Interleukin-15 enhances proinflammatory T-cell responses in patients with MS and EAE

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Abstract

Objective

We posit that interleukin-15 (IL-15) is a relevant contributor to MS pathobiology as this cytokine is elevated in the CNS and periphery of patients with MS. We aim to investigate (1) the impact of IL-15 on T lymphocytes from patients with MS and (2) the *in vivo* role of IL-15 using the experimental autoimmune encephalomyelitis (EAE) mouse model.

Methods

We compared the impact of IL-15 on T lymphocytes obtained from untreated patients with MS (relapsing-remitting, secondary progressive, and primary progressive) to cells from age/sex-matched healthy controls (HCs) using multiparametric flow cytometry and *in vitro* assays. We tested the effects of peripheral IL-15 administration after EAE disease onset in C57BL/6 mice.

Results

IL-15 triggered STAT5 signaling in an elevated proportion of T cells from patients with MS compared with HCs. This cytokine also enhanced the production of key proinflammatory cytokines (interferon γ , granulocyte-macrophage colony-stimulating factor [GM-CSF], IL-17, and tumor necrosis factor) by T cells from both MS and controls, but these effects were more robust for the production of IL-17 and GM-CSF in T-cell subsets from patients with MS. At the peak of EAE disease, the proportion of CD4⁺ and CD8⁺ T cells expressing CD122⁺, the key signaling IL-15 receptor chain, was enriched in the CNS compared with the spleen. Finally, peripheral administration of IL-15 into EAE mice after disease onset significantly aggravated clinical scores and increased the number of inflammatory CNS-infiltrating T cells long term after stopping IL-15 administration.

Conclusions

Our results underscore that IL-15 contributes to the amplification of T-cell inflammatory properties after disease onset in both MS and EAE.

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Glossary

EAE = experimental autoimmune encephalomyelitis; GM-CSF = granulocyte-macrophage colony-stimulating factor; HC = healthy control; IFN = interferon; IL = interleukin; PBMC = peripheral blood mononuclear cell; PMS = progressive MS; PPMS = primary progressive MS; RRMS = relapsing-remitting MS; SPMS = secondary progressive MS; TNF = tumor necrosis factor.

Interleukin-15 (IL-15) is a potential contributing factor to the pathobiology of MS.^{1–3} CNS cells (astrocytes and microglia) and peripheral blood cells (monocytes and B lymphocytes) from subjects with MS express elevated levels of IL-15 compared with controls.^{1–4} We demonstrated that human CD8⁺ T lymphocytes pre-exposed to IL-15 exhibit an increased ability to kill oligodendrocytes in vitro and to migrate across an in vitro human model of the blood-brain barrier.² Although IL-15 and IL-2 share the same receptor signaling chains, IL-15 displays unique properties and targets a broader range of cells.^{5,6}

The effects of IL-15 on disease induction have been previously examined in experimental autoimmune encephalomyelitis (EAE).^{7–9} Although aggravated EAE was reported for IL-15^{−/−} mice compared with controls,^{8–10} definitive conclusions are hindered by the abnormal immune system in IL-15^{−/−} mice^{11,12} (i.e., reduced numbers of several immune cell subsets). Therefore, this model does not closely reflect human MS pathobiology.

We sought to investigate whether IL-15 shapes T-cell responses in patients with MS and the underlying mechanisms of such IL-15-mediated effects. We herein report that in both patients with relapsing-remitting MS (RRMS) and progressive MS (PMS), IL-15 induces signaling in a greater proportion of CD4⁺ and CD8⁺ T lymphocytes compared with age/sex-matched healthy controls (HCs). We also show that IL-15 more potently increases proinflammatory properties (e.g., IL-17 production) by MS T lymphocytes. Moreover, we establish using the EAE model that elevated peripheral IL-15 levels contribute to disease progression after the onset of symptoms. Thus, IL-15 represents a novel, relevant therapeutic target in MS.

Methods

Ethics

Written informed consent was obtained from every donor before sample collection, in accordance with institutional guidelines; these studies were approved by the Centre Hospitalier de l'Université de Montréal research ethics committee (approval numbers BH 07.001 and HD 07.002). Untreated patients with MS were recruited from the MS-CHUM Clinic between 2016 and 2019. Each untreated patient was diagnosed according to the 2010 and 2017 revised McDonald criteria¹³ and classified as RRMS, secondary progressive (SPMS), or primary progressive (PPMS) at the time of blood

collection by a highly trained MS neurologist. Untreated patients with MS were separated into 2 groups: RRMS and PMS, the latter including patients with SPMS and PPMS. T lymphocytes from patients with SPMS and PPMS exhibited similar properties and responses in our assays. Age- and sex-matched healthy volunteers were used as controls. To account for the differences in age and sex between the 2 MS cohorts, each untreated MS group was compared with a different group of age/sex-matched HCs.

