Caspase-1 Regulates the Inflammatory Process Leading to Autoimmune Demyelination

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Caspase-1 Regulates the Inflammatory Process Leading to Autoimmune Demyelination

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T cell-mediated inflammation is considered to play a key role in the pathogenic mechanisms sustaining multiple sclerosis (MS). Caspase-1, formerly designated IL-1β-converting enzyme, is crucially involved in immune-mediated inflammation because of its pivotal role in regulating the cellular export of IL-1β and IL-18. We studied the role of caspase-1 in experimental autoimmune encephalomyelitis (EAE), the animal model for MS. Caspase-1 is transcriptionally induced during EAE, and its levels correlate with the clinical course and transcription rate of proinflammatory cytokines such as TNF-α, IL-1β, IFN-γ, and IL-6. A reduction of EAE incidence and severity is observed in caspase-1-deficient mice, depending on the immunogenicity and on the amount of the encephalitogenic myelin oligodendrocyte glycoprotein (MOG) peptide used. In caspase-1-deficient mice, reduced EAE incidence correlates with defective development of anti-MOG IFN-γ-producing Th1 cells. Finally, pharmacological blockade of caspase-1 in Biozzi AB/H mice, immunized with spinal cord homogenate or MOG35–55 peptide, by the caspase-1-inhibitor Z-Val-Ala-Asp-fluoromethylketone, significantly reduces EAE incidence in a preventive but not in a therapeutic protocol. These results indicate that caspase-1 plays an important role in the early stage of the immune-mediated inflammatory process leading to EAE, thus representing a possible therapeutic target in the acute phase of relapsing remitting MS.


The caspase family comprises thus far 13 different cysteine proteases that are mainly involved in the apoptotic pathway (1). Among them, caspase-1, formerly named IL-1β-converting enzyme, which is activated by caspase-11-mediated proteolytic cleavage (2), is less involved in the apoptotic cascade but is prominent in inflammation because of its pivotal role in regulating the cellular export of proinflammatory cytokines such as IL-1β. Caspase-1 is elevated in intestinal macrophages during inflammatory bowel disease (3) and in a variety of organs, including the brain, in response to bacterial LPS administration (4). Further evidence on the role played by caspase-1 in inflammation comes from studies on caspase-1-deficient (−/−) mice and caspase-1 pharmacological inhibitors. Caspase-1−/− mice display an alteration in the export of several proinflammatory cytokines, namely IL-1β, IL-1α, IL-6, and TNF-α, although neither IL-1α nor IL-6 nor TNF-α are substrates for caspase-1 (5). Furthermore, caspase-1 proteolytically activates IL-18, and caspase-1−/− mice have also reduced serum levels of IL-18 and IFN-γ in response to LPS administration (6). Caspase-1−/− mice are resistant to LPS-induced endotoxic shock (7) and to the induction of experimental pancreatitis (8). In vivo pharmacological inhibition of caspase-1 protects mice from TNF-α-induced liver failure (9) and collagen-induced arthritis (10).

MS* is an immune-mediated demyelinating disease of the CNS of unknown etiology (11). The pathological hallmark of the disease is the presence within the CNS of inflammatory infiltrates containing few autoreactive T cells and many pathogenic nonspecific mononuclear cells (12). It is currently believed that Ag-specific T cells provide the organ specificity of the pathogenic process and regulate the recirculation within the CNS of activated mononuclear cells releasing inflammatory myelinotoxic substances. These latter cells can be activated in the periphery by polyclonal inflammatory stimuli, thus determining disease recurrence (12, 13). Proinflammatory cytokines participate either in Ag-specific T cell activation or in peripheral activation of nonspecific mononuclear cells. TNF-α, IFN-γ, and IL-6 levels increase before disease relapses (13, 14). An increased number of disease relapses was observed in MS patients treated with IFN-γ (15). Moreover, TNF-α, IFN-γ, and IL-1β are present in demyelinating plaques (16), and IL-1β has been shown to be a mediator of the inflammatory process sustaining EAE, the animal model for MS (17).

