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IL-13–Mediated Regulation of Learning and Memory

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The role of proinflammatory cytokines in cognitive function has been investigated with both beneficial and possible detrimental effects, depending on the cytokine. More recently, the type 2 IL-4 has been demonstrated to play a role in cognition. In this study, using the Morris water maze task, we demonstrate that IL-13–deficient mice are significantly impaired in working memory as well as attenuated reference memory, both functions essential for effective complex learning. During the learning process, wild-type mice increased the number of $CD4^+$ T cells in the meninges and production of IL-13, whereas neither Morris water maze–trained IL-4 nor trained IL-13–deficient mice were able to increase $CD4^+$ T cells in the meninges. Mechanistically, we showed that IL-13 is able to stimulate primary astrocytes to produce brain-derived neurotrophic factor, which does foster cognitive functions. Moreover, Morris water maze–trained wild-type mice were able to increase astrocyte-produced glial fibrillary acidic protein in the hippocampus, which was impaired in Morris water maze–trained IL-4– and IL-13–deficient mice. Collectively, this study strongly suggests that the Th2 cytokines, not only IL-4 but also IL-13, are involved in cognitive functions by stimulating astrocytes from the meninges and hippocampus. These results may be important for future development of therapeutic approaches associated with neurologic disorders such as Parkinson disease–associated dementia and HIV-associated dementia among others. ***The Journal of Immunology*****, 2017, 198: 000–000.**

It is known that the function of a healthy brain is partially influenced by the immune system (1). However, under normal physiological conditions, lymphocytes are scarce in the CNS, but are able to gain access to the CNS via the meninges or the choroid plexus (2, 3). Previous research focused on cognitive effects (4, 5) of proinflammatory cytokines such as TNF, IL-1, IL-6, and IL-12 (6) found in the hippocampus, an area of the brain associated with spatial learning and memory acquisition (7, 8). The role of adaptive immunity on cognitive function was only recently unveiled (9), with mice deficient of T cells, or IL-4, exhibiting cognitive impairment (10, 11).

It is possible that neuroimmune interactions associated with cognition may be initiated in the meninges, as meningeal T cell numbers increased following Morris water maze (MWM) visuospatial learning and memory tasks (12). Of importance, these T cells produced high levels of IL-4 (12, 13), enabling astrocytes to produce brain-derived neurotrophic factor (BDNF). Based on these studies, and due to the fact that both IL-4 and IL-13 share the IL-4Rα-chain, where IL-4 can signal via the IL-4Rα/γ_c and IL-4Rα/IL-13Rα1, whereas IL-13 can signal via the IL-4Rα/IL-13Rα1, we investigated whether IL-13 contributes to T cell recruitment to the meninges, and whether IL-13 is involved in learning and memory during MWM task.

In this study, we provide evidence for the requirement of IL-13 for optimal acquisition, as MWM-trained IL-13–deficient mice abrogated working memory (WM) and reference memory, whereas this function was attenuated in IL-4–deficient mice. IL-13 deficiency presented altered latency to platform during complex learning (reversal and visible platform phases), whereas trained wild-type (WT) BALB/c mice were able to employ effective learning during both simple (acquisition phase) and complex learning tasks. Moreover, upon stimulation with IL-13, astrocytes showed a significant increase in BDNF production, also confirmed in primary astrocyte cultures. The essential role of IL-13 in the regulation of BDNF and the recruitment of T cells to the meninges, two processes tightly associated with spatial learning and memory, is shown.

Materials and Methods

Animals

Inbred 10- to 16-wk-old IL-4–deficient (14), IL-13–deficient (15), and WT control mice, of BALB/c genetic background, were obtained from the University of Cape Town specific pathogen-free animal facility and kept in individually ventilated cages. All animals were housed in temperature- and humidity-controlled rooms, maintained on a 12 h light/dark cycle and age matched in each experiment.

Animal protocols were approved by the independent Animal Ethics Research Committee at the University of Cape Town (approval no. 012/017).

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The online version of this article contains supplemental material.

Abbreviations used in this article: BDNF, brain-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; MWM, Morris water maze; WM, working memory; WT, wild-type.

