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Neurobiology

IL-21 and IL-21 Receptor Expression in Lymphocytes and Neurons in Multiple Sclerosis Brain

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IL-17–producing CD4$^+$ T cells (Th-17) contribute to the pathogenesis of experimental autoimmune encephalomyelitis and are associated with active disease in multiple sclerosis (MS). In addition to IL-17, Th-17 cells can also express IL-21, IL-22, and IL-6 under Th-17–polarizing conditions (IL-6 and transforming growth factor-β). In this study we investigated IL-21 and IL-21 receptor (IL-21R) expression in MS lesions by *in situ* hybridization and immunohistochemistry. We detected strongly IL-21$^+$ infiltrating cells predominantly in acute but also in chronic active white matter MS lesions in which IL-21 expression was restricted to CD4$^+$ cells. In contrast, IL-21R was much more broadly distributed on CD4$^+$, CD19$^+$, and CD8$^+$ lymphocytes but not major histocompatibility complex class-II$^+$ macrophages/microglia. Interestingly, in cortical areas we detected both IL-21 and IL-21R expression by neurons. These findings suggest role(s) for IL-21 in both the acute and chronic stages of MS via direct effects on T and B lymphocytes and, demonstrated for the first time, also on neurons. *(Am J Pathol 2011, 178:794–802; DOI: 10.1016/j.ajpath.2010.10.043)*

Multiple sclerosis (MS) is the most common neuro-inflammatory demyelinating disease of the central nervous system (CNS), and it affects both the white and gray matter. White matter lesions are characterized by inflammatory infiltration, demyelination and gliosis, with axonal loss, whereas cortical lesions are characterized by demyelination and modest neuronal loss.[1] Interleukin-17 (IL-17)–producing CD4$^+$ T cells (Th-17) cells play important roles in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model of MS,[2] and are also found at high frequency in active MS lesions.[3] However, because IL-17 cytokines seem to be only partially involved in the development of EAE,[4,5] other concomitant Th-17 cell products may contribute to the disease process and thus demand investigation. They include IL-6,[4] IL-21,[6] and IL-22.[7] IL-6 is implicated because EAE was prevented either by its genetic deletion[8] or by monoclonal antibody blockade of its receptor.[9] In MS, IL-6 has been detected predominantly in astrocytes and some macrophages at sites of ongoing demyelination[10] and also in inactive plaques.[11] By contrast, the mainly mucosal IL-22 does not appear to be directly involved in the pathogenesis of EAE, because IL-22 knock-out mice are fully susceptible.[7]

Involvement of IL-21 in EAE is controversial; its administration before EAE induction leads to NK cell activation and exacerbated disease,[12] and other studies have shown that IL-21 also leads to Th-17 activation and IL-17 expression *in vitro*.[13,14] However, blockade of IL-21 before or after EAE induction reportedly accelerated EAE, with more severe inflammatory infiltration, because of reduction of regulatory T cells.[15,16] Studies in IL-21 and/or IL-21 receptor (IL-21R) knock-out mice have also yielded apparently conflicting evidence as to the role of IL-21 in EAE. In one study, the absence of IL-21 prevented Th-17 cell activation and ameliorated EAE[6]; however, in three subsequent studies in IL-21$^{-/-}$ and/or IL-21R$^{-/-}$ mice, IL-21 was not required for

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disease promotion and may even have partially protected against EAE.[15,17,18]

IL-21 expression has not yet been studied in MS CNS tissue, but studies examining T-cell clones derived from patients with MS have identified a subset of myelin basic protein–specific T cells with a pro-inflammatory phenotype that express IL-21.[19] In addition, polymorphisms in *IL-21R* showed associations in EAE and MS in a recent genetic study.[20] *IL-21* polymorphisms have been associated with systemic lupus erythematosus[21] and diabetes mellitus, whereby a polymorphism in the IL-21R was also studied.[22] Expression studies have shown that IL-21 is up-regulated in gastrointestinal inflammation, notably in Crohn's and celiac diseases.[23,24] Taken together, these studies strongly implicate IL-21 in several autoimmune diseases, including MS.

Here, we present the first study on the expression of IL-21/IL-21R mRNA and protein in acute, chronic, and inactive MS lesions by *in situ* hybridization and immunohistochemistry. We show expression of IL-21 by the majority of $CD4^+$ T cells and of IL-21R by the majority of T and B cells in acute and chronic MS lesions. We therefore propose direct and pleiotropic roles of IL-21 in MS pathogenesis. We also detected expression of IL-21 and IL-21R in neurons in the cortical gray matter, which may implicate IL-21 in modulating neuronal responses to inflammation and neuronal injury, an observation that warrants further exploration.

Materials and Methods

Tissue Specimens

All brain tissues were obtained with informed consent and Research Ethics Committee approval from the UK Multiple Sclerosis Tissue Bank, the Thomas Willis Oxford Brain Collection, and NeuroResource, UCL Institute of Neurology. Both paraffin and frozen brain tissues (see Supplemental Tables S1 and S2 at *http://ajp.amjpathol.org*) were used for this study, obtained at autopsy from patients with MS (25 cases, 36 blocks) and control patients with other neurologic diseases [13 cases, 13 blocks; subacute sclerosing panencephalitis (SSPE), *Herpes simplex* encephalitis (HSE), acute disseminated encephalomyelitis (ADEM), and stroke] or nonneurologic diseases (8 cases, 9 blocks). We typed white matter lesions by staining with oil red O (ORO; for frozen tissue) or for paraffin-embedded tissue, Luxol fast blue (LFB), anti–major histocompatibility complex (MHC) class II and H&E to identify the centers and borders of the lesions, their activity, hypercellularity (inflammatory cell infiltration), and areas of hypocellularity (loss of parenchymal cells in inactive lesions). In all cases, the clinical diagnosis had been made during life and confirmed neuropathologically at autopsy.

