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HLA-DR expression on myeloid cells is a potential prognostic factor in patients with high-risk neuroblastoma

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Abbreviations: DC, dendritic cell; HR, high-risk; LR, low-risk; MDSC, myeloid-derived suppressor cell; PBMC, peripheral blood mononuclear cell

The adaptive immune system has been reported to play a dual role in many cancers, on one hand inhibiting tumor growth and, on the other hand, promoting disease progression, escape from cancer immunosurveillance and relapse. We have previously reported that the suppression of the adaptive immune response associated with high levels of myeloid-derived suppressor cells (MDSC) was evident in patients with low-risk neuroblastoma. Here, we report the results of a pilot study demonstrating that the amounts of HLA-DR positive or negative myeloid cells in the peripheral blood might predict disease outcome among individuals affected by high-risk neuroblastoma.

Neuroblastoma accounts for 7–8% of pediatric cancers, totaling about 9 cases/million children/year in the US. The Children's Oncology Group stratifies patients into a low-risk (LR) and a high-risk (HR) category based on age at diagnosis, International Neuroblastoma Staging System, tumor histopathology, DNA index, and *MYCN* amplification status. Treatment is tailored according to risk. Patients with LR neuroblastoma respond well to therapy,[1] whereas those with a HR profile generally have a poor long-term survival[2] and are refractory to multimodal therapy. Therefore, understanding why HR neuroblastoma patients respond differently to therapy is critical not only to avoid the overtreatment of those HR patients who are likely to respond to treatment, but also to develop therapeutic strategies that could overcome resistance to therapy. Multiple biomarkers have been suggested to predict the prognosis of neuroblastoma, including *MYCN* amplification, DNA ploidy, loss of chromosomes 1p and 11q, gain of chromosome 17q, as well as expression of proteins like TrkA (transforming tyrosine kinase) and MDR (multi drug resistance). Very recently, CD133 has been associated with the resistance of neuroblastoma cells to chemotherapy, in vitro.[3] Age has been shown to be an important prognostic factor, such that patients older than 18 mo are classified in the HR group and usually have a worse prognosis than younger individuals (generally included in the LR group).[4–7] Age also determined the development status of the adaptive immune system. Indeed, children with more than 1 y of age usually have a well-developed adaptive immune system as compared with neonates and younger children, who rather exhibit a well-developed innate immune system. Interestingly, several groups reported that cytokines/chemokines such as interleukin-1β (IL-1β), chemokine (C-X-C motif) ligand 12 (CXCL12), and CXCL4, which are involved in innate immune responses, play a critical role in the neuronal differentiation that is associated with LR neuroblastoma.[8–10] These observations suggest that a well-developed adaptive immune system may have a paradoxical role in the progression of neuroblastoma, being associated with poor, rather than improved, outcome. This is also the case of other neoplasms, in which adaptive immune responses play a dual function as they exert an antineoplastic activity on the one hand, and mediate tumor editing on the other. Such an editing of malignant cells by the adaptive immune system has been associated with disease relapse in many cancers.[11–15] In this respect, we have previously reported that patients with LR neuroblastoma exhibit high levels of HLA-DR$^-$ myeloid-derived suppressor cells (MDSCs) along with a diminished adaptive immune response as compared with their HR counterparts.[16] These findings suggests that MDSCs suppress adaptive immune responses in LR neuroblastoma patients.

In the present study, we sought to determine whether the amounts of circulating HLA-DR$^-$ or HLA-DR$^+$ myeloid cells may predict disease outcome in HR neuroblastoma patients. We analyzed the peripheral blood of patients with HR neuroblastoma

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Table 1. Patient characteristics

Patient	Disease category	Age at the time sample collected	Treatment rcvd	Current status
H01	Healthy volunteer	40m	NA	Healthy
H06	Healthy volunteer	16m	NA	Healthy
04NB	HR-G	22m	C, S, Rad, ASCT, mAb+RA	Healthy 36 min off Rx
17NB	HR-G	21m	C, S, Rad, Tan ASCT, mAb+RA	Healthy 24 min off Rx
03NB	HR-P	54m	C, S, Rad, Tan ASCT, RA	Relapse, deceased
18NB	HR-P	20m	C, S, Rad, Tan ASCT, mAb+RA, cyto/topo, Irino/Tmz, Avastin	Relapse, deceased
19NB	HR-P	17m	C, S, Rad, Tan ASCT, mAb+RA, Irino/Tmx, I-MIBG	Relapse, Cont Rx

Abbreviations: C, Chemo per ANBL0532; S, Surgery; Rad, Radiation; ASCT, Autologous stem cell transplant; Tan, Tandem; mAb, monoclonal antibody ch14.18; RA, cis retinoic acid; NB, neuroblastoma; HR, High risk; LR, Low risk; C*, 2 chemo given to relieve spinal compression; HR-G, high risk good prognosis; HR-P, high risk poor prognosis

