

# Association of High-Mobility Group Box-1 With Th Cell-Related Cytokines in the Vitreous of Ocular Sarcoidosis Patients

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**PURPOSE.** High-mobility group box-1 (HMGB1) is a nonhistone DNA-binding nuclear protein released from necrotic cells, which is also secreted by activated leukocytes and acts as a primary proinflammatory cytokine. In this study, we compared vitreous HMGB1 levels in ocular sarcoidosis with those in noninflammatory vitreoretinal diseases and evaluated its association with Th cell-related and proinflammatory cytokines.

**METHODS.** The study group consisted of 24 patients with ocular sarcoidosis. The control group consisted of 27 patients with proliferative diabetic retinopathy (PDR) and 24 with idiopathic epiretinal membrane (ERM). Vitreous fluid samples were obtained at the beginning of vitrectomy. Vitreous levels of HMGB1 and IL-1 $\beta$ , IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN- $\gamma$ , soluble CD40 ligand (sCD40L), and TNF $\alpha$  were measured.

**RESULTS.** High-mobility group box-1 was detected in the vitreous of 23 of 24 patients (95.8%) with ocular sarcoidosis. Mean vitreous level of HMGB1 was the highest in the sarcoidosis group, followed by the PDR and ERM groups, with significant differences between the three groups. In the sarcoidosis group, vitreous levels of IL-6, IL-10, IL-31, IFN- $\gamma$ , sCD40L, and TNF $\alpha$  were significantly higher than those in the idiopathic ERM group, and IFN- $\gamma$  and sCD40L were significantly higher than those in the PDR group. Vitreous HMGB1 level correlated significantly with IL-10, IFN- $\gamma$ , and sCD40L levels but not with IL-6, IL-17, IL-31, or TNF $\alpha$  levels.

**CONCLUSIONS.** The vitreous level of HMGB1 is elevated in ocular sarcoidosis and is associated with vitreous levels of Th1- and regulatory T-related cytokines, but not with proinflammatory or Th17-related cytokines.

**Keywords:** sarcoidosis, uveitis, cytokine, inflammation

Sarcoidosis is a chronic systemic disease of unknown etiology, characterized by noncaseating granuloma formation in multiple tissues and organs.<sup>1,2</sup> Although the mediastinal and hilar lymph nodes, as well as the lungs, are affected most commonly (50%–90%), ophthalmic involvement is not rare and occurs in 30% to 60% of patients with sarcoidosis.<sup>1,3–5</sup> Granulomas that are the fundamental feature of sarcoidosis consist of macrophages and epithelioid cells encircled by lymphocytes, in which some macrophages and epithelioid cells form multinucleated giant cells with loss of phagocytic capacity.

CD4<sup>+</sup> T-helper (Th) cells play a critical role in maintenance of homeostasis. However, inappropriate differentiation of Th cells is responsible for various disorders. T-helper 1 cells produce mostly IL-2 and IFN- $\gamma$ , and they promote organ-specific autoimmune diseases. T-helper 2 cells are involved in humoral immunity by producing IL-4, IL-5, IL-13, and IL-31.<sup>6</sup> T-helper 17 cells preferentially produce IL-17A, IL-17F, IL-21, and IL-22<sup>7</sup> and are involved in the development of multiple chronic inflammatory diseases.<sup>8</sup> In sarcoidosis, pathogenic Th cells are involved in the formation of granulomas.<sup>9</sup>

High-mobility group box-1 (HMGB1), a major member of damage-associated molecule patterns, is a nonhistone DNA-

binding nuclear protein that is highly conserved across species (99% amino acid identity among mammals).<sup>10</sup> Necrotic cells release HMGB1 that recruits inflammatory cells to the site of injury and promotes cell division to replace dying cells by activating immune system. High-mobility group box-1 is also secreted by activated leukocytes and dendritic cells (DCs) and acts as a primary proinflammatory cytokine in the extracellular environment in an autocrine/paracrine manner.<sup>11–13</sup> Extracellular HMGB1 transmits signals via putative receptors Toll-like receptor (TLR)2, TLR4, and TLR9<sup>13</sup> and the receptor for advanced glycation end product (RAGE), a member of the Ig superfamily.<sup>14,15</sup> TLRs are mainly expressed on immune cells but are also expressed on tissue cells. Uveal cells,<sup>16</sup> RPE cells,<sup>17</sup> retinal photoreceptor cells,<sup>18</sup> and astrocytes<sup>19</sup> express TLRs in intraocular tissues. The signals via these receptors activate the extracellular signal-regulated kinase 1 and 2 (ERK1/2) signaling pathway and the transcription factor NF- $\kappa$ B, promoting the upregulation of proinflammatory cytokines. In ocular diseases, increased vitreous level of HMGB1 and in situ localization of HMGB1 and RAGE expression in vascular endothelial cells and stromal cells in fibrovascular epiretinal membranes have been



TABLE 1. Clinical Background and Vitreous Levels of HMGB1 in Individual Patients With Ocular Sarcoidosis

