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ORIGINAL ARTICLE

Research and Analysis of Molecules such as CD28, CD45RA, CD45RO, CD38, HLA-DR, and CD57 on T Cells in Multiple Myeloma

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SUMMARY

Background: **For many years it has been postulated that the immune system controls the progress of multiple myeloma (MM). However, the phenotypes of T cells in MM remain to be elucidated. In this study, we compared the phenotypes of T cells, which were obtained from the peripheral blood, in MM patients with those in healthy donors (HD). The expression of CCR7, CD57, CD28, HLA-DR, CD38, CD45RA, and CD45RO were assessed on T cells from MM patients and HDs using multicolor flow cytometry (MFC).**
Methods: **For this study, 17 newly diagnosed MM patients were selected, and 20 healthy people were selected as a control group. MFC was used to detect the markers on T cells.**
Results: **We detected significant increases in the expression levels of HLA-DR, CD38, and CD57on $CD8^+$ T cells, significant decreases in the expression levels of CD28 and CD45RA on $CD8^+$ T cells, and a decrease of $CD4^+$ effector T cells in MM patients, compared to the HD group.**
Conclusions: **Our study shows that the accumulation of peripheral $CD8^+CD57^+$T cells, $CD8^+CD38^{high}$ T cells, and $CD8^+HLA\text{-}DR^+CD38^{high}$ T cells is reflective of an ongoing antitumor T cell response and a progressive immune dysfunction in MM. During chemotherapy, the recovery of immune function can be monitored by detecting the proportion of activated molecules of T lymphocytes.**
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KEYWORDS

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INTRODUCTION

Multiple myeloma (MM) is a clonal B-cell malignancy characterized by an increased blood calcium level, renal failure, anemia, and bone lesions (CRAB). MM occurs as a consequence of complex interactions between plasma cells (PCs) and a variety of accessory cells in the bone marrow (BM) microenvironment. The immune system plays an active role in controlling the progress of MM. T cells have evolved to mount a protective response against tumor cells. This is particularly governed by the intricate balance between the activation and the inhibitory signals. The activation, exhaustion, and se-

nescence of T cells are very important for the staging and clinical prognosis of MM [1]. Our study focused on the markers of CD28, CD57, CD45RA, CD45RO, CCR7, CD38, and HLA-DR on T cells.

MATERIALS AND METHODS

Subjects

From June, 2021, to October, 2022, 17 newly diagnosed MM patients, according to the International Myeloma Working Group criteria, were admitted to the hospital, and served as the study group. In addition, 20 healthy donors (HDs) were selected as the control group. Peripheral blood samples were obtained from MM patients and from HD patients.

Instruments

The following antibodies were obtained from Becton, Dickinson and Company (BD) for the flow cytometry assays: CD3, CD45, CD8, CD4, CD28, HLA-DR, CD38, CD57, CD45RA, CD45RO, CCR7. Blood samples were lysed with lysing solution (BD). Data were acquired on Flow Cytometer (Beckman Coulter Navios) and analyzed using Kaluza software (Gating Strategy Figure 1).

Statistical analysis

Data were analyzed using SPSS Statistics vs25.0 software (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). Groups were compared using the independent samples *t*-test. Data were reported as means ± SE. A p-value < 0.05 denoted a statistically significant difference.

RESULTS

Co-stimulation marker of CD28 and differentiation marker of CD45RA

$CD4^+CD45RA^+CD28^+$ and $CD8^+CD45RA^+CD28^+$ T cells were lower in the MM group than in the HD group (19.26 ± 18.84% versus 25.20 ± 12.04%, $p > 0.05$ and 10.80 ± 14.25% versus 21.17 ± 12.19%, $p < 0.05$) (Figure 2a). The cytotoxic T cells ($CD8^+CD28^+$) in the MM group were significantly decreased compared to those in the HD group (37.12 ± 29.84% versus 59.54 ± 16.89%, $p < 0.05$). The suppressor T cells ($CD8^+CD28^-$) in the MM group were significantly increased compared to those in the HD group (62.93 ± 29.74% versus 40.09 ± 16.86%, $p < 0.05$) (Figure 2b).

