig. 5, the MFI of GzmK in CD8$^+$ T cells was positively correlated with the MFI of TNF-α in lymphocytes (Pearson R 0.4522, *P*-value = 0.0157) and the MFI of IFN-γ in lymphocytes (Pearson R 0.4636, P-value = 0.0225). Furthermore, the MFI of GzmK in senescent CD8$^+$ T cells was positively correlated only with the MFI of TNF-α in lymphocytes (Pearson R 0.5334, P-value = 0.0035).

4. Discussion

In this study, the serum GzmK level was significantly increased in the active BD group compared to the inactive BD group and HC group.

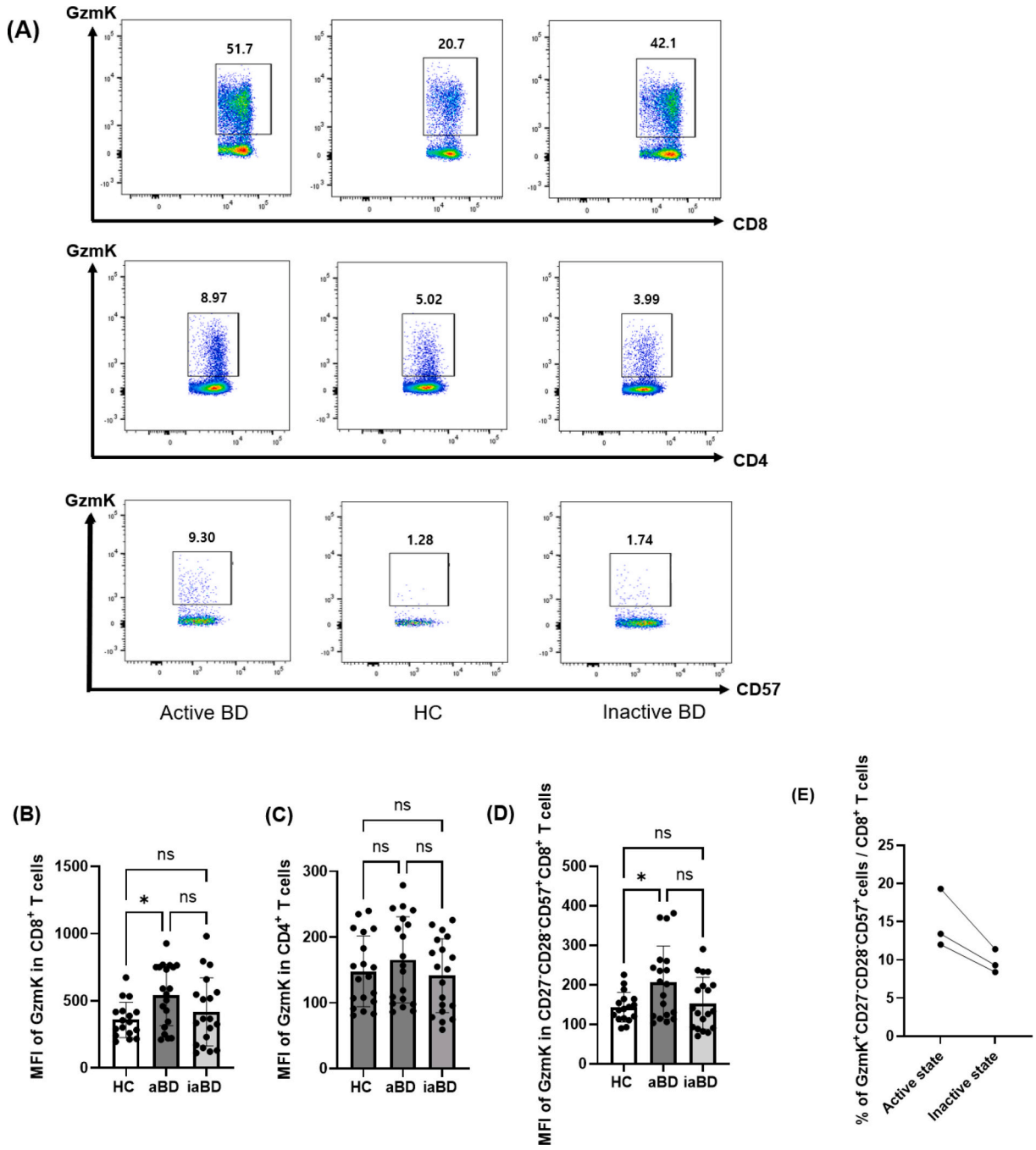


Fig. 3. Flow cytometric analysis of GzmK$^+$ T cells in BD patients and healthy controls. (A) Representative flow cytometric dot plots illustrating GzmK expression in CD8$^+$, CD4$^+$, and senescent CD8$^+$ T cell subsets. (B—D) The mean fluorescence intensity (MFI) of (B) GzmK in CD8$^+$ T cells, which were significantly higher in the aBD group than in the HC group ($p = 0.0432$), (C) MFI of GzmK in CD4$^+$ T cells (ns), and (D) MFI of GzmK in CD27$^-$CD28$^-$CD57$^+$CD8$^+$ T cells (senescent CD8$^+$ T cells) were significantly elevated in the aBD group compared to the HC ($p = 0.0345$). Statistical significance across the three groups was determined using Kruskal-Wallis test followed by Dunn's multiple comparison test. (E) Longitudinal changes in the proportion of senescent GzmK$^+$ cells within the CD8$^+$ T cell population in the three individual patients tracking the transition from an active state to an inactive state. Data are presented as mean ± SD. *p < 0.05, ns: not significant. Abbreviations: MFI, mean fluorescence intensity; BD, Behçet's disease; aBD, active BD group; iaBD, inactive BD group; HC, healthy control; Gzm, granzyme.

Moreover, importantly, GzmK$^+$ CD8$^+$ T cells and GzmK$^+$CD27$^-$CD28$^-$CD57$^+$CD8$^+$ T cells (GzmK$^+$ senescent CD8$^+$ T cells) were significantly higher in the active BD group than in the inactive BD group and HC group.

Gzms have recently been implicated in various cellular functions beyond immune cell-mediated killing. Each of the human Gzms (A, B, K, H, and M) has distinct roles, primarily in cytotoxicity and immune regulation (Bade et al., 2005a). GzmB is the most well-studied and is a key inducer of apoptosis in target cells. It plays a crucial role in the immune system's ability to destroy virus-infected cells; cancer cells; and cells presenting abnormal proteins (Bratke et al., 2005). Over-activated NK cells; mainly the CD56^{dim} NK cell subset; and CD8+ T cells are known to secrete GzmB and perforin; and these molecules contribute to tissue damage and inflammation in areas affected by BD (Chiang et al., 2013; Cooper et al., 2001). In BD, increased activity of GzmB$^+$CD8$^+$ T cells has been observed, particularly in patients with severe symptoms,