All mice were treated in strict adherence with approved protocols (N13043NAs and N17031NAs) from the CRCHUM Institutional Committee for the Protection of Animals and the Canadian Council on Animal Care.

Data availability

Data not provided in the article because of space limitations can be shared at the request of other investigators for purposes of replicating procedures and results.

Results

CD122 is similarly expressed by CD4⁺ and CD8⁺ T cells from patients with MS and HCs

CD8⁺ T lymphocytes from patients with MS are more responsive to IL-15 than their counterparts from HC.² However, the underlying mechanisms that control IL-15's impact on MS T lymphocytes have not been identified. We sought to determine whether this is due to elevated expression of the cognate receptor. The IL-15 signaling receptor is composed of 2 chains: CD132 and CD122. CD132 (also known as common γ chain, γ_c) is shared by many cytokines and expressed by most lymphocytes.¹⁴ In contrast, CD122 is specific to IL-15 and IL-2.⁵ We assessed the expression of CD122 (IL-2/IL-15R β) on ex vivo peripheral blood mononuclear cells (PBMCs) from untreated patients with MS and age/sex-matched HCs (figure 1A). The percentages of CD4⁺ and CD8⁺ T-cell subsets (naïve, central memory, and effector memory) expressing CD122 were similar or slightly higher, without reaching significance, in patients with RRMS and PMS compared with their respective age/sex-matched HCs (figure 1B). As CD122 is shared by IL-15 and IL-2, we also measured the expression of CD25 (IL-2R α), the high-affinity IL-2 receptor chain (figure e-1, [links.elsevier.com/NXI/A356](https://www.elsevier.com/links/links.elsevier.com/NXI/A356)). CD4⁺ and CD8⁺ T lymphocytes from patients with RRMS and PMS contained similar or slightly elevated proportions of CD25⁺ cells compared with matched HCs without reaching statistical significance (figure e-1, A and B). Overall, our

results show that the proportions of CD4⁺ and CD8⁺ T cells expressing CD122 or CD25 are not significantly different in patients with MS compared with age/sex-matched HCs. Our results corroborate our previous report on the expression of CD122 by global CD4⁺ and CD8⁺ T cells.² Therefore, factors other than CD122 expression contribute to the altered IL-15 responsiveness in T lymphocytes from patients with MS.

IL-15 triggers pSTAT5 in a greater proportion of T lymphocytes from patients with MS than HCs

We next inquired whether a more robust signaling could contribute to the enhanced IL-15 responsiveness of T lymphocytes from patients with MS.² As IL-15 primarily triggers STAT5 in T lymphocytes,⁵ we compared the IL-15-induced STAT5 phosphorylation (pSTAT5) in patients with RRMS and PMS with age/sex-matched HCs. Ex vivo PBMCs were incubated with increasing concentrations of IL-15 for 15 minutes (peak response to IL-15¹⁵) before being analyzed by flow cytometry (figure 2A). The proportions of central memory CD4⁺ and CD8⁺ T lymphocytes expressing pSTAT5 on IL-15 stimulation were significantly greater in RRMS compared with age/sex-matched HCs (figure 2, B and C); similar results were obtained for naive counterparts, almost reaching significance. Moreover, all CD4⁺ and CD8⁺

T-cell subsets from patients with PMS exhibited significantly elevated proportions of pSTAT5 on IL-15 stimulation compared with matched HCs (figure 2, B and C). In contrast, IL-2 induced pSTAT5 in similar proportions of T lymphocytes from patients with RRMS and PMS compared with their respective age/sex-matched HCs (figure e-1C, links.lww.com/NXI/A356). Although IL-2 and IL-15 share common signaling receptor chains, only the IL-15-triggered pSTAT5 was increased in T lymphocytes from patients with MS compared with HCs, particularly in patients with PMS.

IL-15 favors the production of key proinflammatory cytokines

It is well established that T lymphocytes contribute to the pathobiology of MS by multiple mechanisms including the release of key inflammatory cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-17, and interferon (IFN) γ .¹⁶ IL-15 increases the production of IL-17,¹⁷ tumor necrosis factor (TNF),¹⁸ and IFN γ ² by human T cells. Moreover, STAT5 signaling has been shown to promote GM-CSF production by mouse and human T cells.^{19,20} We therefore investigated whether the enhanced IL-15 responsiveness of T lymphocytes from patients with MS augments the production of these inflammatory cytokines by

Figure 1 CD122 is similarly expressed by T lymphocytes from patients with MS and healthy controls

