Increased CD200 expression in acute myeloid leukemia is linked with an increased frequency of FoxP3+ regulatory T cells

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CD200 is a type-1a transmembrane cell-surface glycoprotein that is normally expressed in critical tissues such as the central nervous system and testis, as well as certain leukocytes, including T and B lymphocytes, where its role is to promote peripheral tolerance and protect immune privileged sites.1 CD200 has no known intracellular signaling motif, but induces immunosuppression through engagement with CD200R, a cell-surface receptor homolog, which is expressed on leukocytes of myeloid lineage, including mast-cells, macrophages, basophils, dendritic cells as well as certain T-cell populations.2 CD200, which is frequently overexpressed in acute myeloid leukemia (AML) patient blasts and associated with a worse outcome,3 has the potential to induce the formation of CD4+ CD25+ FoxP3+ regulatory T cells (Tregs),4 a subset of immunosuppressive T cells that are linked with a poor prognosis in AML.5 Most importantly, Tregs have been documented to suppress the anti-leukemia response in vitro from AML patients,6 suggesting that these cells have an important role in regulating AML patient tumor immunity. We therefore investigated whether CD200 protein expression on human AML blasts was associated with an increased frequency of Treg cells in AML using a cohort of 40 AML patients (at the point of diagnosis before any treatment; Supplementary Table S1 for patient demographic). AML blast CD200 protein expression and patient Treg frequency was analyzed by flow cytometry (for full gating strategy, refer to Supplementary Figure S1). The data show that CD200 blast expression level correlated significantly with the frequency of Treg cells (Figure 1a). This association was found to be independent of white blood cell count (Figure 1b), suggesting that CD200 expression on AML blasts promotes Treg formation. We also examined whether CD200 expression was differentially expressed on AML blasts with a putative leukemic stem cell phenotype (CD34+ CD38-),7 but could find no evidence for this within the six samples examined (Supplementary Figure S2).

Given that the depletion of Tregs has been shown to improve T-cell-mediated therapy, as well as improving antitumor activity in leukemia patients that are in complete remission,8,9 we investigated whether Tregs from CD200hi patients were functionally immunosuppressive. We examined the ability of purified Tregs to suppress naive responder T-cell (CD4+ CD25−) proliferation following CD3/CD28 co-stimulation (refer to Supplementary Materials and Methods). The data demonstrate that Tregs isolated from CD200hi AML patients were capable of suppressing T-cell proliferation at responder to Treg ratios of >1 to 0.01 (Figure 1c). Reciprocal analysis of Tregs from CD200lo patients (Figure 1d). In fact, Treg frequencies in CD200lo AML patients could not be carried out because of the extremely low frequencies of these cells in CD200lo patients (Figure 1d). In fact, Treg frequencies in CD200lo AML patients were uniformly lower than in healthy donor controls, suggesting that CD200 has an influence on Treg induction in this context.

Since mouse models have suggested that CD200-induced Tregs are likely to mediate Th1 immunosuppression,10 a cytokine response with a prognostic link in leukemia,11 we measured the Th1 cytokine response (TNFs, IL2 and IFNγ; production) in CD200hi patients before and after Treg depletion. Representative flow cytometric plots confirmed Treg depletion using magnetic separation (Figure 2a). However, removal of Tregs alone was insufficient to significantly improve the Th1 cytokine response as detected by intracellular cytokine staining (Figure 2b). Elsewhere we report that, in addition to its known influence on Treg production, CD200 is also directly immunosuppressive through engagement with CD200R.
on T cells in AML. However, in the present study we show that removal of Treg cells alone is unlikely to show effect in these assays when large numbers of CD200⁺ blast cells are present. These data would predict that Tregs have little impact on immunosuppression at diagnosis when the disease burden is high, as the dominant mode of T-cell suppression would be mediated by CD200⁺ blast cells directly inhibiting Th1 responses. Conversely, following chemotherapy, the reduction of tumor burden would be predicted to make Treg-mediated immunosuppression cells the dominant factor.

In summary, these findings illustrate a clear correlation between blast CD200 expression level and the frequency of immunosuppressive Treg cells. Previous studies of human AML have also reported increased frequencies of Tregs in untreated disease as well as during regeneration following treatment, however, the link with CD200 expression has not previously been established. Our data also indicate that while Tregs in these patients may be functionally immunosuppressive, they may only become influential following cytoreduction. Indeed, several studies have identified that increased frequencies of Tregs are associated with relapse in myeloid malignancy. Furthermore, phase-I studies using anti-CD200 monoclonal antibody immunotherapy have shown that blocking CD200 is sufficient to reduce the Treg frequency in chronic lymphocytic leukemia patients, illustrating that blocking CD200 may be therapeutically advantageous in AML.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

SJC designed and performed the experiments/analyzed all data and co-wrote the manuscript. RKH provided statistical guidance. ECYW provided biological insight. AKB provided resources and clinical insight. SM, RLD and AT contributed to experimental design and co-wrote the manuscript.

SJC Coles, RK Hills, ECY Wang, AK Burnett, S Man, RL Darley and A Tonks

1Department of Haematology, Institute of Cancer and Genetics, Cardiff University, Wales, UK and
2Institute of Infection and Immunity, School of Medicine, Cardiff University, Wales, UK

E-mail: Tonksa@cf.ac.uk

*These authors contributed equally to this work.
Expression of CD200 on AML blasts directly suppresses memory T-cell function

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Previous studies have shown that immunosuppression in acute myeloid leukemia (AML) is associated with changes in the adaptive immune compartment. Such changes include the suppression of memory T-cell function and the suppression of Th1 cytokine (TNF-α, IL-2 and IFN-γ)-producing cells. A suppressed immune response in AML is associated with a worse patient outcome and increased risk of relapse, as well as increased risk of infection impairing patient recovery. The over-expression of the immunosuppressive ligand CD200 is also associated with an increased risk of relapse in AML (hazard ratio 1.7); an observation consistent with a hypothesis in which CD200 inhibits clearance of tumors,8,9 suggesting that CD200-mediated Th1 suppression is a central mechanism in cancer immunomodulation.

The ability to simultaneously produce TNF-α, IL-2 and IFN-γ is an important indicator of T-cell quality in anti-tumor/viral responses. We therefore simultaneously measured the frequencies of TNF-α-, IL-2- and IFN-γ-producing CD4+ memory cells, as well as CD8+ memory cells between CD200hi, CD200lo and healthy donors (data not shown). CD200 has also been reported to mediate suppression of the Th1 response in chronic lymphocytic leukemia as well as solid tumors, suggesting that CD200-mediated Th1 suppression is a central mechanism in cancer immunomodulation.

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