The senescent marker of CD57

$CD4^+$ senescent T cells ($CD28^-CD57^+$) and $CD8^+$ senescent T cells ($CD28^-CD57^+$) were higher in the MM group than in the HD group (15.49 ± 17.92% versus 4.82 ± 4.52%, $p < 0.05$ and 27.93 ± 17.11% versus 20.78 ± 10.69%, $p > 0.05$) (Figure 3).

The activation markers of HLA-DR and CD38

$CD8^+CD38^{high}$ T cells were higher in the MM group than in the HD group (11.06 ± 14.01% versus 3.89 ± 4.54%, $p < 0.05$) (Figure 4a).

$CD4^+CD38^{high}$ T cells in the MM group were significantly decreased compared to those in the HD group (7.85 ± 6.26% versus 12.19 ± 5.16%, $p < 0.05$) (Figure 4a).

$CD8^+CD38^{high}$ $HLA\text{-}DR^+$ T cells were higher in the MM group than in the HD group (9.07 ± 8.08% versus 4.12 ± 4.72%, $p < 0.05$) (Figure 4b).

$CD4^+$ and $CD8^+$ effector T cells

$CD4^+$ effector T cells ($CCR7^-CD45RO^+$) in the MM group were significantly decreased compared to those in the HD group (24.28 ± 17.42% versus 33.02 ± 9.22%, $p < 0.05$).

$CD8^+$ effector T cells ($CCR7^-CD45RO^+$) in the MM group were slightly increased compared to those in the HD group (11.32 ± 15.96% versus 6.20 ± 2.63%, $p > 0.05$) (Figure 5).

DISCUSSION

MM results from the consequences of complex reactions between PCs and a variety of accessory cells in the bone marrow (BM) microenvironment. The immune system plays an active role in controlling the progress of MM. T cells have evolved to mount protective responses against tumor cells. This is particularly governed by the intricate balance between the activation and inhibitory signals.

For the activation, differentiation, and survival of naive T cells, three essential signals must be required, such as costimulatory receptors and cytokine receptors on the surface of T cells. CD28 is expressed by naive T cells after antigen recognition, that may bind to B7 proteins to provide co-stimulatory signals. According to CD28 expression, CD8 T cells can be subdivided into cytotoxic ($CD28^+$) and suppressor ($CD28^-$) T cells. However, continuous T cell stimulation and activation lead to gradual loss of CD28. Downward regulation of CD28 is an important marker of senescent T-cells, and $CD8^+$-$CD28^-$ senescent T cells have an immunosuppressive function in tumor. In our study, $CD8^+$ $CD28^+$ cytotoxic T cells were decreased and $CD8^+$ CD28- suppressor T cells were increased in MM patients.

$CD57^+$ T cells are closely related to tumor immunity. The expression of CD57 on T cells is known to be associated with cytotoxic activity, enhanced effector functions, and late-differentiated phenotype. The CD57 antigen is commonly used to identify populations of late-differentiated 'senescent' cells [2]. The frequency of CD57 expression is related to the clinical prognosis of various cancers. In the late phases of differentiation, CD57 has been found to increase on $CD4^+$ and $CD8^+$ T cells. CD57 identifies terminally differentiated cells with decreased proliferative responses in $CD8^+$ T lym-

