Impaired natural killer regulatory cells in multiple sclerosis

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Introduction

In recent years, the horizon of innate immune cells has been redesigned with the identification of innate lymphoid cells (ILC). ILC are now regarded as the innate, more phylogenetically ancient mirror of T cells and thus, based on transcription factors and cytokines they produce, ILC are divided in subfamilies similar to T helper cell subsets. However, whether ILC include the innate correlate of T regulatory cell subsets has not been clarified yet. Natural killer (NK) cells are type 1 ILC innate cells with prominent antitumoral and antiviral functions, which they exert through direct cytotoxicity. Recent evidence has shown that the CD56bright NK cells subset (NKregs) may shape adaptive immune responses by interacting in particular with T cells. In MS, expansion of CD56bright NK cells has been associated to response to different treatments (daclizumab, interferon-beta) and to remission in pregnancy; however it is not known what function they exert in physiologic conditions and whether it is impaired in MS. Aim of this study was to dissect the function of CD56bright NK regulatory cells in healthy subjects (HS) and assess whether it is impaired in MS

Methods

CD56bright NK regulatory cells and CD56dim NK cells, isolated from peripheral blood of HS and untreated patients with Clinically Isolated Syndrome (CIS) or MS (CIS/MS), were pre-activated with the proinflammatory cytokines interleukin-12 and -15 and cultured in presence of autologous CD4+ T cells stimulated with anti-CD3 and anti-CD28 beads. Proliferation of T cells in coculture and cytotoxicity of CD56bright NK cells towards autologous T cells were evaluated by quantification of DNA and flow cytometry, respectively. Selective blocking of NK activating receptors was performed to identify those involved in CD56bright NK cells -T cell interactions. Lytic enzymes mediating the effect of CD56bright NK cells were evaluated by real-time PCR, intracellular staining and the use of selective inhibitors. Phenotype of CD56bright NK cells and expression of HLA-E (ligand to the inhibitory ligand NKG2A) on CD4+ T cells were evaluated by flow cytometry.

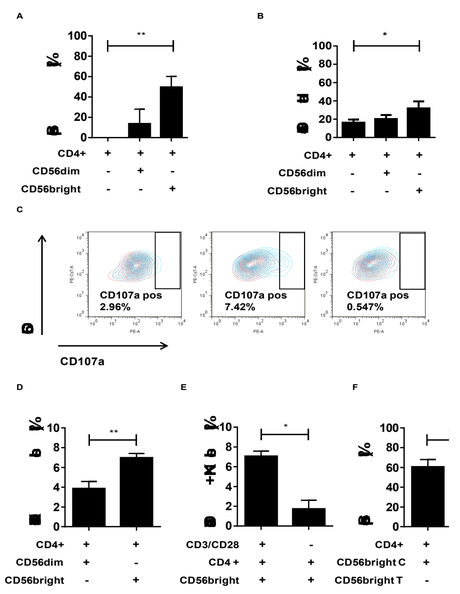

Figure 1 CD56bright NK cells suppress autologous CD4+ T cell proliferation through direct cytotoxicity

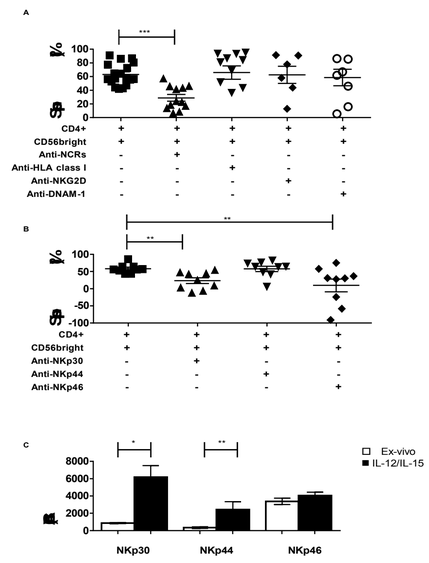

Figure 2 The activating receptors NKp30 and NKp46 are required for the suppressive function of CD56bright NK cells

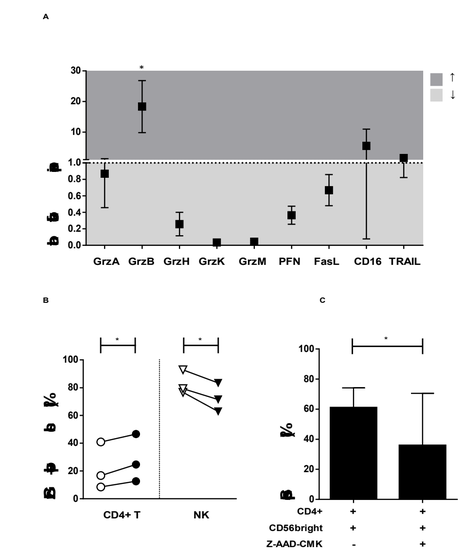

Figure 3 Granzyme B mediates the regulatory function of CD56bright NK cells

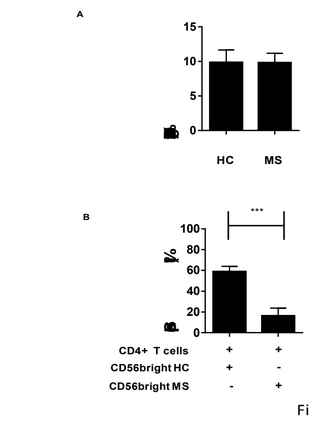

Figure 4

Figure 4 CD56bright NK cells from CIS/MS patients do not suppress autologous T cell proliferation

