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Emerging data suggest that polymorphonuclear leukocytes (PMNLs) can play an important role in Ag-dependent immune responses. Therefore, we have assessed the involvement of these cells in the development of an organ-specific autoimmune disease, experimental autoimmune encephalomyelitis (EAE), in the mouse. Depletion of peripheral blood PMNLs beginning day 8 after immunization significantly delayed and in some cases totally prevented the development of clinical EAE in mice. Depletion of PMNLs beginning 1 day before sensitization and continuing until day 7 postimmunization had no effect on the subsequent development of EAE, suggesting that depletion alters the efferent but not the afferent arm of the immune response. In vitro studies showed that lymphoid cells from mice protected from EAE by PMNL depletion beginning on day 8 poststimulation proliferated in response to specific Ag to a level equal to cells from sensitized animals treated with control serum, again indicating that treatment was not affecting the afferent limb of the immune response. Further evidence that PMNL may be necessary in initiating the pathology of EAE was seen in passive transfer experiments where PMNL-depleted recipients of MBP-specific lymphoid effector cells developed EAE much less effectively than did animals treated with control Ab. Taken together, these data indicate that PMNLs play a critical role in the effector phase of the development of the clinicopathologic expression of EAE in mice. The Journal of Immunology, 1998, 161: 6421–6426.

Polymorphonuclear leukocytes (PMNLs) form the first line of immune defense and as such are the first cell type to arrive at sites of inflammation and infection. In response to chemotactic factors released at these sites, PMNLs migrate from the bloodstream through the vascular endothelium to the inflammatory site (1). Once at the site, PMNLs release a variety of agents, including degradative enzymes and products of the oxidative burst, in response to appropriate stimulation in an attempt to resolve the inflammatory response (2, 3). In recent years, we have demonstrated that blood PMNLs are capable of significant de novo RNA and protein synthesis (4). Other studies have shown that PMNLs can up-regulate the expression of cytokines such as the IL-1 receptor antagonist (5), enzymes such as the 5-lipoxygenase (6), integral membrane proteins such as the 5-lipoxygenase-activating protein (7), transcription factors such as c-fos (8, 9), and chemokines such as IL-8 (10–12) and macrophage inflammatory protein-1α (MIP-1α) (11–13). The latter indicates that IL-8 and MIP-1α are the major chemokines produced by PMNLs (10, 11, 13). These studies, along with others, have significantly contributed to the changing perception that, in addition to playing a role as an effector cell, PMNLs can influence the afferent limb of the immune response (14, 15).

In addition to the above data, over the last few years it has become apparent that PMNLs may control the influx of different leukocyte subpopulations in various models of inflammation and immunity. For instance, depletion of PMNLs in mice using the anti-PMNL hybridoma RB6-8C5 dramatically reduces the number of lymphocytes infiltrating tumors (16). When rats are selectively depleted of PMNLs using the hybridoma RP-3, inhibition of both the priming and effector phases of delayed-type hypersensitivity (DTH) associated with a significant reduction in mononuclear cell recruitment occurs (17, 18). Furthermore, PMNLs are required for the recruitment of CD4+ T cells to s.c. sites upon administration of IL-8 (19). Another study shows that depletion in rats of PMNLs using RP-3 abrogates tumor-inhibitory CD8+ effector T cell generation (20).

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system (CNS) that serves as an experimental model of multiple sclerosis (21). The pathophysiology and pathogenesis of EAE is still not clearly understood; however, following active or passive induction of disease, there is a substantial cellular infiltrate into the CNS. T lymphocytes, both CD4+ and CD8+, B cells (22), macrophages (23), as well as occasional plasma cells can be found in the perivascular space, meninges, and parenchyma of the CNS. PMNLs are also present, but rare, in the guinea pig and rat with acute EAE; however, massive PMNL infiltration is present in more severe lesions in the monkey and dog (24) and in rats with...
hyperacute EAE (25–27). PMNL are uncommon in murine EAE lesions. There is an absolute requirement for CD4+ T cells to initiate disease, but how the cascade of other cells is orchestrated, and which cell(s) or cell product(s) is responsible for CNS damage and clinical signs, has not been defined.

