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Author(s)	Hasebe, Rie; Suzuki, Akio; Yamasaki, Takeshi; Horiuchi, Motohiro
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Regular Article

Temporary upregulation of anti-inflammatory cytokine IL-13 expression in the brains of CD14 deficient mice in the early stage of prion infection

Rie Hasebe, Akio Suzuki, Takeshi Yamasaki, Motohiro Horiuchi

Laboratory of Veterinary Hygiene, Graduate School of Veterinary Medicine, Hokkaido University, Nishi 9, Kita 18, Kita-ku, Sapporo 060-0818, Japan

Correspondence to Motohiro Horiuchi

Address: Laboratory of Veterinary Hygiene, Graduate School of Veterinary Medicine,
Hokkaido University

Nishi 9, Kita 18, Kita-ku, Sapporo 060-0818, Japan

Tel & Fax: +81-11-706-5293

E-mail: horouchi@vetmed.hokudai.ac.jp

1 **Abstract**

2 CD14 deficient (CD14^{-/-}) mice survived longer than wild-type (WT) C57BL/6J
3 mice when inoculated with prions intracerebrally, accompanied by increased expression
4 of anti-inflammatory cytokine IL-10 by microglia in the early stage of infection. To
5 assess the immune regulatory effects of CD14 in detail, we compared the gene
6 expression of pro- and anti-inflammatory cytokines in the brains of WT and CD14^{-/-}
7 mice infected with the Chandler strain. Gene expression of the anti-inflammatory
8 cytokine *IL-13* in prion-infected CD14^{-/-} mice was temporarily upregulated at 75 dpi,
9 whereas *IL-13* gene expression was not upregulated in prion-infected WT mice.
10 Immunofluorescence staining showed that IL-13 was mainly expressed in neurons of
11 the thalamus at 75 dpi. These results suggest that CD14 can suppress IL-13 expression
12 in neurons during the early stage of prion infection.

Keywords: prion disease, CD14, IL-13, pathogenesis

13 **1. Introduction**

14

15 Prion diseases are fatal neurodegenerative disorders including scrapie in sheep and
16 goats, bovine spongiform encephalopathy in cattle, chronic wasting disease in cervids,
17 and Creutzfeldt-Jakob disease in humans. These diseases are characterized in the central
18 nervous system (CNS) by deposition of the disease-specific form of prion protein
19 (PrP^{Sc}), vacuolation of neurons and neuropil, astrocytosis and microglial activation.
20 Despite little recruitment of adaptive immune cells into the CNS in prion diseases,
21 innate immunity is reported to be involved in the pathogenesis of prion diseases [1].

22 CD14 is expressed by monocytes and macrophages as a
23 glycosylphosphatidylinositol-anchored membrane form and a soluble form [2,3]. The
24 binding of pathogen-associated molecular patterns to CD14 clusters transmembrane
25 proteins including toll-like receptors (TLR) 2 and 4 to induce gene expression of
26 pro-inflammatory mediators [4,5]. We recently showed that CD14 has a progressive role
27 in the neuropathogenesis of prion diseases [6]. CD14 knockout (CD14^{-/-}) mice survived
28 longer than wild-type (WT) mice after intracerebral inoculation with prions, with
29 reduced deposition of PrP^{Sc} and prion infectivity in the early to middle stage of the
30 infection. Our data showed that expression of an anti-inflammatory cytokine IL-10 was
31 increased in brains of CD14^{-/-} mice in the early stage of prion infection, accompanied by
32 accelerated microglial activation. This suggests that CD14 has suppressive roles in
33 anti-inflammatory responses and microglial activation in prion-infected mouse brain.
34 Further analyses of influence of CD14-deficiency on the inflammatory condition in
35 prion-infected mouse brains could provide valuable information regarding the immune

36 regulatory effects by CD14 molecule. Thus, we compared the gene expression profiles
37 of pro- and anti-inflammatory cytokines in prion-infected WT and CD14^{-/-} mice.

38

39 **2. Materials and Methods**

40

41 *2.1 Mice*

42

43 Six-week-old female C57BL/6J WT mice (Japan Clea Inc.) and CD14^{-/-} congenic
44 mice B6.129S-Cd14^{<tm1Fmm>/J} (Jackson Laboratories) were inoculated intracerebrally
45 with 2.5% brain homogenates from scrapie Chandler strain-infected WT mice or from
46 age-matched uninfected WT mice for a negative control. All animal procedures were
47 approved by the Institutional Animal Care and Use Committee of the Graduate School
48 of Veterinary Medicine, Hokkaido University.

