CD161 intermediate expression defines a novel activated, inflammatory and pathogenic subset of CD8+ T cells involved in multiple sclerosis

B. Nicol, M. Salou, I. Vogel, A. Garcia, E. Dugast, J. Morille, S. Killens, E. Charpentier, A. Donnant, S. Nedellec, et al.

CD161 intermediate expression defines a novel activated, inflammatory and pathogenic subset of CD8$^+$ T cells involved in multiple sclerosis

P981

B. Nicol[1,2], M. Salou[1,2], I. Vogel[2], A. Garcia[2,3], E. Dugast[2], J. Morille[2], S. Killens[2], E. Charpentier[4,5], A. Donnant[4,5], S. Nedellec[6], M. Jacq-Foucher[3], F. Le Frère[3], S. Wiertlewski[7], A. Bourreille[8], S. Brouard[2], L. Michel[2,7], L. David[2,9], P.-A. Gourraud[2], N. Degauque[2], A. Nicot[2], L. Berthelot[2], D.A. Laplaud[1,10]

[1]Université de Nantes, [2]CRTI - Inserm 1064, [3]CHU Nantes, [4]Inserm U 1127 UMR 1087, [5]CNRS UMRS 6291, [6]SFR François Bonamy, Cellular and Tissue Imaging Core Facility (MicroPICell), [7]Neurology, [8]Gastro-Enterology, CHU Nantes, [9]iPSC Core Facility, SFR François Bonamy, UMS INSERM 016, [10]CRTI, Neurology, CHU Nantes, Nantes, France

Objective: CD8 T cells are the most numerous infiltrating T cells in central nervous system (CNS) lesions of multiple sclerosis (MS) and several lines of evidence support their role in tissue damage. However, to date the precise phenotype of the circulating CD8 T cells that may be recruited from the periphery to invade the CNS remains largely undefined. It has been suggested that Tc17 lymphocytes may be involved and in humans, these cells are characterized by the expression of CD161. Whereas CD8+ T cells with a high CD161 expression, representing mainly Mucosal Associated Invariant T cells, are likely not involved in the disease process, CD8+ T cells with CD161 intermediate expression constitute a unique, recently described subset of CD8+ T cells. Yet, its role in neuro-inflammation has not been investigated.

Methods. CD8$^+$CD161^{int} T cells from the blood of sex- and age-matched Relapsing Remitting (RR) untreated MS patients (n=35) and Healthy Volunteers (HV, n=30) were compared by flow cytometry (for frequency and phenotype). Their frequency and phenotype were also compared between the blood and the cerebrospinal fluid (CSF) in MS patients. Their transcriptional profile was analyzed by Fluidigm technology using Biomark^{TM} HD (n=4 for HV and n=7 for MS). Their ability to transmigrate through an *in vitro* blood-brain barrier (hCMEC/D3 cell line) have been studied and their presence and phenotype in CNS lesions were also studied by immunofluorescence staining on post-mortem CNS samples. Their *in vivo* behavior has been studied in an Graft-Versus-Host Disease (GVHD) model using NOD-*scid* gamma (NSG) mice.

The frequency of CD8$^+$CD161^{int} T cells is decreased in the blood and enriched in the CSF and CNS of MS patients