These previous observations in vivo suggest that PMNLs may play a key role in orchestrating the recruitment of mononuclear cells to various extravascular sites and, as a consequence, may play a greater role than previously thought in the development of chronic inflammation. The potential of PMNLs to control mononuclear cell recruitment as well as their ability to produce potentially damaging mediators could have important implications in the pathogenesis of EAE and possibly multiple sclerosis. Therefore, we have investigated the possibility that PMNLs play a critical role in the development of EAE.

Materials and Methods

Mice

Female SJL/J mice age 7–14 wk were used throughout the experiments. The mice were bred at the Animal Breeding Establishment of the Australian National University under specific pathogen-free conditions. They were housed in conventional mouse rooms and given food and water ad libitum.

Ags, adjuvant, and immunization

Ags used were either mouse spinal cord homogenate (MSCH), myelin basic protein (MBP) peptide 89–101, or bovine MBP. MSCH was prepared from allogeneic spinal cord as a mix of four parts spinal cord and one part saline. Following homogenization it was freeze-dried and stored in a desiccator. MBP peptide 89–101 was a kind gift of Dr. Anand Gautam (Division of Cell Biology, John Curtin School of Medical Research, Canberra, Australian Capital Territory, Australia), and bovine MBP was prepared following the method of Elyar et al. (28). CFA contained 0.5 mg/ml Mycobacterium butyricum plus Mycobacterium tuberculosis H37Ra at 4 mg/ml. For immunization with MSCH, each mouse received 6 mg in CFA. The emulsion contained equal volumes of MSCH (4 mg in 50 μl saline) and CFA, and each mouse received 120 μl emulsion injected s.c. into the two hind foot pads, 50 μl/foot pad and 20 μl in the nape of the neck. Two hours before and two days after the injection of MSCH emulsion, the mice received an i.v. injection of 4 mg of pertussis in 250 μl of PBS. Pertussis, a crude extract of Bordetella pertussis-infected cells, was a gift from Dr. Jack Munoz (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD). For immunization with MBP peptide 89–101, 150 μg was injected/mouse in the same volume of CFA emulsion as for MSCH, and mice were treated with pertussis as above.

Clinical assessment of EAE

Mice were observed daily until day 20 for clinical signs of EAE. Disease severity was scored on a scale of 0 (asymptomatic) to 5 (moribund) (29). No detectable signs of EAE was designated a score of 0; slight weakness of the tail was designated 1; definite tail and partial hind limb paralysis was designated 2; tail paralysis and moderate hind limb paralysis was designated 3; complete paralysis of the tail and hind limbs often associated with incontinence was designated 3.5; paralysis of tail and hind limbs with moderate forelimb weakness was designated 4; and total paralysis of hind and forelimbs was designated 5.

Passive transfer of EAE

Donor mice (13-wk-old SJL/J) were immunized with guinea pig or bovine MBP in CFA. Each mouse received 50 μl in each hind foot and 20 μl s.c. in the scuff of the neck. The total dose of MBP was 400 μg/mouse. Animals were killed 10 days postimmunization and the draining lymph nodes harvested. Single cell suspensions were prepared and cells were cultured in Linbro six-well plates, 4 ml per well, at a concentration of 4 × 10^6/ml in RPMI 1640 plus 10% FCS, 5 × 10^-5 M 2 ME, nonessential amino acids, Na pyruvate, glutamine, penicillin, streptomycin, and neomycin, and 50–100 μg/ml MBP or 2 μg/ml Con A. Cells were harvested 4 days later, washed, and transferred to recipient mice in a volume of 400 μl at the indicated concentrations. In some experiments (indicated in Results), donor mice were given two doses of pertussis as for active immunization and cells harvested and cultured in the same manner.

Lymphocyte proliferation assays

Mice immunized with MBP peptide 89–101 were followed for clinical disease until day 17 postimmunization and then draining lymph nodes and spleen were taken for proliferation assays (30). Single-cell suspensions were prepared and cells were cultured in 200 μl volumes in 96-well round-bottom plates at a concentration of 4 × 10^5/ml with added peptide at a concentration of 1 or 10 μg/ml. After 48 h of culture, 1 μCi [3H]thymidine was added per well; the cultures were harvested 18 h later and assessed for incorporation of [3H]thymidine.