Case No.	Age, y	Sex	Complications*	Inflammation Scores in AS†	Inflammation Scores in PS‡	Treatment§	Vitreous HMGB1 Level (ng/mL)
Sar1	69	Female	ERM	2	1	-	6.9
Sar2	46	Female	VO, CME	0	2	Steroid	20.2
Sar3	47	Female	CME	0	1	Steroid	6.5
Sar4	71	Female	VO	2	2	Steroid	7.4
Sar5	71	Female	CME, VO	2	2	Steroid	10.6
Sar6	68	Female	VH, DR	0	2	Steroid	5.8
Sar7	65	Female	ERM	1	2	-	5.8
Sar8	76	Female	VO	1	2	-	5.6
Sar9	73	Female	VO	1	2	-	4.5
Sar10	72	Male	RD	1	1	-	9.7
Sar11	69	Male	VH, DR	3	4	-	12.0
Sar12	41	Female	ERM	4	2	Steroid	6.1
Sar13	74	Male	VH, DR	1	4	-	8.9
Sar14	62	Male	VO	1	1	-	9.8
Sar15	68	Male	CME, ERM	0	1	-	8.0
Sar16	68	Male	CME, ERM	0	0	-	0
Sar17	61	Male	VO	0	2	Steroid	31.1
Sar18	77	Female	VO	0	3	-	33.4
Sar19	76	Female	VO	0	3	-	52.5
Sar20	76	Female	VH	0	2	Steroid	5.7
Sar21	89	Female	ERM	1	1	Steroid	20.1
Sar22	64	Female	ERM	0	1	Steroid	6.1
Sar23	55	Female	VO	0	2	-	5.4
Sar24	65	Female	CME	0	0	Steroid	7.8

DR, diabetic retinopathy; RD, retinal detachment.

\* Complications requiring vitrectomy.

† Ocular inflammation scores based on the degree of cells and flare in the anterior segment (AS).<sup>26</sup>

‡ Ocular inflammation scores based on the degree of vitreous haze in the posterior segment (PS).<sup>27</sup>

§ Systemic treatment.

demonstrated in patients with proliferative retinopathy (PDR).<sup>20,21</sup>

Experimental autoimmune uveoretinitis (EAU), which is induced in susceptible rodents by active immunization with retinal autoantigens, is an animal model of endogenous uveitis including sarcoidosis.<sup>22</sup> Expression of HMGB1 on the iris, ciliary body, and retina is elevated in eyes developing EAU, and the level of HMGB1 in the aqueous humor is increased.<sup>23</sup> Furthermore, expressions of HMGB-1 and RAGE are observed in inflammatory cells infiltrating the anterior chamber, vitreous cavity, and subretinal space. Because HMGB1 released from retinal cells into extracellular matrix and intraocular fluid serves as a danger signal, administration of HMGB1 antagonists attenuates EAU via mechanisms including inhibition of uveitogenic T-cell proliferation and their IFN- $\gamma$  and IL-17 production.<sup>24</sup> Thus, it is possible that HMGB1 plays a role in granulomatous lesion formation in sarcoidosis. In this study, we measured vitreous levels of HMGB1 in patients with ocular sarcoidosis and compared them with those in patients with PDR and other vitreoretinal diseases. We also evaluated the association between HMGB1 level and levels of proinflammatory and Th cell-related cytokines in the vitreous.

## MATERIALS AND METHODS

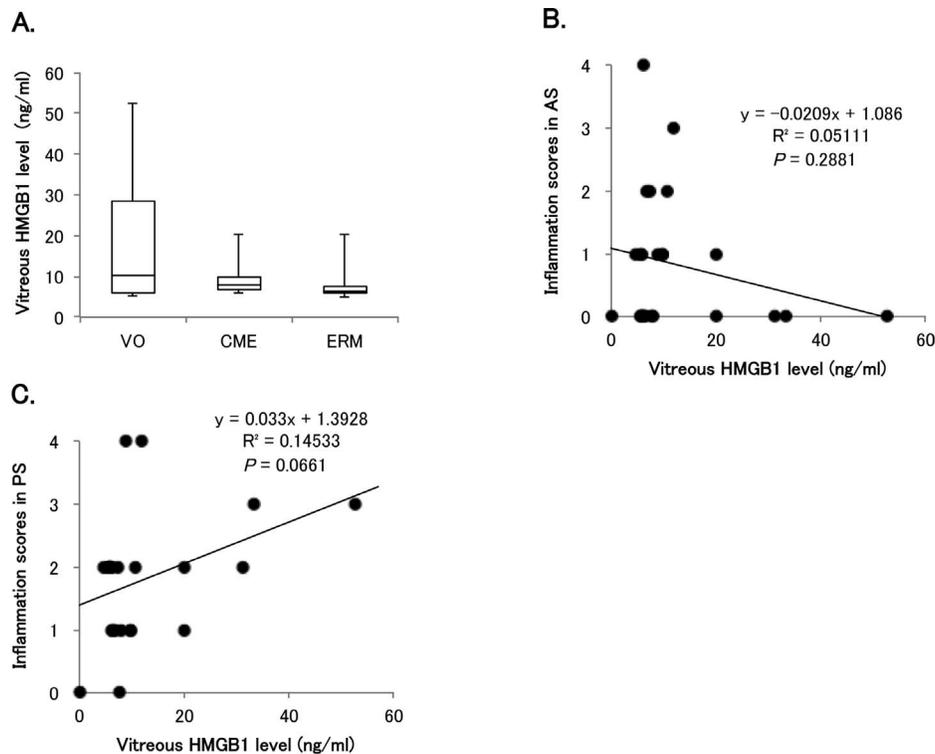
### Subjects

The study group consisted of 24 patients with ocular sarcoidosis who underwent vitrectomy for complications between January 1, 2013 and December 31, 2015 at the National Defense Medical College. The complications requiring

vitrectomy are summarized in Table 1. Counting multiple complications, the most frequent complication was vitreous opacity (VO) in 10 cases, followed by epiretinal membrane (ERM) in 7 cases, cystoid macular edema (CME) in 6 cases, and vitreous hemorrhage (VH) in 4 cases. Because 11 sarcoidosis patients had active uveitis, vitrectomy was performed while they were on systemic corticosteroid therapy. None of the patients were treated with immunosuppressive agents or anti-TNF $\alpha$  monoclonal antibodies. Ocular inflammation was scored on a scale of 0 to 4 based on the degree of cells and flare in the anterior segment<sup>25</sup> and that of vitreous haze in the posterior segment.<sup>26</sup> In sarcoidosis patients with VH, inflammation scores in the posterior segment obtained before the development of VH were used in analysis. The exclusion criteria included previous vitrectomy, prior intravitreal therapies, trauma, and infectious endophthalmitis. The control group consisted of 27 patients with PDR and 24 patients with idiopathic ERM. The age (mean  $\pm$  SD) was 66.8  $\pm$  10.9 years (range, 41–89 years) in the sarcoidosis group, 54.9  $\pm$  13.2 years (range, 31–83 years) in the PDR group, and 61.8  $\pm$  11.9 years (range, 39–87 years) in the ERM group. The sex (male/female) ratio was 7/17 in the sarcoidosis group, 18/9 in the PDR group, and 12/12 in the ERM group. The PDR group was significantly younger ( $P < 0.05$ ) and consisted of more males ( $P < 0.05$ ) compared with the sarcoidosis or ERM group.

