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Expression and Function of B7 Family Molecules in Hematologic Malignancies

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Introduction

B7 family molecules, which play an important role in the immune response by costimulating or coinhibiting T cells via antigen-T-cell receptor interactions, are expressed not only on professional antigen-presenting cells but also on some tumor cells. We previously reported that the expression of B7.2 and B7-H2 on leukemic cells was associated with poor prognosis in acute myeloid leukemia (AML)[1], and that B7-H1 molecules on tumor cells could deliver negative signals through PD-1 and other receptors on tumor-specific cytotoxic T lymphocytes (CTLs), resulting in the inhibition of antitumor immune responses[2,3]. Multiple myeloma (MM) and myelodysplastic syndromes (MDS) are hematologic malignancies with poor prognosis, characterized by monoclonal growth of plasma cells (myeloma cells) and cytopenias with a risk of progression to AML, respectively. Furthermore, in these diseases, T-cell dysfunction has been reported, which may weaken antitumor immune responses in patients. In the current study, we investigated whether functional B7.2, B7-H1, and B7-H2 molecules are expressed on tumor cells in the hematologic malignancies MM and MDS and, if so, whether these molecules are associated with the pathophysiology of these diseases.

Methods and Results

First, using flow cytometry (FCM), we examined the expression of B7 molecules, defined as B7.2, B7-H1, and B7-H2 in this study, on 14 human myeloma cell lines (HMCLs) and fresh plasma cells in bone marrow samples from 15 hematologically normal individuals (controls), 21 monoclonal gammopathy of undetermined significance (MGUS) patients, and 50 MM patients. The expression of B7.2, B7-H1, and B7-H2 molecules was detected in 7, 3, and 9 of 14 HMCLs, respectively. The percentages of $B7.2^+$, $B7\text{-}H1^+$, and $B7\text{-}H2^+$ cells in CD38-highly positive plasma cells were markedly higher in MM patients than in MGUS patients or controls (**Table 1**). B7.2 and B7-H2

Table 1 Expression of B7 family molecules on plasma cells from controls and MGUS and MM

	Control (n=15)	MGUS (n=21)	MM (n=50)
B7.2	15.7 ± 7.1	20.1 ± 21.8*	38.6 ± 30.4*
B7-H1	1.0 ± 1.2	2.7 ± 4.8	12.5 ± 19.8*
B7-H2	0.2 ± 0.5	0.4 ± 1.2	2.9 ± 9.6*

Data represent the percentage of B7-positive cells in plasma cell (%, mean ± SD).
*Significant difference ($P<0.05$) when the percentages of B7 expression were compared with those in hematologically normal controls.
The expression levels of B7.2, B7-H1, and B7-H2 in MM were significantly higher than those in MGUS ($P<0.05$).

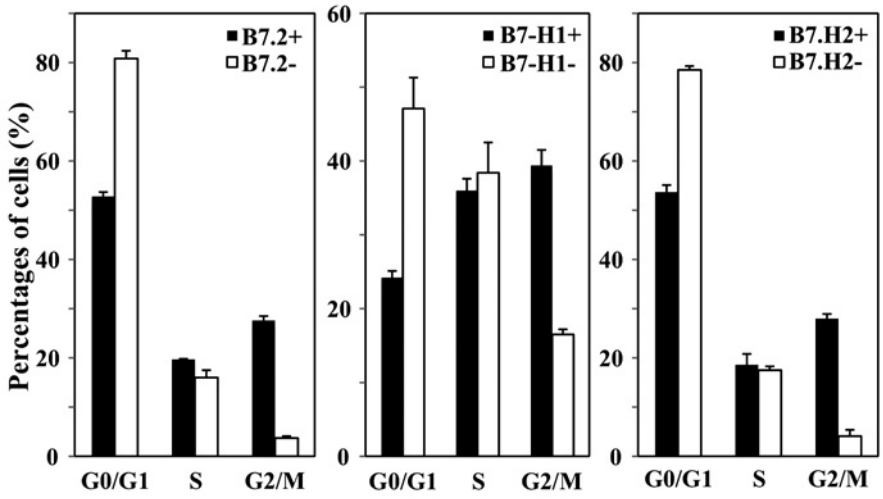

Fig. 1 Growth advantage of $B7^+$ myeloma cells. The cell cycle of $B7.2^+$ or $B7.2^-$ KMS-27 cells (left) , of $B7\text{-}H1^+$ or $B7\text{-}H1^-$ RPMI8226 cells (middle), and of $B7\text{-}H2^+$ or $B7\text{-}H2^-$ KMS-27 cells (right) was analyzed using FCM. Data are mean ± SD of three independent experiments.