49

50 *2.2 Quantitative RT-PCR*

51

52 The brains were sliced using the AltoTM Brain Matrix (Robaz Surgical Instrument
53 Co.) at a thickness of 1 mm. The thalamus, hippocampus and combined regions of
54 internal capsule(ic), fimbria of hippocampus (fi) and cerebral peduncle (cp) were
55 dissected from the slice around the bregma -1.82 mm [7] under a stereoscopic
56 microscope. Total RNA was extracted using TRIZOL[®] reagent (Thermo Scientific).
57 Gene expression was analyzed by quantitative RT-PCR using the TaqMan Gene
58 Expression Assay as described previously [6]. TaqMan Gene Expression Assays used

59 here were IFN- γ (*ifng*, Mm99999056_m1), IL-1 β (*il1 β* , Mm01336189_m1), IL-6 (*il6*,
60 Mm99999064_m1) and Nos2 (*nos2*, Mm01309902_m1) for pro-inflammatory
61 cytokines, and IL-4 (*il4*, Mm00445259_m1), IL-13 (*il13*, Mm00434204_m1), IL-10
62 (*il10*, Mm99999062_m1), TGF- β (*tgfb β* , Mm03024053_m1) and IL-1 receptor antagonist
63 (*il1ran*, Mm00446186_m1) for anti-inflammatory cytokine.

64

65 2.3. Immunofluorescence staining

66

67 For PrP^{Sc} specific staining, anti-mouse prion protein mouse monoclonal antibody
68 (mAb) 132 was prepared as described previously [8]. Immunofluorescence staining for
69 cytokines and neural markers was performed as described previously [6]. Dilutions of
70 antibodies were 1:800 for CD68 (rat mAb, clone FA-11), 1:200 for CD11b (rat mAb,
71 clone M1/70), NeuN (mouse mAb, clone A60), IL-13 (rabbit polyclonal Ab, Abcam, no.
72 ab79277), and IL-13R α 1 (rabbit polyclonal Ab, Abcam, no. ab106732), 1:500 for GFAP
73 (rat mAb, clone 2.2B10), 1:1000 for GFAP (rabbit polyclonal Ab, Dako, no. Z033401)
74 and 1:2000 for all Alexa Fluor-labeled antibodies (Thermo Scientific).

75

76 3. Results

77

78 3.1. Gene expression of IL-13 is temporarily up-regulated in CD14^{-/-} mice in the early 79 stage of prion infection

80

81 Expression of an anti-inflammatory cytokine IL-10 was increased, whereas a
82 pro-inflammatory cytokine IL-1 β was decreased in certain areas of CD14^{-/-} mouse

83 brains in the early stage of prion infection, suggesting that inflammatory condition was
84 shifted to anti-inflammatory status in the absence of CD14 [6]. Here we analyzed
85 inflammatory condition in the brains more in detail by comparing gene expression of
86 pro- and anti-inflammatory cytokines between WT and CD14^{-/-} mice by quantitative
87 RT-PCR. The thalamus, hippocampus, and the area combined with internal capsule,
88 fimbria of hippocampus and cerebral peduncle were chosen, since difference in the
89 microglial activation between WT and CD14^{-/-} mice was prominent in these areas at 60
90 and 75 dpi (Fig. 1).

91 *IL-13* gene expression at 75 dpi was higher in prion-infected CD14^{-/-} mice than in
92 prion-infected WT mice in all examined areas, and these differences were statistically
93 significant for the thalamus and hippocampus (Figs. 2B & 2D). In the regions of ic, fi
94 and cp, *IL-13* gene expression was detected in 2 of 3 prion-infected-CD14^{-/-} mice at 75
95 dpi (Fig. 2C). However, in prion-infected WT mice and mock-infected mice, *IL-13*
96 expression was below the detection limit (Fig. 2C). Interestingly, the temporarily
97 upregulated *IL-13* gene expression in CD14^{-/-} mouse brains was restored to the
98 steady-state level at 90 dpi and thereafter (Figs. 2B-2D). In the hippocampus, gene
99 expressions of pro-inflammatory cytokines were significantly higher in prion-infected
100 WT mice than in prion-infected CD14^{-/-} mice: *IL-1β* at 120 dpi and *IL-6* at 90 dpi (Fig.
101 2D). Cytokine genes significantly upregulated by prion infection both in WT and
102 CD14^{-/-} mice were as follows: *IL-1β* at 75 and 120 dpi, *IL-6* at 75 dpi, *TGF-β* at 75 dpi
103 and *IL1 receptor antagonist (IL1ran)* at 75 and 120 dpi in the thalamus (Fig. 2B), and
104 *IL-6* at 75 dpi, *IFN-γ* at 75 dpi and *TGF-β* at 75 and 90 dpi in the hippocampus (Fig.
105 2D). The gene expressions of *IL-4* and *IL-10* were below the limit of detection in all
106 samples (data not shown).