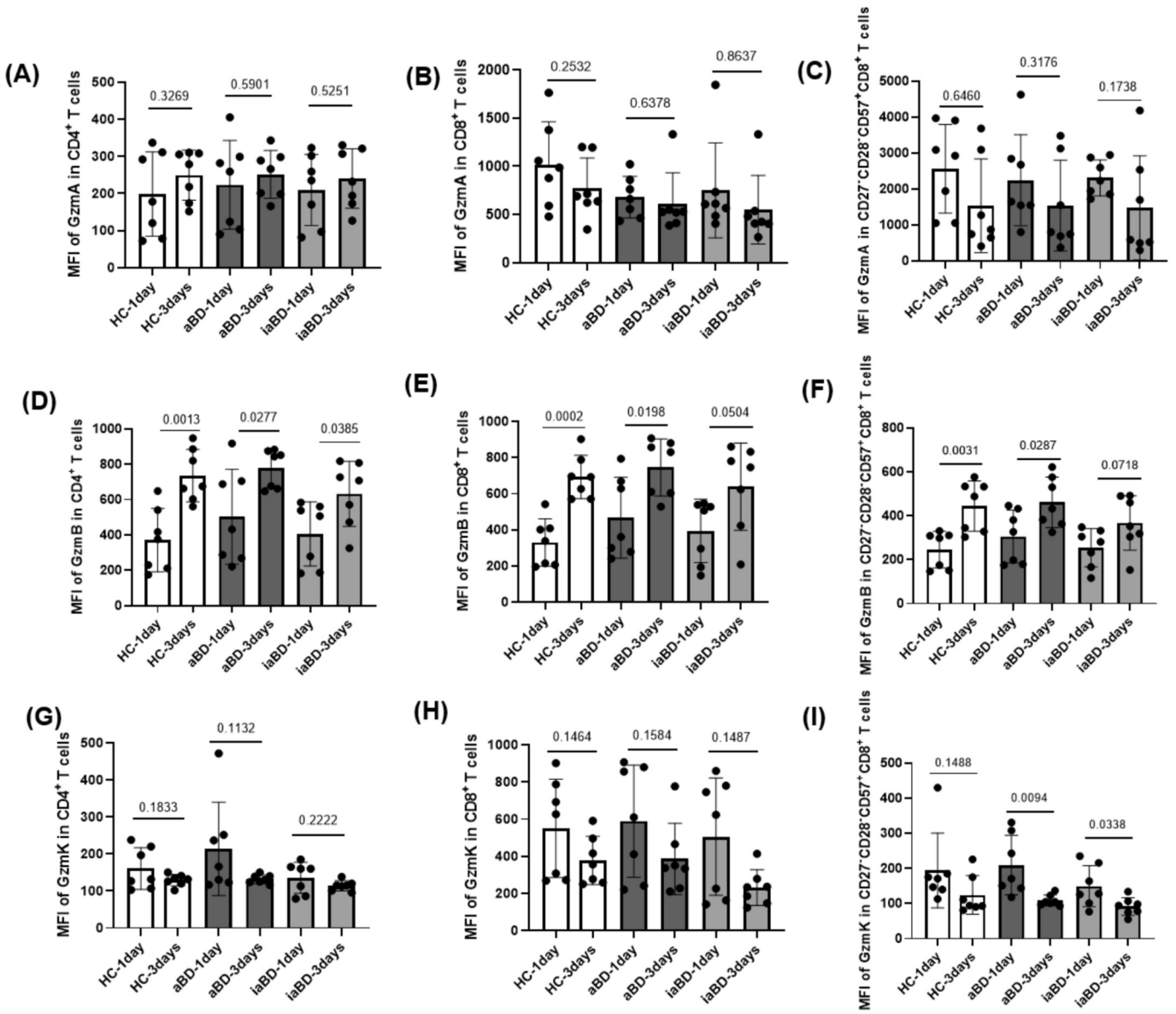
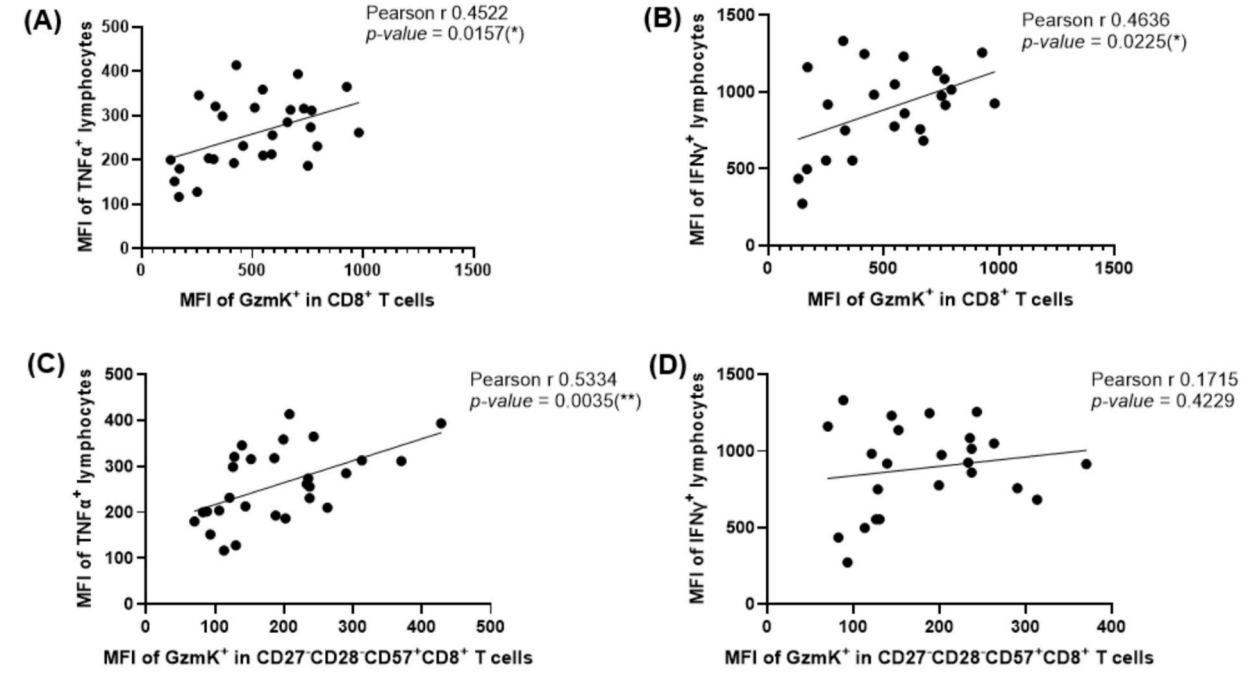