Ex vivo PBMCs obtained from untreated patients with MS and age/sex-matched healthy controls (HCs) were analyzed by flow cytometry for CD3, CD4, CD8, CD45RA, CD45RO, CCR7, CD25, and CD122. The expression of CD122 (IL-2/IL-15 β chain) was determined on naive, central memory (CM), and effector memory (EM) subsets of CD4⁺ and CD8⁺ T lymphocytes. Gating strategy was performed as previously described.¹⁵ (A) Representative contour plots (black FMO/isotype, red CD122) on CM CD4⁺ T cells from 1 RRMS donor. (B) Percentages of cells expressing CD122 are shown for each subset of CD4⁺ and CD8⁺ T lymphocytes and compared between untreated patients with MS and HCs; each dot represents 1 donor. Patients with RRMS and PMS (including SPMS and PPMS) were analyzed separately and compared with respective age/sex-matched HCs (RRMS: n = 13, PMS: n = 9–10). Naive T lymphocytes are shown in green, CM T lymphocytes in red, and EM T lymphocytes in blue. Results are expressed as mean $\pm$ SEM. IL = interleukin; PBMC = peripheral blood mononuclear cell; PMS = progressive MS; PPMS = primary progressive MS; RRMS = relapsing-remitting MS.

Figure 2 IL-15 triggers pSTAT5 in greater percentages of T lymphocytes from patients with MS compared with controls

(A) Ex vivo PBMCs incubated in the presence of increasing concentrations of IL-15 were stained for CD3, CD4, CD8, CCR7, CD45RA, CD45RO, and pSTAT5 and analyzed by flow cytometry. Representative contour plots of typical pSTAT5 expression in CD4⁺ T cells, untreated or stimulated with 0.1, 0.5, and 1.0 ng/mL of IL-15 for 15 minutes, are shown. (B) Dose-response of IL-15 triggering pSTAT5 for each T-cell subset of CD4⁺ and CD8⁺ (naive, CM, and EM) in HCs and patients with MS (RRMS $n = 9$ and PMS $n = 10$). (C) For the 1.0 ng/mL IL-15 dose, the percentage of pSTAT5 expressing cells is illustrated for each T-cell subset and donor group. Results are expressed as mean $\pm$ SEM. One-way analysis of variance followed by the Fisher least significant difference post hoc test comparing pSTAT5 expression by each T-cell subset from patients with MS vs HCs #0.05 < p < 0.1, * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001. CM = central memory; EM = effector memory; IL = interleukin; PBMC = peripheral blood mononuclear cell; PMS = progressive MS; RRMS = relapsing-remitting MS.

unpolarized or Th1/Tc1 or Th17/Tc17 polarized CD4⁺ and CD8⁺ T cells (representative IFN γ and IL-17 production detected after polarization shown in figure e-2A, links.lww.com/NXI/A357). To determine the direct effects of IL-15 on T lymphocytes, we activated purified naive CD4⁺ and CD8⁺ T cells with α -CD3/ α -CD28 in the absence of antigen-presenting cells. We observed elevated percentages of Th1/Tc1 cells producing IFN γ in the presence of IL-15 (figure 3A); however, we did not detect differences between patients

with MS and HCs (figure 4A). In contrast, IL-15 significantly increased the proportion of IL-17⁺ cells in Th0 and Th17 conditions in patients with RRMS, but not in HCs (figures 3B and 4B). IL-15 also significantly enhanced the proportion of IL-17⁺ cells in the Th17 condition for patients with PMS. The IL-15-mediated increase, illustrated as Δ percentage of cytokine⁺ cells (percentage cytokine⁺ cells in the presence of IL-15 minus percentage cytokine⁺ cells without IL-15) (figure 4B, right panel), shows that Th0 from RRMS and Th17 from

Figure 3 IL-15 stimulation increases the production of cytokines by T lymphocytes under polarizing conditions

Naive CD4⁺ and CD8⁺ T lymphocytes were cultured under polarizing conditions or not in the presence or absence of IL-15 and subsequently treated with phorbol myristate acetate + ionomycin + brefeldin A for 5 hours before being stained for CD4, CD8, IFN γ , IL-17, GM-CSF, and TNF. (A–D) Representative contour plots of T cells expressing the cytokine in the absence/presence of IL-15-producing IFN γ (A), IL-17 (B), GM-CSF (C), or TNF (D). GM-CSF = granulocyte-macrophage colony-stimulating factor; HC = healthy control; IFN = interferon; IL = interleukin; TNF = tumor necrosis factor.

PMS exhibited the greatest IL-15-mediated increase in IL17 production. The percentage of IL-17-producing CD8 T lymphocytes starting from naive cells was too low for most donors to investigate the impact of IL-15.