### Ethics Statement

The study was approved by the Institutional Review Board of National Defense Medical College. Eligible participants were informed about the purpose and experimental procedure of the study and signed a copy of the Institutional Review Board-



**FIGURE 1.** Association between vitreous levels of HMGB1 in patients with ocular sarcoidosis and their ocular findings. (A) Box plots of vitreous levels of HMGB1 in ocular sarcoidosis patients undergoing vitrectomy for VO, CME, or ERM. There were no significant differences between VO, CME, and ERM. (B, C) Vitreous level of HMGB1 was not associated with inflammation scores in (B) anterior segment (AG) and (C) posterior segment (PG).

approved consent form prior to participation. The study was conducted in accordance with the Declaration of Helsinki and HIPAA regulations.

### Sample Collection

Approximately 0.2 to 0.5 mL of undiluted vitreous fluid was sampled using a 25-G vitreous cutter inserted into the mid-vitreous cavity at the beginning of surgery before active infusion. The vitreous samples were transferred to sterile tubes and stored at  $-70^{\circ}\text{C}$  until analysis. No complication associated with vitreous sampling was observed.

### High-Mobility Group Box-1 Measurement

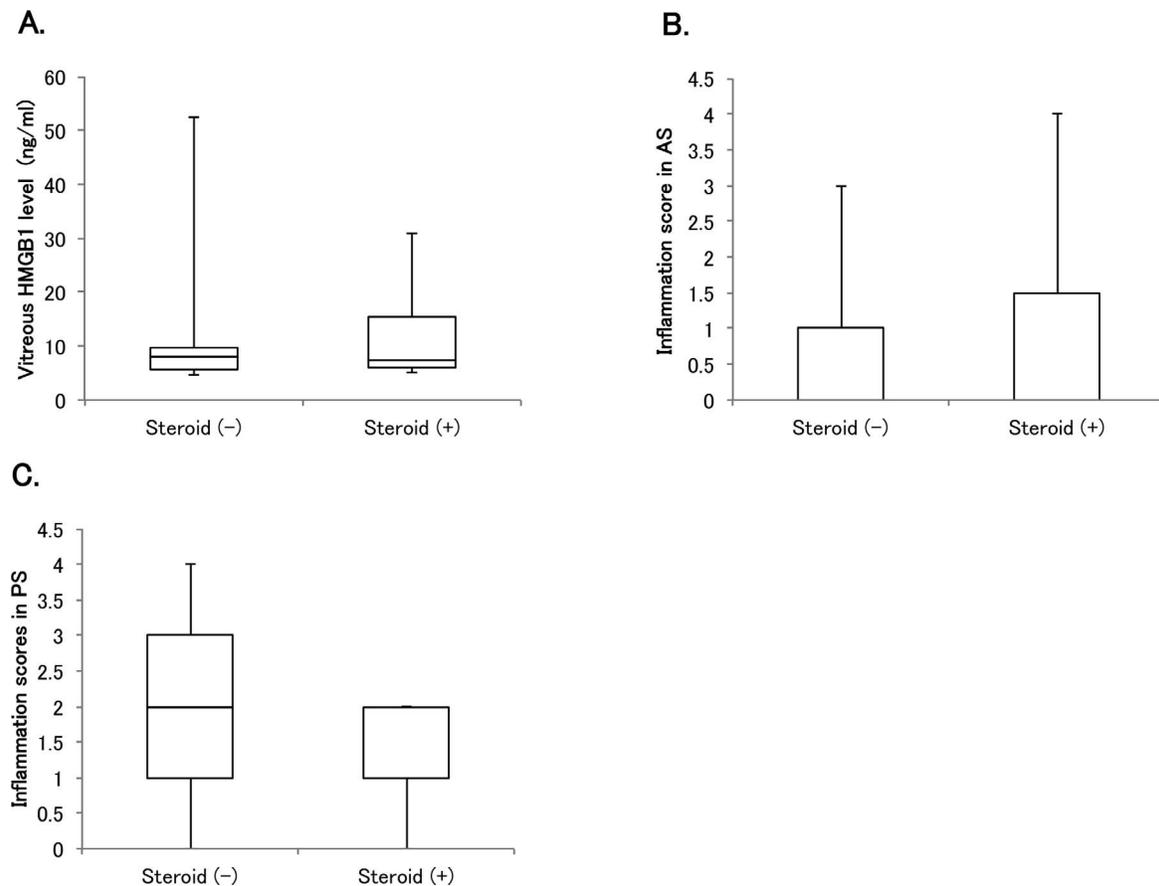
Level of HMGB1 in the vitreous fluid was measured using ELISA kits (Shino-Test, Inc., Tokyo, Japan) according to the manufacturer's instructions. In brief, 100  $\mu\text{L}$  buffer was added to each well of the microtiter plate, followed by 10  $\mu\text{L}$  standard positive control or sample in the respective wells. After a 24-hour incubation at  $37^{\circ}\text{C}$ , the wells were washed five times, and 100  $\mu\text{L}$  peroxidase-linked anti-HMGB1 monoclonal antibody was added to each well. After incubation for 2 hours at room temperature, the wells were washed five times, and 100  $\mu\text{L}$  substrate solution (prepared 5 minutes before use) was added to each well. After incubation for 30 minutes at room temperature, the reaction was arrested by adding 100  $\mu\text{L}$  stop solution (0.35 M  $\text{H}_2\text{SO}_4$ ). Optical density was measured at 450 nm, with 620 nm as a reference, using a microplate reader. The lower limit of detection according to our standard curves was 4.2 to 5.2 ng/mL. Cross-reaction with HMGB2 was less than 2%.