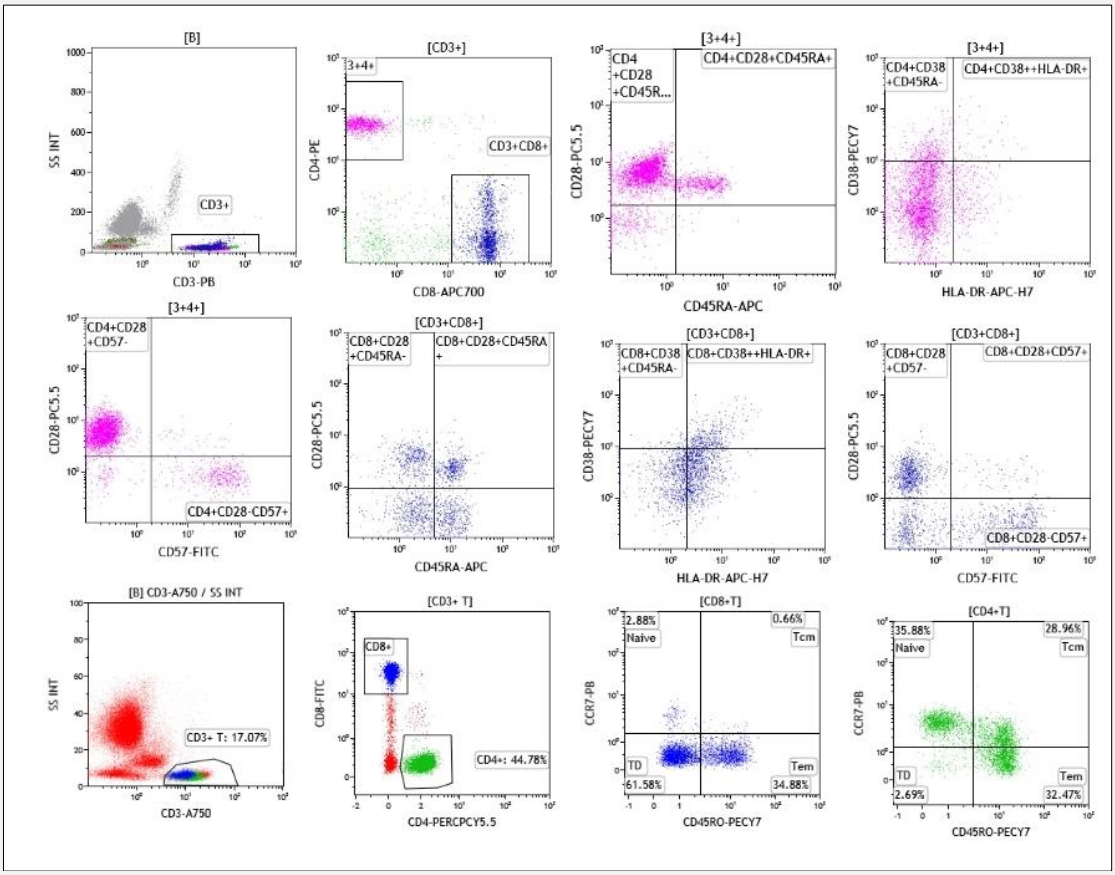

Figure 1. Gating strategy.