We observed that IL-15 significantly increased the proportions of GM-CSF⁺ and TNF⁺ cells in Th17/Tc17-polarized T cells from all donors (figures 3, C and D and 4, C and D). Notably, the impact of IL-15 on GM-CSF was more significant on Tc17 cells (mean Δ percentage: 19.1%) from patients with PMS compared with age/sex-matched HCs (mean Δ percentage: 8.5%) (figure 4C, right panel). We observed that IL-15 boosted the amount of secreted GM-CSF and TNF in the supernatants of several T-cell populations from both healthy and MS donor groups (figure e-2B, [links.lww.com/NXI/A357](https://www.lww.com/NXI/A357)). Notably, Tc17 cells from RRMS produced greater amounts of GM-CSF and TNF than cells from HCs, regardless of IL-15 addition (figure e-2B). IL-15 increased the expression of GM-CSF in Th0/Tc0 and TNF in most Th0/Tc0 and Th1/Tc1 to similar levels in all donors (figure e-2, C and D). Overall, we showed that IL-15 significantly augments the expression of key mediators of the Th1/Tc1 and Th17/Tc17 signature. Such IL-15-mediated effects are more robust in specific T-cell subsets and conditions in patients with MS compared with age/sex-matched HCs (IL-17 production by RRMS Th0 and PMS Th17 and GM-CSF production by PMS Tc17).

CD122⁺ T cells from EAE mice display proinflammatory properties

We have previously shown that T cells from patients with MS are exposed to elevated levels of IL-15 both in the periphery

and CNS.^{1,2} Moreover, IL-15 significantly boosts key inflammatory cytokines produced by human CD4⁺ and CD8⁺ T lymphocytes (figures 3 and 4). These T-cell subsets and cytokines play central roles in the pathobiology of the EAE mouse model.^{16,21} Therefore, we sought to evaluate the presence of potential IL-15 responder T lymphocytes, which express the CD122 receptor, at the peak of EAE (EAE score 3.0–4.0). Using cell surface markers CD45 and CD11b, we identified microglia (CD45^{low}CD11b⁺), macrophages (CD45^{hi}CD11b⁺), and nonmyeloid leukocytes (CD45^{hi}CD11b[−]) in the CNS of these mice. Then, we identified CD4⁺ and CD8⁺ T lymphocytes by gating on nonmyeloid leukocytes (CD45^{hi}CD11b[−]) as illustrated in figure 5A. At the peak of disease (EAE score 3.0–4.0; day 14–19 postimmunization), the majority of CD4⁺ and CD8⁺ T lymphocytes that have infiltrated the CNS (pooled brain + spinal cord for each mouse) of EAE mice expressed CD122, and these proportions were significantly greater compared with their splenocyte counterparts (figure 5B). As the broadly used short phorbol myristate acetate/ionomycin/brefeldin A in vitro stimulation downregulated the CD122 surface expression, we characterized T lymphocytes from mice that received an intraperitoneal injection of brefeldin A 5 hours before being killed for organ collection and flow cytometry assessment. The in vivo production of the proinflammatory cytokines IFN γ and GM-CSF was compared in CD122⁺ vs CD122[−] T lymphocytes obtained from the CNS and spleen from EAE mice at disease peak (figure 5, C and D). Among CNS-infiltrating CD4⁺ and CD8⁺ T lymphocytes, significantly higher proportions of CD122⁺ cells produced IFN γ and GM-CSF compared with their CD122[−] counterparts

Figure 4 IL-15-mediated increase in the production of cytokines by T lymphocytes is enhanced in MS

Naive CD4 and CD8 T lymphocytes were cultured under polarizing conditions or not in the presence or absence of IL-15 and subsequently treated with phorbol myristate acetate + ionomycin + brefeldin A for 5 hours before being stained for CD4, CD8, IFN γ , IL-17, GM-CSF, and TNF. (A–D) Percentages of Th0, Th1, Th17, Tc0, Tc1, or Tc17 cells (as indicated on each graph) producing IFN γ (A), IL-17 (B), GM-CSF (C), or TNF (D) in untreated patients with MS (RRMS $n = 7$ –9 and PMS $n = 6$ –8) compared with respective age/sex-matched HCs. Graphs on the left show percentage of cytokine producing cells by subset and donor groups and graphs on right-hand side show IL-15-induced effects (percentage of cytokine + cells in the presence of IL-15 minus percentage cytokine + cells in the absence of IL-15) for each condition and donor group. Each dot represents 1 donor. Results are expressed as mean $\pm$ SEM. Student 2-tailed paired t test or Wilcoxon test comparing IL-15 $^{-}$ vs IL-15 $^{+}$ stimulation for each cell subset and donor subset p values are shown in color; 1-way analysis of variance followed by the uncorrected Fisher least significant difference test or Friedman test for comparison of MS vs HC p values shown in black. #0.05 < p < 0.1; * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001. GM-CSF = granulocyte-macrophage colony-stimulating factor; HC = healthy control; IFN = interferon; IL = interleukin; PMS = progressive MS; RRMS = relapsing-remitting MS; TNF = tumor necrosis factor.