Lesions were classified into three groups: i) acute lesions with hypercellularity due to infiltration with lymphocytes and numerous macrophages (frozen tissue: macrophages staining strongly with ORO; paraffin-embedded tissue: macrophages staining with LFB or for anti–proteolipid protein); ii) chronic active lesions, typically with hypercellular active borders with strongly staining ORO^+ or LFB^+ macrophages and demyelinated hypocellular/inactive centers with very few ORO^- or LFB^- macrophages; iii) inactive lesions, characteristically showing demyelination, severe hypocellularity due to the significant loss of parenchymal cells, and only minimal inflammatory infiltration staining negative with ORO or LFB.

In Situ *Hybridization*

IL-21R and IL-21 oligoprobes were obtained from Exiqon labeled with Digoxigenin.[25] Sections were processed for *in situ* hybridization cytochemistry as previously described.[26] In brief, frozen tissue sections (10 μm) were fixed for 11 minutes with 4% paraformaldehyde followed by treatment with proteinase K (Sigma-Aldrich, Dorset, UK) for 20 minutes at room temperature under RNase-free conditions. Hybridization was performed overnight at 56°C with the digoxigenin-3′–coupled IL-21R linked nucleic acid oligoprobe (5′-GAGCGCCAGGAGATATTATACTGT-3′) and at 54°C with the digoxigenin-3-5′–coupled IL-21 linked nucleic acid oligoprobe (5′-ATGAAGGGCATGTTAGTCTGTGTT-3′). After washing, the sections were incubated with antidigoxigenin alkaline phosphatase–conjugated Fab fragments (1:2000; Roche Diagnostics) overnight at 4°C and visualized with the use of Nitro-Blue Tetrazolium Chloride/5-Bromo-4-Chloro-3′-Indolyphosphate p-Toluidine Salt (Roche Diagnostics) chromogenic reaction. Positive controls with fresh frozen tonsil showed specific signals in lymphocytes and epithelial cells (data not shown). As a negative control, a digoxigenin-3′–coupled non-complementary linked nucleic acid oligoprobe (5′-GTGTAACACGTCTATACGCCCA-3′) was used under the same conditions as the IL-21 and IL-21R linked nucleic acid oligoprobes on fresh frozen tonsil (data not shown) and consecutive sections of MS brains (Figures 1B, 4B, 5C, and 6C).

Immunohistochemistry

Paraffin-embedded sections (6 μm thick) were deparaffinized followed by hydration with graded alcohol solutions. Endogenous peroxidase was blocked by incubation with 0.75% hydrogen peroxide for 15 minutes, followed by antigen unmasking with the use of heated citrate buffer (pH, 6). Sections were stained with mouse anti–IL-21 (1/400; 1F1A2B2C2; Novo Nordisk, Denmark). Slides were developed with the Envision kit by incubation for 1 hour at room temperature with the horseradish peroxidase anti-rabbit/mouse complex (Dako, Cambridgeshire, UK) as described by the manufacturer. Staining was performed with diaminobenzidine (Dako) as chromogenic substrate for up to 5 minutes.

For double labeling, we used anti–IL-21 mouse monoclonal or IL-21R rabbit polyclonal (ab13268; Abcam) antibodies, followed by staining with the Tyramide Signal Amplification kit with Alexa Fluor 488–anti-mouse IgG (Invitrogen, Paisley, UK). For IL-21 staining, sections were blocked with anti-mouse IgG antibody (1/200) to prevent background staining. After washing, slides were then incubated overnight with the following sequential primary antibodies: mouse anti-CD4 (1/10; 4B12; Novocastra, UK), mouse anti-CD8 (1/20; kindly donated by Dr Margaret Jones, John