We evaluated the role of caspase-1 in EAE. We found that caspase-1 mRNA blood levels parallel those of proinflammatory cytokines, such as IL-1β, IL-6, TNF-α, and IFN-γ, during EAE and peak at the time of maximal EAE severity. A reduction of EAE incidence and severity was observed in caspase-1−/− mice depending on the immunogenicity and on the amount of the encephalitogenic MOG peptide used. Finally, pharmacological blockade of caspase-1 reduced the incidence of EAE, induced either with SCH or MOG35–55 peptide, in a preventive but not therapeutic...
protocol. These results indicate that caspase-1 plays an important role in the immune-mediated pathogenic events leading to EAE and might represent a suitable therapeutic target of the active phase of the immune-mediated inflammatory demyelination.

Materials and Methods

Mice and immunizations

Female B6/OlaHsd mice, 4–6 wk old, were purchased from Harlan U.K. (Blackthorn, U.K.). Female C57BL/6 mice, 4–6 wk old were obtained by Charles River (Calco, Italy). Female (SV129 × C57BL/6)F1 mice, hereafter designated (SV129 × B6)F1, 4–6 wk old, were obtained by The Jackson Laboratory (Bar Harbor, ME). Caspase-1−/− mice had been obtained as previously described (5). Briefly, chimeric mice were obtained by injection of embryonic stem cells, in which the caspase-1 gene was disrupted and replaced with a neomycin resistance gene cassette, into C57BL/6 blastocysts. The chimeric males were then mated with C57BL/6 females. Homozygous mice with two copies of the disrupted caspase-1 gene were identified by Southern blot of genomic DNA, and the absence of caspase-1 mRNA in caspase-1−/− mice was confirmed by RT-PCR analysis. Homozygous mice were then interbred and used for the experiments. All animals were housed in specific pathogen-free conditions and treated according to the guidelines of the Animal Ethical Committee of our Institute. Mice were immunized with IFA (Difco, Detroit, MI) supplemented with 4 mg/ml Mycobacterium tuberculosis (strain H37Ra; Difco) and MOG35−55 (Multiple Peptide Systems, San Diego, CA), MOG40–55 (Roche Milano Ricerche, Milan, Italy), or SCH from Biozzi AB/H mice. Two immunization schedules were used for peptides: a single injection of 200 μg or two injections of 300 μg peptide 7 days apart. For SCH immunization, 1 mg of Ag was given twice, at days 0 and 7. All injections were followed by i.p. administration of 500 ng pertussis toxin (Sigma, St. Louis, MI) the same day and 48 h later. Body weight and clinical score (0 = healthy, 1 = flaccid tail, 2 = ataxia and/or paresis of hind limbs, 3 = paralysis of hind limbs and/or paresis of forelimbs, 4 = tetraparesis, 5 = moribund or death) were recorded daily.

Miniosmotic pumps

Miniosmotic pumps (Alzet 2001, Alza, Palo Alto, CA) were implanted s.c. in the dorsal flank of mice. The mean fill volume of pumps was ~220 μl, and the mean pumping rate was ~1 μl/h, delivering continuously for ~10 days. Pumps were filled with 50 mg/ml of the caspase-1 inhibitor Z-Val-Val-Ala-Asp-fluoromethylketone (Bachem, Bubendorf, Switzerland) or two injections of 300 μg peptide 7 days apart. For SCH immunization, 1 mg of Ag was given twice, at days 0 and 7. All injections were followed by i.p. administration of 500 ng pertussis toxin (Sigma, St. Louis, MI) the same day and 48 h later. Body weight and clinical score (0 = healthy, 1 = flaccid tail, 2 = ataxia and/or paresis of hind limbs, 3 = paralysis of hind limbs and/or paresis of forelimbs, 4 = tetraparesis, 5 = moribund or death) were recorded daily.