Figure 5 Enrichment of CD122+ T cells in the CNS of EAE mice

Splenocytes and CNS-infiltrated leukocytes were collected from EAE mice at disease peak and analyzed by flow cytometry. (A and B) Cells were stained for CD45, CD11b, CD3, CD4, CD8, and CD122. Representative contour plots of CNS cells illustrating the detection of microglia (CD45^{low}CD11b⁺), macrophages (CD45^{hi}CD11b⁺), and nonmyeloid leukocytes (CD45^{hi}CD11b⁻) and gating for CD4⁺ and CD8⁺ T lymphocytes within CD45^{hi}CD11b⁻ cells. (B) Assessment of CD122 expression by T lymphocytes from EAE mice at disease peak. Representative contour plots illustrating CD122 expression on CNS-infiltrating CD4⁺ T lymphocytes in EAE. Percentages of CD4⁺ and CD8⁺ T lymphocytes expressing CD122 in the CNS (white bars) and the spleen (black bars) $n = 20$. Results are expressed as mean $\pm$ SEM. One-way ANOVA followed by the Sidak multiple comparison test comparing percentage of CD122⁺ CD4⁺ or CD8⁺ T lymphocytes CNS vs spleen $^{**}p < 0.01$, $^{****}p < 0.0001$. (C and D) Cells were collected from EAE mice at disease peak 5 hours after the in vivo injection of brefeldin A and stained with CD45, CD11b, CD3, CD4, CD8, CD122, IFN γ , and GM-CSF. Representative contour plots gated on CD4⁺CD122⁻ or CD4⁺CD122⁺ T lymphocytes illustrating the expression of GM-CSF (C) or IFN γ (D) in the CNS. Percentage of CD122⁻ (white bars) and CD122⁺ (gray bars) CD4⁺ and CD8⁺ T lymphocytes expressing GM-CSF (C) or IFN γ (D) in the CNS and the spleen, $n = 9-13$. Results are expressed as mean $\pm$ SEM. One-way ANOVA followed by the Sidak multiple comparison test comparing percentage of CNS or splenic GM-CSF⁺ in CD122⁻ vs CD122⁺ CD4⁺ or CD8⁺ T lymphocytes. Friedman test followed by the Dunn test comparing percentage of CNS or splenic IFN γ ⁺ in CD122⁻ vs CD122⁺ CD4⁺ or CD8⁺ T lymphocytes $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$; $^{*}p < 0.01$, $^{****}p < 0.0001$. ANOVA = analysis of variance; EAE = experimental autoimmune encephalomyelitis; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; TNF = tumor necrosis factor.

(figure 5, C and D). Finally, a higher proportion of splenic CD122⁺ CD4⁺ T lymphocytes produced IFN γ or GM-CSF compared with CD122⁻ counterparts, whereas only the percentage of splenic CD122⁺ CD8⁺ T lymphocytes producing GM-CSF was elevated compared with CD122⁻ counterparts (figure 5, C and D). Overall, our results highlight that at the peak of EAE disease, within CNS-infiltrating T lymphocytes, proinflammatory CD4⁺ and CD8⁺ T lymphocytes are enriched among those carrying CD122 and are thus susceptible to IL-15.

Peripheral administration of the active form of IL-15 increases EAE severity

To definitively establish the role of IL-15 in EAE and to mimic the elevated IL-15 levels we and others detected in the blood

of patients with MS,^{2,22} we investigated the impact of increasing peripheral levels of IL-15 after disease onset. We used the same approach as published by others and injected the biologically active form of IL-15 precomplexed with IL-15Ra.^{23,24} We used a physiologic approach to increase IL-15 levels in vivo using the IL-15/IL-15Ra complex.²⁵ This complex is the biologically active form of the cytokine²⁵ and mimics what is found in mouse and human biological fluids.²⁶ At disease onset (EAE score 1.0–1.5), mice were randomized into 2 groups and received a daily injection of the IL-15/IL-15Ra complex or the control IL-15Ra for 8 consecutive days. EAE clinical scores from 3 pooled experiments are represented (figure 6A). IL-15/IL-15Ra-treated mice exhibited a clear and significant worsening of disease severity compared

Figure 6 Peripheral administration of the active form of IL-15 increases EAE severity