Differential staining of PBL

Peripheral blood smears were air-dried, then stained with Diff-Quik (Sigma/Aldrich, Castle Hill, New South Wales, Australia), and differential counts were performed.

Results and Discussion

Experiments were initially conducted to establish conditions under which the anti-granulocyte Ab RB6-8C5 would deplete mice of PMNL. Eleven-week-old SJL/J mice were bled and total white blood cell counts and differential counts were performed. Six mice per group were then given 200 μg RB6-8C5 or an isotype-control Ab (GL113) i.p. All mice were then bled again on days 1, 2, and 3, given another dose of RB6-8C5 or GL113 after the day-3 bleed, and then bled again on day 4. *, Statistically significantly different from the control values at p < 0.05 (Students t test).

FIGURE 1. Effect of treatment of mice with anti-granulocyte Ab RB6-8C5 on the level of circulating PMNL. Eleven-week-old SJL/J mice were bled and total white blood cell counts and differential counts were performed as described in Materials and Methods. Six mice per group were then given 200 μg RB6-8C5 or an isotype-control Ab (GL113) i.p. All mice were then bled again on days 1, 2, and 3, given another dose of RB6-8C5 or GL113 after the day-3 bleed, and then bled again on day 4. *, Statistically significantly different from the control values at p < 0.05 (Students t test).
the mice were given 80 or 200 μg spinal chord homogenate and heat-killed Bordetella pertussis, and on days 8, 10, and 12 after immunization. They were bled for determination of percentage of PMNL on days 7, 9, 11, and 14 as described above. Development of EAE was not assessed in these animals because of the possibility that repeated bleeding for PMNL determination could alter the course of disease. Baseline counts of immunized mice before the first Ab injection are represented as day 7 in Fig. 2. PMNL levels at this time are double that for nonimmunized mice, i.e., 20–30% of total white count compared with 10–15% (see day 0 in Fig. 1). Twenty-four hours after administration of either 80 or 200 μg of RB6-8C5, PMNL levels had dropped to less than 3% compared with 25% in control Ab-treated mice. At the higher dose of RB6-8C5 (200 μg), PMNL levels remained at or below 3% through day 14. At the lower dose of RB6-8C5, PMNL levels were still significantly lower than levels in control animals (which appeared to increase steadily through day 14) but had recovered to approximately baseline levels. Taken together, these data indicate that the anti-granulocyte treatment was capable of decreasing the level of circulating PMNL during induction of EAE.

Experiments were next performed to assess the effect of PMNL depletion on the development of clinical EAE in the SJL/J mouse. EAE was induced by immunization with 20% spinal chord homogenate and heat-killed B. pertussis, and on days 8, 10, and 12 the mice were given 80 or 200 μg i.p. of RB6-8C5 or the isotype-matched control, GL113. The results of this experiment (Table I) indicate that the number of animals developing clinical disease and the severity of disease were significantly lower in the PMNL-depleted mice than in the isotype-matched control mice, and the onset of disease was also delayed. A second experiment was conducted using an identical treatment regimen but only treating with the higher dose of RB6-8C5. In this experiment, there was complete protection from clinical disease in animals treated with RB6-8C5. These data indicate that PMNL play an important role in the development of EAE.

In experiment 1 (Table I), animals that did not develop clinical EAE were killed at day 17, and the spinal cords were examined for histopathologic lesions of EAE. A minimum of 35 sections per mouse were examined and the lesions quantified. The results are presented as the number of mice (without clinical disease) that showed lesions and the mean number of lesions per section for each group (Table II). In both treatment groups, there were some mice that showed no histopathologic signs of EAE. However, the majority in each group did have lesions in the absence of clinical signs of EAE. The number of lesions in the treated groups, however, were significantly less than in the control-treated animals. Taken together, these data indicate that treatment with RB6-8C5 not only reduces the incidence of clinical EAE but also reduces the extent of inflammation in the CNS.