107

108 *3.2. IL-13 expression is upregulated in neurons of the thalamus in CD14^{-/-} mice only in*
109 *the early stage of prion infection*

110

111 To confirm the results of the gene expression analysis, we examined IL-13
112 expression by immunofluorescence staining (Fig. 3A). In prion-infected CD14^{-/-} mice at
113 75 dpi, IL-13 was most frequently expressed in the thalamus with occasional expression
114 also observed in the hippocampus, amygdala, ic, fi and cp. IL-13 expression in CD14^{-/-}
115 mice appeared to decrease at 90 dpi and was undetectable at 120 dpi. IL-13
116 immunoreactivity was also detected in prion-infected WT mice at 75 dpi, however, it
117 was much less frequent than in prion-infected CD14^{-/-} mice. Although IL-13
118 immunoreactivity was detected in the hippocampus of mock-infected WT and CD14^{-/-}
119 mice, it was weaker and occurred less frequently than in the hippocampus of
120 prion-infected CD14^{-/-} mice. Quantitative analysis showed that IL-13-positive areas in
121 the thalamus of prion-infected CD14^{-/-} mice were much larger than those of
122 prion-infected WT mice at 75 dpi (Fig. 3B). The positive areas in prion-infected CD14^{-/-}
123 mice decreased after 90 dpi, as observed microscopically. Double immunofluorescence
124 staining showed that IL-13-positive cells differed among the analyzed brain regions (Fig.
125 3E). In the thalamus, IL-13 was mostly expressed in neurons, only occasionally in
126 microglia and rarely in astrocytes. IL-13 also appeared to be expressed in neurons in the
127 hippocampus (data not shown). However, in the combined regions of ic, fi, and cp,
128 IL-13 was mostly expressed in microglia, only occasionally in astrocytes, and rarely in
129 neurons.

130

131 *3.3. Expression of IL-13 receptor is upregulated in the middle stage of infection both in*

132 *WT and CD14^{-/-} mice*

133

134 IL-13 signaling is recognized via a heterodimeric transmembrane receptor complex
135 composed of an IL-4 receptor α (IL-4R α) and an IL-13 receptor $\alpha 1$ (IL-13R $\alpha 1$) [9,10].
136 Therefore, we assessed if IL-13R $\alpha 1$ expression was also altered in the absence of CD14
137 (Fig. 3C). In contrast to IL-13, expression and distribution of IL-13R $\alpha 1$ appeared to be
138 similar between prion-infected WT and CD14^{-/-} mice. IL-13R $\alpha 1$ immunoreactivity was
139 detected in the ic, fi, and cp at 75 dpi. IL-13R $\alpha 1$ -positive areas were found to spread
140 into the corpus callosum and thalamus at 90 dpi. However, IL-13R $\alpha 1$ -positive cells in
141 the corpus callosum and thalamus were almost absent at 120 dpi both in WT and
142 CD14^{-/-} mice. Quantitative analysis suggested that the IL-13 $\alpha 1$ -positive area in WT and
143 CD14^{-/-} mice at 90 dpi were similar, although variation was observed in the
144 prion-infected CD14^{-/-} mice (Fig. 3D). The cells mainly expressing IL-13R $\alpha 1$ were the
145 same as those expressing IL-13: neurons in the thalamus and microglia in the cerebral
146 peduncle (Fig. 3F).

147

148 *3.4. IL-13 expression is not completely associated with PrP^{Sc} deposition*

149

150 IL-13 immunoreactivity was most frequently observed in neurons of the thalamus
151 where PrP^{Sc} first appear in the Chandler-infected mouse brains [6]; therefore, we
152 assessed if there was any association of PrP^{Sc} deposition with IL-13 expression. At 75
153 dpi, PrP^{Sc} was distributed mainly in the lateral ventral part of the thalamus in CD14^{-/-}
154 mice, the same region where IL-13 was preferentially detected (Fig. 4A). In this area,
155 PrP^{Sc} was occasionally detected in IL-13-positive cells (Fig. 4A). Furthermore, PrP^{Sc}
156 was detected in the same part of the thalamus in WT and CD14^{-/-} mice, with its

157 detection being more in WT mice than in CD14^{-/-} mice as previously reported [6],
158 whereas IL-13 was rarely expressed (Fig. 4B). A tendency for the concurrence of PrP^{Sc}
159 with IL-13 expression was also observed in the hippocampus of CD14^{-/-} mice (Fig. 4D).
160 However, the presence of PrP^{Sc} did not appear to induce IL-13 in the cerebral cortex of
161 prion-infected CD14^{-/-} mice. Indeed, IL-13 immunoreactivity was hardly detected in the
162 cerebral cortex of CD14^{-/-} mice, although the levels of PrP^{Sc} accumulated in the cerebral
163 cortex appeared to be at least as high as those observed in the hippocampus (Figs 4C &
164 4D).