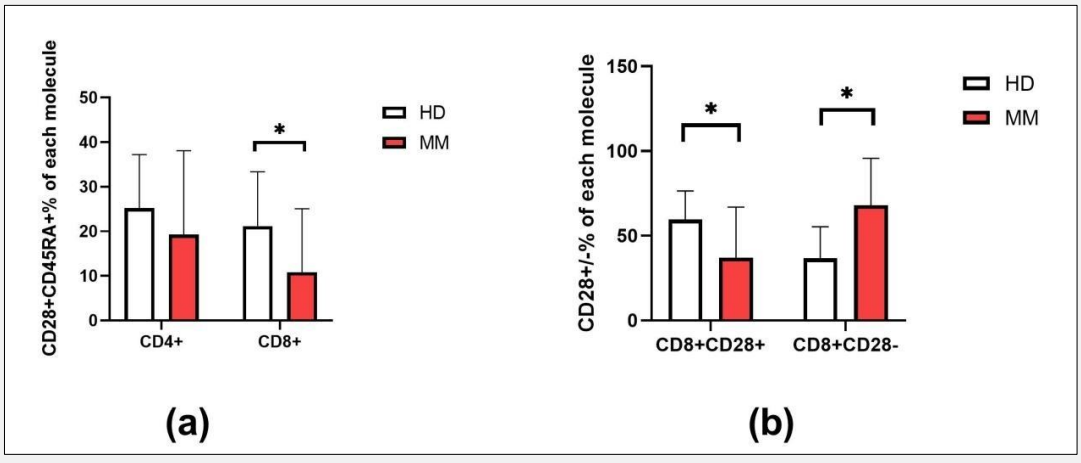

Figure 2. The level of CD28 and CD45RA on T cells in two groups.

a. $CD4^+CD45RA^+CD28^+$ T cells in two groups. b. $CD8^+CD28^+$ cytotoxic T cells and $CD8^+CD28^-$ suppressor T cells in two groups, * $p < 0.05$.

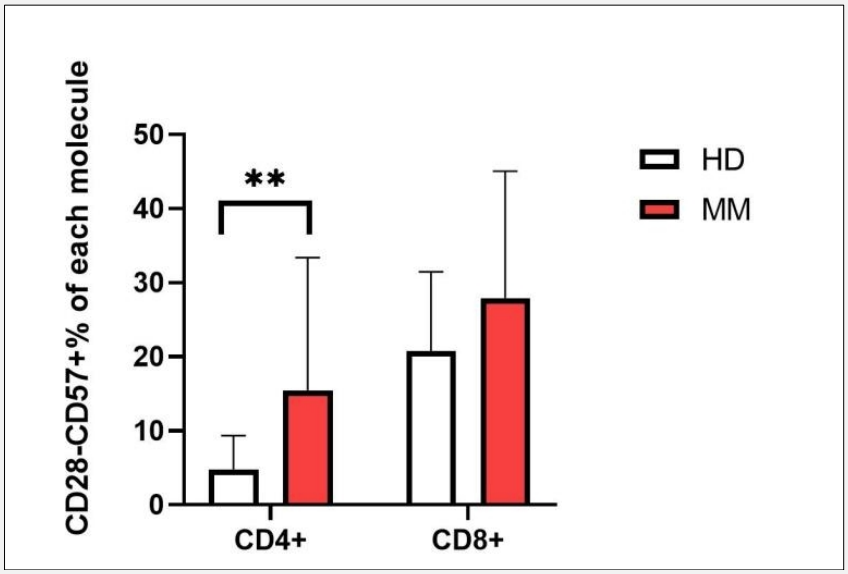

Figure 3. $CD4^+CD28^-CD57^+$ senescent T cells in two groups. $CD4^+$senescent T cells ($CD28^-CD57^+$) and $CD8^+$ senescent T cells ($CD28^-CD57^+$) in two groups, * p < 0.05.