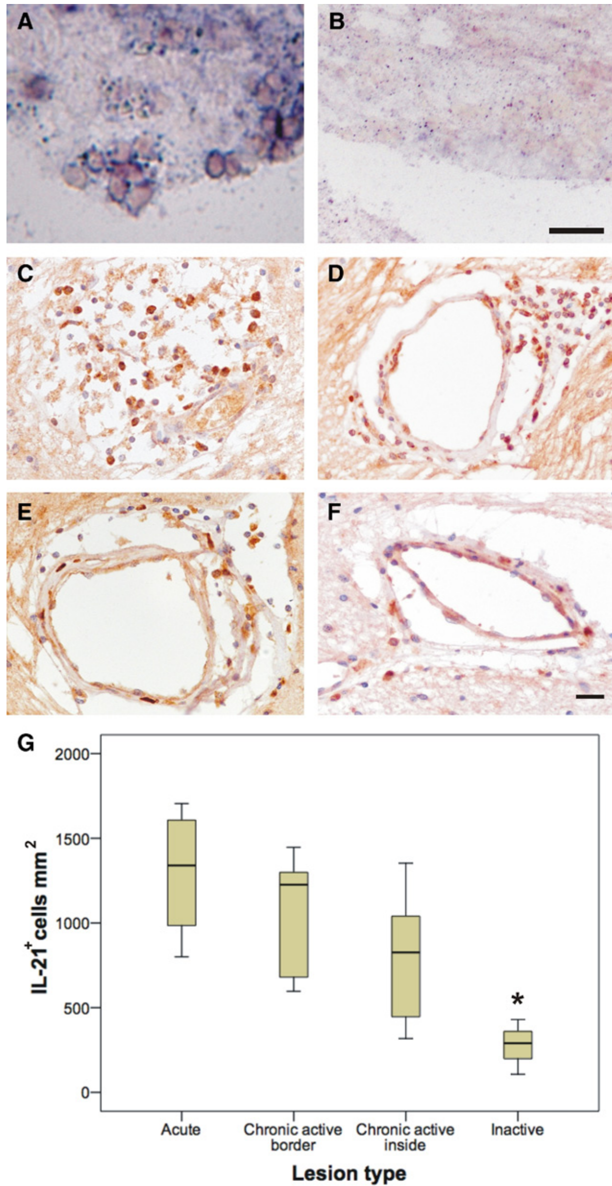

Figure 1. IL-21 is predominantly expressed in acute and chronic active MS lesions. **A:** High-magnification image of IL-21 mRNA expression in an active border of a chronic active MS lesion visualized by *in situ* hybridization with the use of a specific probe for IL-21. **B:** Negative control that used a scrambled oligoprobe on a consecutive section from the same cuff. **C-F:** Light microscopy analysis of IL-21 immunostaining in perivascular cuffs of lesions with different activity. **C**: Acute lesion, (**D**) active border of a chronic active lesion, (**E**) inactive center of a chronic active lesion, (**F**) an inactive lesion. Scale bars = 30 μm. **G:** Density of IL-21$^+$ cells in MS lesions of different activity. There is a statistically significant difference of $^*P < 0.05$ between the density of IL-21$^+$ cells in inactive lesions and in each of the other lesion types.

Radcliffe Hospital, Oxford, UK), rabbit anti–glial fibrillary acidic protein (1/1000; DAKO), sheep anti-carbonic anhydrase isoenzyme II (1/300; Serotec), rat anti-MHC class II (1/100; Serotec), or mouse anti-microtubule associated protein (1/200; M-1406; Sigma, UK). Appropriate secondary antibodies included Cy3 anti-rat Ig (1/100; Jackson Laboratory, Suffolk, UK) and Alexa Fluor 568–anti-rabbit (1/200) or anti-sheep Ig (1/200; both Invitrogen) for 4 hours. As negative controls for each second layer in sequential stainings, the same procedure as mentioned previously was followed except that the sequential primary antibodies were omitted. No staining was seen in these sections appropriate to the sequential secondary antibody.

Data Acquisition

Areas of different activity as defined above were used for quantitation, from acute, chronic active, and inactive lesions and from control brains (see Supplemental Table S1 at *http://ajp.amjpathol.org*). Digital images of tissue sections were captured with an Olympus light microscope BX41 and a Zeiss camera (Zeiss, Thornwood, NY). Multiple images of different perivascular cuffs were acquired, and their areas were calculated by the Axion Image software (Zeiss). IL-21$^+$ cells with lymphocytic morphology were counted by eye within predefined areas of each perivascular cuff, and their densities were expressed per mm^2. In each lesion, three to six cuffs with a total area $>2 \times 10^5$ μm^2 were counted. To investigate colocalization of IL-21 or IL-21R proteins with lymphocyte subpopulations (CD4$^+$ T cells, and of IL-21R with CD4$^+$ or CD8$^+$ T cells and CD19$^+$ B cells), multiple images were acquired with the use of a confocal microscope (Bio-Rad, Hercules, CA). For counting percentages of CD4$^+$ cells in lesion areas of different activity, multiple images were taken from all of the cuffs, and their cells were counted with ImageJ software. To quantify and