Fig. 4. Changes in granzyme A, B, and K expression levels (MFI) in T cell subsets following in vitro T-cell receptor (TCR) stimulation. MFIs of GzmA in CD4 T cells and in CD8 T cells and MFI of GzmB in CD4 T cells and in CD8 T cells were increased after 3-day stimulation (A, D, E, F) except for MFI of GzmA in $CD8^+$ T cells (B) and their senescent subset (C). MFI of GzmK were decreased in $CD4^+$ T cells, $CD8^+$ T cells, and senescent $CD8^+$ T cells (G, H, I). Baseline MFI values before stimulation are presented in Fig. 3. Data are expressed as mean ± SD and *p*-values are directly indicated above the bars. ns indicates no significant difference. Abbreviations: MFI, mean fluorescence intensity; BD, Behçet's disease; aBD, active BD group; iaBD, inactive BD group; HC, healthy control; Gzm, granzyme.

where these cells contribute to tissue damage through their cytotoxic and pro-inflammatory actions (Yang et al., 2018; Hu and Guan, 2023) (Cavers et al., 2022). Studies have also shown a link between genetic factors such as ERAP1 variants and altered $CD8^+$ T cell function; which modulates granzyme B release and impacts the immune response (Aubert et al., 2024).

However, in this study, the serum level of GzmK rather than GzmB was significantly higher in the active BD group than in the HC and inactive BD groups. Currently, the pro-inflammatory effects of GzmK are being revealed. Granzyme K is known to promote inflammation by cleaving pro-IL-1β, which leads to the release of active IL-1β and amplifies pro-inflammatory signalling (Kaiserman et al., 2022). Additionally; Granzyme K activates protease-activated receptor 1 (PAR1) on endothelial and immune cells; initiating downstream signalling pathways that enhance cytokine production; immune cell recruitment; and endothelial activation; thus intensifying the inflammatory response (Cooper et al., 2011). Elevated GzmK has been implicated in inflammatory diseases; such as in acute lung inflammation (Bratke et al., 2008); atopic dermatitis (Turner et al., 2023); psoriasis (Richardson et al., 2024); septic condition (Rucevic et al., 2007); viral infection (Bade et al., 2005b); and thermal injury (Turner et al., 2019). In this study; Fig. 2 demonstrates that the dominant source of the elevated GzmK is $CD8^+$ T cells rather than $CD4^+$ T cells and other lymphocytes including NK cells. Jonsson et al. have reported that majority of the $CD8^+$ T cells in regional RA synovial fluid and tissue showed GzmK expression (Jonsson et al., 2022). Also, in our study, $GzmK^+$ $CD8^+$ T cells were significantly higher in the PBMC of the active BD group than HC and inactive BD groups. This result suggests that not only the tissue $GzmK^+$ $CD8^+$ T cells but also the circulating $GzmK^+$ $CD8^+$ T cells were increased in autoinflammatory diseases. In our cellular source analysis, the 'other cells' population, which likely includes NK cells, also showed GzmK expression. Given that NK cells are known innate producers of Gzms, they may contribute to the total pool of serum GzmK. However, in this study, the most significant expansion of $GzmK^+$ cells during the active stage of BD