165

166 **Discussion**

167 In the current study, we showed that IL-13 expression was upregulated in the
168 absence of CD14 at the early stage of prion infection. The major cell type that expressed
169 IL-13 in CD14^{-/-} mouse brains was neurons. Considering that microglia are major cells
170 expressing CD14 in the brain, the interaction of neurons with CD14-expressing
171 microglia may suppress IL-13 expression in neurons. Alternatively, soluble CD14 may
172 act as a suppressor of IL-13 expression in neurons. This theory is supported by a
173 previous study suggesting suppression of IL-13 production from human dendritic cells
174 by soluble CD14 molecule [11]. In the thalamus of CD14^{-/-} mice at 75 dpi, IL-13
175 expression appeared to be concurrent with deposition of PrP^{Sc}. This observation leads us
176 to speculate that subtle changes in neurons of the thalamus directly stimulate IL-13
177 expression to evoke certain protective responses. However, IL-13 expression was not
178 induced in the cerebral cortex of prion-infected CD14^{-/-} mice, suggesting that the
179 induction of IL-13 expression cannot be simply explained by the deposition of PrP^{Sc}.
180 Furthermore, IL-13 expression was markedly decreased after 90 dpi, whereas PrP^{Sc}
181 deposition increased toward the terminal stage [6]. Thus, humoral factors temporarily

182 induced in the early stage of prion infection may stimulate IL-13 expression in the
183 brains of CD14^{-/-} mice. IL-4 is one of the factors that stimulates IL-13 expression [12].
184 However, gene expression of IL-4 was below the detection limit in both WT and
185 CD14^{-/-} mice (data not shown).

186 IL-13 was originally reported as a T cell-derived cytokine that down-regulates
187 pro-inflammatory responses [13,14]. Although IL-13 signaling is reported to have both
188 neuroprotective and neurotoxic effects in vivo [15,16,17], it has been thought to have a
189 neuroprotective role in some neurodegenerative disorders. The levels of IL-13 in the
190 cerebrospinal fluid of multiple sclerosis patients were related to reduced amount of A β ,
191 better performance in neurological test and GABAA-mediated cortical inhibition [18].
192 Intracerebral microinjections of IL-4 and IL-13 reduced A β accumulation in APP23
193 mice and promoted phagocytosis and the increased expression of neprilysin in neurons,
194 which leads to degradation of A β [19]. A previous study showed that the survival time
195 of IL-13 deficient mice was shortened compared with WT mice following intracerebral
196 inoculation with a low dose of Chandler strain; however, no difference was observed
197 with a high dose inoculation [20]. Low IL-13 expression in prion-infected WT mouse
198 brains, as shown in the current study, may be one of the reasons why the IL-13 knockout
199 had a limited effect on the survival time. Nevertheless, the increased prion susceptibility
200 of IL-13^{-/-} mice indicates the possible involvement of IL-13 in the pathogenesis of prion
201 diseases.

202 This study, along with our previous work, has shown that CD14 deficiency evokes
203 temporal anti-inflammatory condition in the brain in the early and middle stage of prion
204 infection. It is of interest to clarify whether anti-inflammatory responses reduce the
205 production of pro-inflammatory cytokines. In the previous study, IL-1 β -positive area in
206 the internal capsule of prion-infected CD14^{-/-} mice was smaller than that of

207 prion-infected WT mice at 60 dpi [6]. However, gene expressions of pro-inflammatory
208 cytokines at 75 dpi did not show this tendency (Fig. 2), although lower expressions of
209 IL-1 β and IL-6 in CD14^{-/-} mice were occasionally observed in the hippocampus (Fig.
210 2D). The apparent discrepancy in the gene and protein expression of IL-1 β in the
211 current and previous study may be due to a difference of the sampling area: 10
212 μ m-thickness for immunofluorescence staining and approximately 1 mm-thickness for
213 gene expression analysis, which is the limitation of thin-slice using the AltoTM Brain
214 Matrix. Thus, any difference in the IL-1 β expression observed by immunofluorescence
215 staining might be overwhelmed by collecting tissue from a larger area.

216 The mechanism for the temporal upregulation of anti-inflammatory cytokines in
217 prion-infected CD14^{-/-} mice remains to be elucidated. However, temporal induction of
218 anti-inflammatory milieu slows disease progression by interfering with prion
219 propagation. This indicates that immunomodulation that evokes long-lasting
220 anti-inflammatory responses may be a candidate for therapeutics of prion diseases.

221

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231

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305

306

307

308

309 Figure Legends

310 Fig. 1. Expression and distribution of microglial markers in the brains of WT and
311 CD14^{-/-} mice infected with the Chandler strain. (A) CD11b. (B) CD68. Six-week-old
312 female WT and CD14^{-/-} mice were inoculated intracerebrally with scrapie Chandler
313 strain. For a negative control, mice were inoculated with brain homogenate prepared
314 from uninfected WT mice. The brains were harvested at 60, 75, 90, 120 dpi and the
315 terminal stage for frozen blocks and subjected to immune fluorescent staining. The
316 samples were prepared from two mice for each group. Representative figures from the
317 thalamus are shown for each time point. Figures for the mock-infected mouse brains at
318 60 dpi are shown as a control. Red, CD11b (A) and CD68 (B). Blue, nuclei. Bars show
319 50 μm. Insets show a summary of the antigen distribution, plotted as black dots on
320 illustrations of The Mouse Brain at the level of bregma -1.82 mm [7].