Morris water maze

Cognitive function was investigated in mice ($n = 10$) using the MWM for 8 d. Mice were given four 5-min trials a day for 4 consecutive days for training to locate a plexiglass circular platform (10 cm in diameter), which was placed ~0.5 cm below water level in an open circular 123-cm-diameter MWM. The water and room temperature were kept constant between 21 and 22 and 20 and 24°C, respectively (16). BALB/c mice used in this study have poor vision (17) and cannot fully see shapes and objects, although they can distinguish light from darkness (18), and therefore a light source was placed behind the MWM, parallel to the platform position to serve as an external distal cue, where lux by the platform quadrant measured 1 and other quadrants had a lux of 0. During the acquisition phase of the task, each mouse was allowed a maximum of 60 s to locate and climb onto the platform. Once the mouse had located the platform, it was given ~10 s to remain on the platform before returning to its home cage with infrared heat lamps. Mice that failed to locate the platform within 60 s were gently guided to the platform and allowed to acclimatize for 10 s before returning to the home cage. On the fifth day, a probe trial was performed with the platform removed to test reference memory (19). Each mouse was given a maximum of 60 s in the MWM. On days 6 and 7, the platform was placed in the quadrant opposite the original training quadrant, and the mouse was retrained for four sessions each day. On day 8 mice were introduced to the pool with a visible platform in a third quadrant, placed ~0.5 cm above water level. This quadrant was different from the first two training quadrants. Mice were tested on four trials from varying points of entry. Data were recorded using the EthoVision XT 8 automated tracking system (Noldus Information Technology, Leesburg, VA). Statistical analysis was performed using ANOVA and the Bonferroni post hoc test or Student *t* test. Groups were counterbalanced, that is, run in alternating order on successive training days. All MWM testing was performed between 9:00 AM and 3:00 PM during the lights-on phase. Representative experiments are shown out of at least three independently performed in each case.

FACS of meningeal and hippocampal isolates

Flow cytometry was used to determine T cell and astrocyte populations in single-cell preparations of either the meninges and/or hippocampus after stimulation with 20 μg/ml anti-CD3 and GolgiStop (BD Pharmingen) in complete media: IMDM (Life Technologies/Invitrogen, Carlsbad, CA), 10% FCS, penicillin/streptomycin on ice. Samples were stained with an Ab mix (MACS buffer plus 2% inactivated rat serum), 2% anti-FcγII/III (clone 2.4G2), anti-CD4 (clone GK1.5; BD Pharmingen), anti-CD3 (clone 500A2; BD Pharmingen), and anti–glial fibrillary acidic protein (GFAP; clone GA5; Abnova) for 30 min on ice, and then fixed in 2% paraformaldehyde before being permeabilized (saponin containing permeabilization buffer) for 1 h at 4°C and read by a BD FACS Fortessa machine (BD Biosciences, San Diego, CA).

The data were analyzed by FlowJo (Tree Star, Ashland, OR) and graphed with GraphPad Prism software. Unless otherwise stated, Abs were from BD Pharmingen.

Astrocyte primary culture

Mouse astrocyte cultures were prepared from 1-d-old mouse neonates as follows. Brains were excised and placed in ice-cold DMEM/F-12 (Lonza). Neocortical tissue was passaged through 40-μm nylon mesh cell strainers with sterile plastic plungers, and then triturated gently through a 5-ml serological pipette 20 times and passed through a 40-μm filter. To obtain purified astrocytes, flasks were rinsed with DMEM/F-12 (Lonza) before media changes (every 2 d for the first week) to eliminate nonadherent cells, and were cultured for 3 wk until they reached confluence and dendrites were developed. Cultures were then stimulated with either IL-4 (eBioscience) or IL-13 (eBioscience) (10 ng/ml) and compared with nonstimulated cells. Supernatants were collected after 36 h for sandwich ELISA.

ELISA analysis

Single-cell suspensions of cytokine-stimulated primary astrocyte cultures or single-cell suspensions from either the meninges or hippocampus of MWM-trained and nontrained mice were analyzed for BDNF levels according to the manufacturer's instructions (Promega, Madison, WI). Plates were developed using a Versamax microplate spectrophotometer (Molecular Devices).

Immunocytochemistry

Coverslips were coated with poly-L-lysine for 1 h at room temperature before being rinsed with sterile H_2O, three times each for 5 min. Coverslips were allowed to dry completely and were sterilized under UV light for 4 h. Primary astrocytes were grown on glass coverslips and later rinsed briefly in PBS, followed by samples being fixed in acetone for 10 min at room temperature. Samples were washed twice with ice-cold PBS, and cells were incubated with 1% BSA (Roche Diagnostics) in PBS for 30 min to block unspecific binding of the Abs, and later incubated with diluted Alexa Fluor 488 anti-mouse IgG Ab (1:1000; Invitrogen) for 1 h at room temperature. The cells were washed three times in PBS for 5 min during each wash and incubated in the dark. Samples were examined by confocal microscopy (ZEN lite imaging software; LSM 510; Carl Zeiss).

Meninges immunofluorescence

Whole-mount meninges attached to the skull where fixed in 1:1 ethanol/acetone solution for 20 min at −20°C. The dura/arachnoid was then dissected from the skullcap and samples were incubated in wells with PBS containing 1% inactivated rat serum and 1% anti-FcγII/III (clone 2.4G2) for 1 h with appropriate dilutions of primary Abs, that is, anti–IL-4 PE (1:50, lot. 3221956; BD Pharmigen) and anti–IL-4 PE (1:50, clone eBio13A; eBioscience), in the dark. Samples were washed 10 min in PBS, followed by 1:8000 Hoechst (33342; Thermo Fisher Scientific) reagent for 10 min and another 10 min wash in PBS. Samples where then mounted on slides with Mowiol antifade mounting medium under coverslips. Samples were examined by confocal microscopy (ZEN lite imaging software; LSM 510; Carl Zeiss).