From disease onset and for 8 days, EAE mice were injected IP with either the IL-15/IL-15Ra complex or IL-15Ra alone (used as a control) and followed for clinical score over numerous days. A subset of mice (not included in the clinical scoring) were killed 9 or 14–15 days after treatment initiation for flow cytometry analysis of the CNS and spleen. (A) Clinical scores are shown as mean $\pm$ SEM. Two-way ANOVA and Bonferroni test IL-15/IL-15Ra complex vs IL-15Ra alone day 8–26. * to **** p values: * p < 0.05 (days 8, 18, 19, and 22–26), ** p < 0.01 (days 20 and 21), *** p < 0.001 (days 15–17), **** p < 0.0001 (days 9–14), n = 18–22 per group. (B) Number of splenocytes (upper graph) and percentage of CD4⁺ and CD8⁺ T lymphocytes among splenocytes (lower graph) at day 9 (D9) and 15 (D15) posttreatment initiation. Unpaired t test D9-number of splenocytes IL-15/IL-15Ra complex vs IL-15Ra ** p < 0.01. One-way ANOVA followed by the Fisher LSD post hoc test comparing percentage of CD8⁺ T lymphocytes IL-15/IL-15Ra complex vs IL-15Ra ** p < 0.01. (C) Percentages of CD4⁺ and CD8⁺ T lymphocytes within CNS-infiltrating CD45^{hi}CD11b⁺ cells in each group, day 15 (upper graph). Relative number of CNS cells per mouse for each group, day 15 (lower graph). One-way ANOVA followed by the Fisher LSD post hoc test comparing IL-15/IL-15Ra complex vs IL-15Ra * p < 0.05. (D) Relative numbers of CD4⁺ and CD8⁺ T lymphocytes within the CNS producing IFN γ , IL-17, GM-CSF, or granzyme B for each group, day 15. One-way ANOVA followed by the Fisher LSD post hoc test number of mediator-producing CD4⁺ or CD8⁺ T lymphocytes IL-15/IL-15Ra complex vs IL-15Ra # p = 0.0686, * p < 0.05, ** p < 0.01. Results are expressed as mean $\pm$ SEM n = 3–8 for (B and D). ANOVA = analysis of variance; EAE = experimental autoimmune encephalomyelitis; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; LSD = least significant difference; TNF = tumor necrosis factor.

with control mice from day 9 posttreatment up to the end of the monitoring period (up to 20 days after treatment); mice injected with PBS only had similar clinical scores to IL-15Ra only (data not shown). Increasing peripheral IL-15 levels thus triggered long-lasting effects beyond the treatment period.

To uncover the impact of IL-15 on immune cells, we performed flow cytometry analysis on spleen and CNS (pooled spinal cord and brain) cells. The number of splenocytes was

significantly increased on day 9 (1 day after the last injection) in the IL-15/IL-15Ra-treated mice compared with controls, but came back to similar levels at day 15 (figure 6B). The proportion of CD8⁺ T lymphocytes within splenocytes was significantly greater in the IL-15/IL-15Ra compared with the control group at day 9 (figure 6B). Our results correlate with a previous report showing induced proliferation of CD8⁺ T lymphocytes on IP injection of the IL-15/IL-15Ra complex.²⁴ We compared CNS-infiltrating T lymphocytes in both

groups at day 15 posttreatment initiation, which was 1 week after the treatment regimen was stopped (figure 6C). We observed that the percentage within nonmyeloid CNS leukocytes and the relative number of CD4⁺ T lymphocytes per CNS were significantly increased in the IL-15/IL-15-Rα-treated group compared with the controls (figure 6C).

We evaluated the percentages of mediator-producing T lymphocytes in the spleen and CNS of EAE mice at day 15 posttreatment initiation. We observed that the percentage of splenic CD8⁺ T lymphocytes producing IFNγ was significantly increased in the IL-15/IL-15-Rα group compared with the controls, whereas the proportions of CD4⁺ T lymphocytes producing this cytokine were similar in both groups (figure e-3A, links.lww.com/NXI/A358). The percentages of splenic CD4⁺ or CD8⁺ T lymphocytes producing IL-17, GM-CSF, or granzyme B were similar in both groups (figure e-3A). Among the CNS-infiltrating CD4⁺ and CD8⁺ T lymphocytes, the percentages of IFNγ, GM-CSF, or granzyme B were similar between both groups (figure e-3B). Furthermore, the percentage of IL-17–producing CD4⁺ T lymphocytes was elevated in the IL-15/IL-15-Rα group compared with the control group, without reaching significance. However, we observed a significantly higher number of CNS-infiltrating CD4⁺ T lymphocytes producing IL-17, GM-CSF, or granzyme B in IL-15/IL-15-Rα-treated mice compared with controls (figure 6D). The transitory increase of IL-15 levels (8 days) was sufficient to cause a long-term effect on EAE disease pathobiology (figure 6A) and an increased number of CNS-infiltrating inflammatory CD4⁺ T lymphocytes (figure 6D). Overall, our results highlight that a transient increase of peripheral IL-15 levels has a long-term impact on EAE pathobiology.