### Cytokine Measurements

Cytokines comprising IL-1 $\beta$ , IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN- $\gamma$ , soluble CD40 ligand (sCD40L), and TNF $\alpha$  in the vitreous fluid were measured by the Bio-Plex Pro Human Th17 Cytokine Assays (Bio-Rad Laboratories, Inc., Tokyo, Japan). In brief, the samples were diluted fourfold with the diluting solution, and centrifuged at 10,000g for 5 minutes. Fifty microliters supernatant was used for the cytokine assays in accordance with the manufacturer's instructions. The lower limits of detection according to our standard curves were 0.27 to 0.28 pg/mL for IL-1 $\beta$ , 1.3 to 4.3 pg/mL for IL-4, 0.9 to 2.6 pg/mL for IL-6, 2.0 to 3.7 pg/mL for IL-10, 1.5 to 2.0 pg/mL for IL-17A, 1.8 to 8.0 pg/mL for IL-17F, 5.0 to 20.5 pg/mL for IL-21, 4.6 to 5.0 pg/mL for IL-22, 7.0 to 26.1 pg/mL for IL-23, 1.2 to 1.4 pg/mL for IL-25, 2.8 to 4.8 pg/mL for IL-31, 3.3 to 7.8 pg/mL for IL-33, 3.2 to 3.9 pg/mL for IFN $\gamma$ , 3.2 to 7.4 pg/mL for sCD40L, and 0.3 to 2.4 pg/mL for TNF $\alpha$ .

### Statistical Analysis

High-mobility group box-1 and cytokines in vitreous fluid were analyzed by detection rate for each group (%) and level (ng/mL or pg/mL). The samples below detection limits were graded as "not detected," and the levels were assigned a numerical value of 0 pg/mL for statistical analysis. Detection rate was compared by Pearson's  $\chi^2$  test. Because the levels of HMGB1 and cytokines were not normally distributed, statistical comparisons were performed using the nonparametric Steel-Dwass multiple comparison test. Pearson's correlation coefficient test was used to examine the association between two parameters.  $P < 0.05$  was considered significant.



**FIGURE 2.** Effects of systemic corticosteroid therapy in ocular sarcoidosis patients at the time of vitrectomy on vitreous HMGB1 level and inflammation scores. Box plots of (A) vitreous level of HMGB1, (B) inflammation score in the anterior segment (AG), and (C) inflammation score in posterior segment (PG). No significant differences were found between patients treated with systemic corticosteroids and those not treated with corticosteroids.

## RESULTS

### Vitreous HMGB1 in Ocular Sarcoidosis and Association With Ocular Findings

Patient background and vitreous levels of HMGB1 in individual patients with ocular sarcoidosis are shown in Table 1. High-mobility group box-1 was detected in the vitreous of 23 of 24 patients with ocular sarcoidosis, and the level was apparently higher in patients complicated with VO than in those with CME or ERM (counting multiple complications), but with no significant differences (Fig. 1A). Vitreous level of HMGB1 showed an apparent positive relation with posterior segment inflammation score but not with anterior segment score (Figs. 1B, 1C), although the correlation was not statistically significant. At the time of vitrectomy, sarcoidosis patients treated with systemic corticosteroids and those not treated with corticosteroids did not differ significantly in vitreous HMGB1 level or anterior or posterior segment inflammation score (Fig. 2).

### Comparison of Detection Rates for Vitreous Cytokines and HMGB1 in Sarcoidosis, PDR, and Idiopathic ERM

Figure 3 compares the detection rates of cytokines and HMGB1 in the vitreous between sarcoidosis, PDR, and idiopathic ERM. Although IL-6 was detected in all samples in the three groups,

there were significant differences in detection rates for IL-4, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-31, IFN- $\gamma$ , sCD40L, TNF $\alpha$ , and HMGB1. Detection rates for IL-4, IL-17A, IL-21, IL-22, IL-31, sCD40L, and TNF $\alpha$  were significantly higher in the PDR group than in the sarcoidosis or ERM group, whereas the rates for IL-10, IL-17F, IFN- $\alpha$ , and HMGB1 were significantly higher in the sarcoidosis group than in the PDR or idiopathic ERM group.

### Comparison of Vitreous Levels of Cytokines and HMGB1 in Sarcoidosis, PDR, and Idiopathic ERM

Vitreous levels of HMGB1 in the sarcoidosis, PDR, and idiopathic ERM groups are shown in Table 2 and Figure 4. The median and mean  $\pm$  SD of vitreous HMGB1 were 7.59 and  $12.1 \pm 11.8$  ng/mL in the sarcoidosis group, 6.01 and  $5.64 \pm 2.64$  ng/mL in the PDR group, and 4.51 and  $3.26 \pm 2.41$  ng/mL in idiopathic ERM group. Vitreous HMGB1 level was highest in sarcoidosis, followed by PDR and then idiopathic ERM. There were statistical differences between the sarcoidosis and ERM groups ( $P < 0.0001$ ), between the PDR and ERM groups ( $P < 0.0001$ ), and between the sarcoidosis and PDR groups ( $P = 0.0357$ ; Fig. 4). Next, the Th-related and proinflammatory cytokines with detection rates higher than 25% were selected, and the vitreous levels of these cytokines were compared between the three groups (Fig. 5). In sarcoidosis, vitreous levels of IL-6, IL-10, IL-31, IFN- $\gamma$ , sCD40L, and TNF $\alpha$  were significantly higher compared with idiopathic ERM, whereas