Histology

Isolated brain tissue samples were fixed in a neutral buffered formalin solution and embedded in paraffin and cut into 5-μm sections. These sections were stained with anti-CD3 (clone 500A2; BD Pharmingen) for identification of meningeal T cells. Cells were identified from individual mice ($n = 5$) and Nikon NIS elements software was used to identify ±10 sections per mouse.

Results

IL-13 is essential for WM

IL-4 is responsible for $CD4^+$ T cell differentiation of Th2 and has recently been shown to be involved in cognitive learning. IL-13 is produced by Th2 cells, shares the IL-4Ra chain with IL-4, and can be involved in Th2 effector functions. Hence, IL-13 could be a possible candidate for cognitive functions. This was investigated in a loss of function approach using IL-13–deficient mice tested for spatial learning and memory tasks by MWM (19) (Fig. 1A). WT BALB/c mice, IL-4–deficient BALB/c mice, and IL-13–deficient BALB/c mice were trained on the MWM, and all three strains showed similar distances swimming, ruling out other behavior factors, besides cognition, as possible contributors to any differences observed across the groups (Fig. 1B). When tested on a task for WM for 4 d, both $IL\text{-}4^{-/-}$ and $IL\text{-}13^{-/-}$ mice took significantly longer time than did WT mice to locate the submerged platform (Fig. 1C). This difference became even more robust during the reversal (Fig. 1D) and visible (Fig. 1E) phases of the task. Interestingly, although IL-4 deficiency led to IL-4–attenuated WM, IL-13 deficiency shows abrogated WM. Statistical differences between IL-4–deficient and IL-13–deficient mice during learning demonstrate overall longer latencies to platform by IL-13–deficient mice for complex learning (reversal phase), with no differences between IL-4–deficient and IL-13–deficient mice during simple learning (acquisition phase) (Fig. 1G). Taken together, these results suggest that IL-13 is involved in WM (Fig. 1C).

IL-13 influences $CD4^+$ T cell accumulation in the meninges during MWM training

In an attempt to better understand the cellular and molecular mechanisms underlying the observed behavioral beneficial effect of IL-13 in learning, T cell responses in the meninges, before and after MWM training, were examined. T cells, stained with an immunofluorescent Ab against CD3, were identified in the subarachnoid spaces of the meninges in histological sections (Fig. 2A). Meningeal cells from MWM-trained and nontrained mice were examined