321

322 Fig. 2. Cytokine gene expression in the brains of WT and CD14^{-/-} mice infected with
323 prion. (A) Regions subjected to the analysis: thalamus, hippocampus, and combined
324 regions of ic, fi and cp. Total RNA was extracted from each region at 75, 90, and 120
325 dpi and subjected to quantitative RT-PCR. (B-D) Relative expression of cytokine genes
326 in the thalamus (B), the region of ic, fi and cp (C) and the hippocampus (D). Expression
327 level of the corresponding gene of the mock-infected mice at 75 dpi was defined as 1.

328 When gene expression in the mock-infected WT mice was below the detection limit,
329 expression level at 40 cycles was tentatively defined as 1 for the calculation of relative
330 fold changes. Graphs show the mean with standard deviations ($n = 3$), except for
331 mock-infected CD14^{-/-} mice at 75 and 120 dpi ($n = 2$). * $p < 0.05$, student's t -test. ND:
332 not determined.

333

334 Fig. 3. Expression and distribution of IL-13 and IL-13R α 1 in the brains of WT and
335 CD14^{-/-} mice infected with prion. (A, C) Expression and distribution of IL-13 (A) and
336 IL-13 R α 1 (C) at 75, 90, and 120 dpi. Frozen sections at the level of bregma -1.82 mm
337 were subjected to immunofluorescence staining using rabbit polyclonal antibodies
338 against IL-13 and IL-13R α 1. Representative figures from the thalamus are shown. Red,
339 IL-13 or IL-13R α 1. Blue, nuclei. Bars, 20 μ m. The insets show a summary of the
340 distribution, plotted as black dots on illustrations of The Mouse Brain at the bregma
341 -1.82 mm [7]. The amygdala and corpus callosum are circled on the insets in (A) and
342 (C), respectively. (B, D) Quantitative results. Graphs show the quantification of positive
343 areas for IL-13 (B) and IL-13R α 1 (D), measured by Imaris ver 7.6.1. The number of
344 examined mice was two, except for the mock-infected CD14^{-/-} mouse at 90 and 120 dpi
345 ($n = 1$). (E, F) Double immunofluorescence staining of IL-13 (E) at 75 dpi or IL-13 R α 1
346 (F) at 90 dpi with neuronal and glial cell markers in the thalamus and the cerebral
347 peduncle. Green: NeuN (neurons), CD11b (microglia) or GFAP (astrocytes). Red: IL-13
348 or IL-13 R α 1. Blue: nuclei. Bars, 10 μ m. Arrows, double-positive cells for IL-13 or
349 IL-13R α 1 with any of the markers.

350

351 Fig. 4. Distributions of PrP^{Sc} and IL-13 in Chandler-infected WT and CD14^{-/-} mouse
352 brains. Frozen sections at 75 dpi were used. Because GdnSCN treatment for the

353 PrP^{Sc}-specific detection weakened the IL-13 immunoreactivity, sections were first
354 subjected to staining for IL-13. After the fixation with 4% paraformaldehyde for 10 min,
355 sections were treated with 5 M GdnSCN for 10 min and PrP^{Sc}-specific
356 immunofluorescence staining was performed [6,21]. (A) Thalamus of CD14^{-/-} mouse.
357 (B) Thalamus of WT mouse. (C) Cerebral cortex of CD14^{-/-} mouse. (D) Hippocampus of
358 CD14^{-/-} mouse. Green, PrP^{Sc}. Red, IL-13. Blue, nuclei. Bars, 20 μ m.