Semiquantitative RT-PCR for cytokines

Blood samples were obtained from mice by tail bleed every week. RNA was recovered from these samples in guanidinium thiocyanate by acidic phenol extraction. A T-promed first strand kit was used for the reverse transcription of total RNA into cDNA (Ready-to-go kit, Pharmacia, Uppsala, Sweden). PCR amplification (30 cycles: 1 min 95°C, 1 min 55°C, 1 min 72°C) of cDNA sequences specific for caspase-1 and cytokines were performed using 20 pmol of each primer, 200 mM concentrations of each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, and 2.5 U Taq polymerase. Amplified PCR products were hybridized with the specific labeled oligonucleotide probe (caspase-1 and cytokines) and then labeled probe obtained from a plasmid containing the mouse GAPDH cDNA, followed by analysis on a PhosphorImager (Molecular Dynamics, Sunnyvale, CA; Image Quant Software, version 3.3). Values were normalized against the GAPDH gene. For intertrial comparisons, the normalized intensities were further corrected with the use of the normalized intensity of the housekeeping gene resulting from RT-PCR amplification of a cDNA clone derived from mouse LPS-activated splenocytes (positive control). The following primers and probes were used: caspase-1 (product: 343 bp), antisense 5′-GTTTGGAGACTGAAAGAGACA-3′, sense 5′-GAGATGTTGAGAAGTGGAAAAGTGA-3′, probe 5′-TGAAGAACACTGCAAGGCAAAGG-3′; IL-1β (product: 563 bp), antisense 5′-CAGCGAGATGATAGTTCTTCTCTTCTT-3′, sense 5′-ATGGAACATTGTTGAGAAGGACACT-3′; IL-6 (product: 634 bp), antisense 5′-TTCAGAATGCTGCTGCTGGATTCTACACT-3′, sense 5′-AGCTCTCAGGCGCCCTTGAC-3′; TNF-α (product: 373 bp), antisense 5′-GATTAGGATCGAATTGGCTCAGTGGTG-3′, sense 5′-TTCTG TCTACTGAACTGCGGTATGCTGGTC-3′; probe 5′-GGCGTGTGCGAAGGGCTGGTGGCCTTG-3′; IFN-γ (product: 450 bp), antisense 5′-ACACTGATCCTTGCTCGT-3′, sense 5′-CAGCTCCTTTCTCCTGCT-3′; probe 5′-TCTTCCACACAAGCAG-3′; probe 5′-TCTCAGAAGGACACT-3′. 

Ap-specific proliferation assays

For T cell proliferation assays, draining lymph nodes were removed, and 4 × 10^5 lymph node cells per well were cultured in 96-well culture plates (Costar, Cambridge, MA) in synthetic HL-1 medium (Ventrex Laboratories, Portland, ME) supplemented with 2 mM l-glutamine and 50 μg/ml gentamicin (Sigma, St. Louis, MO) and serial concentrations (1, 3, 10 μM) of MOG35−55 peptide. Cultures were incubated for 3 days in 5% CO2 in air and pulsed for 2 h before harvesting with 1 μCi [3H]TdR (40 Ci/mmol, Pharmac Biotech, Amersham, Cologno Monzese, Italy). Incorporation of [3H]TdR was measured by liquid scintillation spectrometry.

Intracytoplasmatic staining for cytokine production

Lymph node cells (6 × 10^5 cells/well) were cultured in 96-well plates in synthetic HL-1 medium with 10 μM MOG35−55. After 72 h of culture, cells were harvested, washed, and resuspended for additional 72 h in RPMI 1640 supplemented with 2 mM l-glutamine, 50 μg/ml gentamicin, 50 μM 2-ME (Fluka Chemical, Ronkonkoma, NY), and 10% FCS (Sigma). After culture, living cells separated on a Ficoll gradient were restimulated with PMA (1 μg/ml) and ionomycin (50 ng/ml) for 4 h at 37°C, with 10 μg/ml brefeldin A (Novartis, Basel, Switzerland) added for the last 2 h to prevent loss of newly synthesized cytokines. After fixation with 4% paraformaldehyde for 20 min at room temperature, cells were stained for IFN-γ and IL-4 using the method of Openshaw et al. (20) and Galbiati et al. (21). Cells were washed, preincubated for 10 min with PBS/FCS/saponin, and then incubated with FITC ant mouse IFN-γ (XMGI2, Pharmingen, San Diego, CA) and PE rat anti mouse IL-4 (1B11, Pharmingen) or with isotype controls FITC- and PE-labeled rat IgG1, x (B3-34, Pharmingen). After 30 min, cells were washed twice with PBS/FCS/saponin and then with PBS containing 5% FCS without saponin to allow membrane closure. Cell membranes were then stained with Cy-Chrome-labeled anti-CD4 (L3T4, Pharmingen) for 15 min at room temperature. Analysis was performed with a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) equipped with CellQuest software, and 50,000 events were acquired.

