Serological Evidence for an Inflammatory Response in Murine Scrapie

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Transmissible spongiform encephalopathies (TSEs) are initiated by a novel kind of agent that produces characteristic degenerative changes in the brain without a detectable systemic inflammatory response or serological changes. A murine scrapie model was evaluated for changes in plasma concentration of serum amyloid P component (SAP), a protein that is up-regulated in infected and/or injured mice during the acute phase response (APR). C57BL10 and IRW mice inoculated with scrapie brain developed clinical scrapie 125–150 days later. At this time, concentration of plasma SAP increased in most of them. The SAP level increased ≳3-fold in >80% of the scrapie-affected C57BL10 mice and IRW male mice. A similar increase was found in <3% of respective nonscrapie control mice. The up-regulation of mouse SAP during clinical scrapie provides evidence for the activation of a systemic APR in TSE, a serological change that may be clinically useful.

Scrapie, a transmissible spongiform encephalopathy (TSE) of sheep and goats, is the original and prototype TSE. Its causative agent has been adapted to the laboratory mouse, in which the clinical and pathological features of the experimental disease mimic TSEs found in other animals, including sheep (scrapie) and cattle (bovine spongiform encephalopathy, or mad cow disease) and also the TSE diseases of humans, such as Creutzfeldt-Jacob disease and Kuru. The mechanism responsible for the neurodegeneration in these spongiform encephalopathies is unclear but apparently involves the accumulation in the brain of a disease-associated protease K–resistant protein referred to as PrP-res [1]. Diagnosis requires biochemical or histological analysis of affected tissues, and typical signs of inflammation usually are not detectable either clinically or morphologically [2, 3]. There is no known blood test that correlates with the presence of a TSE. Such a test, though, would be helpful for the screening of potentially affected animals, in eradication efforts, or for corroborating an antemortem diagnosis of TSE that otherwise requires biopsy and processing of affected tissues.

Serum amyloid P component (SAP) is one of a variety of acute phase response (APR) elements in mouse plasma (including leukocytosis, complement, and serum amyloid A [SAA]) that are induced by tissue injury and inflammation [4]. SAP is a pentraxin, a family of conservatively evolved proteins with similar structure and function that are widely expressed in vertebrates, as well as in invertebrates [5]. Pentraxins frequently participate in the APR by the action of circulating cytokines (interleukin [IL]–1 and IL-6) that usually up-regulate the hepatic synthesis and the serum level of the protein as part of the inflammatory response [6–8]. A homologue of mouse SAP is human C-reactive protein (CRP), the first pentraxin protein identified and the archetype acute phase protein [9, 10]. Human CRP is a sensitive indicator of inflammation, with a >100-fold increase in serum level after infection/injury. Although not as dramatic as human CRP, murine SAP levels also increase in response to inflammation, and SAP is known as the acute phase pentraxin of mice [11, 12]. In the present study, we quantified SAP in the blood of mice affected with scrapie, to determine whether an APR could be detected in a TSE, a disease without known serological changes. Serum levels of SAP were found to be elevated in most mice affected with experimental scrapie.

Materials and Methods

Mice. C57BL10 and IRW mice (≥20 females and 20 males of each strain) and C57BL10 PrP<sup>−/−</sup> mice (obtained from the Rocky Mountain Laboratories’ animal production unit, Hamilton, MT) were inoculated intracerebrally with 50 μL of a 1% brain homogenate containing an ID<sub>50</sub> of 10<sup>5.0</sup> of mouse adapted Chandler strain scrapie agent (scrapie brain) [13]. For controls, an equal number of mice of each strain were inoculated with a 1% homogenate of healthy mouse brain (normal brain). IRW mice were tested because their PrP levels are known to be lower than those of C57BL10 mice [14].