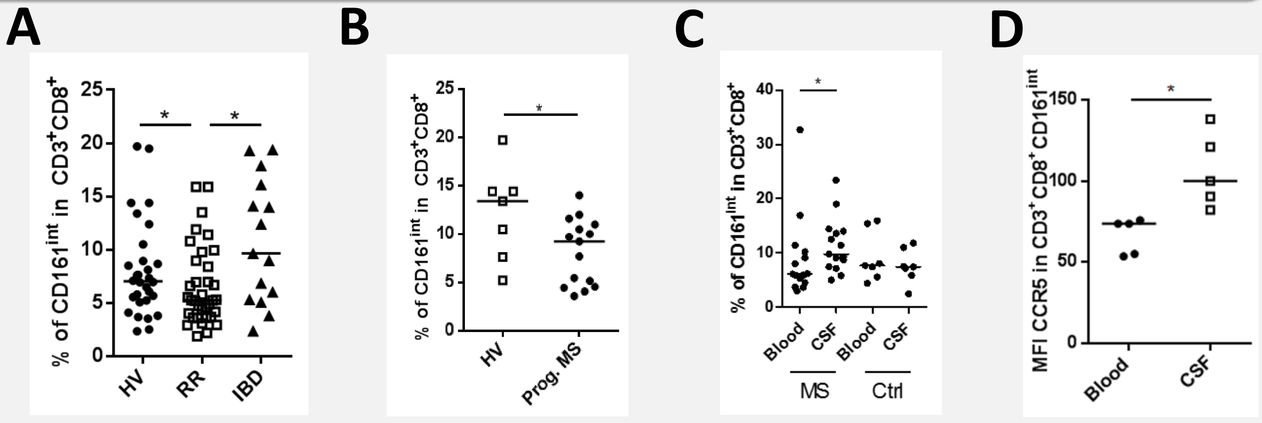

Figure 1: (A) Frequency of CD8$^+$CD161^{int} T cells in the blood of HV (n=30), RR-MS patients (n=35) and IBD patients (n=15). (B) Frequency of CD8$^+$CD161^{int} T cells in the blood of prog. MS and age-matched HV (C) Frequency of CD8$^+$CD161^{int} T cells in the blood and the CSF of MS patients (n=15) and controls with non-inflammatory neurological diseases (n=6). (D) MFI of CCR5 in the blood and the CSF of MS patients (n=5). (E) Example of CD8$^+$CD161$^+$ staining in a perivascular infiltrate from a multiple sclerosis lesion. Blue: DAPI, gray: CD8, red: CD161. White arrows indicate CD8$^+$CD161$^+$ cells and red arrow indicates one CD8^{neg}CD161$^+$ cell. (F) Percentage of CD8 T cells expressing CD161 in a set of eight multiple sclerosis lesions characterized by the absence of Mucosal Associated Invariant T cells (CD3$^+$CD161$^+$Vα7.2$^+$ cells).

CD8$^+$CD161^{int} T cells display a specific and activated phenotype with homing properties

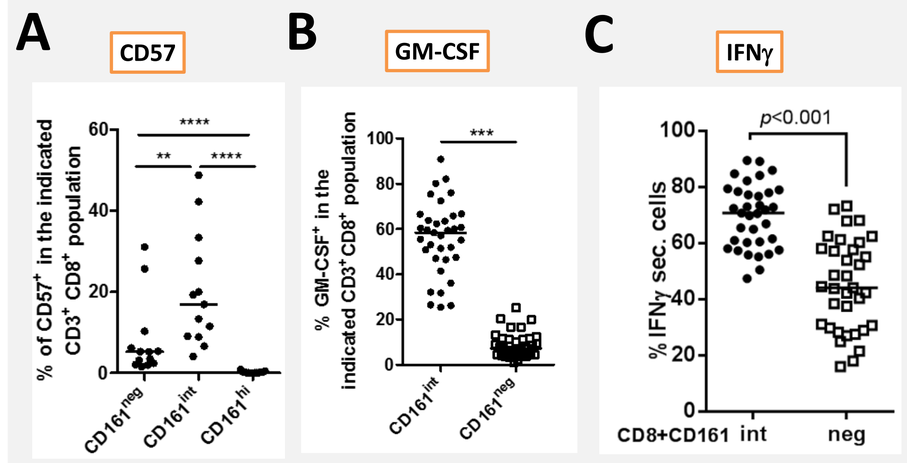

Figure 2: (A) Frequency of CD57+CD8$^+$CD161^{neg}, CD161^{int} or CD161^{hi} T cells in HV (n=24, 10 and 13 respectively). (B) Frequency of GM-CSF secreting cells in CD8$^+$CD161^{neg} and CD161^{int} T cells (C) Frequency of IFNg secreting T cells in CD8$^+$CD161^{neg} and CD161^{int} T cells (D) Frequency of CD49d and PSGL-1 expressing CD8+CD161neg, CD161int and CD161high T cells in HV (E) Ratio of the frequency of CD3$^+$CD8$^+$ CD161^{neg}, CD161^{int}, or CD161^{hi} in the transmigrated fractions as compared to the total non-transmigrated fraction under inflamed conditions (n=12).