ELISA for IFN-γ secretion

IFN-γ was quantified by two-site sandwich ELISA using polyclonal microtiter plates (Falcon 3012) coated with AN-18,17,7,4mAb in carbonate buffer (100 μg/ml) previously described (21). Samples (50 μl/well) diluted in 10-fold serial dilution (PBS containing 5% FCS and 1% gelatin) were incubated together with 50 μl peroxidase-conjugated XMGI2.12 mAb. After overnight incubation at room temperature, bound peroxidase was detected by 3′,3′5′-tetramethylbenzidine (Fluka Chemical), and absorbance was read at 450 nm with an automated microplate ELISA reader (MR5000, Dynatech Laboratories, Chantilly, VA). IFN-γ was quantified from two to three titration points using standard curves generated by purified recombinant mouse IFN-γ, and results were expressed as cytokine concentration in ng/ml. Detection limit was 15 pg/ml.

Neuropathological features in EAE mice

At the time of sacrifice, mice were transcardially perfused with 4% paraformaldehyde. Brains and spinal cords were removed and postfixed in the same fixative for 2–4 h, washed in PBS, and then embedded in paraffin. Tissue sections were cut at 4 μm on a microtome and stained for histological examination. Hematoxylin and eosin staining was used to reveal the general neuropathology of EAE mice, Luxol Fast Blue staining was used to reveal demyelinated areas, and Bielschowsky staining was used to detect axonal loss. Macrophages were stained using peroxidase-labeled BS-I solution (PBS containing 5% FCS and 1% gelatin) were incubated together with 50 μl peroxidase-conjugated XMGI2.12 mAb. After overnight incubation at room temperature, bound peroxidase was detected by 3′,3′5′-tetramethylbenzidine (Fluka Chemical), and absorbance was read at 450 nm with an automated microplate ELISA reader (MR5000, Dynatech Laboratories, Chantilly, VA). IFN-γ was quantified from two to three titration points using standard curves generated by purified recombinant mouse IFN-γ, and results were expressed as cytokine concentration in ng/ml. Detection limit was 15 pg/ml.

The number of perivascular inflammatory infiltrates was calculated and expressed as the numbers of inflammatory infiltrates per mm2, whereas demyelinated areas and axonal loss were expressed as the percentage of...
FIGURE 1. Caspase-1 and proinflammatory cytokine mRNAs are up-regulated during the course of EAE in blood from C57BL/6 mice immunized with 200 μg of MOG35–55. A, Caspase-1 mRNA levels. B, mRNA levels of IL-1β ( ), IL-6 ( ), IFN-γ ( ), and TNF-α ( ) from the same mice. mRNA levels were measured by a semiquantitative RT-PCR on blood samples obtained from the tail vein of mice once a week after immunization as indicated on x-axis. C, Clinical course of EAE in blood donors (n = 12 mice). Each circle represents the mean EAE score registered. Bars represent the SE.

damaged area per mm² (22). T cells and macrophages were counted and expressed as the number of cells per mm².

Statistical analysis

Data are expressed as mean ± SE. Student’s t test for unpaired data was used to compare cytokine and caspase-1 mRNA levels and the cytokine protein levels. EAE cumulative score was calculated by summing up each individual score registered in any group of mice studied during the follow-up period levels. Comparison between cumulative scores was performed using Student’s t test. A χ² test was used to compare EAE incidence in the different groups of mice. p < 0.05 was considered significant.