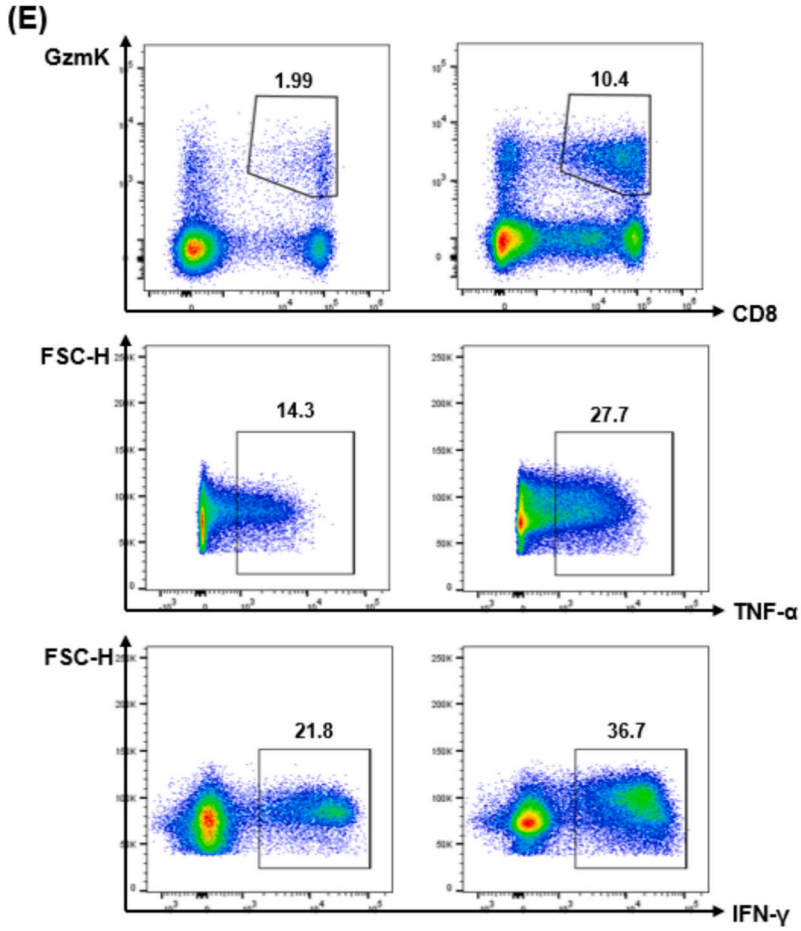


Fig. 5. Correlation between GzmK$^+$ CD8$^+$ T cell subsets and cytokine levels. (A, B) MFI of GzmK in CD8$^+$ T cells showed a positive correlation with the expression (MFI) of TNF-α and IFN-γ measured within the total lymphocyte population (gated by FSC/SSC). (C, D) GzmK$^+$CD27$^-$CD28$^-$CD57$^+$CD8$^+$ T cells were positively correlated with the MFI of TNF-α in total lymphocytes but not with IFN-γ. Each dot in (A–D) represents an individual sample. (E) Representative flow cytometry plot illustrating the correlation analysis. Abbreviations: MFI, mean fluorescence intensity; Gzm, granzyme.

were observed within the $CD8^+$ T cell compartment. Furthermore, the strong correlation between the senescent phenotype and TNF-α production suggests that highly differentiated T cells, rather than the general NK cell population, are the primary drivers of the GzmK-mediated inflammatory loop in BD.

Interestingly, these $GzmK^+$ $CD8^+$ T cells were positively correlated with the expression of TNF-α^+ lymphocytes and IFN-γ^+ lymphocytes of the BD patients. Both TNF-α and IFN-γ are potent pro-inflammatory cytokines, indicating that $GzmK^+$ $CD8^+$ T cells could interact with various inflammatory cytokines to initiate diverse immune responses. This association implies that these cells might play a role in modulating and amplifying inflammation while exerting direct cytotoxicity in their activated state.

Classic cytotoxic CD8 T cells, often referred to as cytotoxic T lymphocytes, are known to express large amounts of GzmB and perforin, which enter target cells and induce apoptotic caspase cascades (Buggert et al., 2023). Also; there are non-migratory tissue $CD8^+$ T cell populations called resident memory T cells; typically identified by the expression of CD103 and CD69 (Szabo, 2023). However; recent studies have revealed that the common $CD8^+$ T cell subset in tissues affected by autoimmune diseases does not clearly fall into any of these categories. Thus; there has been an attempt to classify T cells according to their stages of differentiation based on Gzms (Bratke et al., 2005). Our findings support the idea of classifying $CD8^+$ T cells based on their GzmK expression in autoimmune diseases, particularly by their pro-inflammatory roles.