SAP assay. Blood samples for the assay were collected in heparinized microhematocrit capillary tubes (American Scientific Products) from the retro-orbital plexus of individually identified mice. An occasional plasma sample was lost during centrifugation of the capillary tubes. Duplicate 2-fold dilutions of plasma samples were quantified for SAP levels by using a solid-phase ELISA immuno-
Figure 1. Serum amyloid P (SAP) concentration in individual IRW female mice inoculated with normal mouse brain (top, controls) or with scrapie-affected brain (bottom). Mean SAP level is indicated (I) on days 50, 110, 130, and 150 after inoculation and was increased in mice only after clinical scrapie was detected. Mice that were clinically normal at time of bleeding (●) and mice with clinical scrapie (×) are shown. After development of clinical scrapie on day 150, SAP levels were significantly higher than those of scrapie-affected mice on day 130 or of control mice on day 150. The no. of mice on day 0 of the experiment is indicated by n, although not all mice were represented on each day, because of occasional death of the animals or loss of plasma sample.

Results

The SAP levels were determined in plasma samples obtained monthly before clinical scrapie occurred (day 125) and weekly thereafter. SAP levels of IRW mice are normally higher in female than in male mice, because of sex hormone control of SAP concentration in this mouse strain (J.E.C. and M.J.R., unpublished data). The SAP plasma level did not change in IRW female control mice (figure 1, top) or in IRW male control mice (figure 2, top) inoculated with normal brain during the course of the experiment. In IRW mice inoculated with scrapie brain, SAP levels were stable during the first 110 days in both females (figure 1, bottom) and males (figure 2, bottom); however, during the onset and progression of clinical scrapie (days 125–150), SAP levels increased in both females (figure 1, bottom) and males (figure 2, bottom). Note that a similar mean SAP level was achieved in both male and female IRW mice during clinical scrapie, although males started at a lower level. Figures 1 and 2 show SAP levels up to day 150, when all mice were affected with scrapie and a few had died. Shortly thereafter, the mice with terminal scrapie were killed, although some lived for another 2 weeks. Although the mean SAP level in scrapie-affected IRW females on day 150 was more than twice the SAP level before clinical signs appeared, many individual values were within the normal range. Figure 3 shows the typical heterogeneity of scrapie incubation periods and SAP responses that were found in 3 female IRW mice. During clinical scrapie, plasma SAP concentration did not change in 1 mouse, although signs of clinical scrapie became progressively worse (1+ to 4+).
In the other 2 mice, plasma SAP concentration increased with the progression of clinical scrapie, but in 1 mouse it subsequently decreased before death. This terminal decrease in SAP levels occurred in ~25% of scrapie-affected mice of all strains. To compensate for the asynchronous, and even divergent, kinetics of the SAP response during clinical scrapie, we calculated the relative increase of SAP that occurred after day 110 in each individual scrapie mouse and also in each individual control mouse (table 1). In both the scrapie brain- and normal brain-inoculated groups, we compared the highest SAP level observed during the time of clinical scrapie (from day 130 to day 165) with the average preclinical SAP level (derived by averaging the concentrations on days 50 and 110). By following results obtained from individual IRW female mice, a ≥2-fold increase occurred in 17 (85%) of 20 scrapie-affected mice, whereas a similar increase was found in only 1 (5%) of 20 control mice (table 1). When individual male IRW mice were followed, 100% of scrapie-affected mice developed a ≥2-fold SAP increase during clinical scrapie, in contrast to only 15% of the control mice during that same time period (table 1). In fact, a ≥3-fold increase was not seen in any of the IRW control mice but was found in 15 (83%) of 18 scrapie-affected males and in 9 (45%) of 20 scrapie-affected females (table 1).

C57BL10 mice inoculated with normal mouse brain did not show signs of scrapie and had no detectable increase in plasma SAP concentration (figure 4, top) during the course of the experiment. SAP levels in C57BL10 mice inoculated with scrapie mouse brain also were constant until day 100, but SAP then increased as signs of clinical scrapie appeared on about day 130 (figure 4, bottom). Because the SAP levels/responses in males and females were similar, the combined results are shown. On day 130, 5 mice without signs of clinical scrapie had SAP levels >20 μg/mL, a SAP concentration that was higher than any of the earlier SAP determinations. The incubation period
for clinical scrapie varied from 125 to 145 days, but by day 150 all mice showed signs of the disease. At day 150 and later, many affected mice had SAP levels within the normal range (figure 4, bottom), although higher levels had been observed earlier. Again, we calculated individual changes in SAP concentration by following individual mice (scrapie-affected mice and control mice) and compared mean SAP levels before day 101 with the highest level found during the period of clinical scrapie (days 130–165). A 3-fold increase in SAP was observed in 26 (81%) of 32 scrapie-affected mice, whereas only 1 (2.6%) of 38 control mice showed such an elevation (table 1).