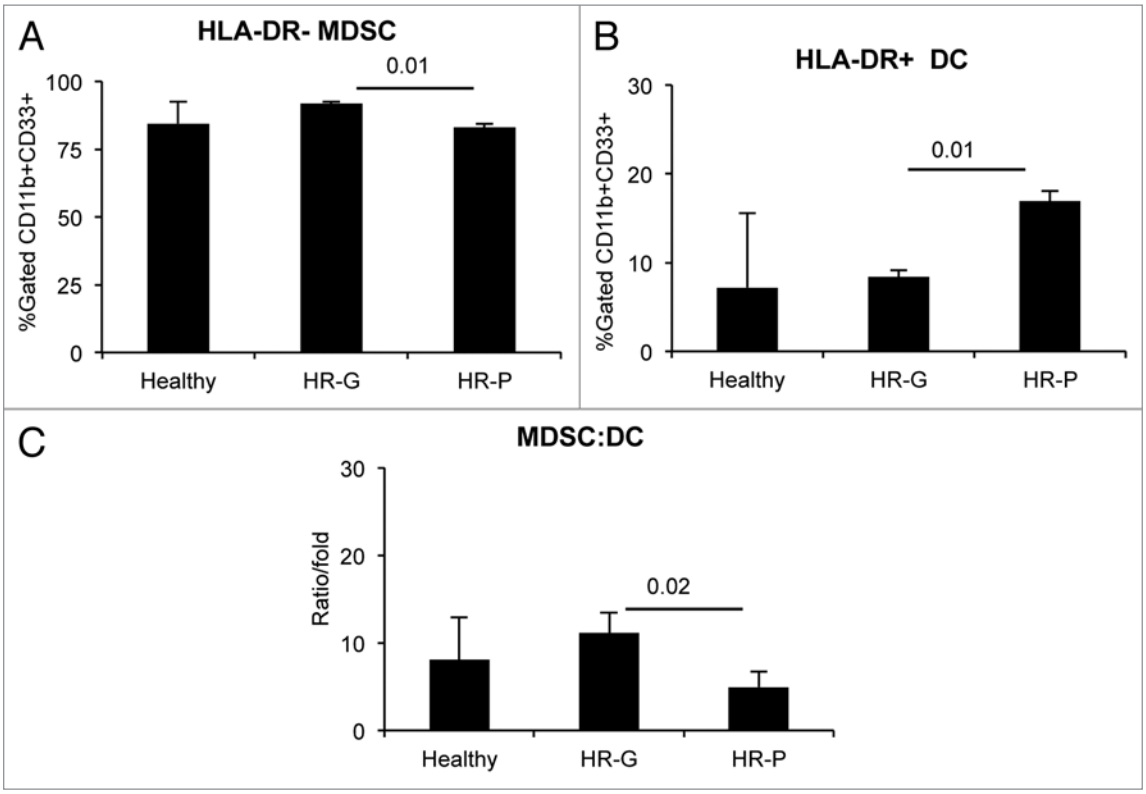

Figure 1. Levels of HLA-DR$^-$ or HLA-DR$^+$ myeloid cells in the blood of high-risk neuroblastoma patients. (**A and B**) Frequency of CD33$^+$CD11b$^+$HLA-DR$^-$ myeloid cells [HLA-DR$^-$ myeloid-derived suppressor cells, MDSCs, (**A**)] and CD33$^+$CD11b$^+$HLA-DR$^+$ myeloid cells [HLA-DR$^+$ dendritic cells, DCs, (**B**)] in the blood of healthy volunteers as well as patients with high-risk (HR) neuroblastoma who either responded (HR-G) or were refractory (HR-P) to therapy. (**C**) Ratio between HLA-DR$^-$ and HLA-DR$^+$ myeloid cells (MDSC:DC ratio) in the blood of healthy children of HR neuroblastoma patients of the indicated group.

and compared the cellular profiles of individuals who responded to therapy or were refractory to treatment. As pediatric neuroblastoma is a very rare disease, a limited number of patients were available for the collection of fresh blood sample for the analysis of MDSCs. We also included in the study 2 healthy volunteers for comparison purposes. Patient characteristics and treatment modalities are summarized in **Table 1**. Thus, blood was collected at diagnosis from these patients, peripheral blood mononuclear cells (PBMCs) were isolated and subjected to 3-color immunostaining followed by the analysis of CD33$^+$CD11b$^+$ myeloid cells, as previously described by our group.[16,17] Statistical comparisons between groups were made using unpaired, 2-tailed Students t-tests, with p values < 0.05 being considered as statistically significant. Interestingly, patients who responded well to therapy (n = 2) showed significantly higher levels of HLA-DR$^-$ myeloid cells, i.e., MDSCs, as compared with those who were refractory to therapy (n = 3) (**Fig. 1A**, p = 0.01).

A reverse correlation was observed on the levels of circulating HLA-DR$^+$ myeloid cells, i.e., dendritic cells (DCs). Thus, patients who responded to therapy showed significantly lower levels of DCs than individuals who were refractory to treatment (**Fig. 1B**, p = 0.01). Finally, patients who responded to therapy showed a greater ratio of HLA-DR$^-$ to HLA-DR$^+$ myeloid cells, MDSC:DC ratio, than children who failed to do so (**Fig. 1C**, p = 0.02). These data suggest that the circulating levels of HLA-DR$^-$ myeloid cells may constitute a prognostic/predictive indicator of disease outcome in patients with HR neuroblastoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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