$GzmK^+$ $CD8^+$ T cells are shown to accumulate in murine models and humans as they age (Mogilenko et al., 2021). Additionally; Duquette et al. reported that $GzmK^+$ levels were low in cord blood; suggesting GzmK could be upregulated with immune experiences (Duquette et al., 2023). The $CD27^-CD28^-CD57^+$ phenotype suggests that these $CD8^+$ T cells are highly differentiated or senescent; typically arising after repeated activation. The loss of costimulatory molecules CD27 and CD28 is a hallmark of transition from naive to terminally differentiated effector cells due to chronic antigenic stimulation. Combined with the expression of CD57; a definitive marker of replicative senescence characterized by reduced proliferative capacity and shortened telomeres; this phenotype represents a state of functional exhaustion and cellular aging. In this study; these senescent $CD8^+$ T cells were higher in GzmK in patients with active BD. This trend has also been discussed in RA (Jonsson, 2024). Thus; senescence due to aging or early senescence in inflammatory disease could be related to elevated GzmK levels. In our stimulation assay; GzmK expression significantly decreased after TCR-mediated activation with anti-CD3/CD28 antibodies; a trend distinct from the upregulation seen in GzmA and GzmB. While this suggests that GzmK upregulation in BD is not a primary result of abnormal TCR signalling; it points toward an alternative regulatory mechanism. Recent studies indicate that $GzmK^+CD8^+$ T cells can function as innate-like effectors; where their phenotype is driven by homeostatic or proinflammatory cytokines such as IL-12; IL-15; or IL-18 rather than direct antigen engagement. Therefore; the accumulation of $GzmK^+$ senescent $CD8^+$ T cells in active BD may be sustained by the chronic cytokine-rich environment; further fueling the autoinflammatory cascade through a TCR-independent; cytokine-responsive pathway. Senescent $CD8^+$ T cells are associated with reduced proliferative capacity but increased cytotoxic function and inflammatory cytokine; SASP production; including IL-6; IL-1α; IL-1β; and TNF-α (Ohtani, 2022). In BD; chronic tissue inflammation is often associated with elevated levels of TNF-α; which recruits additional immune cells to sites of inflammation (Al-Obeidi and Nowatzky, 2023). Therefore, it makes sense with the positive correlation between senescent $GzmK^+$ $CD8^+$ T cells and TNF-α^+ expression in patients with BD in this study. $GzmK^+$ senescent $CD8^+$ T cells could contribute to local tissue damage through their cytotoxic activity, which stimulates more TNF-α production by macrophages and other immune cells in response to the damage. This subset of cells might be crucial in sustaining the inflammatory cascade in BD by a focused role within TNF-α-centered inflammatory environments. Given their characteristics as terminally differentiated $CD8^+$ T cells, they may act more prominently in specific inflammatory conditions or areas of tissue damage.