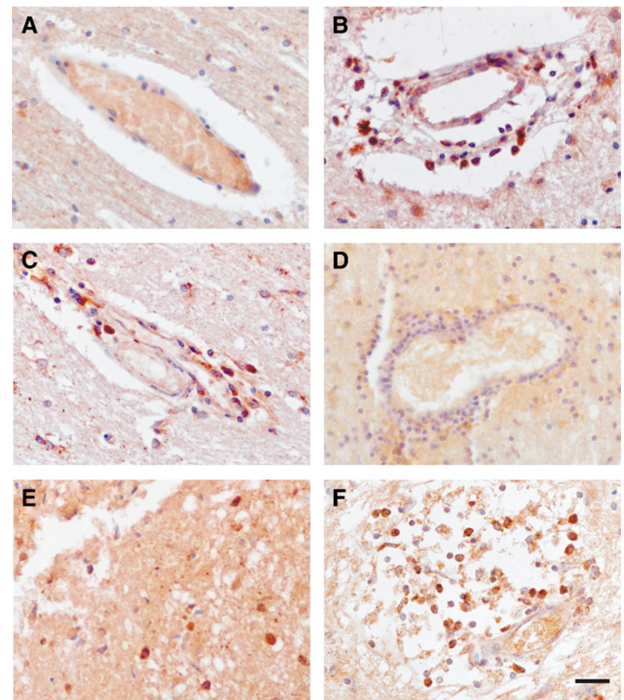

Figure 2. IL-21 expression in other neuro-inflammatory diseases. IL-21 expression was not detected lymphocytes within control brain (**A**). In the neuro-inflammatory conditions of SSPE (**B**) and HSE (**C**), significant numbers of IL-21$^+$ lymphocytes were observed in perivascular spaces. Surprisingly, in ADEM (four cases), few, if any, IL-21$^+$ lymphocytes were observed (**D**). In stroke (**E**), few IL-21$^+$ lymphocytes were observed in perivascular spaces, but high infiltration of IL-21$^+$ lymphocytes in the parenchyma of the white and gray matter was observed. **F:** An acute MS lesion is shown for comparison (same as in Figure 1). Scale bar = 50 μm.

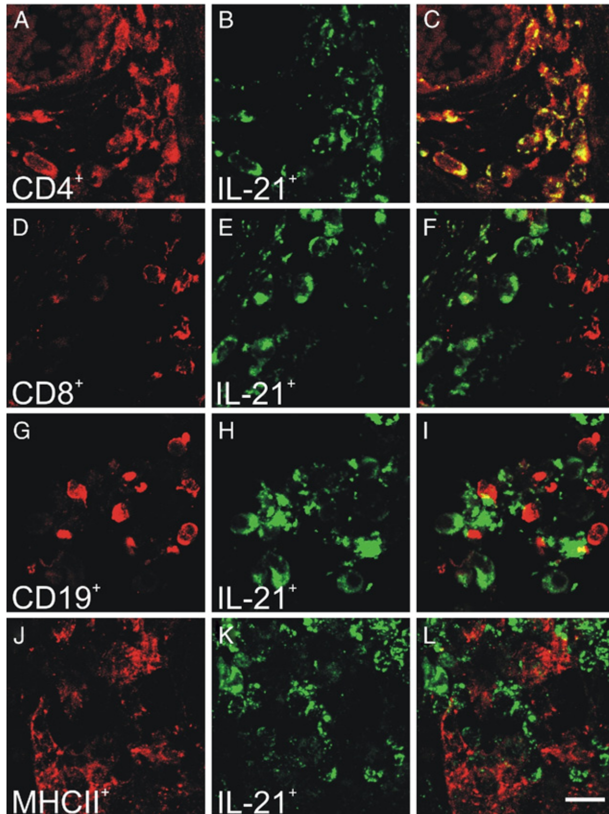

Figure 3. IL-21 is restricted to CD4$^+$ cells in acute MS lesions. A high percentage of CD4$^+$IL-21$^+$ cells are associated with acute and chronic active MS lesions but not with inactive lesions. Double-immunofluorescence staining in the perivascular space of active areas of MS lesions was analyzed by confocal microscopy for IL-21 staining (**B**, **E**, **H**, and **K**; green), CD4$^+$ (**A**; Th cells, red), CD8$^+$ (**D**; cytotoxic lymphocytes, red), CD19$^+$ (**G**; B lymphocytes, red), and MHCII$^+$ (**J**; macrophages/microglia). The overlays (**C**, **F**, **I**, and **L**) show specific expression of IL-21 in CD4$^+$ cells in panel **C** (but not in the other cell types). Scale bar = 20 μm.

compare IL-21 and IL-21R protein expression in neurons gray matter cortical areas from MS tissue cases and controls were measured, taking images of neurons and calculating the intensity of signal via semiquantitative microdensitrometry with the use of ImageJ software.

Statistical Analysis

Statistical significances were calculated with Student's *t*-test for unequal variances and single analysis of variance with multiple comparisons (Tukey's test) as appropriate. Data are presented as mean ± SEM. $*P$ value < 0.05 denotes statistically significant differences.

Results

Most CD4$^+$ T Cells Infiltrating Active MS Lesions Are IL-21$^+$

We first studied the expression of IL-21 mRNA and protein in MS lesions. In white matter lesions, IL-21 mRNA expression was detected specifically in perivascular cells, which showed the disposition and morphology typical of lymphocytes (Figure 1A). Next, we compared IL-21 mRNA expression in lesions of different activity and also confirmed our findings independently by staining for IL-21 protein in an overlapping series of samples. We observed high densities of IL-21$^+$ cells within perivascular areas, whether in acute MS lesions (Figure 1C), active borders of chronic active MS lesions (Figure 1D), or their inactive interiors (Figure 1E). However, the densities of IL-21$^+$ cells (Figure 1F) were significantly higher in acute (P = 0.01) or chronic active (P = 0.03) than in inactive MS lesions; in the latter, they did not differ significantly between the active borders and inactive interiors (P = 0.21) (Figure 1G). In control white matter, no IL-21$^+$ lymphocytes were detected.