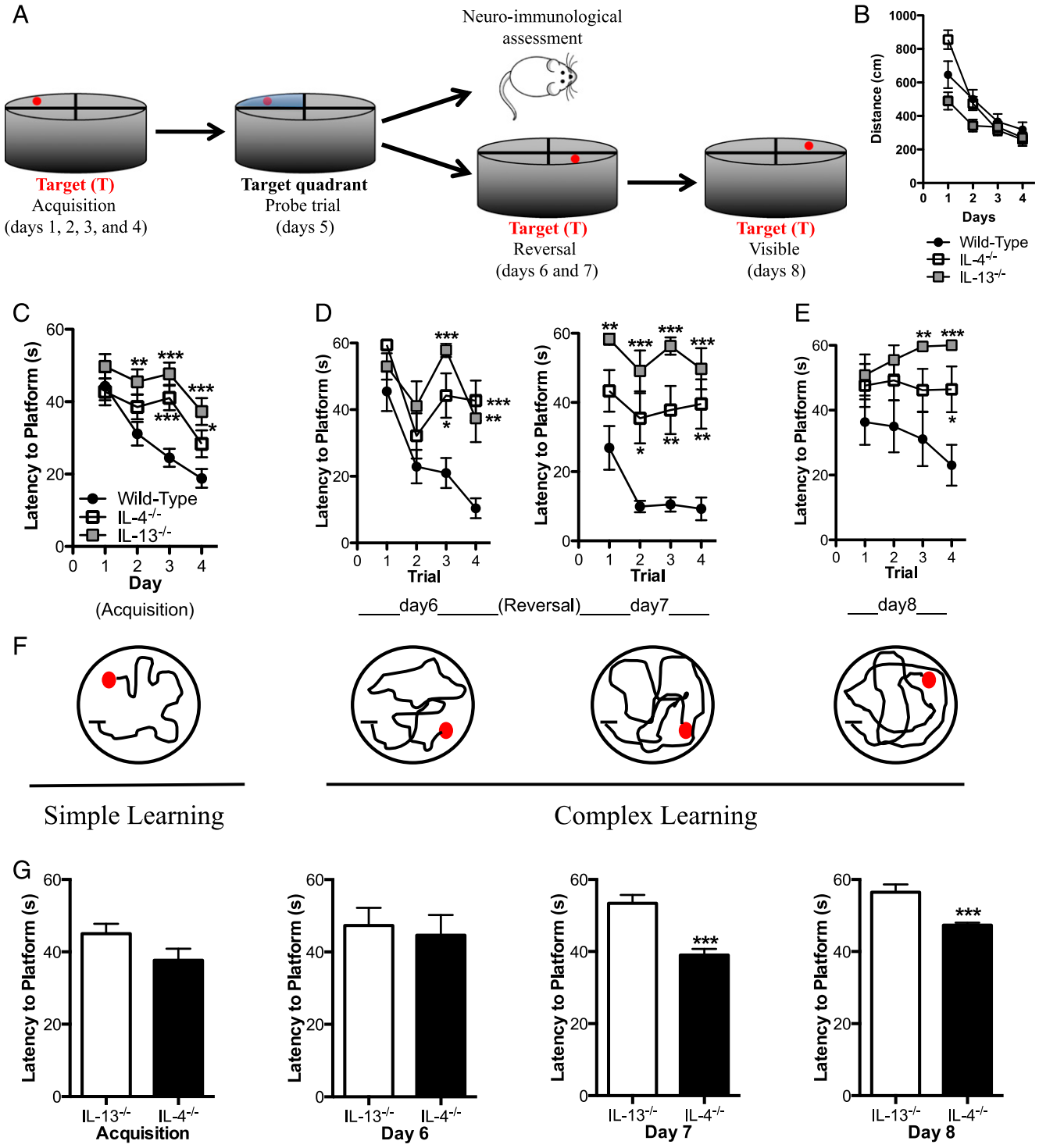


FIGURE 1. Deletion of IL-13 is associated with abrogated spatial learning. (**A**) Time-course experiment indicating time points of WM and reference memory tasks, including ex vivo neuroimmunological assessment conducted following behavioral task. (**B**) Mice were tested on the distance they swam as a control factor for noncognitive differences, with all groups swimming similar distances during the acquisition phase of the task. (**C**–**E**) BALB/c male mice (16 wk old) were monitored during the MWM task. Both IL-4$^{-/-}$ and IL-13$^{-/-}$ mice had longer latencies to platform location during the acquisition (C), reversal (D), and the visible platform (E) phases of the task compared with WT mice. IL-13$^{-/-}$ mice navigation through the MWM is shown schematically to become more haphazard during complex learning phase of the task, compared with simple learning task (**F**). Latency to platform by IL-4$^{-/-}$ and IL-13$^{-/-}$ mice was compared, showing significantly longer latencies by IL-13$^{-/-}$ mice during (**G**) complex learning tasks of (D) reversal phase (days 6 and 7) and (E) visible platform phase (day 8). Two-way repeated measures ANOVA was used for statistical analyses, with a Bonferroni post hoc test used for individual time point comparisons (n = 10 mice per group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Results are representative of three independent experiments.

by FACS analyses, with a series of gates to allow identification of CD4$^+$CD3$^+$ T cells (Fig. 2B). WT MWM-trained mice showed an ~45% increase of CD3$^+$CD4$^+$ T cells (5.1–7.4%) from the meningeal tissue compared with untrained mice (Fig. 2C), demonstrating that CD4$^+$ T cells do accumulate within the meninges area during MWM training. In contrast, neither IL-4–deficient nor IL-13–deficient mice showed any significant increases of CD3$^+$CD4$^+$ T cells during MWM training (Fig. 2C). This suggests that both IL-4

