HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2015 May 01.

Published in final edited form as:

J Invest Dermatol. 2014 November ; 134(11): 2839–2842. doi:10.1038/jid.2014.248.

DC-HIL^{+} CD14^{+} HLA-DR$^{no/low}$ Cells Are a Potential Blood Marker and Therapeutic Target for Melanoma

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TO THE EDITOR

Melanoma growth and metastasis depend on a battle between the cancer's invasive properties and the host's capacity to counter such attributes. Immunosuppression is a potent promoter of cancer progression that not only counters host control of tumor spread but also prevents anti-cancer treatments from achieving their full benefit (Ilkovitch and Lopez, 2008). Because CD11b^{+}Gr1^{+} cells are most potent at suppressing T-cell function (Frey, 2006), their exponential proliferation in cancer patients severely limits efficacy of immunotherapy (Diaz-Montero *et al.*, 2009).

We discovered the DC-HIL receptor to potently inhibit effector T-cell function following binding to syndecan-4 (SD-4) on these cells (Chung *et al.*, 2007a; Chung *et al.*, 2007b). In a submitted accompanying article, we showed that melanoma-bearing (but not tumor-free) mice harbors an expanded population of DC-HIL-expressing CD11b^{+}Gr1^{+} cells and that functional blockade of DC-HIL on these cells via gene deletion or specific Ab abrogates their suppressor function, making DC-HIL a marker for immunosuppressive CD11b^{+}Gr1^{+} cells and a powerful promoter of melanoma growth.

Since CD14^{+}HLA-DR$^{no/low}$ cells are the human equivalent of mouse CD11b^{+}Gr-1^{+} cells (Filipazzi *et al.*, 2007), we posited that blood CD14^{+}HLA-DR$^{no/low}$ cells in melanoma patients express DC-HIL and that such expression makes them immunosuppressive. Thus we examined blood frequencies of CD14^{+}HLA-DR$^{no/low}$ cells and their DC-HIL expression,

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CONFLICT OF INTEREST

The authors state no conflict of interest.

in cases of: melanoma with varying clinical stages (0-IV) (n=62), dysplastic nevi (in which melanocytes are abnormal but not malignant (n=12)), and healthy donors (n=21) (Figure 1a and Supplementary Table S1). Compared to healthy donors, all cases of melanoma exhibited elevated blood $CD14^{+}HLA\text{-}DR^{no/low}$ cells (Figure 1b), consistent with a prior report (Filipazzi *et al.*, 2007). Whereas blood $CD14^{+}HLA\text{-}DR^{no/low}$ cells in healthy donors had little-to-no expression of DC-HIL (0.1 ± 0.1% $DC\text{-}HIL^{+}$ cells among PBMCs), all cases of metastatic melanoma (stages III/IV) displayed high-level DC-HIL expression on these cells (2.9 ± 0.9% and 2.6 ± 0.6%, respectively; *t* test $p=0.001$ vs. healthy donors) (Figure 1c). Intermediate levels of DC-HIL expression were seen in blood $CD14^{+}HLA\text{-}DR^{no/low}$ cells of melanoma confined to skin (stages 0/I-II). Dysplastic nevi showed lower expression than skin-restricted melanoma, but higher than for healthy donors ($p=0.01$). Thus blood levels of $DC\text{-}HIL^{+}CD14^{+}HLA\text{-}DR^{no/low}$ cells correlated with cancer progression, particularly in advanced stages. Other myeloid cells thought to have suppressor function ($CD14^{+}IL\text{-}4R\alpha^{+}$, $CD14^{neg}CD11b^{+}CD15^{+}$, and $CD14^{neg}IL\text{-}4R\alpha^{+}CD15^{+}$) also expressed DC-HIL at a range of 30–75% (Supplementary Figure 1).