Interestingly, we also found significant numbers of perivascular IL-21$^+$ cells in two other neuro-inflammatory conditions, SSPE (Figure 2B) and HSE (Figure 2C). By contrast, in stroke (Figure 2E), infiltrating IL-21$^+$ cells were seen mostly in the ischemic parenchyma of the white and gray matter, suggesting a possible role of IL-21 in inflammatory/ischemic-mediated tissue injury. Interestingly, in ADEM (Figure 2D), an acute monophasic neuro-inflammatory disease with abundant CD4$^+$ T-cell lymphocytes in perivascular cuffs,[27] few, if any, IL-21$^+$ cells were observed in any of the four cases, suggesting different pathogenic mechanisms.[28]

IL-21 Is Restricted to CD4$^+$ Cells and Is Associated with Acute and Chronic Active MS Lesions

We next identified the immune cell subpopulations expressing IL-21 in MS lesions, by co-staining for CD4$^+$ (Th; Figure 3, A–C) or CD8$^+$ (cytotoxic) T cells (Figure 3, D–F), CD19$^+$ (B cells; Figure 3, G–I), and MHC class II$^+$ cells (microglia/macrophages; Figure 3, J–L). Among these, IL-21 expression was restricted to CD4$^+$ cells and not observed in CD8$^+$, CD19$^+$, or MHCII$^+$ cells (Figure 3). Approximately 67% to 75% of the CD4$^+$ cells were IL-21$^+$ in acute and in both active borders and interiors of chronic active MS lesions but in only 26% of CD4$^+$ T cells in inactive lesions ($P < 0.01$; Table 1). These data show

Table 1. CD4$^+$ Cells Predominantly Express IL-21 in Acute and Chronic Active MS Lesions

	MS lesion category			
	Acute (% ± SEM)	Chronic active (border) (% ± SEM)	Chronic active (inside) (% ± SEM)	Inactive (% ± SEM)
CD4$^+$ cells that are IL-21$^+$	75.3 ± 4.6	73.8 ± 4.7	66.8 ± 4.3	26 ± 6.4*

For each lesion category, three to five lesions with 75 ± 20 cells/area were measured (see Supplemental Figure S1 at *http://ajp.amjpathol.org*). Higher percentages of IL-21$^+$CD4$^+$ T cells within active and chronic active lesions are shown, in contrast to inactive lesions in which there is a low frequency of IL-21$^+$CD4$^+$ cells.
$*P < 0.001$.

that a high proportion of $CD4^+$ T cells express IL-21 in the acute (acute lesions) and chronic (chronic active lesions) phases of MS, and this proportion declines only in inactive MS lesions ($P < 0.01$). As observed with single immunostaining (see above), there was no difference in the percentage of $IL21^+CD4^+$ cells between the active borders and the inactive interiors of chronic active lesions ($P = 0.693$). These findings suggest that IL-21 plays a role in maintaining a pro-inflammatory environment in chronic active MS lesions.

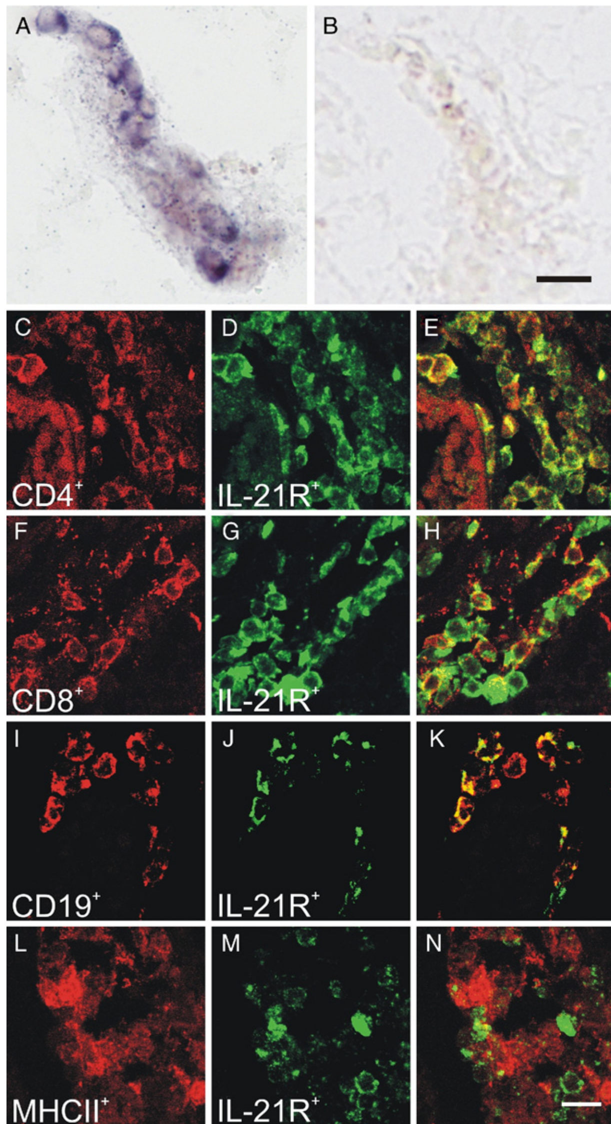

Figure 4. High expression of IL-21R on $CD4^+$ and $CD8^+$ T cells and $CD19^+$ B cells in acute and chronic active MS lesions. **A:** Perivascular cuff showing high expression of IL-21R mRNA in perivascular lymphocytes in an active area of a white matter MS lesion. **B:** Negative control that used the scrambled oligoprobe in a consecutive section. Double-immunofluorescence staining in the perivascular region of active areas of MS lesions was analyzed by confocal microscopy for IL-21R expression (**D**, **G**, **J**, and **M**; green) in $CD4^+$ (**C**; Th cells, red), $CD8^+$ (**F**; cytotoxic lymphocytes, red), $CD19^+$ (**I**; B lymphocytes, red), and MHCII class II (**L**; macrophages/microglia, red). The overlays (**E**, **H**, **K**, and **N**) show expression of IL21R in $CD4^+$ (**E**), $CD8^+$ (**H**), and $CD19^+$ lymphocytes (**K**) and no expression in $MHCII^+$ macrophages/microglia (**N**). Scale bars = 20 μm.