Discussion

Accumulating evidence supports a key role for IL-15 in chronic inflammatory diseases including MS.^{2,27–30} In the present study, we establish that IL-15 contributes to the amplification of inflammatory T-cell responses after disease onset in both patients with MS and EAE mice. We demonstrate that despite similar expression of the CD122/IL-15Rβ chain (figure 1), CD4⁺ and CD8⁺ T-cell subsets from both patients with RRMS and PMS exhibit an enhanced response to IL-15, but not IL-2, compared with age/sex-matched HCs as documented by the STAT5p response (figure 2 and figure e-1, links.lww.com/NXI/A356). IL-15 enhances more strongly the production of IL-17 by CD4⁺ T lymphocytes and the production of GM-CSF by CD8⁺ T lymphocytes from patients with MS compared with HCs (figures 3 and 4). Finally, we establish that a transient boost of peripheral IL-15 levels after symptom onset causes a sustained increase in EAE disease severity and an elevated number of IL-17, GM-CSF, or granzyme B–producing CD4⁺ T lymphocytes in the CNS (figure 6).

Our results demonstrate that expression of CD122 does not contribute to the altered responses of T lymphocytes from

patients with MS (figure 1). Others reported an enhanced expression of IL-15R by CD4⁺ T cells from patients with RRMS and SPMS compared with HCs, but they did not specify which receptor chain was assessed.⁴ We observed similar or elevated proportions of CD25⁺ T lymphocytes in MS compared with HC (figure e-1, links.lww.com/NXI/A356). This is in line with other reports showing either enhanced proportions of CD25⁺CD4⁺ and CD25⁺CD8⁺ effectors T lymphocytes in patients with RRMS or no change of CD25 expression in CD4⁺ T lymphocytes obtained from patients with RRMS and SPMS.^{31,32} We did not assess regulatory T lymphocytes among the CD25⁺CD4⁺ T lymphocyte population, which would require additional identifying markers (FoxP3⁺ CD127^{low}). However, we do not believe that this population significantly contributes to the greater proportions of CD25⁺CD4⁺ T lymphocytes we observed in patients with PMS compared with HCs (figure e-1) since several groups demonstrated that patients with MS have an unaltered number of regulatory T cells compared with controls.³³

We show that the IL-15–induced STAT5 signaling is enhanced in most T-cell subsets from both patients with RRMS and PMS compared with HCs (figure 2). Total unphosphorylated STAT5 and baseline pSTAT5 have been shown to be similar in T lymphocytes from MS and HC³⁴; notably, we did not detect differences in baseline pSTAT5 between donor groups (data not shown). Therefore, we can rule out that elevated availability of STAT5 contributes to the enhanced STAT5p triggered by IL-15 in patients with MS. Notwithstanding upregulated CD25 expression in some MS T-cell subsets (figure e-1B, links.lww.com/NXI/A356), IL-2–induced pSTAT5 signaling was similar in all T-cell subsets from patients with MS and controls (figure e-1C). Thus, IL-15 and IL-2, despite their shared receptor chains, have distinct biological impacts.⁵ Notably, another cytokine using CD132 as a coreceptor, IL-7, triggered pSTAT5 in higher proportions of CD4⁺ and CD8⁺ memory T lymphocytes from patients with RRMS compared with controls.³⁴ Moreover, increased pSTAT5 levels in autoreactive CD4⁺ T lymphocytes have been associated with a more aggressive disease course in patients with systemic lupus erythematosus.³⁵

The mechanisms contributing to the enhanced IL-15–triggered STAT5 phosphorylation we observed in T lymphocytes from patients with MS remain unknown. Signaling responses of T lymphocytes are shaped by the composition and abundance of lipid rafts.³⁶ Notably, T lymphocytes from patients with autoimmune disease (e.g., systemic lupus erythematosus) contain more cholesterol and GM1 (lipid raft maker) than cells from healthy donors.³⁶ Moreover, higher expression of lipid rafts on murine CD8 T lymphocytes has been shown to correlate with greater responses to IL-2 and IL-15, including pSTAT5.³⁷ Additional studies will be necessary to investigate whether the composition and abundance of lipid rafts in T lymphocytes of patients with MS is altered compared with HCs and how such alterations affect their responses to cytokines. Overall, these

studies and our work underscore the potential central role of altered STAT5 signaling in response to multiple cytokines in T lymphocytes from patients with autoimmune diseases.