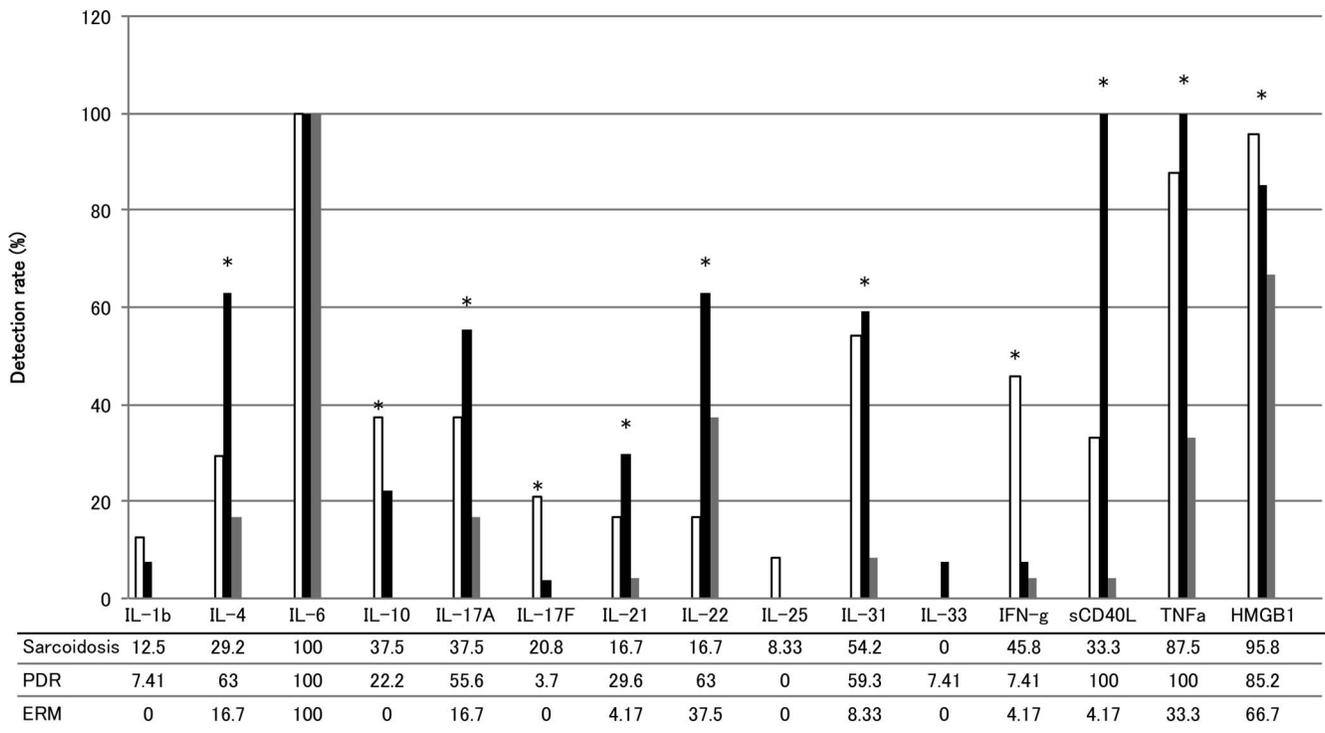


FIGURE 3. Detection rates of vitreous cytokines and HMGB1 in sarcoidosis, PDR, and idiopathic ERM groups. Detection rates of vitreous IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-25, IL-31, IL-33, IFN-γ, sCD40L, TNFα and HMGB1 in sarcoidosis (white bars), PDR (black bars), and idiopathic ERM (gray bars) are shown. Detection rates were compared by Pearson's  $\chi^2$  test. \* $P < 0.05$ .

IFN-γ and sCD40L levels were significantly higher compared with PDR.

### Correlation of Levels of HMGB1 With Th-Related Cytokines in the Vitreous of Sarcoidosis

Figure 6 presents the correlation of vitreous level of HMGB1 with those of cytokines with detection rates higher than 25% in the sarcoidosis group. A significant correlation was observed between HMGB1 and IL-10 ( $y = 0.4861x + 1.1015$ ,  $R^2 = 0.21446$ ,  $P < 0.05$ ), IFN-γ ( $y = 1.7679x + 5.9252$ ,  $R^2 = 0.18966$ ,  $P < 0.05$ ), and sCD40L ( $y = 2.1663x - 6.4664$ ,  $R^2 = 0.47905$ ,  $P < 0.0005$ ). No correlation was observed between HMGB1 and IL-4, IL-6, IL-17A, IL-31, or TNFα.

### DISCUSSION

High-mobility group box-1 has been identified as a critical molecule involved in many inflammatory conditions such as sepsis,<sup>27</sup> rheumatoid arthritis,<sup>28</sup> intestinal inflammatory disorder,<sup>29</sup> stroke,<sup>30</sup> and lung inflammatory diseases.<sup>31,32</sup> In the eye,

HMGB1 may be released from (1) inflammatory cells infiltrating the eye, such as macrophages, DC, and lymphocytes<sup>11-13</sup>; (2) parenchymal cells such as residential microglia,<sup>33</sup> astrocytes,<sup>34</sup> and Müller cells<sup>34</sup> by interaction with pathogenic T cells; and (3) tissue cells injured by intraocular inflammation. It