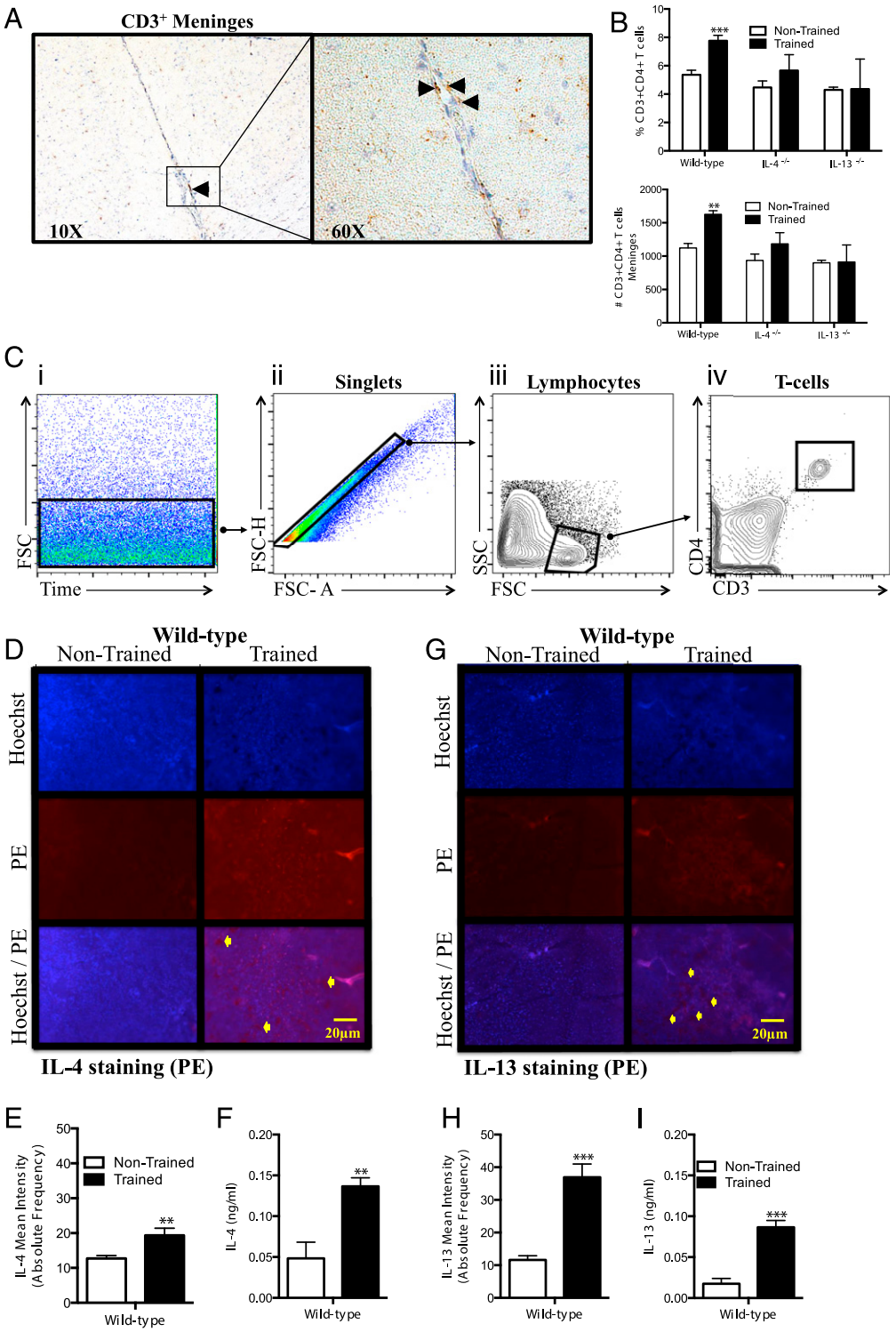


FIGURE 2. IL-4 and IL-13 influence T cell accumulation in the meninges of MWM-trained mice. (**A**) Whole-brain histological sections (original magnification, ×100 or ×600) were labeled for CD3 that was visualized using 1,4-diamino-2-butanone substrate (K3466; Dako), counterstained with Mayer's hematoxylin. Meningeal subarachnoid spaces occupied by $CD3^+$ T cells are presented, and arrowheads point to $CD3^+$ T cells. (**Ci–iv**) Meningeal single-cell suspensions were examined by FACS for $CD3^+CD4^+$ T cells in nontrained and MWM-trained mice, (**B**) showing significantly increased $CD3^+CD4^+$ T cells by WT trained mice but not IL-4–deficient and IL-13–deficient mice. Experiments were performed three separate times, with meninges from individual mice ($n = 4$ mice per group; one-way ANOVA, $^{***}p < 0.001$, with Bonferroni post hoc test). These results were pooled and an average result is presented. Following MWM training, whole-mount meninges (dura/arachnoid) of trained and nontrained mice were labeled for (**D**) anti–IL-4 PE or (**G**) anti–IL-13 PE (scale bars, 20 µm). Mean intensity of expression was determined in 10 various sections of a sample ($n = 4$ mice per group), revealing an increase in both (**E**) IL-4 and (**H**) IL-13 for trained, compared with nontrained, mouse samples. This result was confirmed by sandwich ELISA where single-cell suspensions of dura/arachnoid from MWM-trained and nontrained mice investigated for (**F**) IL-4 and (**I**) IL-13 production revealed an increase in both by trained mice ($n = 4$ mice per group; unpaired t test, $^{**}p < 0.01$, $^{***}p < 0.001$). Results are representative of three independent experiments.

and IL-13 may play a significant role in T cell accumulation in meningeal spaces. To support this hypothesis, the cytokine profile of meningeal (dura/arachnoid) cells from WT mice after MWM training was examined. Of interest, these cells expressed significantly higher levels of IL-4 (Fig. 2D, 2E) and IL-13 (Fig. 2G, 2H) following MWM training, whereas IL-4/13$^{-/-}$ gene–deficient mice showed no IL-4 and IL-13 production before and after MWM training (Supplemental Fig. 1A). Moreover, this result was confirmed by sandwich ELISA, where both IL-4 (Fig. 2F) and IL-13 (Fig. 2I) showed significant increases with MWM training. Taken together, this suggests that both cytokines are produced during the process of learning and are required for effective WM (Fig. 1).