Results

Caspase-1 mRNA is up-regulated in the course of EAE

C57BL/6 mice immunized with MOG30–55 were bled once a week for 5 weeks after immunization. RT-PCR was performed on blood samples to determine caspase-1, IL-1β, IL-6, TNF-α, and IFN-γ mRNA levels. Caspase-1 mRNA levels increased almost 8-fold (Fig. 1A) during EAE from basal levels recorded at day 7 p.i. This increase paralleled disease severity, peaking 4 wk p.i. (day 27 p.i., 52.9 ± 16.8 AU; p = 0.01 vs day 7 p.i.) (Fig. 1A) when EAE clinical score reached its maximum (Fig. 1C). Caspase-1 mRNA level increase paralleled that of the caspase-1 substrate IL-1β, which showed a 2-fold increase (day 27 p.i., 3.3 ± 0.9 AU; p = 0.03 vs day 7 p.i.) (Fig. 1B); IL-6 mRNA increased almost 8-fold (day 27 p.i., 15.4 ± 7.6 AU; p = 0.04) (Fig. 1B); TNF-α mRNA increased almost 3-fold (day 27 p.i., 12.2 ± 5.4; p = n.s. vs day 7 p.i.) (Fig. 1B), and IFN-γ mRNA 4-fold (day 27 p.i., 1.6 ± 0.5 AU; p = 0.02 vs day 7 p.i.) (Fig. 1B). These data indicate that caspase-1 is up-regulated at the transcriptional level during the course of EAE and that this up-regulation parallels that of proinflammatory cytokines such as IL-1β, IFN-γ, TNF-α, and IL-6.

Caspase-1–/- mice are less susceptible to EAE induction

To further explore the role of caspase-1 in EAE, we studied the susceptibility of caspase-1–/- mice to MOG peptide-induced EAE. We first analyzed EAE susceptibility of (SV129 × B6)F1 and C57BL/6 mice, which have a genetic background comparable with that of the caspase-1–/- mice using 200 μg of MOG35–55. The two groups of mice showed similar disease courses (Fig. 2A, Table 1) confirming results previously reported (23).

To analyze EAE susceptibility in caspase-1–/- mice, we used two different amounts (200 μg once or 300 μg twice, 7 days apart) of two MOG overlapping peptides (MOG35–55 and MOG40–55). MOG peptides were chosen because of their previously described difference in immunogenicity and encephalitogenicity in C57BL/6 mice (24). Low dose immunization (200 μg) with MOG40–55 failed to induce EAE in caspase-1–/- mice (0 of 12 sick animals) compared with C57BL/6 mice (11 of 12) (p < 0.0001) (Fig. 2B, Table 1). Caspase-1–/- mice developed EAE when immunized with the low dose (200 μg) of MOG35–55 (Fig. 2A, Table I) or the high dose (600 μg) of MOG40–55 (Fig. 2B, Table I); the incidence, however, was lower and the onset delayed compared with (SV129 × B6)F1 or C57BL/6 mice. Therefore, caspase-1–/- mice are less susceptible to MOG-induced EAE compared with C57BL/6 or (SV129 × B6)F1 mice. However, caspase-1–/- mice can develop EAE when the immunogenicity of the encephalitogenic peptide is increased.
Inhibition of the Th1 response in caspase-1−/− mice

To elucidate the possible causes of reduced susceptibility of caspase-1−/− mice to EAE, we analyzed mice immunized with 200 µg of MOG40–55 peptide in which the most striking difference between caspase-1−/− and C57BL/6 was observed (Fig. 2B, Table I). Five caspase-1−/− mice and 5 C57BL/6 mice were sacrificed 10 days after immunization with MOG40–55, and cells from the draining lymph nodes were obtained. Proliferation assays to MOG40–55 showed no impairment in the responsiveness of T cells from caspase-1−/− compared with C57BL/6 mice (Fig. 3A). We detected, however, decreased levels of IFN-γ secreted in the supernatant of MOG40–55 restimulated cells (Fig. 3B) from caspase-1−/− compared with C57BL/6 mice (p = 0.01). Intracytoplasmic staining for IFN-γ and IL-4 of MOG40–55 restimulated cells showed reduced IFN-γ-producing cells and no induction of IL-4-producing cells (Fig. 3, C–E). These results suggest that cells from caspase-1−/− mice efficiently present the encephalitogenic peptide to T cells but are defective in Th1 development.