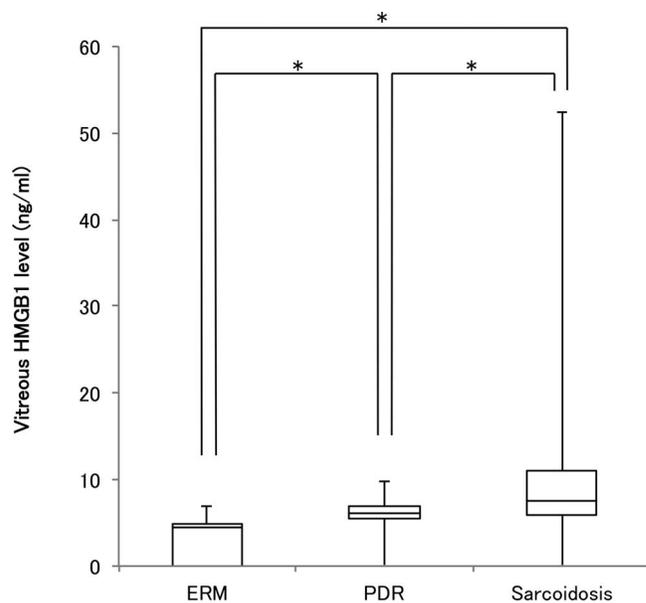
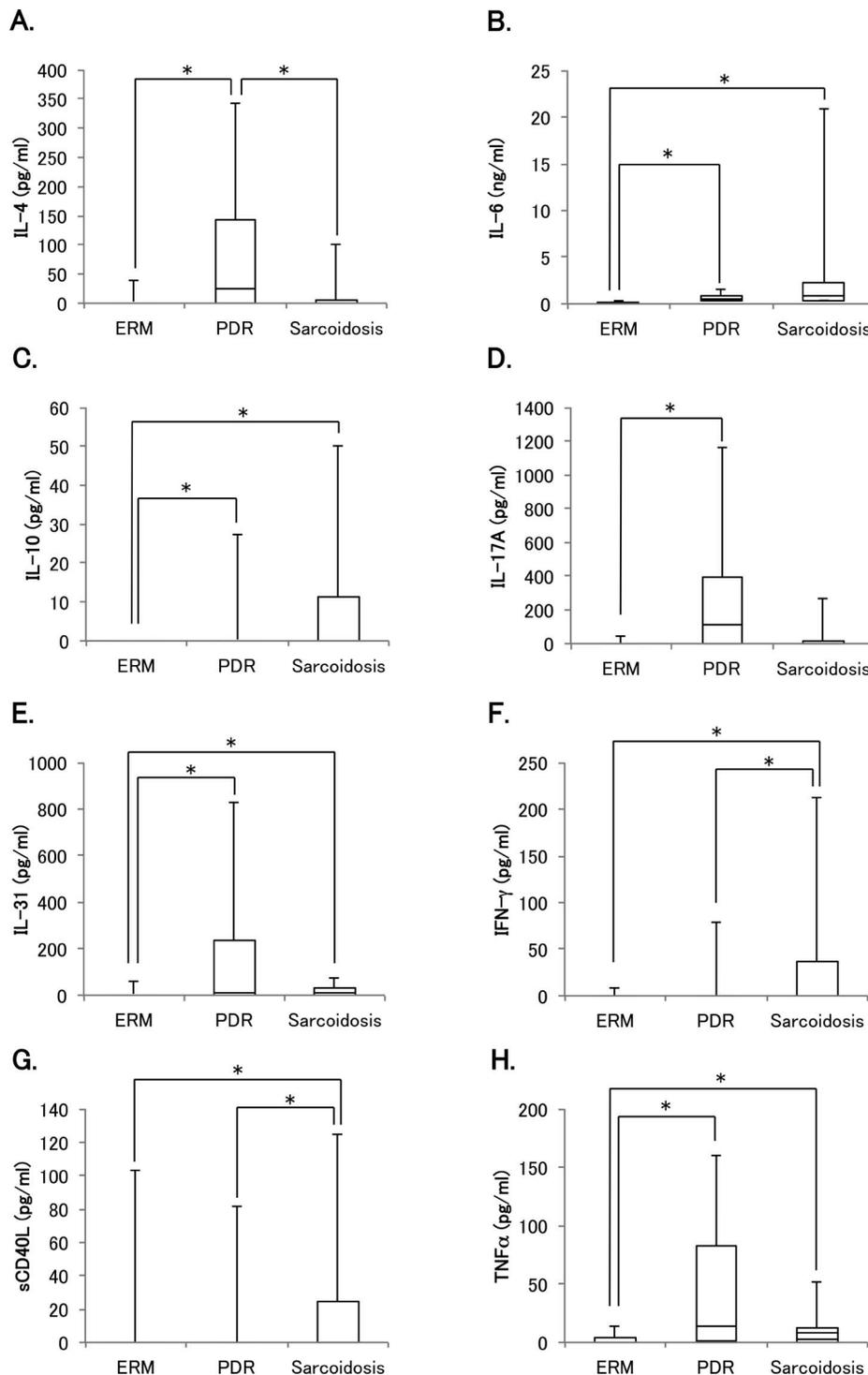


FIGURE 4. Vitreous levels of HMGB1 in sarcoidosis, PDR, and idiopathic ERM groups. Vitreous level of HMGB1 was significantly higher in the sarcoidosis group than in the PDR or ERM groups. \* $P < 0.05$ .