To determine whether melanoma was the cause of the elevated blood levels, we followed a new cohort of 9 patients with stage 0 melanoma and assayed for % $DC\text{-}HIL^{+}CD14^{+}HLA\text{-}DR^{no/low}$ cells in their PBMCs (Figure 1d), at 0, 1, 3, and 6 months after excision of the melanoma. At the time of resection (0 month), all subjects except one (subject M83) exhibited higher levels than healthy controls (0.3 to 12.8%) (Supplementary Table S2). Across the 3-month follow-up, these elevated levels declined significantly in 8 patients (Wald test, $p=0.045$) to an average of 0.4 %, close to that of 6 normal controls (Supplementary Table S3). Interestingly, in the case of one patient (M71), the % $DC\text{-}HIL^{+}CD14^{+}HLA\text{-}DR^{no/low}$ cells that declined a month post-resection climbed back to a high level at 3 months, which coincided with discovery of a new melanoma *in situ* (stage 0), and then fell back after resection of this second melanoma. We concluded that melanoma is responsible (directly or indirectly) for acquisition of DC-HIL expression by $CD14^{+}HLA\text{-}DR^{no/low}$ cells. Because our mouse studies showed IFN-γ and IL-1β to induce DC-HIL expression by $CD11b^{+}Gr1^{+}$ cells, we speculate similar mechanisms for human $CD14^{+}HLA\text{-}DR^{no/low}$ cells.

Do $CD14^{+}HLA\text{-}DR^{no/low}$ cells from melanoma patients suppress T-cell function and is DC-HIL responsible for that function? $CD14^{+}HLA\text{-}DR^{no/low}$ cells isolated from melanoma patients (vs. healthy donors) were cocultured with autologous T-cells activated by anti-CD2/CD3/CD28 Ab (Figure 2a). $CD14^{+}HLA\text{-}DR^{no/low}$ cells from melanoma patients inhibited IFN-γ production by autologous T-cells dose-dependently and almost completely, whereas corresponding cells from healthy donors were weakly immunosuppressive.

Treatment with anti-DC-HIL mAb (but not control IgG) restored the T-cell IFN-γ response dose-dependently (up to 80%) (Figure 2b). Moreover, treatment of total (unfractionated) PBMCs from melanoma patients with anti-DC-HIL mAb (but not with control IgG) enhanced the IFN-γ response, and this enhancement correlated positively with melanoma staging (Figure 2c), but negatively with IFN-γ levels from IgG-treated PBMCs (Figure 2d).

Our outcomes indicated that neutralizing DC-HIL's T cell-suppressive function could be beneficial to melanoma patients. Among currently available treatments for melanoma, the most closely related to a DC-HIL antagonist are humanized mAb directed against CTLA-4 (ipilimumab) or PD-1 (lambrolizumab). Both treatments have been shown to prolong survival of patients with metastatic melanoma (Hamid *et al.*, 2013; Hodi *et al.*, 2010), presumably by blocking the inhibitory functions of CTLA-4 and PD-1, respectively. However, their benefits have been limited by development of autoimmune disease causing dermatitis, hepatitis, colitis, and in many cases, death (Hodi *et al.*, 2010), making the search for even better treatments important.

Our mouse studies showed that, unlike DC-HIL, the ligands for CTLA-4 (CD80 and CD86) and for PD-1 (PD-L1) are not critically involved in the T-cell suppressor function of myeloid cells. Moreover, both CTLA-4 and PD-1 are expressed by most activated T-cells and regulate development of autoreactive T-cells via regulatory T-cell function (Gattinoni *et al.*, 2006). By contrast, SD-4 (the DC-HIL ligand) is expressed by only a restricted population of effector T-cells, with no impact on regulatory T-cell function (Chung *et al.*, 2013). Finally, CTLA-$4^{-/-}$ or PD-$1^{-/-}$ mice develop spontaneous autoimmune diseases (Nishimura *et al.*, 1999; Tivol *et al.*, 1995) causing early death, while DC-$HIL^{-/-}$ or syndecan-$4^{-/-}$ mice survive without observable autoimmune diseases (unpublished data). These differences suggest strategies neutralizing DC-HIL function may restore T-cell function in melanoma patients via mechanisms different from CTLA-4 or PD-1 blockers.

In sum, the positive correlation between % blood DC-$HIL^{+}CD14^{+}HLA\text{-}DR^{no/low}$ cells and advancing melanoma stage, this parameter's quick decline after resection of early melanoma, and the restoration by anti-DC-HIL mAb of the T-cell IFN-γ response in melanoma patients constitute strong bases for developing these cells as a useful biomarker and therapeutic target for melanoma. Our results should be confirmed by large, multi-centers studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Irene Dougherty and Megan Randolph for technical and administrative assistance, respectively. This research was supported by VA merit award and NIHRO1 grant (AI064927-05).