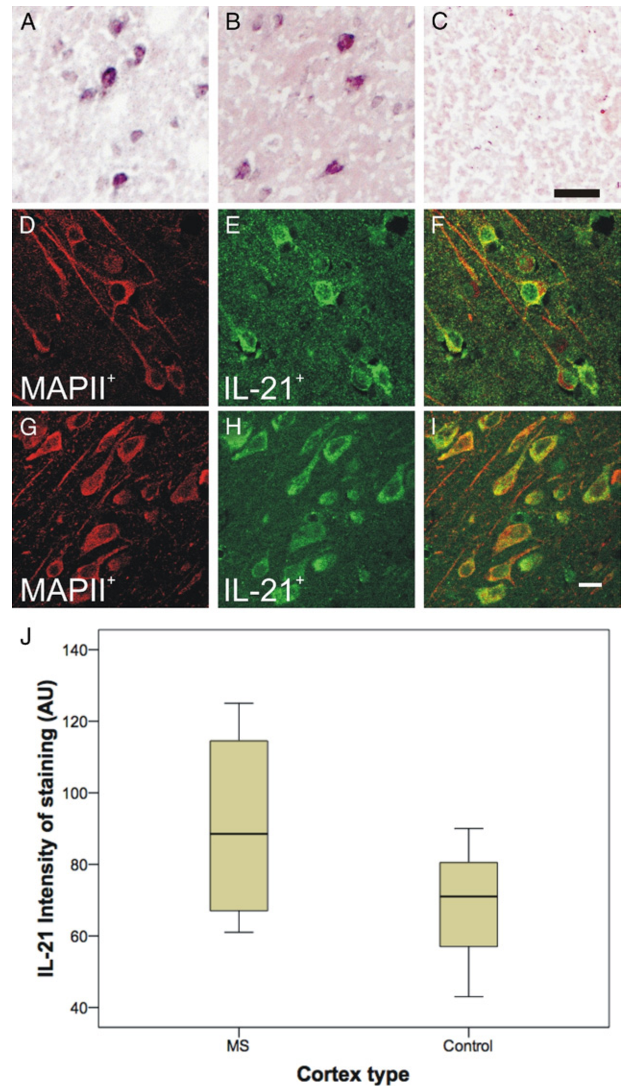

Figure 5. IL-21 mRNA and protein expression in cortical areas from MS and control brain. **A–C:** *In situ* hybridization with a specific probe for IL-21. MS (**A**), normal (**B**); negative control with the use of a non-complementary oligoprobe on a consecutive section of the MS tissue (**C**). **D–I:** Double immunofluorescence staining analyzed by confocal microscopy staining for IL-21 (**E** and **H**; green), MAPII (microtubule associated protein) (**D** and **G**; for neurons, red), taken from cortical areas from MS (**D–F**) and normal brain (**G–I**). Overlays show a similar degree of IL-21 staining in MS and normal brain. Scale bars: 30 μm (**A–C**); 10 μm (**D–I**). **J:** Semiquantitative microdensitometry of $IL-21^+$ neurons in MS cortical neurons and normal control tissue. The difference in IL-21 intensity between normal and MS brains is not statistically significant ($P > 0.05$).

IL-21R Is Highly Expressed in $CD4^+$ and $CD8^+$ T Cells and in $CD19^+$ B Cells in Acute and Chronic Active MS Lesions

Next, we checked for cells capable of responding to IL-21 via its cognate receptor. *In situ* hybridization and immunohistochemistry detected strong signals for IL-21R mRNA and protein, respectively, in white matter MS lesions, specifically in perivascular cells (again showing typical lymphocytic disposition and morphology), predominantly in active areas of acute and chronic active MS lesions (Figure 4A). With the use of double-labeling immunohistochemistry in MS lesions showed IL-21R label-

Table 2. IL-21R Is Predominantly Expressed in $CD4^+$, $CD8^+$, and $CD19^+$ Cells in Acute and Chronic Active MS Lesions

	MS lesion category			
Cell type	Acute	Chronic active (border)	Chronic active (inside)	Inactive
$CD4^+$	76.5 ± 5.3	76 ± 2.5	79.2 ± 5.3	40 ± 5.7*
$CD8^+$	63.3 ± 7.1	60.6 ± 7.6	51.2 ± 7.8	34.3 ± 7
$CD19^+$	68.3 ± 5.1	67 ± 7.5	59 ± 8.2	24 ± 1*

For each cell type within a lesion category, three to five lesions with 45 ± 4 cells/area were measured (see Supplemental Table S1 at *http://ajp.amjpathol.org*). Values are percentage (± SD) of $IL\text{-}21R^+$ cells.
*$P < 0.05$.

ing in $CD4^+$ (Figure 4, C–E), $CD8^+$ (Figure 4, F–H), and $CD19^+$ (Figure 4, I–K) cells, but not on MHC class II^+ macrophages/microglia (Figure 4, L–N). Specifically, in the case of $CD4^+$ cells, IL-21R was detected in approximately 75% to 79% of the cells in acute, active border and within chronic active MS lesions versus only 40% in inactive lesions ($P < 0.05$; Table 2). We noted parallel proportions of $IL\text{-}21R^+$ $CD8^+$ and $CD19^+$ cells within active MS lesions (Table 2), although the reduction in $CD8^+$ cells within inactive MS lesions did not achieve significance ($P > 0.05$).