IL-13–stimulated astrocytes enhance BDNF production that contributes to effective reference memory

To further characterize possible memory impairments of IL-4– and/or IL-13–deficient mice, we examined the time spent in the area where the platform was located (Fig. 3Ai), as well as time required for mice to reach the area where the platform was initially located (Fig. 3Aii), along with the number of crossings the mice made through the area where the platform was submerged (Fig. 3Aiii). Both IL-13– and IL-4–deficient mice performed poorly on these parameters when compared with WT mice. Interestingly, IL-13–deficient mice spent significantly longer times in quadrant compared with IL-4–deficient mice. This correlated with longer latencies to the platform by IL-13–deficient mice later seen in the complex learning phase of the task during reversal and visible platform (Fig. 1D, 1E).

Because IL-4 and IL-13 deficiency showed impaired reference memory, and BDNF is known to play a significant role in both memory consolidation and long-term potentiation (20), we tested whether IL-13 is able to induce BDNF, as previously shown for IL-4 (12). For this, we cultured primary astrocytes derived from 1-d-old mouse neonates and verified them by confocal images using the astrocyte marker GFAP (Fig. 3Bii). Astrocytes were stimulated with either IL-4 or IL-13 for 36 h and BDNF production measured by sandwich ELISA. As expected, IL-4 stimulation resulted in the production of BDNF compared with nonstimulated astrocytes (Fig. 3Bi). Of note, IL-13 stimulation resulted in similar increase of BDNF, demonstrating that IL-13 stimulates BDNF production in astrocytes (Fig. 3Bi). This explains that both IL-4 and IL-13 play a significant role in BDNF expression, responsible for both memory consolidation and long-term potentiation.

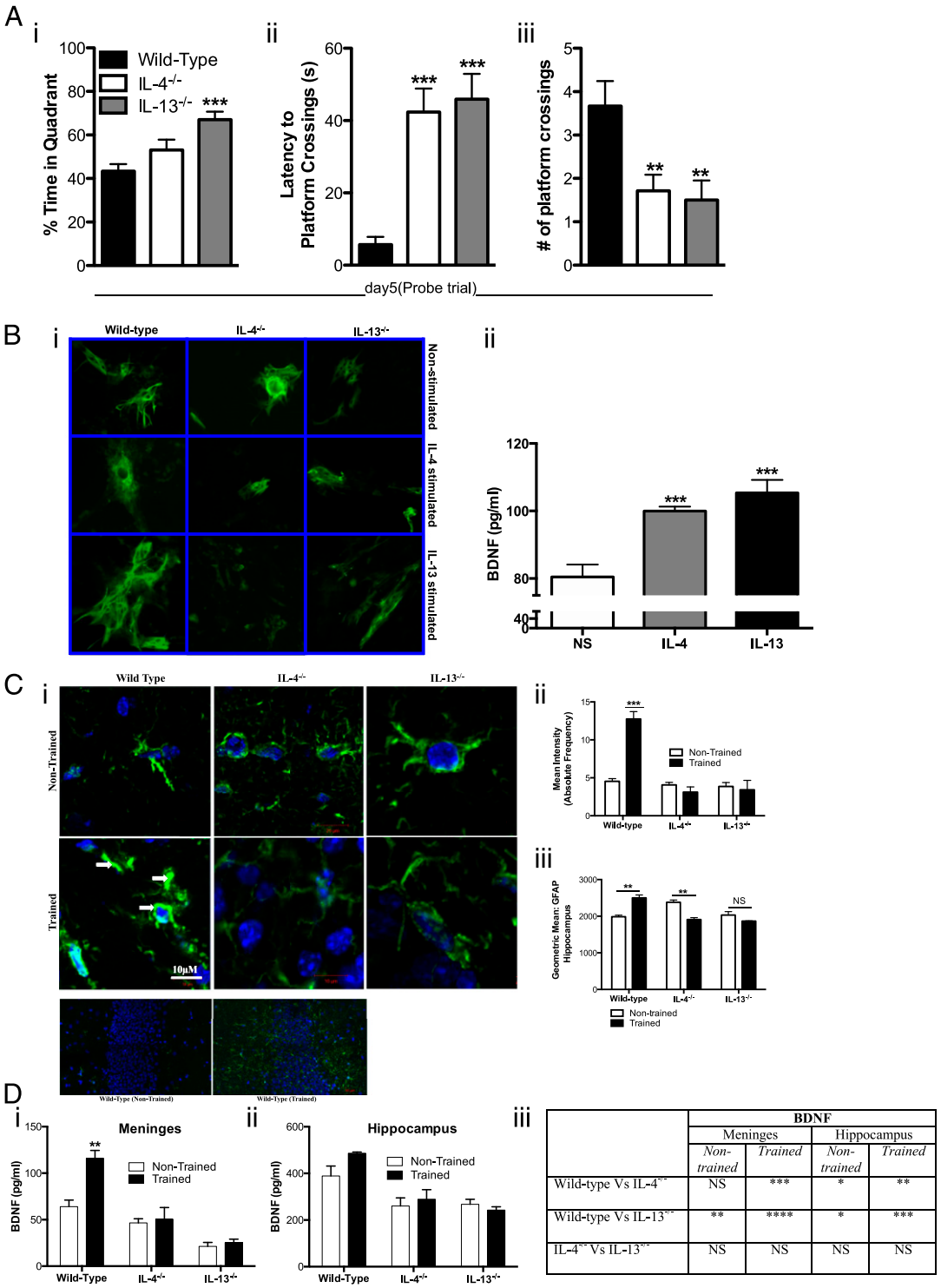
The hippocampus is also known to play a role in spatial learning and memory acquisition (7, 8). Hence, we examined GFAP expression from hippocampus-derived astrocytes by confocal microscopy from MWM-trained and nontrained mice. Trained WT mice showed an increase in GFAP expression by astrocytes, which was downregulated in trained IL-4–deficient and IL-13–deficient mice (Fig. 3Ci, ii). This was supported by similar results using FACS analyses (Fig. 3Ciii). Taken together, these results show that IL-4 and IL-13 are essential for memory consolidation as demonstrated by an increase in BDNF production by astrocytes, including astrocytes from hippocampus.