Table I.  EAE in (SV129 × B6)F1, C57BL/6, and caspase-1−/− mice

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>MOG Peptide (amount)</th>
<th>EAE Incidence (%</th>
<th>EAE Onset (range)</th>
<th>Maximum EAE Score (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(SV129 × B6)F1</td>
<td>MOG35–55 (200 µg)</td>
<td>4/4</td>
<td>20.0 ± 3.7</td>
<td>1.9 ± 0.6</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>MOG35–55 (200 µg)</td>
<td>6/6</td>
<td>19.2 ± 3.0</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>MOG40–55 (200 µg)</td>
<td>11/12</td>
<td>17.3 ± 2.6</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Caspase-1−/−</td>
<td>MOG40–55 (600 µg)</td>
<td>5/7</td>
<td>27.8 ± 2.4</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>MOG40–55 (200 µg)</td>
<td>0/12</td>
<td>NA**</td>
<td>NA**</td>
</tr>
</tbody>
</table>

* EAE onset is expressed as day post immunization ± SE.
** EAE score is expressed as mean maximum score ± SE.
† MOG40–55 has been administered twice 1 wk apart (300 + 300 µg).
‡ p < 0.0001 vs C57BL/6 mice immunized with 200 µg MOG40–55. p = 0.03 and p = 0.006 vs caspase-1−/− mice immunized with 200 µg MOG35–55 or 600 µg MOG40–55, respectively.

Caspase-1 inhibitor administration prevents but does not treat EAE

Pharmacological blockade of EAE with caspase-1 inhibitors was tested in Biozzi AB/H mice immunized with SCH or MOG35–55. In Biozzi mice, SCH induces a very aggressive relapsing-remitting EAE (25), whereas MOG35–55 induces a more chronic progressive disease. We administered vehicle or Z-Val-Ala-DL-Asp-fluoromethylketone, a highly specific, cell-permeable, and irreversible inhibitor of caspase-1-like proteases (26) to Biozzi mice before immunization with SCH or MOG35–55. Because this caspase-1 inhibitor is a peptide with a very short half-life in vivo, we implanted s.c. mini-osmotic pumps able to continuously release the inhibitor for 10 days. Continuous administration of the caspase-1 inhibitor induced a clearcut suppression of either SCH-induced or MOG35–55-induced EAE compared with vehicle-treated mice (Table II). The cumulative EAE score, representing the disease burden, was significantly lower in caspase-1 inhibitor-treated mice than in vehicle-treated mice in both immunization protocols (Table II).
However, the mean day of onset and the mean clinical score of sick animals were comparable between groups (Table II). In the SCH-induced group, two animals for each treatment were sacrificed for neuropathology examination. Animals treated with the inhibitor had less infiltrates per mm² than did vehicle-treated controls (0.4 and 1.6 vs 6.6 and 3.1, respectively) (Fig. 4, A and B), less demy-