Several reports suggest that STAT5 contributes to T cell-mediated neuroinflammation. Mice carrying *stat5*-deficient CD4⁺ and CD8⁺ T lymphocytes developed EAE with a lower incidence and disease severity due to impaired production of GM-CSF by autoreactive CD4⁺ T lymphocytes and reduced expansion of pathogenic Th17 cells.²⁰ Moreover, the interplay between Th17 cells and IL-15 has been suggested in human inflammatory diseases such as psoriasis and rheumatoid arthritis.^{17,38–40} IL-15 increased the percentage of IL-17-producing CD4⁺ T lymphocytes from patients with MS, but it had no impact on such production by T lymphocytes from HCs in our conditions (figure 4B). The boosting impact of IL-15 on the proportion of TNF or IFN γ -producing T lymphocytes was similar in MS and HC. Remarkably, IL-15's capacity to enhance GM-CSF production by T lymphocytes was more prominent in patients with PMS than in cells from HCs (figure 4C). Further studies will be necessary to delineate the relationship between the elevated STAT5 signaling and the upregulation of inflammatory cytokines by T lymphocytes in patients with MS.

Others have postulated that IL-15 is beneficial to EAE based on the aggravated disease in IL-15-deficient mice compared with wild-type controls.^{7–9} However, the immune system of IL-15-deficient mice exhibits multiple impairments such as reduced numbers of several immune cell subsets (e.g., natural killer cells and CD8 T lymphocytes),^{11,12} some of which are comprised of potential regulatory immune cell subsets. Therefore, the worse disease observed in IL-15-deficient mice could be due to multiple factors beyond the sole absence of IL-15. One group found that injection of IL-15 alone before EAE symptom onset tends to alleviate disease severity, albeit very modestly.¹⁰ This discrepancy with our results may be due to several methodological differences such as mouse strain (SJL/J), dose, and form of IL-15 (IL-15 alone). We recognize these differences; nevertheless, we believe that our experimental approach more closely models the human disease and provides solid evidence that IL-15 enhances the severity of the ongoing disease.

Collectively, our results point to the deleterious role of IL-15 in MS and its animal model EAE. Our in vivo data support IL-15 as an appealing therapeutic target given its impact after disease onset. Finally, the universality of the enhanced IL-15-triggered responses in T lymphocytes from all kinds of patients with MS (RRMS and PMS) underlines that this cytokine represents a common deleterious factor that could potentially be targeted in most patients with MS.

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Disclosure

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Cyril Laurent, PhD	Department of Neuroscience, Faculty of Medicine, Université de Montréal; Centre de recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Québec, Canada	Conducted experiments, analyzed and interpreted data, and wrote the manuscript
Gabrielle Deblois, MSc	Department of Neuroscience, Faculty of Medicine, Université de Montréal; Centre de recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Québec, Canada	Conducted experiments and analyzed and interpreted data
Marie-Laure Clénet, PhD	Department of Neuroscience, Faculty of Medicine, Université de Montréal; Centre de recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Québec, Canada	Conducted experiments and analyzed and interpreted data
Ana Carmena Moratalla, MSc	Department of Neuroscience, Faculty of Medicine, Université de Montréal; Centre de recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Québec, Canada	Conducted experiments

Appendix (continued)

Name	Location	Contribution
Negar Farzamkia, BSc	Department of Neuroscience, Faculty of Medicine, Université de Montréal; Centre de recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Québec, Canada	Wrote the manuscript
Marc Girard, MD	Multiple Sclerosis CHUM Clinic, Department of Neuroscience, Faculty of Medicine, Université de Montréal; Centre de recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Québec, Canada	Was involved in the collection of human samples and clinical characterization of patients
Pierre Duquette, MD	Multiple Sclerosis CHUM Clinic, Department of Neuroscience, Faculty of Medicine, Université de Montréal; Centre de recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Québec, Canada	Was involved in the collection of human samples and clinical characterization of patients
Alexandre Prat, MD, PhD	Multiple Sclerosis CHUM Clinic, Department of Neuroscience, Faculty of Medicine, Université de Montréal; Centre de recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Québec, Canada	Was involved in the collection of human samples and clinical characterization of patients
Catherine Larochelle, MD, PhD	Multiple Sclerosis CHUM Clinic, Department of Neuroscience, Faculty of Medicine, Université de Montréal; Centre de recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Québec, Canada	Was involved in the collection of human samples and clinical characterization of patients and provided important scientific input
Nathalie Arbour, PhD	Department of Neuroscience, Faculty of Medicine, Université de Montréal; Centre de recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Québec, Canada	Designed the study, analyzed and interpreted the data, wrote the manuscript, and secured funding

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