![FIGURE 2](image)

To determine whether protection against EAE by PMNL depletion was related to the type of Ag used (whole spinal cord contains a number of different neuroantigens), the immunodominant MBP peptide for the SJL/J mouse, MBP 89–101, was used to induce EAE. Mice were immunized with 150 μg of peptide in CFA and given pertussis as described in Materials and Methods. The mice were then treated with either GL113 or RB6-8C5 (200 μg/dose) on days 8 and 12 after sensitization. All seven control Ab-treated mice developed EAE, with a mean day of onset of day 12 and mean maximal clinical score of 3. None of four mice treated with RB6-8C5 developed clinical EAE.

Data in the literature indicate that depletion of rodents of PMNL inhibits both the priming and the effector phase of experimental DTH, implying that the observed inhibition of active EAE in the present study could be due to either inhibition of sensitization to the Ag, to inhibition of the effector phase, or a combination of both. Although Ab treatment of mice was delayed until day 8 after sensitization, the possibility remained that this treatment with RB6-8C5 could have affected the level of specific Ag priming in those animals. Therefore, we next addressed the question of whether PMNLs play a role in the afferent limb (sensitization phase) of the disease. Mice were treated as described above using MBP peptide, and four of the control Ab-treated mice (mouse numbers 1–4) and all of the RB6-8C5-treated mice (mouse numbers 5–7) were killed on day 17 postsensitization and assayed for proliferation against MBP peptide in vitro. Fig. 3 shows the results for both spleen and draining lymph node proliferation, which indicated that treatment of mice with RB6-8C5 had no significant effect on sensitization of T lymphocytes to MBP peptide.

Table I. Effect of depletion of peripheral blood granulocytes on the development of clinical EAE

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Sick/Total</th>
<th>Mean Day of Onset*</th>
<th>Mean Maximum Clinical Score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt. 1 GL113&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8/13</td>
<td>12.6 ± 0.5</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>RB6-8C5 (80 μg)</td>
<td>5/12</td>
<td>14.0 ± 0.7</td>
<td>1.0 ± 0.4*</td>
</tr>
<tr>
<td>RB6-8C5 (200 μg)</td>
<td>3/12</td>
<td>15.0 ± 0.5*</td>
<td>0.8 ± 0.4*</td>
</tr>
<tr>
<td>Expt. 2 GL113</td>
<td>9/10</td>
<td>14.7 ± 0.6</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>RB6-8C5 (200 μg)</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SEM.
<sup>b</sup> Treatment regimen as follows: EAE was induced by immunization with 20% spinal chord homogenate and heat-killed Bordetella pertussis, and on days 8, 10, and 12 the mice were given 80 or 200 μg i.p. of RB6-8C5 or the isotype-matched control, GL113 Ab.
<sup>c</sup> Statistically significantly different from the control values at p < 0.05 (Students t test).

Table II. Effect of depletion of granulocytes on histopathology in the CNS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. with Lesions/Total</th>
<th>Lesions/Section&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL113&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3/3</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>RB6-8C5 (80 μg)</td>
<td>5/7</td>
<td>0.2 ± 0.1*</td>
</tr>
<tr>
<td>RB6-8C5 (200 μg)</td>
<td>6/9</td>
<td>1.1 ± 0.5*</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SEM.
<sup>b</sup> Treatment regimen as follows: EAE was induced by immunization with 20% spinal chord homogenate and heat-killed Bordetella pertussis, and on days 8, 10, and 12 the mice were given 80 or 200 μg i.p. of RB6-8C5 or 200 μg i.p. of the isotype-matched control, GL113. Animals that had not developed clinical signs of EAE by day 17 were sacrificed, and the spinal cords were examined for histopathological lesions of the CNS.
<sup>c</sup> Statistically significantly different from the control values at p < 0.05 (Students t test).
This result was confirmed in experiments in which mice were depleted of PMNL beginning 24 h before sensitization and depletion maintained for up to 8 days, after which time, the treatment with RB6-8C5 ceased. These animals developed EAE to the same extent as control animals receiving GL113 under the same conditions (data not shown). Because the number of circulating PMNL increases rapidly following cessation of RB6-8C5 treatment (data not shown) the results of these experiments suggest that the presence of PMNL is not required during the sensitization phase of the disease, thus supporting the in vitro data described above.