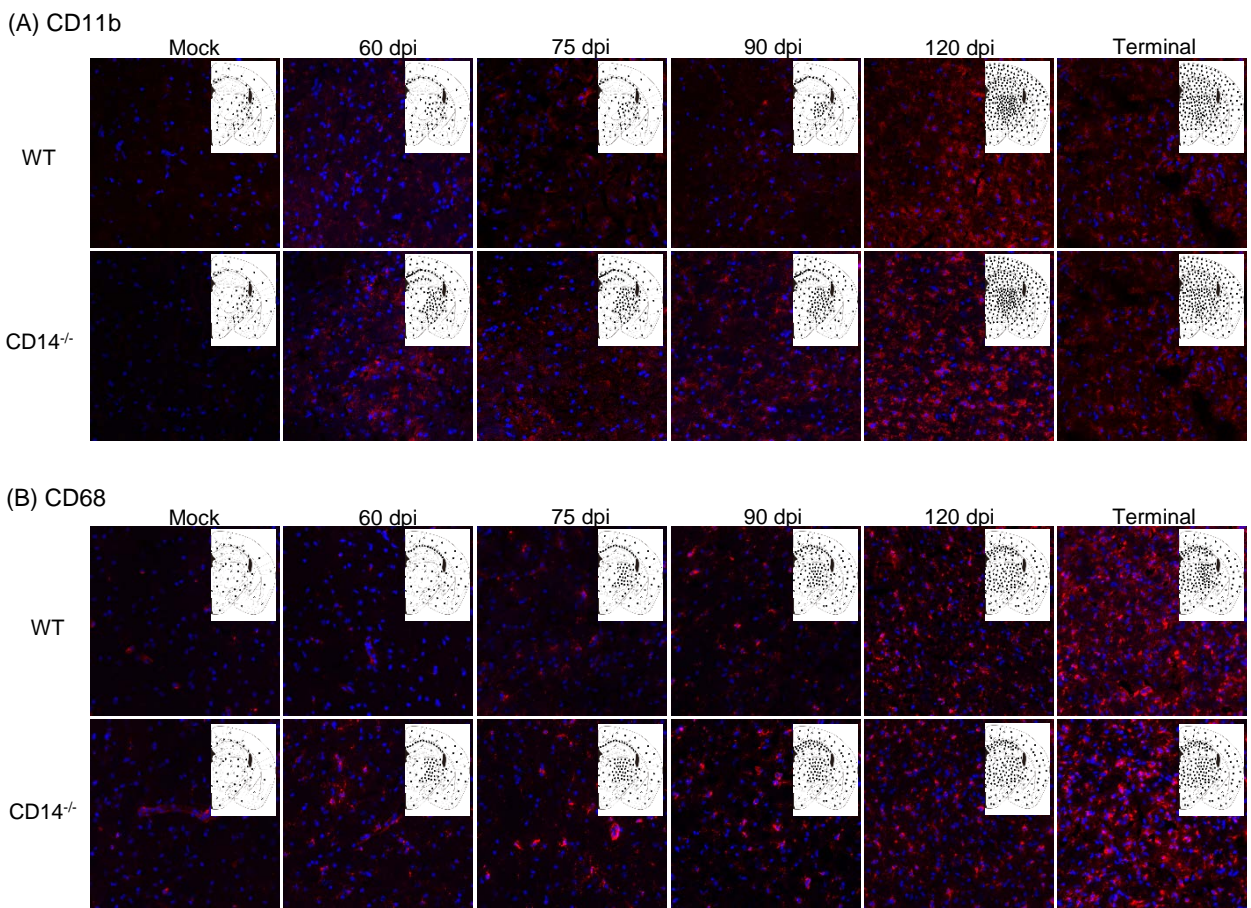


Fig. 1. Expression and distribution of microglial markers in the brains of WT and CD14^{-/-} mice infected with the Chandler strain. (A) CD11b. (B) CD68. Six-week-old female WT and CD14^{-/-} mice were inoculated intracerebrally with scrapie Chandler strain. For a negative control, mice were inoculated with brain homogenate prepared from uninfected WT mice. The brains were harvested at 60, 75, 90, 120 dpi and the terminal stage for frozen blocks and subjected to immune fluorescent staining. The samples were prepared from two mice for each group. Representative figures from the thalamus are shown for each time point. Figures for the mock-infected mouse brains at 60 dpi are shown as a control. Red, CD11b (A) and CD68 (B). Blue, nuclei. Bars show 50 μ m. Insets show a summary of the antigen distribution, plotted as black dots on illustrations of The Mouse Brain at the level of bregma -1.82 mm [7].