Discussion

In this study, we demonstrate a central role for type 2 cytokines in the regulation of cognitive function. We show that, similarly to IL-4, IL-13 is necessary for T cell accumulation in the meninges during learning (21). Although the role of other immune cells is not excluded as significant, to render our claims unequivocal, the total CD4$^+$ T cell numbers in the meninges show a numerical as well as proportional increase of T cells within the meninges following MWM training. We further show that in WT mice, the performance of cognitive tasks leads to the accumulation of IL-4 and IL-13 in the meninges. Consequently, we demonstrated that IL-13 deficiency abrogated spatial learning, resulting in severe cognitive impairment. Consistent with the important role of IL-4 in the regulation of cognitive function, a critical role for IL-13 in the maintenance of effective cognitive function was also unprecedentedly uncovered, whereby the latter fostered an astroglial phenotype and triggered production of BDNF by astrocytes, supportive of cognitive function. We show correlational evidence of low BDNF by ELISA associated with deletion of IL-13 in the meninges, with no significant differences observed in the hippocampus of WT trained mice.

A beneficial role of IL-4 on cognitive function has recently been shown, and its ability to drive myeloid cell activation away from classical inflammation with increased production of TNF and IL-12 was reported to be instrumental in this process (12). A central tenet of these findings was the establishment of a tight link between IL-4, the phenotype of meningeal myeloid cells, and the resulting performance in the MWM (22). Numerous proinflammatory cytokines associable with classical activation of myeloid cells have been shown to interfere with learning and memory (4, 6, 23–25). IL-13 is a cytokine tightly linked to IL-4, as both share a signaling complex driving complementary and even redundant functions (26). For instance, similarly to IL-4, IL-13 has been ascribed an anti-inflammatory potential and the ability to drive alternative activation of myeloid cells (22, 27). Thus, considering the growing body of evidences for an anticognitive role of proinflammatory cytokines and a more procognitive role of type 2 immune responses (24, 28), more information on the role of IL-13 on cognition in particular and CNS in general is warranted. In this study, we show that IL-13 deficiency substantially impaired T cell recruitment to the meninges during cognitive training, where under steady-state conditions, all groups showed similar numbers of CD4$^+$ T cells that only shifted with MWM training in WT but not IL-4$^{-/-}$ or IL-13$^{-/-}$ mice. Given the reported striking Th2 skewing of the T cells recruited to the meninges following training (12), a possible explanation to this lack of T cell recruitment to the meninges could be an impaired development of Th2 cells in these mice (15). This process has been strongly associated with the mitigation of innate inflammatory responses during learning and translates into efficient cognitive function, as demonstrated by a severe cognitive impairment of these mice in the MWM.

After WT mice were trained in MWM, a significant increase in the production of IL-4 and IL-13 could be observed in the meningeal tissues. These findings are in line with a previous observation that IL-4 production is strikingly increased in meningeal cells following MWM training. Importantly, we now show that, similarly to IL-4, IL-13 production is substantially enhanced in the meninges of MWM-trained mice. Hence, we propose that this increase in type 2 cytokines in the meninges of MWM-trained mice led a potential alternative mode of activation of meningeal myeloid cells and the resulting efficient cognitive function. This hypothesis is currently under investigation in our laboratory. Our data demonstrate a severe cognitive impairment as apparent in IL-4–deficient mice as in IL-13–deficient mice. Additionally, our data showing an increase of IL-13 in the meninges of WT trained mice compared with nontrained WT mice, coupled to increased recruitment of T cells, indirectly indicates that the recruited T cells might contribute to the reported elevated IL-13. This, however, does not exclude the possibility of other cell types contributing to IL-13 increases observed in the meninges of WT trained mice. Furthermore, we found that production of BDNF, a major factor in hippocampal function that associates with learning (12, 29, 30), is elicited in astrocytes by IL-4 or IL-13. Consistent with previously published