Importantly, Fig. 3(E) illustrates that the frequency of these senescent $GzmK^+$ $CD8^+$ T cells decreased substantially as clinical symptoms improved in the followed active BD patients. While the limited number of patients followed longitudinally ($n = 3$) remains a constraint for a definitive correlation analysis, this dynamic shift provides preliminary but direct evidence that this cell population is closely associated with disease progression and clinical transition, rather than being a merely static phenotypic marker. Also, this subset of cells is considered a promising therapeutic target because its cytotoxic effects are primarily directed at diseased or infected cells, while it generally has less impact on healthy cells (Cigalotto and Martinvalet, 2024). This renders it particularly valuable in autoimmune diseases.

This study's limitation is its emphasis on only the phenotypic aspects of senescent immune cells, leaving precise functional studies and mechanisms of cell accumulation necessary to determine the contribution of immunosenescence to BD pathogenesis. Understanding the impact of cytotoxic molecules and proinflammatory cytokines released by these senescent T cells on inflammation in BD remains a crucial area for further exploration. Furthermore, due to the relatively small sample size ($n = 19$ per group), this study was not sufficiently powered to conduct a formal statistical subgroup analysis to compare high-GzmK and low-GzmK aBD patients also to inactive BD patients. Also, our active BD cohort consisted primarily of patients with mucocutaneous manifestations, with limited representation of major organ involvement; specifically, only two cases presented with ocular lesions, and no cases of vascular, gastrointestinal, or central nervous system involvement were included in the current series. While mucocutaneous symptoms are the most prevalent features of BD, the lack of diverse organ involvement means our findings may not be fully representative of all BD clinical subtypes. And, the longitudinal portion of our study was limited to three patients who transitioned between active and inactive states during the observation period. Although these initial observations align with our cross-sectional findings, a more robust, long-term follow-up study with a larger cohort is necessary to fully establish GzmK as a predictive biomarker for disease flare or treatment response.

In conclusion, an increased GzmK serum level, and more numerous $GzmK^+$ $CD8^+$ T cells and senescent $GzmK^+$ $CD8^+$ T cells were observed in the active BD group than that in the other groups. This could be a hallmark of BD inflammation.

CRediT authorship contribution statement

Heera Lee: Writing – original draft, Software, Investigation, Data curation, Conceptualization. **Soo-Jin Lee:** Investigation, Formal analysis, Data curation. **Young Joon Park:** Software, Resources, Conceptualization. **Han Seul Kim:** Formal analysis, Data curation. **Eun-So Lee:** Supervision, Investigation, Conceptualization. **Sun Park:** Writing – review & editing, Validation, Supervision, Investigation, Conceptualization.

Fundings

This study was supported by the National Research Foundation of Korea (NRF-2022R1A2C2008603 and RS-2022-NR074607) funded by the Ministry of Science and ICT of Korea.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by Juhee Kang from the "Clinical faculty research support program" of the Institute of Medical Science, School of Medicine, Ajou University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imbio.2026.153176.

Data availability

Data will be made available on request.

References

Al-Obeidi, A.F., Nowatzky, J., 2023. Immunopathogenesis of Behcet's disease. Clin. Immunol. 253, 109661. https://doi.org/10.1016/j.clim.2023.109661.

Aubert, A., Jung, K., Hiroyasu, S., Pardo, J., Granville, D.J., 2024. Granzyme serine proteases in inflammation and rheumatic diseases. Nat. Rev. Rheumatol. 20, 361–376. https://doi.org/10.1038/s41584-024-01109-5.

Bade, B., Boettcher, H.E., Lohrmann, J., Hink-Schauer, C., Bratke, K., Jenne, D.E., Virchow Jr., J.C., Luttmann, W., 2005a. Differential expression of the granzymes a, k and m and perforin in human peripheral blood lymphocytes. Int. Immunol. 17, 1419–1428. https://doi.org/10.1093/intimm/dxh320.