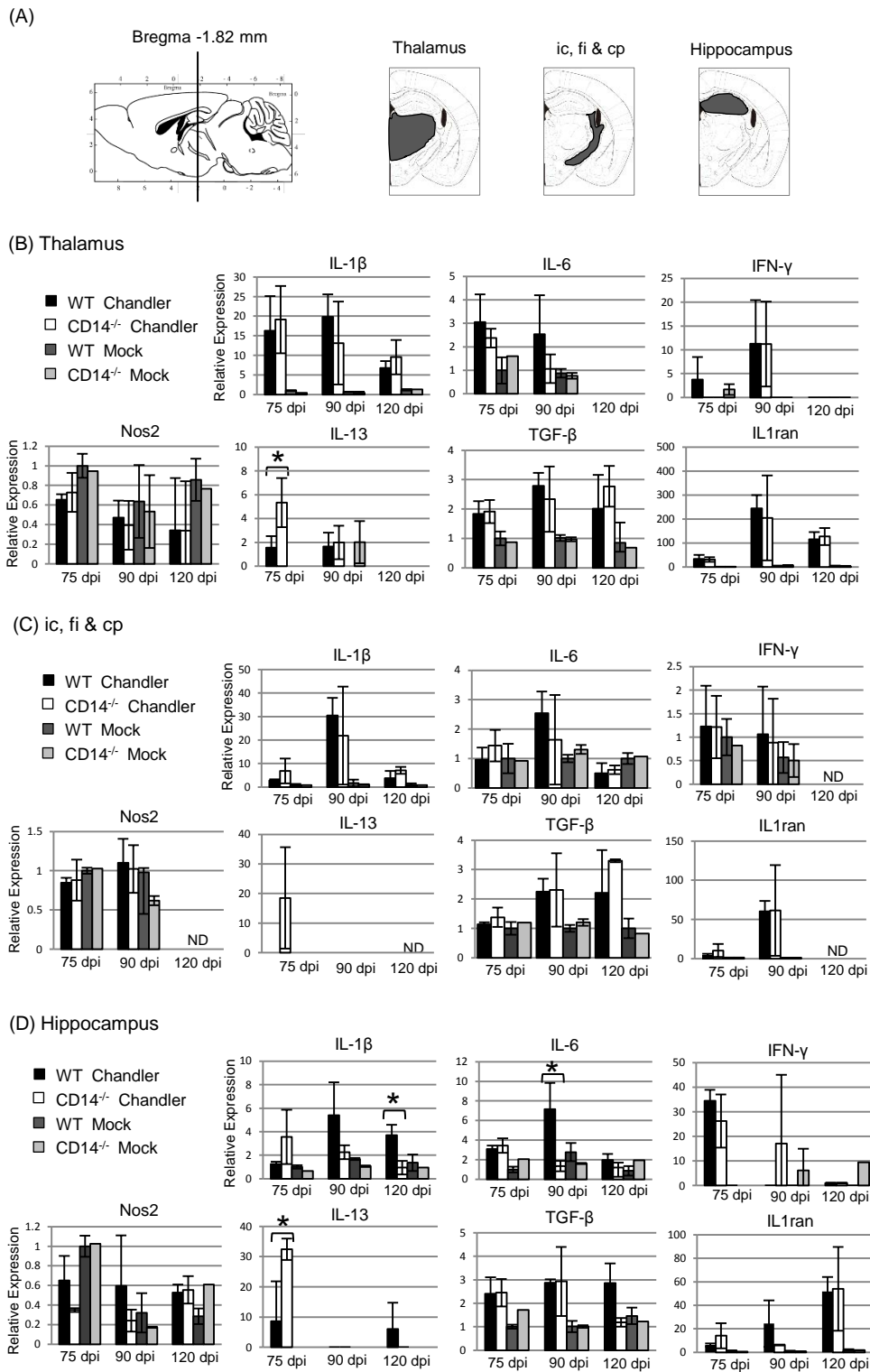


Fig 2. Cytokine gene expression in the brains of WT and CD14^{-/-} mice infected with prion. (A) Regions subjected to the analysis: thalamus, hippocampus, and combined regions of ic, fi and cp. Total RNA was extracted from each region at 75, 90, and 120 dpi and subjected to quantitative RT-PCR. (B-D) Relative expression of cytokine genes in the thalamus (B), the region of ic, fi and cp (C) and the hippocampus (D). Gene expression level of the mock-infected mice at 75 dpi was defined as 1. When gene expression in the mock-infected WT mice was below the limit of detection, expression level at 40 cycles was tentatively defined as 1 for the calculation of relative fold changes. Graphs show the mean of three samples with standard deviations, except for mock-infected CD14^{-/-} mice at 75 and 120 dpi (n = 2). * p<0.05, student's *t* test. ND: not determined.