Abbreviation used

PBMCs	peripheral blood monocytes
SD-4	Syndecan-4

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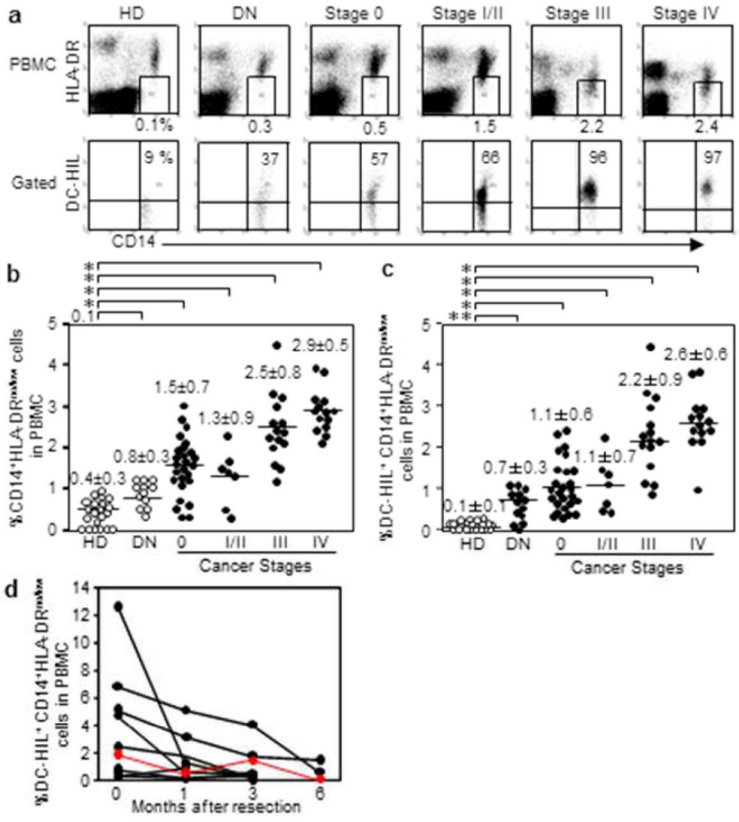

Figure 1. Positive correlation between DC-HIL^{+}CD14^{+}HLA-DR$^{no/low}$ cells and melanoma stage

PBMCs from melanoma patients (stages 0-IV) or dysplastic nevus (DN), and from healthy donors (HD) were analyzed for CD14 vs. HLA-DR expression, in which CD14^{+}HLA-DR$^{no/low}$ cells are indicated (%). These cells were FACS-gated and examined for expression of DC-HIL vs. CD14. Data shown are representative of each group **(a)**. % CD14^{+}HLA-DR$^{no/low}$ (**b**) or % DC-HIL^{+}CD14^{+}HLA-DR$^{no/low}$ cells/PBMC (**c**) in each cohort is summarized (mean % ± sd). Statistical significance for each stage was calculated by comparison with HD. (**d**) % blood DC-HIL^{+}CD14^{+}HLA-DR$^{no/low}$ cells/PBMCs was assayed at indicated times post-resection in 9 patients with stage 0 melanoma (data for patient M71 are in red), *p<0.001 and **p<0.01.