To determine whether PMNL were involved in the effector phase of EAE, recipient SJL/J mice were treated with either GL113 or RB6-8C5 (200 μg/dose) on days 8 and 12 after sensitization, the RB6-8C5-treated and the control Ab-treated mice were killed on day 17 post sensitization, and (A) lymph node cells and (B) splenocytes were assayed for proliferation against MBP peptide in vitro as described in Materials and Methods.

FIGURE 3. Lack of inhibition of priming of the immune response to MBP 89–101 peptide in PMNL-depleted mice. Mice were immunized with 150 μg of MBP 89–101 peptide in CFA and given pertussigen as described in Materials and Methods. The mice were then treated with either GL113 or RB6-8C5 (200 μg/dose) on days 8 and 12 after sensitization, and spleen cells from the same donors were also cultured with Con A-activated spleen cells as has been done with the rat (31), and this disease was also inhibited by treatment of the recipients with RB6-8C5, indicating that PMNL are required during the effector phase of EAE.

Because of the quantity of guinea pig MBP required for these experiments, subsequent passive transfers were conducted using bovine MBP. In this protocol, donors were immunized with bovine MBP (same dose and adjuvant as with guinea pig MBP) and given two doses of pertussigen as for active induction of disease. Lymph node cells harvested from such animals and cultured as for guinea pig MBP-immunized donors readily transferred EAE to naive recipients. With 10^8 cells/recipient, disease onset occurred as early as day 5 after transfer and progressed rapidly to death (data not shown). Using this passive transfer technique but reducing the number of transferred cells to 5 \times 10^7 cells/recipient to lessen the severity of the disease, we were able to reproducibly evaluate the effect of treating recipients with RB6-8C5 (200 μg/dose) given on days 1, 3, 5, 6, 7, and 8 following transfer of cells. Under these conditions, both control Ab-treated and RB6-8C5-treated animals developed disease, but there was a clear difference in the kinetics of development (Fig. 4A). It should also be noted that Ab treatment was ceased on day 8, and by day 10 the severity of disease in the two groups was the same. This experiment was repeated and Ab given (80 μg/dose) daily from day 1 until day 7. As seen in Fig. 4B, there was also a clear delay in the onset of disease in those mice treated with RB6-8C5, but on the day after cessation of treatment most animals showed severe clinical signs.

Taken together, these data demonstrate that depletion of PMNLs in animals receiving primed EAE effector cells can significantly inhibit the development of clinical signs of the disease. This indicates that the PMNL play an important role in the effector phase of the disease. Other evidence suggesting a role of PMNL in aspects of autoimmune CNS inflammation derives from experiments with IFN-γ receptor knockout mice (IFN-γR^-/-). These mice, which lack the ligand-binding chain for IFN-γ, develop severe EAE when immunized with human MOG35–55 peptide and either die or remain chronically ill (29). The inflammatory infiltrate in the CNS of these animals comprises approximately 25–30% PMNLs. Therefore, experiments were conducted on these mice to determine whether the PMNL present in the lesion were simply bystanders or were playing a role in the pathogenesis of the disease. IFN-γR^-/- mice were treated with either control Ab or RB6-8C5 every other

Table III. Effect of depletion of granulocytes in recipient mice on the development of clinical EAE following passive transfer of MBP-specific effector lymphocytes

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Sick/Total</th>
<th>Mean Day of Onset</th>
<th>Mean Maximum Clinical Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL113^a</td>
<td>5/6</td>
<td>14.4 ± 1.0</td>
<td>2.50 ± 0.5</td>
</tr>
<tr>
<td>RB6-8C5 (200 μg)</td>
<td>2/8</td>
<td>17.5 ± 0.3*</td>
<td>0.75 ± 0.5*</td>
</tr>
</tbody>
</table>

^a Mean ± SEM.