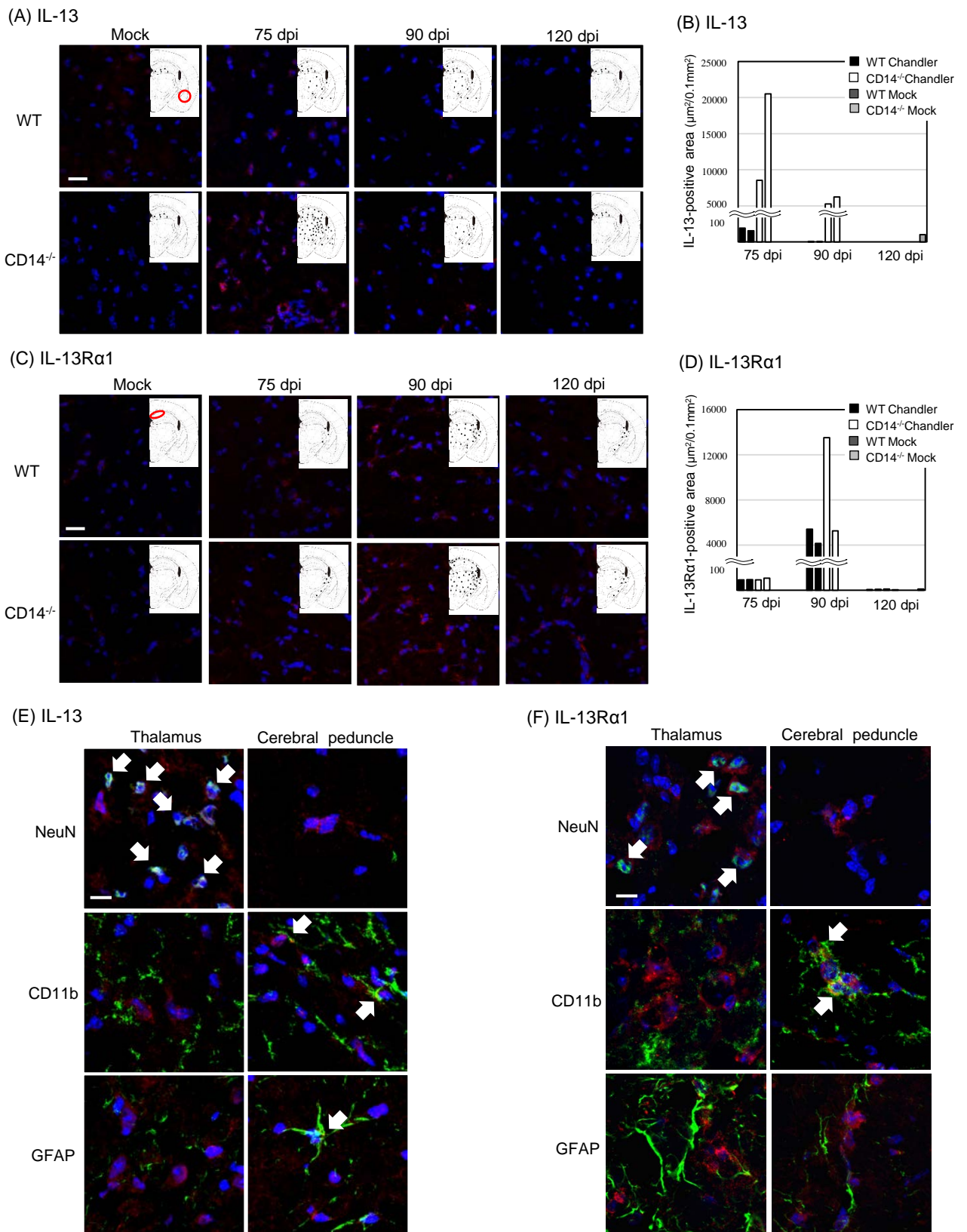


Fig 3. Expression and distribution of IL-13 and IL-13Rα1 in the brains of WT and CD14^{-/-} mice infected with prion. (A, C) Expression and distribution of IL-13 (A) and IL-13 Rα1 (C) at 75, 90, and 120 dpi. Frozen sections were subjected to immunofluorescence staining using rabbit polyclonal antibodies against IL-13 and IL-13Rα1. Representative figures from the thalamus are shown. Red, IL-13 or IL-13Rα1. Blue, nuclei. Bars, 20 µm. The insets show a summary of the distribution, plotted as black dots on illustrations of The Mouse Brain at the bregma -1.82 mm [7]. The amygdala and corpus callosum are circled on the illustrations in (A) and (C), respectively. Graphs show the quantification of positive areas for IL-13 (B) and IL-13Rα1 (D), measured by Imaris ver 7.6.1. The number of examined mice was two, except for the mock-infected CD14^{-/-} mouse at 90 and 120 dpi (n = 1). (E, F) Double immunofluorescence staining of IL-13 (E) at 75 dpi or IL-13 Rα1 (F) at 90 dpi with neuronal and glial cell markers in the thalamus and the area of ic, fi and cp. Green: NeuN (neurons), CD11b (microglia) or GFAP (astrocytes). Red: IL-13 or IL-13 Rα1. Blue: nuclei. Bars, 10 µm. Arrows, double positive cells for IL-13 or IL-13Rα1 and the corresponding markers.

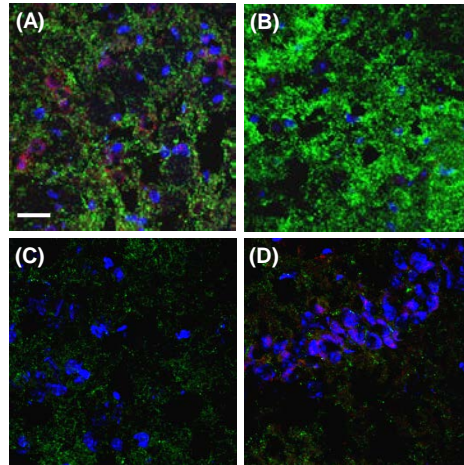


Fig 4. Distributions of PrP^{Sc} and IL-13 in Chandler-infected WT and CD14^{-/-} mouse brains at 75 dpi were used. Because GdnSCN treatment for the PrP^{Sc}-specific detection weakened the IL-13 immunoreactivity, sections were first subjected to staining for IL-13. After the fixation with 4% paraformaldehyde for 10 min, sections were treated with 5 M GdnSCN for 10 min and PrP^{Sc}-specific immunofluorescence staining was performed [6,21]. (A) Thalamus of CD14^{-/-} mouse. (B) Thalamus of WT mouse. (C) Cerebral cortex of CD14^{-/-} mouse. (D) Hippocampus of CD14^{-/-} mouse. Green, PrP^{Sc}. Red, IL-13. Blue, nuclei. Bars, 20 μ m.