IL-21 and IL-21R Are Expressed in Neurons in the Gray Matter and Appear Up-Regulated in MS Lesions

Because cytokines and cytokine receptors have been detected previously in neuronal[29] and glial cells,[30] we checked for IL-21 and IL-21R expression by these cells in MS and control brains. Interestingly, in cortical areas of postmortem MS tissue and control brains *in situ* hybridization showed active synthesis of both IL-21 (Figure 5, A–C) and IL-21R (Figure 6, A–C) in cells with neuronal morphology. This was further verified at the protein level with double immunofluorescence in cortical areas showing IL-21 (Figure 5, A–I) and IL-21R (Figure 6, A–I) in cells co-labeling for the neuronal marker microtubule associated protein. Astrocytes and oligodendrocytes were negative for both IL-21 and IL-21R in MS cortical tissue by *in situ* hybridization and fluorescence immunohistochemistry (data not shown) (see Supplemental Figure S1 at *http://ajp.amjpathol.org*).

On semiquantitative microdensitometry, we noted 34% stronger neuronal IL-21 protein labeling of cortical neurons in MS than in control tissue, but the difference was not significant ($P > 0.05$). Interestingly, expression of IL-21R was more than twofold higher in MS than in control cortical neurons ($P < 0.05$). These findings suggest potential roles for IL-21 both in mediating responses by nonimmune cells and in tissue injury in MS lesions.

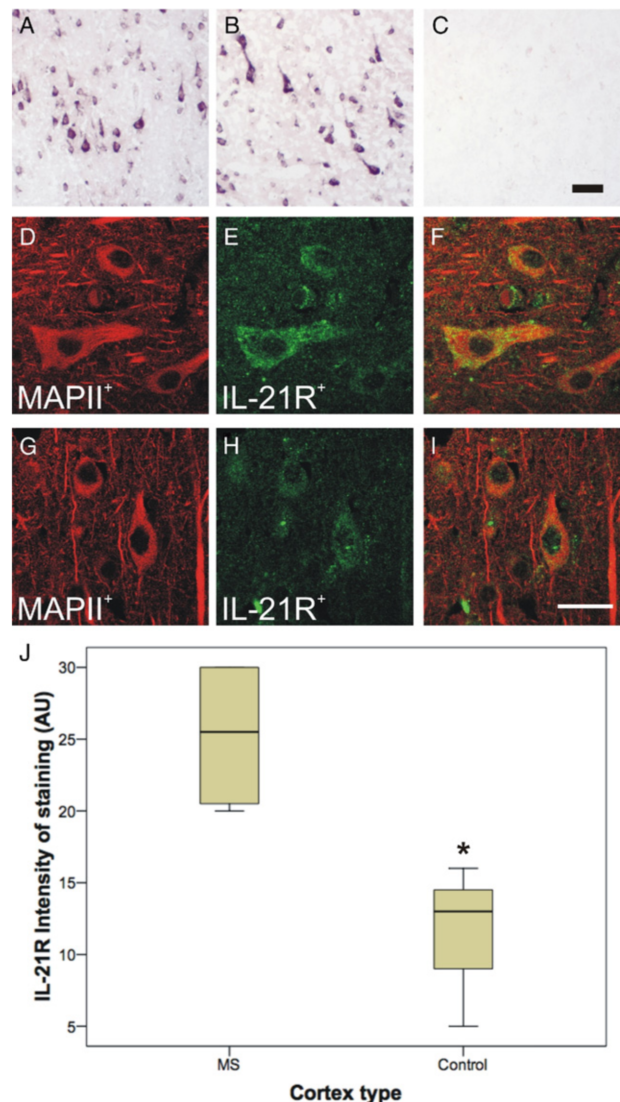

Figure 6. IL-21R mRNA and protein expression in cortical areas from MS and normal brain. **A–C:** *In situ* hybridization with a specific probe for IL-21R. **A:** MS, (**B**) control; (**C**) negative control with the use of a noncomplementary oligoprobe on a consecutive section of the MS tissue. **D–I:** Double immunofluorescence staining analyzed by confocal microscopy staining for IL-21R (**E** and **H**; green), MAPII (microtubule associated protein) (**D** and **G**; for neurons, red), taken from cortical areas from MS (**D–F**) and control brain (**G–I**). Overlays (**F** and **I**) show a similar degree of IL-21R staining in MS and control brain. Scale bar: 30 μm (**A–C**); 15 μm (**E–M**). **J:** Semiquantitative microdensitometry of $IL\text{-}21R^+$ neurons in MS cortical neurons and control tissue. *$P < 0.05$. IL-21R intensity between normal and MS brains.