Because C57BL10 PrP<sup>−/−</sup> mice have a mutant PrP gene, they do not have detectable PrP mRNA sequences or PrP protein in the brain. Furthermore, they do not develop clinical scrapie when inoculated with the scrapie agent. To determine whether SAP levels would increase in the absence of scrapie, male and female C57BL10 PrP<sup>−/−</sup> mice were inoculated with normal (n = 36) or scrapie (n = 32) brain (day 0), and SAP levels were determined in sequential plasma samples obtained until day 365. None of the mice showed signs of clinical scrapie, and there was no significant increase in plasma SAP levels (not shown). SAP levels in the blood of these mice showed great heterogeneity (from 10 to 150 µg/mL), but these levels were consistent for the individual mice for the duration of the experiment. Presumably, this represents the expression of various combinations of SAP genes that resulted from the backcrossing of C57BL10 mice with the original PrP<sup>−/−</sup> mouse (129/ola) [14]. When analyzed by SDS PAGE, the SAP mobility of the C57BL10 PrP<sup>−/−</sup> mice and also of the scrapie-affected IRW and C57BL10 mice was identical to the SAP mobility found in normal control mice.

### Discussion

TSEs are generally regarded as noninflammatory diseases because of their afebrile clinical courses and the absence of detectable changes in the blood usually associated with inflammation (neutrophilia, increased sedimentation rate, etc.). Even in the terminal stages of scrapie, when secondary infection could be anticipated, signs of inflammation/infection are not detectable serologically or histologically [3]. Nevertheless, several studies have found more subtle evidence of a local inflammatory response within the brain of scrapie-affected mice. For example, Betmouni et al. [17] noted the presence of microglial activation and T lymphocyte recruitment in brains of mice affected with scrapie, which are histological changes that are considered to be consistent with inflammation of the brain. In addition, Campbell et al. [18] found increased expression of proinflammatory cytokines in brains of mice affected with the scrapie agent. That is, at the time of expected clinical disease, increased expression (mRNA) of tumor necrosis factor, IL-1α, and IL-1β were detected in the brain but not in other organs. The increased SAP concentration in the blood of scrapie-affected mice found in the present study suggests the presence of an underlying inflammatory process, either inside or outside the central nervous system (CNS), that is capable of triggering hepatic synthesis of SAP. During the APR, increased levels of various cytokines in the blood, IL-1 and especially IL-6, are responsible for the synthesis of SAP by the liver [7, 8]. Campbell et al. [18] did not measure the expression of IL-6 in scrapie-affected mice, although, in their study, the various cytokines

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**Table 1.** Serum amyloid P (SAP) increase in individual mice during clinical phase of scrapie (days 125–165).

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<thead>
<tr>
<th>Mouse strain, brain source&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sex&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. (%) of mice with increased SAP&lt;sup&gt;c&lt;/sup&gt; by amount of increase</th>
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<td>Normal</td>
<td>F + M (38)</td>
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<td>Scrape</td>
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<td>30 (94)&lt;sup&gt;f&lt;/sup&gt;</td>
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**NOTE.** Mice were inoculated (day 0) with normal or scrapie brain. F, female; M, male.

<sup>a</sup> Mice were inoculated intracerebrally with 50 µL of a 1% normal mouse brain homogenate (normal) or 50 µL of a 1% scrapie mouse brain homogenate (Chandler strain) containing an ID<sub>50</sub> of ~10<sup>4</sup>.

<sup>b</sup> No. of mice examined on days 125–165 is given in parentheses. All scrapie-affected mice developed clinical scrapie, although some IRW female and C57BL10 mice died earlier from other causes and were not tallied.