CD8$^+$CD161^{int} T cells display transcriptomic and phenotypic alterations in the blood of MS patients

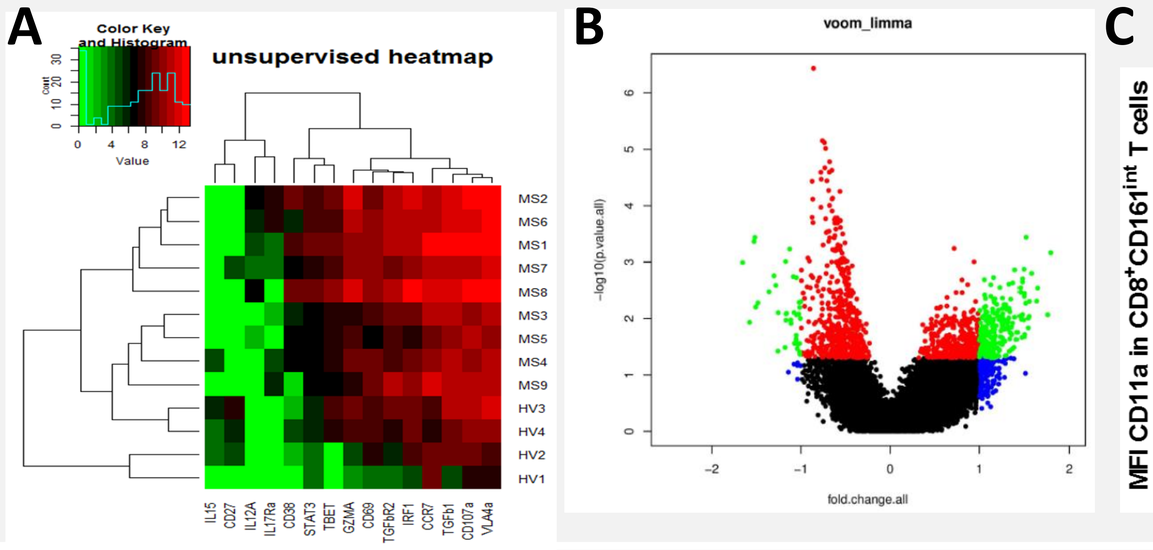

Figure 3: (A) Gene expression analysis of sorted CD8$^+$CD161^{int} T cells in HV (n=4) and MS patients (n=8). Listed genes are significantly differentially expressed between HV and MS patients (B) RNAseq analysis of sorted CD8+CD161int T cells from MS patients (n=8) and HV (n=12), Volcano plot analysis revealed 170 genes differentially regulated in the CD8$^+$CD161^{int} T cells from multiple sclerosis patients versus HV. The volcano plot represents all of the genes found in the multiple sclerosis patients minus the genes found in the HV. In red, the genes with an unadjusted p value < 0.05. In blue, the genes with an expression > log2 and in green, the genes fulfilling both conditions. (C) MFI of PSGL-1 in CD8$^+$CD161^{int} T cells in HV (n=15) and MS patients (n=24). (D) Frequency of MCAM expressing cells in CD8$^+$CD161^{int} T cells after CD3/CD28 stimulation in HV (n=17) and MS patients (n=18) (E°Frequency of DNAM-1High expressing CD8+CD161int T cells from MS patients (n=19) and HV (n=19),