Bade, B., Lohrmann, J., ten Brinke, A., Wolbink, A.M., Wolbink, G.J., ten Berge, I.J., Virchow Jr., J.C., Luttmann, W., Hack, C.E., 2005b. Detection of soluble human granzyme k in vitro and in vivo. Eur. J. Immunol. 35, 2940–2948. https://doi.org/10.1002/eji.200526249.

Bouwman, A.C., van Daalen, K.R., Crnko, S., Ten Broeke, T., Bovenschen, N., 2021. Intracellular and extracellular roles of granzyme k. Front. Immunol. 12, 677707. https://doi.org/10.3389/fimmu.2021.677707.

Bratke, K., Kuepper, M., Bade, B., Virchow Jr., J.C., Luttmann, W., 2005. Differential expression of human granzymes a, b, and k in natural killer cells and during CD8+ t cell differentiation in peripheral blood. Eur. J. Immunol. 35, 2608–2616. https://doi.org/10.1002/eji.200526122.

Bratke, K., Klug, A., Julius, P., Kuepper, M., Lommatzsch, M., Sparmann, G., Luttmann, W., Virchow, J.C., 2008. Granzyme k: a novel mediator in acute airway inflammation. Thorax 63, 1006–1011. https://doi.org/10.1136/thx.2007.091215.

Buggert, M., Price, D.A., Mackay, L.K., Betts, M.R., 2023. Human circulating and tissue-resident memory CD8(+) t cells. Nat. Immunol. 24, 1076–1086. https://doi.org/10.1038/s41590-023-01538-6.

Cavers, A., Kugler, M.C., Ozguler, Y., Al-Obeidi, A.F., Hatemi, G., Ueberheide, B.M., Ucar, D., Manches, O., Nowatzky, J., 2022. Behçet's disease risk-variant HLA-B51/ERAP1-Hap10 alters human CD8 t cell immunity. Ann. Rheum. Dis. 81, 1603–1611. https://doi.org/10.1136/ard-2022-222277.

S.C. Chiang, J. Theorell, M. Entesarian, M. Meeths, M. Mastafa, W. Al-Herz, P. Frisk, K.C. Gilmour, M. Ifversen, C. Langenskiöld, et al., Comparison of primary human cytotoxic T-cell and natural killer cell responses reveal similar molecular requirements for lytic granule exocytosis but differences in cytokine production, Blood 121 (2013) 1345–1356, Doi: https://doi.org/10.1182/blood-2012-07-442558.

Cigalotto, L., Martinvalet, D., 2024. Granzymes in health and diseases: the good, the bad and the ugly. Front. Immunol. 15, 1371743. https://doi.org/10.3389/fimmu.2024.1371743.

Cooper, D.M., Pechkovsky, D.V., Hackett, T.L., Knight, D.A., Granville, D.J., 2011. Granzyme k activates protease-activated receptor-1. PloS One 6, e21484. https://doi.org/10.1371/journal.pone.0021484.

Cooper, M.A., Fehniger, T.A., Caligiuri, M.A., 2001. The biology of human natural killer-cell subsets. Trends Immunol. 22, 633–640. https://doi.org/10.1016/s1471-4906(01)02060-9.

Duquette, D., Harmon, C., Zaborowski, A., Michelet, X., O'Farrelly, C., Winter, D., Koay, H.F., Lynch, L., 2023. Human granzyme k is a feature of innate t cells in blood, tissues, and tumors, responding to cytokines rather than TCR stimulation. J. Immunol. 211, 633–647. https://doi.org/10.4049/jimmunol.2300083.

Emmi, G., Bettiol, A., Hatemi, G., Prisco, D., 2024. Behçet's syndrome. Lancet 403, 1093–1108. https://doi.org/10.1016/s0140-6736(23)02629-6.

Hay, Z.L.Z., Slansky, J.E., 2022. Granzymes: the molecular executors of Immune-Mediated cytotoxicity. Int. J. Mol. Sci. 23. https://doi.org/10.3390/ijms23031833.

Hu, D., Guan, J.L., 2023. The roles of immune cells in Behcet disease. Adv. Rheumatol. 63, 49. https://doi.org/10.1186/s42358-023-00328-w.

Jonsson, A.H., 2024. Granzyme k(+) CD8 t cells in autoimmunity. Best Pract. Res. Clin. Rheumatol. 38, 101930. https://doi.org/10.1016/j.berh.2024.101930.