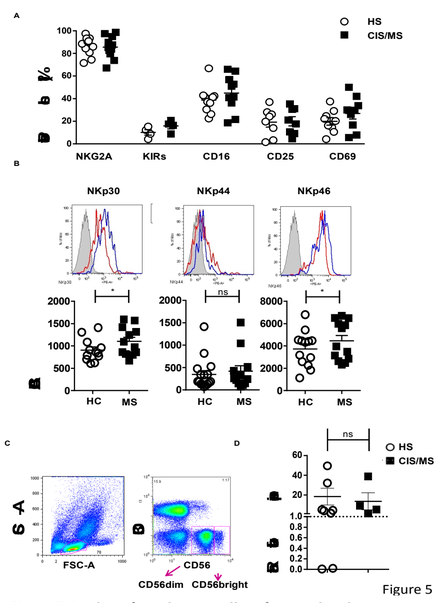

Figure 5

Figure 5 Analysis for relevant cell surface molecules indicate that CD56bright NK cells from CIS/MS patients and HS have similar phenotype with upergulated NKp30 and NKp46 and produce granzyme B

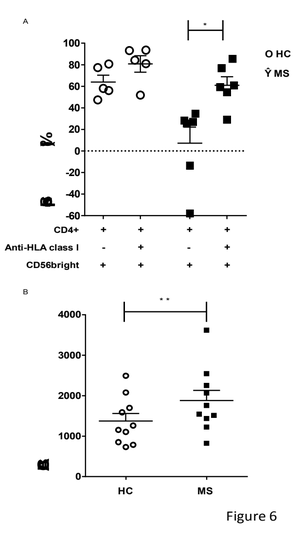

Figure 6

Figure 6 Increased HLA-E on CD4+ T cells inhibits cytotoxicity of CD56bright NK cells from CIS/MS patients

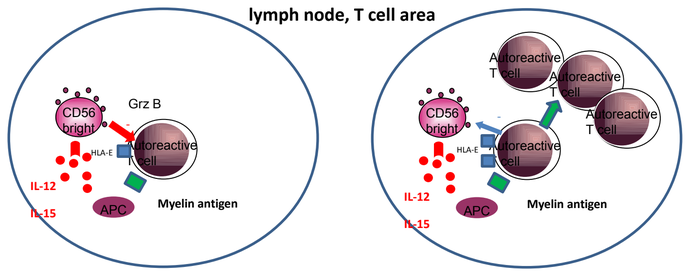

Figure 7 Increased HLA-E on CD4+ T cells inhibits cytotoxicity of CD56bright NK cells from CIS/MS patients

Results

We found that CD56bright NK cells from HS acquire, upon inflammatory cues, the capability of suppressing autologous CD4+T cell proliferation through direct cytotoxicity (Figure 1) which requires engagement of natural cytotoxicity receptors (NCRs) NKp30 and NKp46 (Figure 2) and secretion of granzyme B from CD56bright NK cells (Figure 3). CD56bright NK cells from CIS/MS patients were not decreased in number but had an impaired suppressor function towards autologous T cells (Figure 4). CD56bright NK cells from CIS/MS patients had a phenotype similar to CD56bright NK cells from HS , with upregulated expression of NKp30 and NKp46, and normal production of granzyme B (Figure 5). Blocking HLA class I on T cells did not increase suppressor function of CD56bright NK cells from HS, but restored normal suppressor function in CD56bright NK cells from MS. Accordingly, expression of HLA-E molecule was upregulated in CD4 T cells from CIS/MS patients compared to HS (Figure 6).

Conclusions

The results of this work shed light on the function of the CD56bright NK cell subset in healthy conditions and in MS. First, we demonstrated that CD56bright NK cells are a regulatory population controlling proliferation of CD4+ T cells through a cytotoxic mechanism. Second, we provided evidence that such regulatory mechanism is impaired in CIS/MS (Figure 7). The evidence that the mechanism of action of daclizumab relies, at least in part, on the expansion of CD56bright NK cells had pointed towards a beneficial role of this cell subset. This work shows for the first time an impairment in NK cell-mediated immunoregulation in untreated MS, that is attributable more to the T cells being resistant to NK suppressive action than to intrinsic defects of the NK cells themselves. The findings of the present study provide new insights about the role of “NKregs” cells in the regulation of immune responses and suggest that reverting defective immunoregulation exerted by CD56bright NK cells may be as well a potential therapeutic target in MS.

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