CD8$^+$CD161^{int} T cells are present and able to produce IL-17 in the CNS of MS patients

Overlay

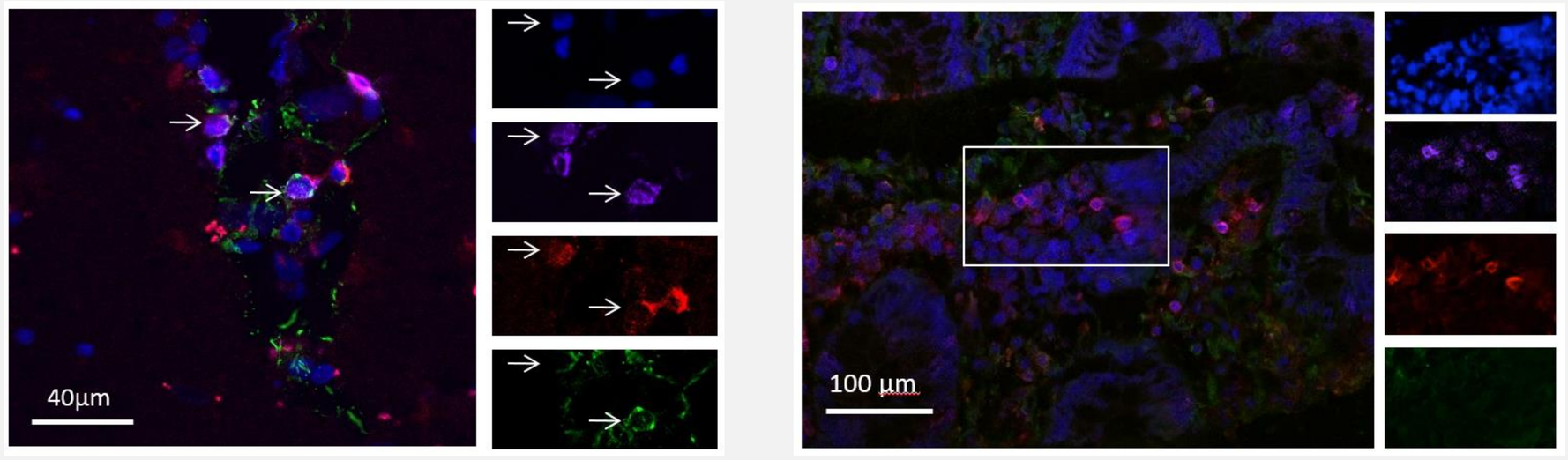

Figure 4: (A) Example of staining of CD8$^+$CD161^{int} T cells secreting IL-17 in a brain multiple sclerosis lesion. Blue: DAPI, purple: CD8, red: CD161, and green: IL-17. (B) Example of the same staining in a colon biopsy from a multiple sclerosis patient. (C) Frequency of IL-17 secreting CD8$^+$CD161^{int} T cells in different tissues in patients with multiple sclerosis. Blood (without stimulation, n=18), blood after four days of stimulation with antiCD3/CD28 (n=18), brain multiple sclerosis lesions (n=7), and gut biopsies from the multiple sclerosis patients (n=5).

Conclusions: We report here that CD8+CD161int T cells present characteristics of effector cells, up-regulate cell-adhesion molecules and have an increased ability to cross the blood-brain barrier and to secrete IFNγ and GM-CSF (and also IL-17 and IL-22, not shown). We further demonstrate that these cells are recruited and enriched in the CNS of MS subjects where they locally and specifically secrete IL-17. In the peripheral blood, RNAseq, RT-PCR and flow cytometry confirmed an increased effector and transmigration pattern of these cells in MS patients compared to healthy controls. Our data demonstrate that intermediate levels of CD161 expression identifies activated and effector CD8+ T cells with pathogenic properties that are recruited to MS lesions. This suggests that CD161 may represent a biomarker and a valid target for the treatment of neuroinflammation.