^b Treatment regimen as follows: EAE was induced by immunization with guinea pig MBP in CFA. Lymph node cells were cultured with Con A as described in Materials and Methods and transferred by i.v. injection into recipients that were then treated on days 4, 8, 10, 12, and 14 with either GL113 or RB6-8C5 (200 μg/dose).
The Ab used in the present study to deplete mice of circulating PMNL does not distinguish between neutrophils and eosinophils. Although neutrophils are the major cell type comprising PMNL, both neutrophils and eosinophils may be involved in the pathogenesis of EAE. The results of previous studies have shown the presence of large numbers of neutrophils in hyperacute EAE in rats (25–27). Moreover, a recent study has demonstrated that a leukotriene B_{4} receptor antagonist inhibits the development of EAE and dramatically reduces the infiltration of eosinophils into the CNS (33). Finally, mice deficient in the IFN-γ receptor develop an acute form of EAE that is characterized by a significant influx of PMNL into the CNS (29). In the present study, we have extended this observation and shown that PMNL play a causal role in the development of EAE in these mice.

No evidence for the involvement of PMNL in the sensitization phase of EAE was obtained. Depletion of PMNL beginning the day before inoculation did not affect the production of MBP-reactive lymphocytes as determined by in vitro proliferation assays or adoptive transfer of EAE into nonsensitized recipients. This is in contrast to the results of several other studies examining the effect of depletion of PMNL on adaptive immune responses. For instance, inhibition of both the priming and effector phases of DTH in the rat was observed when rats were selectively depleted of PMNLs using the hybridoma RP-3 (17, 18). In another study, tumor-inhibitory CD8^{+} effector T cell generation was inhibited upon depletion of rats of PMNLs using RP-3 (20). The reason for the difference between these previous results and our studies with respect to a role for PMNL in priming of the immune response is not clear at this point, but they may relate to differences between the species under investigation (rat vs mouse), to differences in the manner in which the two different Abs function (RP-3 vs RB6-8C5), to spatial differences in the models being tested (foot pad for DTH in the rat vs CNS in the mouse in the present study), or a combination of all of the above.

The results of the present study clearly indicate that PMNL play an important role in the effector phase of EAE; however, the mechanism by which this may be achieved is unclear. PMNL contain large amounts of tissue-modifying enzymes (2). Therefore, the cells may be required for modification of entry points into the CNS that enables EAE effector lymphocytes to enter more easily. Thus, when PMNL are not present, these lymphocytes are prevented from entering the CNS in numbers sufficient to cause clinical signs of EAE. Recent studies may provide further insight into the role of neutrophils in this respect. Of particular relevance to the present study are data indicating that granulocytes may play an important role in regulating the recruitment of T lymphocytes. Using a similar approach to that in the present study, it was shown that PMNL are required for the lymphocyte infiltration into tumors (16) and that PMNLs are required for the recruitment of CD4^{+} T cells to s.c. sites upon administration of IL-8 (19). Although the mechanism by which this may occur during an adaptive immune response is not yet clear, recent in vitro studies indicate that PMNL may release factors from intracellular granules that are chemotactic for lymphocytes, thereby enhancing recruitment of these cells (34). Another possibility is that production of chemokines by PMNL that attract mononuclear cells including T lymphocytes may play an important role in the development of EAE. Recent data have indicated that neutrophils produce the CC chemokine MIP-1α, a chemoattractant for, among other cell types, T lymphocytes (11–13), and the development of EAE in mice is inhibited by blocking Abs against this chemokine (35, 36). Therefore, it is possible that such systems are operating during the effector phase of EAE to regulate lymphocyte recruitment to the CNS.

Table V. Effect of depletion of granulocytes on the development of clinicopathological EAE in IFN-γ receptor-deficient mice

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Sick/Total</th>
<th>Mean Day of Onset ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL113b</td>
<td>4/4</td>
<td>13.0 ± 0.7</td>
</tr>
<tr>
<td>RB6-8C5 (200 μg)</td>
<td>0/4</td>
<td>0</td>
</tr>
</tbody>
</table>

* Treatment regimen as follows: EAE was induced by immunization with MOG 35-55 peptide and on every other day between days 0 and 12, and twice daily from day 12 the mice were given 200 μg i.p. of RB6-8C5 or the isotype-matched control, GL113.