Jonsson, A.H., Zhang, F., Dunlap, G., Gomez-Rivas, E., Watts, G.F.M., Faust, H.J., Rupani, K.V., Mears, J.R., Meednu, N., Wang, R., et al., 2022. Granzyme K(+) CD8 t cells form a core population in inflamed human tissue. Sci. Transl. Med. 14, eabo0686. https://doi.org/10.1126/scitranslmed.abo0686.

Kaiserman, D., Zhao, P., Rowe, C.L., Leong, A., Barlow, N., Joeckel, L.T., Hitchen, C., Stewart, S.E., Hollenberg, M.D., Bunnett, N., et al., 2022. Granzyme k initiates IL-6 and IL-8 release from epithelial cells by activating protease-activated receptor 2. PloS One 17, e0270584. https://doi.org/10.1371/journal.pone.0270584.

D.A. Mogilenko, O. Shpynov, P.S. Andhey, L. Arthur, A. Swain, E. Esaulova, S. Brioschi, I. Shchukina, M. Kerndl, M. Bambouskova, et al., Comprehensive profiling of an aging immune system reveals clonal GZMK(+) CD8(+) T cells as conserved hallmark of inflammaging, Immunity 54 (2021) 99–115.e112, Doi: https://doi.org/10.1016/j.immuni.2020.11.005.

Ohtani, N., 2022. The roles and mechanisms of senescence-associated secretory phenotype (SASP): can it be controlled by senolysis? Inflamm Regen. 42, 11. https://doi.org/10.1186/s41232-022-00197-8.

K.C. Richardson, A. Aubert, C.T. Turner, L. Nabai, S. Hiroyasu, M.A. Pawluk, R.A. Cederberg, H. Zhao, K. Jung, A. Burleigh, et al., Granzyme K mediates IL-23-dependent inflammation and keratinocyte proliferation in psoriasis, Front. Immunol. 15 (2024) 1398120, Doi: https://doi.org/10.3389/fimmu.2024.1398120.

Rucevic, M., Fast, L.D., Jay, G.D., Trespalcios, F.M., Sucov, A., Siryaporn, E., Lim, Y.P., 2007. Altered levels and molecular forms of granzyme k in plasma from septic patients. Shock 27, 488–493. https://doi.org/10.1097/01.shk.0000246905.24895.e5.

Sharma, M., Merkulova, Y., Raithatha, S., Parkinson, L.G., Shen, Y., Cooper, D., Granville, D.J., 2016. Extracellular granzyme k mediates endothelial activation through the cleavage of protease-activated receptor-1. FEBS J. 283, 1734–1747. https://doi.org/10.1111/febs.13699.

Szabo, P.A., 2023. Axes of heterogeneity in human tissue-resident memory t cells. Immunol. Rev. 316, 23–37. https://doi.org/10.1111/imr.13210.

Turner, C.T., Zeglinski, M.R., Richardson, K.C., Zhao, H., Shen, Y., Papp, A., Bird, P.I., Granville, D.J., 2019. Granzyme k expressed by classically activated macrophages contributes to inflammation and impaired remodeling. J. Invest. Dermatol. 139, 930–939. https://doi.org/10.1016/j.jid.2018.09.031.

Turner, C.T., Zeglinski, M.R., Boivin, W., Zhao, H., Pawluk, M.A., Richardson, K.C., Chandrabalan, A., Bird, P., Ramachandran, R., Sehmi, R., et al., 2023. Granzyme k contributes to endothelial microvascular damage and leakage during skin inflammation. Br. J. Dermatol. 189, 279–291. https://doi.org/10.1093/bjd/ljac017.

Wensink, A.C., Kemp, V., Fermie, J., García Laorden, M.I., van der Poll, T., Hack, C.E., Bovenschen, N., 2014. Granzyme k synergistically potentiates LPS-induced cytokine responses in human monocytes. Proc. Natl. Acad. Sci. U. S. A. 111, 5974–5979. https://doi.org/10.1073/pnas.1317347111.

Yang, J.Y., Park, M.J., Park, S., Lee, E.S., 2018. Increased senescent CD8+ t cells in the peripheral blood mononuclear cells of Behcet's disease patients. Arch. Dermatol. Res. 310, 127–138. https://doi.org/10.1007/s00403-017-1802-8.

Zheng, Y., Zhao, J., Shan, Y., Guo, S., Schrodi, S.J., He, D., 2023. Role of the granzyme family in rheumatoid arthritis: current insights and future perspectives. Front. Immunol. 14, 1137918. https://doi.org/10.3389/fimmu.2023.1137918.

Zierhut, M., Mizuki, N., Ohno, S., Inoko, H., Gül, A., Onoé, K., Isogai, E., 2003. Immunology and functional genomics of Behcet's disease. Cell. Mol. Life Sci. 60, 1903–1922. https://doi.org/10.1007/s00018-003-2333-3.