<table>
<thead>
<tr>
<th>Treatment Schedule</th>
<th>EAE Incidence (%)</th>
<th>EAE Onset (range)</th>
<th>Maximum EAE Score (range)</th>
<th>Cumulative Score (days of follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MOG&lt;sub&gt;35-55&lt;/sub&gt; (preventive)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>5/7</td>
<td>22.4 ± 2.9</td>
<td>2.1 ± 0.5</td>
<td>89.5</td>
</tr>
<tr>
<td>Caspase-1 inhibitor</td>
<td>2/7</td>
<td>23.5 ± 5.5</td>
<td>2.2 ± 1.2</td>
<td>32</td>
</tr>
<tr>
<td><strong>SCH (preventive)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>10/10</td>
<td>14.2 ± 0.5</td>
<td>3.7 ± 0.2</td>
<td>282</td>
</tr>
<tr>
<td>Caspase-1 inhibitor</td>
<td>4/10</td>
<td>15.0 ± 0.7</td>
<td>3.6 ± 0.4</td>
<td>82</td>
</tr>
<tr>
<td><strong>SCH (therapeutic)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>10/10</td>
<td>14.5 ± 0.6</td>
<td>3.8 ± 0.3</td>
<td>NA&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caspase-1 inhibitor</td>
<td>9/10</td>
<td>14.3 ± 0.3</td>
<td>3.0 ± 0.3</td>
<td>NA&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Treatment was performed by implantation of mini-osmotic pumps releasing for 10 days vehicle or the caspase-1 inhibitor Z-Val-Ala-Asp-fluoromethylketone (1.2 mg/day) from the day before immunization (preventive) or day 7 (therapeutic) after immunization.

<sup>b</sup> EAE onset is expressed as day post immunization ± SE.

<sup>c</sup> EAE score is expressed as mean maximum score ± SE.

<sup>d</sup> Cumulative score was calculated by summing up each individual score registered during the follow-up period.

<sup>e</sup> <i>p</i> = 0.003 vs vehicle-treated mice.

<sup>f</sup> <i>p</i> = 0.0007 vs vehicle-treated mice.

<sup>g</sup> NA, not applicable because in the therapeutic administration one mouse died during the follow-up period.

FIGURE 4. Neuropathological findings of SCH-induced EAE in two representative Biozzi mice treated with vehicle or caspase-1 inhibitor Z-Val-Ala-Asp-fluoromethylketone, respectively. Spinal cord sections stained with hematoxylin and eosin (×5) are shown in A and B; perivascular infiltrates (arrowheads) are less evident in the caspase-1 inhibitor-treated (A) vs vehicle-treated (B) mouse. Spinal cord sections stained with Luxol Fast Blue (×5) are shown in C and D; demyelinated areas (arrowheads) are less prominent in the caspase-1 inhibitor-treated (C) vs vehicle-treated (D) mouse. Spinal cord sections stained with Bielshowsky staining (×5) are shown in E and F; axonal loss (arrowheads) is decreased in the caspase-1 inhibitor-treated mouse (E) vs the vehicle-treated (F) mouse. Tissue sections of each mouse are from the same spinal cord area and have been obtained serially.
elination (0.6% and 0.8% vs 9.8% and 2.2%, respectively) (Fig. 4, C and D), reduced axonal loss (0.4% and 0.6% vs 8.3% and 1.5%, respectively) (Fig. 4, E and F), less infiltrating CD3⁺ cells/mm² (39.9 and 90.5 vs 291 and 138.2, respectively), and reduced numbers of infiltrating macrophages per mm² (21.8 and 52.5 vs 211.9 and 84.9, respectively).

By contrast, when the mini-osmotic pumps were implanted 7 days p.i. in Biozzi mice immunized with SCH, EAE development was similar in inhibitor- and vehicle-treated mice (Table II). Only 1 of the 10 mice treated with the inhibitor from 7 days p.i. did not develop a full-blown EAE. These results suggest that caspase-1 is crucial in the induction phase of EAE, when peripheral inflammation leads to the recruitment of effector cells into the CNS.

Discussion

The central role of proinflammatory cytokines in orchestrating the pathogenic process leading to EAE has been extensively studied (27–29), but few data are thus far available on the posttranslational mechanisms of regulation of cytokine secretion during this experimental autoimmune CNS-confined disease. We focused our attention on the proteolytic enzyme caspase-1, because previous studies showed that its pharmacological inhibition or its absence (i.e., deletion mutant mice) mainly affects the secretion of proinflammatory cytokines such as IL-1β, IL-1α, IL-6, TNF-α, IL-18, and IFN-γ (5–7). We found that caspase-1 transcription is upregulated during mouse EAE, reaching a peak at maximal disease severity. This induction is higher compared with other proinflammatory cytokines previously shown to be upregulated during EAE (27) and is similar to that found in the Lewis rat EAE (30). These data suggest that proteolytic activation of proinflammatory cytokines is a crucial step in the immune-mediated process leading to EAE.