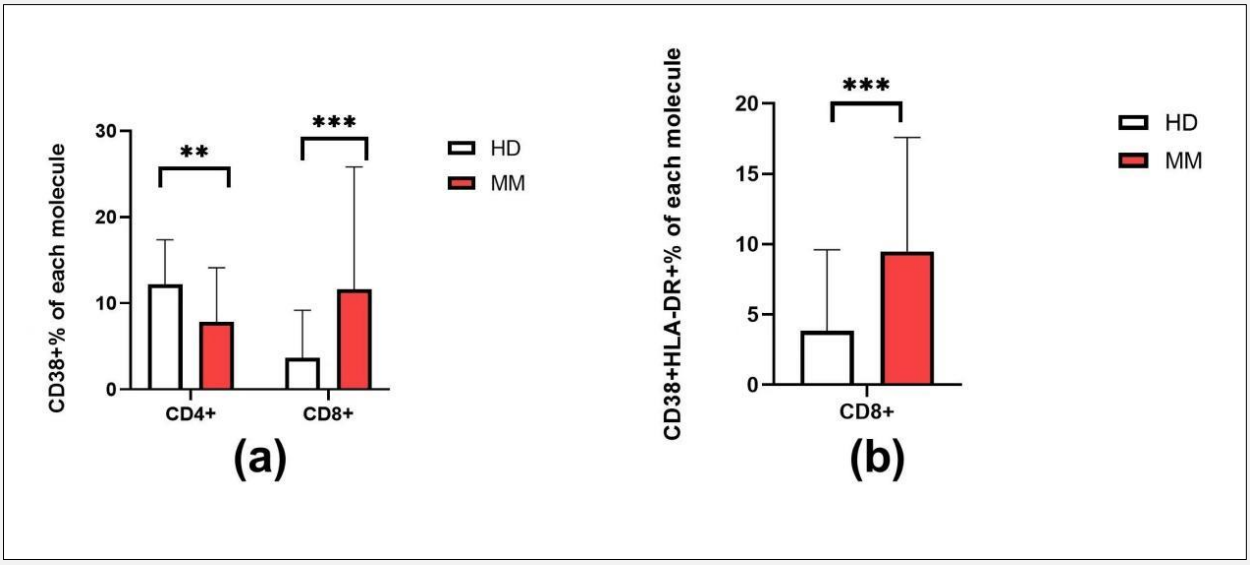

Figure 4. $CD8^+CD38^{high}$ T cells, $CD4^+CD38^{high}$ T cells, and $CD8^+CD38^{high}$ HLA-DR^+ T cells in MM patients (red) and in HD controls (gray).

a. $CD8^+CD38^{high}$ T cells and $CD4^+CD38^{high}$ T cells in two groups. b. $CD8^+CD38^{high}$ HLA-DR^+ T cells in two groups, ** p < 0.01, *** p < 0.001.

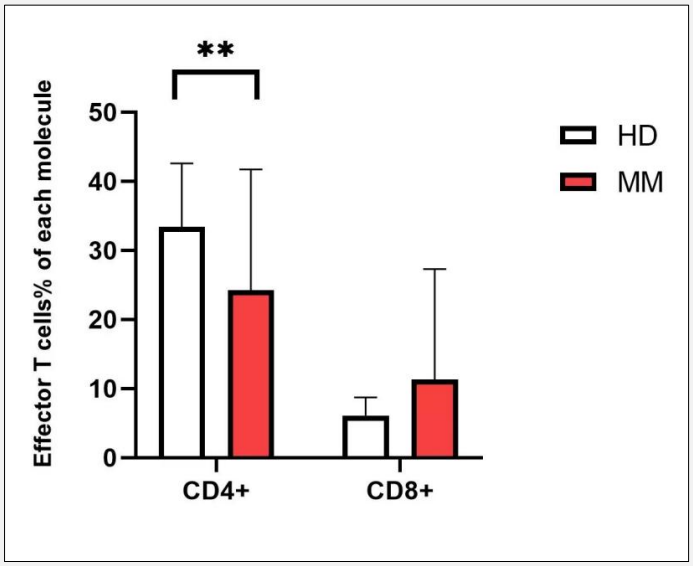

Figure 5. $CD4^+CCR7^-CD45RO^+$ and $CD8^+CCR7^-CD45RO^+$ effector T cells in two groups, ** $p < 0.01$.

phocytes. Senescent markers are more associated with $CD8^+$ T cells than with $CD4^+$ T cells in the human peripheral blood, consistent with our results that CD57 accumulate at a lower frequency for $CD4^+$ T cells [3]. Accumulation of CD57 expression on $CD8^+$ T cells correlated with age-related immunosenescence, chronic viral stimulation, and cancer development [4,5]. So, CD57 should be used as a new strategy against human immune aging, chronic diseases, and various tumors. This is consistent with our results that terminally or memory differentiated cells, such as $CD4^+CD28^-CD57^+$ T cells and $CD8^+CD28^-CD57^+$ T cells were increased in MM patients. The higher level of $CD8^+CD28^+CD57^+$ T cells in MM confirms that the cytotoxicity is weak and the immune suppression is strong.