TABLE 2. Vitreous Levels of HMGB1 in Sarcoidosis, PDR, and Idiopathic ERM Groups

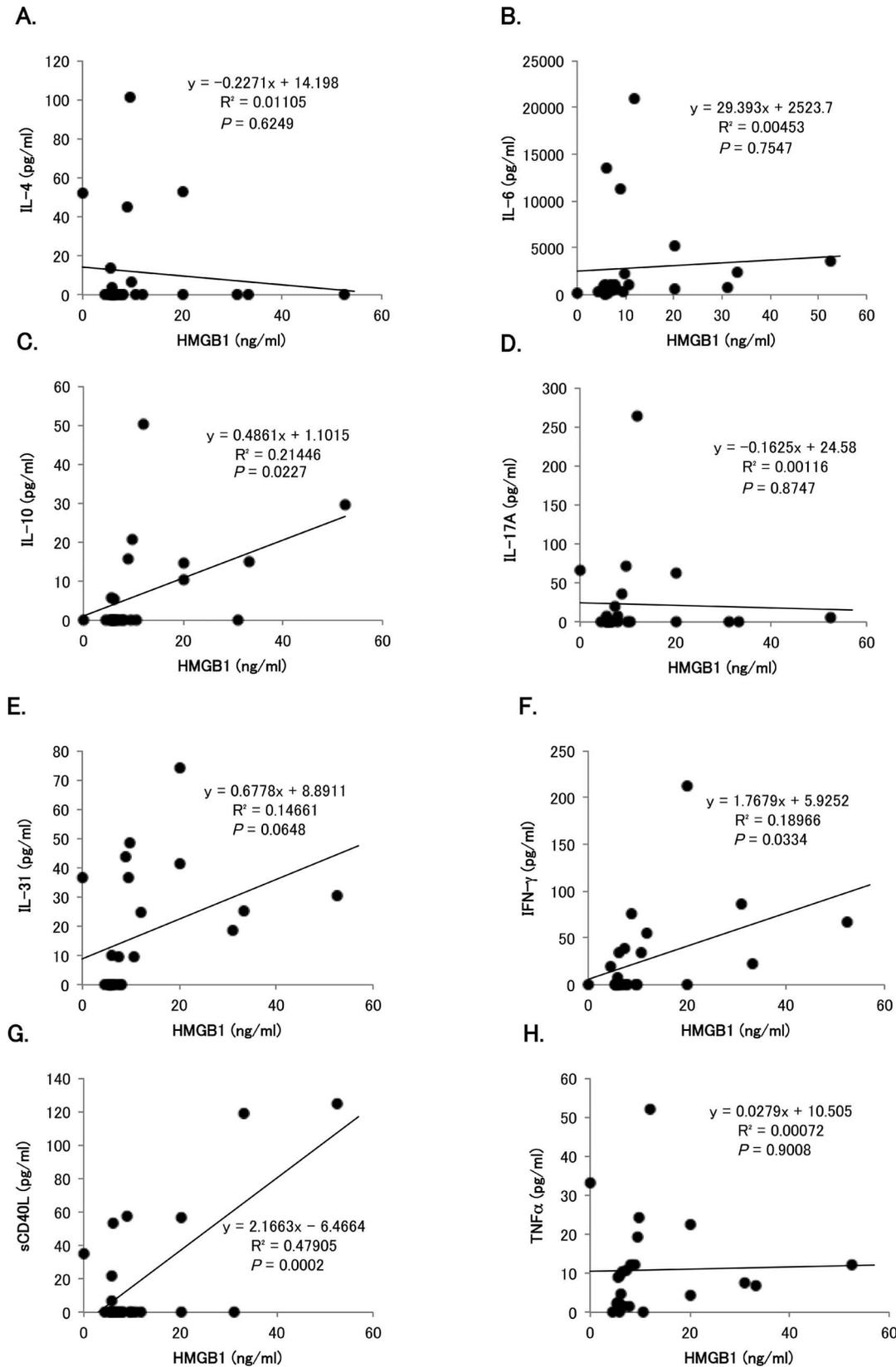
Vitreous Level	Sarcoidosis	PDR	Idiopathic ERM
Highest, ng/mL	52.5	9.84	6.99
Lowest	0	0	0
Median (75%-25%)	7.59 (11.0-5.83)	6.01 (6.96-5.54)	4.51 (4.85-0)
Mean ± SD	12.1 ± 11.8	5.64 ± 2.64	3.26 ± 2.41



**FIGURE 5.** Vitreous levels of Th-related and proinflammatory cytokines in sarcoidosis, PDR, and idiopathic ERM groups. Box plots of vitreous levels of (A) IL-4, (B) IL-6, (C) IL-10, (D) IL-17A, (E) IL-31, (F) IFN- $\gamma$ , (G) sCD40L, and (H) TNF $\alpha$  are shown. In the sarcoidosis group, vitreous levels of IL-6, IL-10, IL-31, IFN- $\gamma$ , sCD40L, and TNF $\alpha$  were significantly higher compared with the idiopathic ERM group, whereas IFN- $\gamma$  and sCD40L were significant higher compared with the PDR group. \* $P < 0.05$ .

is conceivable that HMGB1, together with other cytokines, triggers inflammatory cascades in ocular sarcoidosis to sustain the activation of pathogenic T cells and the damage they invoke in the retina, although the primary mechanism that releases HMGB1 is unknown.

Vitreous levels of HMGB1 in patients with ocular sarcoidosis did not correlate with inflammation scores in the anterior segment and posterior segment. There may be a temporal difference between the progression of uveitis based on clinical findings and elevation of HMGB1 in the vitreous. It has been



**FIGURE 6.** Association between vitreous level of HMGB1 with those of Th-related cytokines in sarcoidosis patients. Correlation of vitreous level of HMGB1 with those of (A) IL-4, (B) IL-6, (C) IL-10, (D) IL-17A, (E) IL-31, (F) IFN- $\gamma$ , (G) sCD40L, and (H) TNF $\alpha$  in patients with ocular sarcoidosis are shown. Vitreous level of HMGB1 was significantly associated with those of IL-10, IFN- $\gamma$ , and sCD40L.

shown that elevation of HMGB1 in intraocular fluids has been shown to precede histopathologic changes in the EAU induced by adoptive transfer of uveitogenic T cells.<sup>35</sup>

There were no significant differences in vitreous HMGB1 level and anterior and posterior segment inflammation scores between patients with ocular sarcoidosis treated with systemic corticosteroids and those without steroid treatment at the time of vitrectomy. These results suggest that vitrectomy was performed in the remission phase of uveitis in most of the sarcoidosis patients, irrespective of systemic treatment with corticosteroids.