	BDNF			
	Meninges		Hippocampus	
	Non-trained	*Trained*	*Non-trained*	*Trained*
Wild-type Vs IL-4$^{-/-}$	NS	***	*	**
Wild-type Vs IL-13$^{-/-}$	**	****	*	***
IL-4$^{-/-}$ Vs IL-13$^{-/-}$	NS	NS	NS	NS

FIGURE 3. Deletion of IL-13 is associated with abrogated learning and memory in the MWM spatial learning task. (**A**) A probe trial was performed on day 5. (**Ai**) IL-13–deficient mice spent more time in the training quadrant and had (**Aii**) longer latencies to platform crossing with (**Aiii**) fewer platform crossings compared with WT mice (n = 10 mice per group). (**B**) Single-cell suspensions of primary astrocytes (stained with FITC for visualization) were stimulated with either IL-4 or IL-13. (**Bi**) Following stimulation, BDNF production was measured by sandwich ELISA, showing increased BDNF by IL-4 and IL-13 stimulation, compared with nonstimulated. (**Bii**) Stimulated primary astrocytes are shown (n = 4 mice per group; one-way ANOVA, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, with Bonferroni post hoc test). Results are representative of three independent experiments. (**Ci** and **Cii**) Hippocampus histological sections were labeled with GFAP (FITC and Hoechst nuclei counterstain), and astrocytes were identified, revealing an increase in WT mice and dampening in IL-4–deficient and IL-13–deficient mice following MWM training. This result is supported by hippocampal single-cell suspensions examined by FACS for GFAP^{+} (FITC) cells in nontrained and MWM-trained mice (**Ciii**) (n = 4 mice per group; unpaired t test, $^{**}p < 0.01$, $^{***}p < 0.001$). Results are representative of three independent experiments. (**Di–iii**) Single-cell suspensions from the meninges or hippocampus were prepared for ELISA and analyzed for BDNF levels showing an increase in BDNF by WT MWM-trained mice, but not IL-4– or IL-13–deficient mice in the meninges (n = 4 mice per group; one-way ANOVA, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$). Results are representative of three independent experiments.

observations (12), we now show that the ability of IL-4 to drive BDNF production is shared with IL-13. These cytokines are also elevated in the meninges of MWM-trained WT mice, but not IL-4–deficient and IL-13–deficient mice that show impaired reference memory. This result clarifies the hypothesis that not only do astrocytes respond to IL-4 or IL-13 for BDNF production to aid memory consolidation (20), but also that IL-4 and IL-13 are essential for spatial learning and memory. A mechanism showing meningeal IL-13 entering the hippocampal parenchyma was not shown in this study. Although the transit of cytokines from the meninges to the hippocampus is difficult to demonstrate empirically (histology, ELISA, and quantitative PCR), we provide evidence for a negative physiological and functional effect, that is, BDNF production influence of IL-13 absence on hippocampal astrocytes, suggesting that IL-13 is required under physiological conditions for the performance of hippocampal astrocytes. Our experiments showed a consistent elevation of hippocampal tissue BDNF following MWM training in vivo. A rather indicative finding in this regard is that of the reduced levels of hippocampal and meningeal BDNF in IL-13–deficient mice (Fig. 3D). This observation strongly argues in favor of an IL-13–regulated production of BDNF in the hippocampus and the meninges.

This further substantiates a redundant role for these type 2 cytokines in the CNS. Importantly, following training, a reduced to inexistent astroglial phenotype was apparent from astrocytes of IL-4–deficient and IL-13–deficient mice as opposed to those of WT mice. Injury to the CNS as a result of learning (31, 32) should lead to a characteristic astroglial response, referred to as an astrocytic scar (33). Astrocytes within the scar undergo a profound phenotypic change, and a consensus now is that these reactive cells are central regulators of the inflammatory response in the CNS (34). With the impairment of such a response in IL-4– and IL-13–deficient mice, the question still remains of whether meningeal type 2 cytokines (IL-4 and IL-13) directly target myeloid cells, astrocytes, or both to mediate their beneficial effects on cognition. Experiments on cell-specific IL-4Rα–deficient mice, which should specifically target a defined class of IL-4/IL-13–responsive cells, are currently underway to address this question.

In summary, the results of this study provide evidence for a shared beneficial role of type-2 cytokines—via a complex neuroimmunological network—on host cognitive function. We describe a neurologic dichotomy that tightly aligns with the division of the immune response; that is, type 1 mediators are anticognitive and type 2 mediators are procognitive. Future studies will be aimed at deciphering the mechanistic bases of this immune-driven regulation of the host cognitive function. Our findings confirming a precognitive role for IL-4 and uncovering a similar role for the sister cytokine IL-13 provide additional support to the already widely reported redundancy of these cytokines in several biological processes.

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Disclosures

The authors have no financial conflicts of interest.

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