Ectonucleotidases, that regulate the extracellular concentration of nucleotides, are also considered pivotal in modulating T cell response [6]. CD38, another critical ectonucleotidase, has got prominence as an important regulator of T cell activation and function [7,8]. The CD38 molecule was reported to be involved in triggering activation and proliferation signals. CD38 responses to stimulation through cytokines, endotoxins, and interferon [9-11]. $CD38^+$ T cells have many functions, such as the cytotoxicity and the immunosuppression. Increased CD38 activity results in $CD8^+$ T cell suppression [12]. High levels of CD38 on $CD4^+$ T cells are associated with regulatory properties [13,14]. More recently, CD38 has been described to be a part of the Treg transcriptional signature [15,16]. On the surface of $Foxp3^+CD4^+$ T cells, high levels of CD38 correlate strongest with $CD4^+$ regulatory T cells [17]. $CD8^+CD38^{high}$ T cells inhibit the proliferation of $CD4^+$ effector T cells. High expression of CD38 has often been found to be associated with several hematological malignancies [18, 19], for example: the pathogenic role of CD38 has been implicated in MM, where tumor cells have a high surface expression of CD38 [20,21]. Likewise, CD38 expression was reported in other hematological tumors. A similar observation has been reported in the cases of esophageal and colorectal cancer patients, where the high levels of CD38 were related with poor survival [22]. Recent findings also indicate that the expression of CD38 can act as a negative regulator. In MM, CD38 is implicated in promoting more aggressive immunosuppressive myeloid-derived suppressor cells (MDSCs) and Tregs [23]. Reported subpopulation of $CD38^+$ Treg was found to be more immunosuppressive than $CD38^-$ Tregs, suggesting an additional mechanism of action for the anti-CD38 antibody used to treat MM patients [24]. The expansion of activated $CD8^+$ T cells is characterized by the expression of CD45RO and HLA-DR antigens [25,26].

It is now recognized that naive T lymphocytes express CD45RA which is lost after activation and replaced by CD45RO. Therefore, $CD45RA^+$ T cells were initially considered to be naive T cells that were the precursors of $CD45RO^+$ T cells that contained the memory T cells [27]. T cell differentiation can be defined by the expression levels of CD27, CD28, and CD45RA. Highly differentiated end-stage T cells ($CD45RA^+CD27^-CD28^-$) also express surface inhibitory receptors such as CD57.

In our study, we confirmed $CD4^+CD28^+CD45RA^+$ naive T cells and $CD8^+CD28^+CD45RA^+$ naive T cells were decreased in MM patients. $CD8^+$ T cells can display a dysfunctional profile, induced by chronic antigen stimulation or by tumors. In our study, $CD8^+CD38^{high}$ T cells, which can inhibit the function of $CD4^+$ effector T cells, were increased. $CD4^+CD38^{high}$ T cells were decreased, because the immunosuppressive effects of $CD38^+$ Tregs were stronger than those of $CD38^-$ Tregs.

CONCLUSION

In summary, immune dysfunction is an important mark for the course of the MM disease. In our study, the MM group presented a higher level of activation markers (HLA-DR, CD38) and senescence marker CD57, a decreased level of the co-stimulation marker CD28, and the differentiation status marker CD45RA on the $CD8^+$ T cells in MM patients. Functional, tumor specific $CD8^+$ cytotoxic T cells and $CD4^+$ effector T cells were decreased. The $CD8^+CD28^-$ population also presented cell senescence which has been increasingly described in many cancers. In many tumors, $CD8^+CD28^-$ T cells have immunosuppression and are resistant to immunotherapy. This provides information regarding the diagnosis and the treatment for MM, and with that, ensures the best outcomes for patients with MM.

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Declaration of Interest:
None of the authors have a conflict of interest to disclose.

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