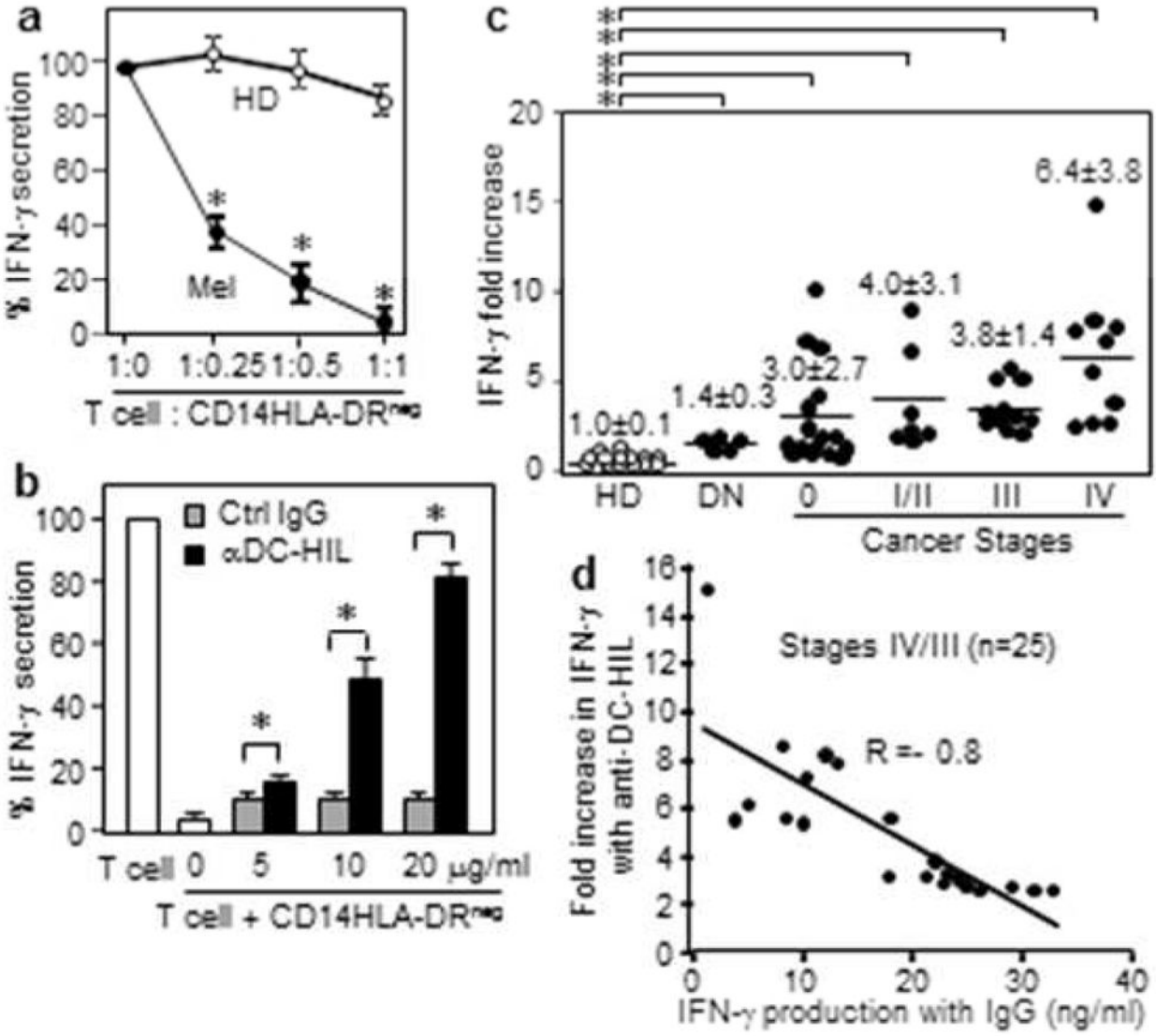

Figure 2. Anti-DC-HIL mAb treatment restored IFN-γ response in melanoma patients

(**a**) $CD14^{+}HLA\text{-}DR^{no/low}$ cells from stage III patient or healthy donor cocultured with T-cells/$HLA\text{-}DR^{+}$ cells (varying ratios) with anti-CD2/CD3/CD28 Ab. (**b**) Effect of anti-DC-HIL or control IgG on IFN-γ secretion by the coculture (1:1 cell ratio) is expressed as IFN-γ amount (%) relative to T-cell culture: 50 and 53 ng/ml for HD and melanoma, respectively (**a**); and 24 ng/ml for (**b**). Representative data of 3 different patients. (**c**) PBMCs from same patients with stages III/IV were cultured with Ab; fold increase in IFN-γ amounts (mAb vs. IgG) is shown with Pearson's correlation coefficient r. (**d**) Same experiments were performed with all samples, and values of fold increase in IFN-γ production plotted to cancer stage. *p<0.001.