Caspase-1⁻/⁻ mice were susceptible to MOG peptide-induced EAE. Disease susceptibility was associated with the number of immunizations and the dose and the MHC-binding affinity of the encephalitogenic MOG-peptide. In our hands, MOG40–55 showed no activity of caspase-1 on the precursor of IL-18 into its mature form by the Ag-specific proliferation assays, but to an impairment in Th1 cell development as indicated by the lower percentage of IFN-γ-producing cells from caspase-1⁻/⁻ vs C57BL/6 mice. The most likely explanation of this finding possibly resides in the cleavage activity of caspase-1 on the precursor of IL-18 into its mature form (6). Although IL-18 in itself does not induce Th1 cell development (31, 32), which is mostly driven by IL-12 (33), Th1 cell development independent from IL-12 could be induced by the cooperative action of IL-18 and other factors (34). These factors may include IFN-γ itself (35) and IL-1αβ (33). Thus, the simultaneous caspase-1-dependent defect in IL-18 and IL-1αβ production could explain the impaired Th1 development and the reduced EAE in caspase-1⁻/⁻ mice. A more aggressive immunization protocol, however, is needed to better understand the role of caspase-1 in the cleavage of pro-IL-1-β and pro-IL-18.

Results obtained with caspase-1 inhibitor administration are consistent with the lower susceptibility to EAE of caspase-1⁻/⁻ mice. The preventive administration of the caspase-1 inhibitor Z-Val-Ala- DL-Asp-fluoromethylketone dramatically reduced both relapsing-remitting or chronic-progressive EAE in two different mouse strains. However, the small proportion (35%) of mice not protected from EAE by caspase-1 inhibitor treatment developed a clinical course of EAE indistinguishable from that of controls. As in inflammatory fluids other proteases (i.e., trypsin, chymotrypsin, elastase, granzyme A) (36) can replace caspase-1 enzymatic activity, an immunization protocol able to induce a potent local inflammation may bypass its requirement. Nevertheless, recent data indicate that Z-Val-Ala-rt-Asp-fluoromethylketone is active also on other cysteine proteases involved in inflammatory reactions but not in the apoptotic cascade, such as caspases-4 and -5 (37).

Administration of Z-Val-Ala-DL-Asp-fluoromethylketone during the effector phase of SCH-induced EAE (i.e., therapeutic protocol) had no effect on the disease course and severity. These data are suggestive for a crucial role played by caspase-1 in the peripheral activation of proinflammatory cytokines during the induction phase of EAE. The lack of efficacy of caspase-1 inhibitors once the inflammatory cascade has already determined CNS infiltration and damage might be also explained by the impaired blood-brain barrier crossing of caspase-1 inhibitors (26).

In conclusion, our results indicate that caspase-1 plays a crucial role in the development of the immune-mediated inflammatory process leading to CNS demyelination. EAE development is impaired in caspase-1⁻/⁻ mice, although the requirement for caspase-1 in the inflammatory phase of EAE can be bypassed. The role of caspase-1 in autoimmune demyelination is further corroborated by the significant reduction of EAE incidence in mice preventively treated with the caspase-1 inhibitor Z-Val-Ala-DL-Asp-fluoromethylketone. However, the inefficacy of caspase-1 inhibitors in blocking ongoing EAE suggests that this enzyme is crucial in the early phase of the inflammatory process leading to immune-mediated demyelination. Thus, caspase-1 could represent both a marker of inflammation sustaining immune-mediated demyelination and a possible therapeutic target of the acute phase of relapsing-remitting MS.

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References