Discussion

The present study showed that IL-21 expression was not detectable in control white matter but was significantly greater in the $CD4^+$ lymphocytes infiltrating acute and chronic active lesions than in inactive lesions (Figures 1 and 2), suggesting that it plays pro-inflammatory role(s)

in MS pathogenesis. However, sample numbers were not sufficient to stratify these results not only by lesion classification but also by the clinical course of the disease. Comparable white matter infiltrates of IL-21$^+$ cells in MS were also observed in SSPE and HSE, suggesting a similar role of IL-21 in these neuro-inflammatory CNS diseases (Figure 3). In intriguing contrast, IL-21$^+$ inflammatory cells were unexpectedly rare in ADEM, an acute monophasic neuro-inflammatory demyelinating disease.[31] This difference suggests that IL-21 is involved in the maintenance of chronic more than acute inflammation in the brain[32] and also in psoriatic skin.[33] Similarly, it seems that Th-17 cells producing IL-21 are not involved in the pathogenesis of ADEM, but they seem to be more important for MS. This is supported by the finding that IL-17 is not increased in the cerebral spinal fluid (CSF) of patients with ADEM,[34] whereas elevated levels can be found in the CSF of patients with early MS.[35]

Moreover, we also demonstrated a significant increase in IL-21R expression within cortical neurons in MS brains, possibly implicating it in modulating neuronal responses/injury. We noted a further striking contrast with the inflammatory pathologies in cerebral infarcts. The IL-21$^+$ cells here were widely distributed in the ischemic parenchyma and were not confined to the perivascular spaces, but they even reached cortical areas, again suggesting that IL-21 might affect neurons. In addition, this detection of a Th-17 cell-related cytokine, IL-21, in SSPE, HSE, and stroke suggests that other cytokines expressed from Th-17 cells, such as IL-17, IL-22, or IL-26,[36] may be involved in these diseases. In fact, lymphocytes expressing IL-17 have been recently detected in stroke.[29]

In vitro studies of IL-21 have shown pleiotropic functions, depending on the activation state of the target cells studied.[37,38] Its restriction to CD4$^+$ T cells mainly in perivascular localization in active regions of white matter lesions in MS, together with the expression of IL-21R on the adjacent CD4$^+$, CD8$^+$, and CD19$^+$ cells (Figure 4), suggests that IL-21 exerts widespread pro-inflammatory influences in MS. IL-21R was not expressed in microglia/macrophages in MS lesions, unlike in rheumatoid synovium,[39] which implies additional specific microenvironmental influences. These novel findings suggest that IL-21 preferentially influences T- and B-cell responses, thus making it an important focus for potential therapeutic interventions in MS. Indeed, recent studies in mice and humans have implicated IL-21 as an important mediator of autoimmune responses[40] and in preventing exhaustion of protective CD8$^+$ lymphocytes in chronic viral infections.[32] Thus, unlike the shorter-lived IL-17,[3] IL-21 may help to sustain pathogenic responses in MS. Indeed, it apparently contributes to autoimmunity by driving cycles of T-cell expansion and death to excess, increasing the probability of generating self-reactive T cells.[40] By contrast IL-6, another important Th-17–polarizing cytokine, which is also expressed by Th-17 cells, was primarily detected in inactive MS lesions expressed by astrocytes.[11] This might indicate that IL-21 produced by Th17 cells is more important than IL-6 for inflammatory activities in active lesions.

Initial studies on human and murine tissues (including CNS tissue) showed IL-21 and IL-21R transcripts only in lymphoid tissue and peripheral blood lymphocytes.[41] However, later studies reported IL-21R expression in fibroblasts from patients with rheumatoid arthritis[39] or inflammatory bowel disease or from healthy controls,[37] and also in keratinocytes from patients with systemic sclerosis.[42] In the present study, the clear neuronal expression of IL-21 and IL-21R in neuronal cells in control and MS brains, newly shown here (Figures 5 and 6), suggests potential for the direct modulatory effects of cytokines on growth, differentiation, and plasticity that are becoming increasingly evident in the nervous system.[43] Such effects have been observed with IL-2,[44] which shares with IL-21 both a common receptor γ chain and a common Janus kinase, signal transducer and activator of transcription 1/signal transducer and activator of transcription 3 activation pathway. If they extend to IL-21, they might contribute to the reported reductions in both neuronal size and density in MS gray matter.[45] However, damaging effects of IL-21 on neurons cannot be excluded. Lymphocytic infiltration of the MS cortex is not conspicuous[46]; the meninges, and leucocortical lesions that span the deeper layers of the cortex, and the subcortical white matter do contain lymphocytes,[45] which may provide a local source of IL-21. In addition to neuronal synthesis, IL-21 might be transported to neuronal cell bodies from white matter lesions where their axons have been damaged. As mentioned previously, numerous IL-21$^+$ cells were detected within and near the cortex in stroke; they might contribute to the fate of neurons during disease progression, as recently proposed for IL-17 in neurons in humans and mice, where it correlates directly with neurotoxicity.[29,47]

In summary, we have demonstrated for the first time the presence and possible role of IL-21 in both CNS white and gray matter in MS. In white matter MS lesions, abundant expression of IL-21 was seen in perivascular lymphocyte infiltrates both in acute lesions and during progression in chronic active lesions; it might have direct effects on both T and B lymphocytes, which both express IL-21R. The unexpected but significant up-regulation of IL-21R expression in neurons within MS tissue implies a role in the neuronal injury and subsequent neurodegeneration that are responsible for most of the disability in MS.

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