Interaction of HMGB1 with TLR2, TLR4, TLR9, and RAGE leads to activation of NF- $\kappa$ B, which results in upregulation of proinflammatory cytokines such as IL-1 $\beta$ , IL-6, and TNF $\alpha$ . The production of proinflammatory cytokines via NF- $\kappa$ B activation is also promoted by these cytokines in an autocrine/paracrine manner. However, the vitreous level of HMGB1 in ocular sarcoidosis was not associated with vitreous level of IL-6 or TNF $\alpha$  in the present study. Other than HMGB1, various ligands for TLRs exist, which also leads to production of proinflammatory cytokines via NF- $\kappa$ B. This would explain the lack of association between vitreous levels of HMGB1 and IL-6 or TNF $\alpha$  in ocular sarcoidosis.

Accumulation of T cells with various cytokine profiles in the eye have been reported in different types of chronic uveitis.<sup>36,37</sup> The CD4/CD8 ratio of T cells infiltrating the vitreous is elevated in ocular sarcoidosis compared with other etiologies of uveitis,<sup>38</sup> and T-cell clones established from the vitreous T cells of ocular sarcoidosis patients produce a large amount of inflammatory cytokines including IL-1 $\alpha$ , IL-6, and IL-8.<sup>39</sup> Nagata et al.<sup>40</sup> reported that vitreous levels of IL-1 $\alpha$ , IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IFN- $\gamma$ , and TNF $\alpha$  were elevated in ocular sarcoidosis patients compared with idiopathic ERM patients. In the present study, although there was no significant difference in vitreous IL-4 level between the ocular sarcoidosis group and the idiopathic ERM group, vitreous levels of IL-6, IL-10, IFN- $\gamma$ , and TNF $\alpha$  were significantly elevated in the ocular sarcoidosis group compared with the idiopathic ERM group (Fig. 5), consistent with the report of Nagata et al.<sup>40</sup>

We previously demonstrated that serum CXCL9 and CXCL10 levels are markedly augmented in patients with ocular sarcoidosis compared with healthy subjects. These results indicate a role of CXCL9 and CXCL10 in the mechanisms that account for migration of CXCR3-expressing activated Th1 cells to the eye. In EAU, retinal cells release HMGB1 into the intraocular fluid via an interaction with pathogenic T cells, and systemic or local administration of anti-HMGB1 antibodies suppresses EAU by inhibiting pathogenic T-cell proliferation and their IFN- $\gamma$  and IL-17 production.<sup>35</sup> It is conceivable that Th1 cells that produce IFN- $\gamma$  promote release of HMGB1 in the vitreous in ocular sarcoidosis and that they are synergistically involved in the development of ocular inflammation.

Although IL-10 is mainly produced by Th2 cells and regulatory T cells and intrinsically plays an immunosuppressive role in most inflammatory conditions, vitreous IL-10 level was elevated in ocular sarcoidosis in this study. Augmented IL-1 $\beta$ , IL-6, and TNF $\alpha$  productions by alveolar macrophages and peripheral mononuclear cells have been reported in patients with sarcoidosis,<sup>41,42</sup> whereas IL-10 is also increased in serum and bronchoalveolar lavage fluid. Up-regulated IL-10 production may be a compensatory response to enhanced expression of proinflammatory cytokines to down-regulate the inflammatory process.

The inflammatory process is involved in the pathogenesis of diabetic retinopathy (DR), and various systemic and local factors are involved in the progression of DR to the proliferative stage.<sup>43,44</sup> Vitreous levels of IL-1 $\beta$ , IL-6, and TNF $\alpha$  are elevated in patients with PDR,<sup>45,46</sup> and we also demon-

strated that vitreous IL-4, IL-6, IL-17A, IL-21, IL-22, IL-31, and TNF $\alpha$  are up-regulated in PDR compared with ERM or macular hole.<sup>47</sup> Because vitrectomy was performed during the remission phase of systemic and intraocular inflammation in most of the ocular sarcoidosis patients in this study, this may partially explain why their vitreous TNF $\alpha$  levels were lower compared with that of patients with PDR. On the other hand, HMGB1 was detected in 66.7% and IL-6 in 100% of the vitreous samples of ERM patients. Interferon- $\gamma$  and TNF $\alpha$  were also detected in the vitreous of ERM, although the detection rates were low. It is possible that unidentified inflammatory process may be involved in the pathogenesis.

Although increased numbers of IL-17A-producing Th17 cells in bronchoalveolar lavage fluid, blood, and granulomatous tissue have been reported in sarcoidosis patients,<sup>48,49</sup> we found no increases in the detection rate or the vitreous level of IL-17A and no association between IL-17A and HMGB1 in the present study. However, a previous study also observed no increase in vitreous IL-17A level in ocular sarcoidosis.<sup>40</sup> It is possible that Th17 cells do not play a pivotal role in the development of ocular inflammation in sarcoidosis with granulomatous formation.

In the present study, HMGB1 was elevated in the vitreous of ocular sarcoidosis patients and correlated significantly with increases in IL-10, IFN- $\gamma$ , and sCD40L, but not with proinflammatory or Th17-related cytokines. These results suggest that HMGB1 released from inflammatory cells infiltrating the eye, resident cells interacting with pathogenic T cells, and/or injured tissue cells modulates inflammatory process by activating pathogenic Th1 cells and IL-10-producing regulatory T cells in the eye of sarcoidosis patients.

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