expression was induced or enhanced by cultivation with tumor necrosis factor (TNF)-α or autologous stromal cells in vitro. Notably, the expression of B7.2, B7-H1, and B7-H2 on myeloma cells was augmented in the refractory stage compared with expression at the initial diagnosis. All of the above findings support the concept that these molecules are associated with disease progression in MM. Second, we investigated whether B7 expression on myeloma cells was associated with their proliferative potential. When the HMCLs KMS-27 and RPMI8226, some of which expressed B7.2/B7-H2 and B7-H1, respectively, were analyzed using FCM, $B7^+$ cells had significantly fewer G0/G1-phase cells and more G2/M-phase cells compared with $B7^-$ cells **(Fig. 1)**. Consistent with these results, when $B7^+$ and $B7^-$ cell populations were isolated and examined, $B7.2^+$, $B7\text{-}H1^+$, and $B7\text{-}H2^+$ HMCLs proliferated more rapidly in liquid cultures and formed more colonies in semisolid cultures. Furthermore, the $B7\text{-}H1^+$ cell population was more resistant to the therapeutic agents melphan and dexamethasone compared with the $B7\text{-}H1^-$ cell population. When B7.2 and B7-H2 expression was induced on RPMI8226 cells by cultivation with TNF-α, the cell cycle was clearly stimulated. Finally, we examined whether B7 molecules on myeloma cells affect T cell immunology. In the mixed lymphocyte-myeloma reaction using KMS-27 expressing both B7.2 and B7-H2 and $CD4^+$ T cells, an antagonistic monoclonal antibody (mAb) against B7.2 decreased the production of interleukin (IL)-10 as well as that of interferon (IFN)-γ and IL-2. Meanwhile, an antagonistic mAb against the inducible costimulator (ICOS), which blocks the B7-H2-ICOS pathways, decreased the production of IL-10 and IFN-γ but not that of IL-2. The finding that both B7.2 and B7-H2 molecules on

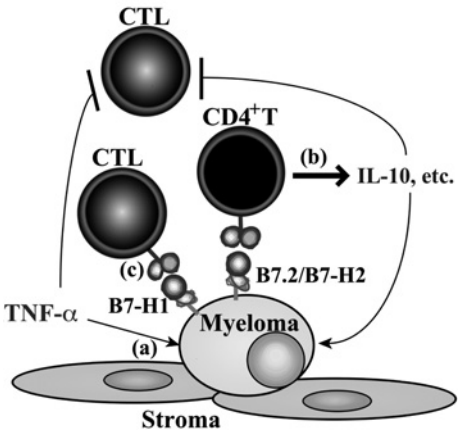

Fig. 2 New insight into the role of B7 molecules in myeloma biology. (a) In the bone marrow environment, TNF-α and stromal cell contact induce the expression of B7.2, B7-H1, and B7-H2 on myeloma cells. TNF-α also inhibits myeloma-specific CTLs. (b) B7.2 and B7-H2 molecules expressed on myeloma cells induce $CD4^+$ T cells to produce soluble factors such as IL-10, which stimulates myeloma cell proliferation and inhibits myeloma-specific CTLs. (c) B7-H1 molecules expressed on myeloma cells inhibit the proliferation of myeloma-specific CTLs. Furthermore, B7-expressing myeloma cells gain an intrinsic proliferative advantage.

myeloma cells enhance IL-10 production is particularly interesting because IL-10 not only reduces the antitumor immune response in general but is also a growth factor for myeloma cells. When B7-H1 expression was induced on RPMI8226 cells by cultivation with IFN-α, the cytotoxic activity of tumor-specific CTLs was inhibited in vitro. These results suggest that B7 molecules on myeloma may confer a growth advantage by inducing the production of the myeloma-stimulatory cytokine IL-10 by $CD4^+$ T cells and by inhibition of myeloma-specific CTLs (**Fig. 2**), in addition to the intrinsic advantage in cell proliferation of $B7^+$ myeloma cells[4].

We also investigated B7-H1 on tumor cells in MDS (MDS blasts). Consistent with the results in MM, our results showed that: 1) B7-H1 expression on blasts occurs more often in the advanced disease stages; 2) B7-H1 expression on blasts may be induced by cytokines derived from the bone marrow environment via nuclear factor κB activation; 3) cell cycling and proliferation are more active in $B7\text{-}H1^+$ blasts than in $B7\text{-}H1^-$ blasts; and 4) B7-H1 molecules on blasts suppress T-cell immunity.

Conclusion

Our results show that functional B7 molecules are expressed on hematologic tumors such as myeloma cells and MDS blasts and that these molecules may be associated with a proliferative advantage of tumor cells and therefore contribute to the pathophysiology of MM and MDS.

References

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