<sup>c</sup> Extremely significant difference (P < 0.001) between scrapie and paired healthy control mice when analyzed by Fisher’s exact test.
Figure 4. Serum amyloid P (SAP) levels in male and female C57BL mice, inoculated either with normal mouse brain (top, controls) or with scrapie mouse brain (bottom). Mean plasma SAP level is indicated (†) and increased only in the group inoculated with the scrapie agent, especially after the appearance of clinical scrapie (×). SAP levels were, however, elevated in several scrapie-inoculated mice on day 130 that were still clinically normal (○). SAP levels of scrapie-affected mice on days 130 and 150 are significantly different from one another and are also significantly different from those of day 100 scrapie-affected mice and all control mice. The no. of mice at the beginning of the experiment is indicated by n, although not all mice are represented on each day, because of occasional death or loss of plasma sample.

that showed an elevated mRNA in brain did not show similar increases in the liver, spleen, or kidneys.

The origin of, or the stimulus for, circulating proinflammatory cytokines responsible for SAP synthesis in mice affected with the scrapie agent could be from lymphoid tissue, a site of early agent replication and PrP accumulation, or the brain itself via a neuroendocrine basis. There is some evidence that induction of APR can originate within the CNS. For example, in rats, psychological stress alone results in a febrile response along with increased levels of IL-6 and corticosterone in plasma [19, 20]. The mechanism by which the brain activates this rat cytokine response is unknown but may involve the hypothalamo-hypophysial-adrenal axis [20]. Furthermore, psychiatric disorders as found in schizophrenic, manic, and depressed persons are associated with a modest APR, as measured by increased blood levels of CRP [21]. There are actually a variety of clinical conditions associated with a modest APR, in which an inflammatory etiology is unproved, such as obesity, diabetes mellitus, physical exertion, and high-altitude exposure [4, 22, 23]. Human CRP is an especially sensitive indicator of inflammation, and asymptomatic subtle increases (<2-fold or <0.5 μg/mL) are significant in predicting increased risk of atherothrombosis, which is presumably due to a smoldering inflammatory process [24]. Therefore, the increased level of murine SAP found with clinical scrapie is indeed a nonspecific response, and, although indicative of a systemic response to inflammation, it is not proof itself.

Mouse strains are known to have different constitutive levels of SAP in their blood [11, 12]. During a lipopolysaccharide-induced inflammatory response, however, all strains achieved similar levels of SAP, so that those strains with low endogenous SAP were actually the high-responder SAP strains in an APR [11]. In similar fashion, during clinical murine scrapie, a more impressive SAP response was found in mice with the lower
endogenous level of SAP, such as C57BL10 mice and male IRW mice. In both of these high-responder groups, SAP levels were significantly elevated over control mice when signs of scrapie were evolving (day 130 and also on day 150), whereas SAP levels in female IRW scrapie, a lower responder because of higher endogenous levels, were not significantly elevated over controls until day 150. Some of the C57BL10 mice inoculated with scrapie even had elevated SAP levels on day 130, before the appearance of clinical scrapie.

We cannot explain the heterogeneity of the SAP response in the scrapie-affected mice or why some mice in a given group were such good responders and others in the same group were poor responders. In addition, the increase in SAP levels frequently did not correlate with severity of clinical scrapie. Perhaps a more sensitive indicator of APR, similar to human CRP, would show a more consistent response, even prior to detectable clinical signs, and correlate better with severity of disease.

SAP levels in scrapie-affected mice were observed to actually decrease as the clinical signs progressed. The terminal decline in SAP level that was seen in ~25% of scrapie-affected mice is puzzling, because there is no reason to expect hepatic failure to occur in this disease. In Campbell et al.’s [18] study, expression of cytokine in scrapie brain did not diminish during the course of the disease, which is a typical finding for an APR. Perhaps the continued formation of PrP amyloid is responsible for SAP leaving the blood, because SAP binds avidly to amyloid fibrils and is a constituent of all amyloids [25]. Thus, when radiolabeled hamster SAP—also called hamster female protein (FP) or FP(SAP)—was injected into the blood of an amyloidotic hamster, the SAP promptly left the intravascular space and accumulated in the amyloid deposit until a dynamic equilibrium was established with the circulating SAP [26].

SAP may even have a role in amyloid formation or in amyloid persistence [27]. In the Syrian hamster, for instance, the sex hormone–enhanced expression of SAP in females results in the rapid accumulation of amyloid [28]. Knockout mice that do not express SAP develop amyloid at a retarded rate [29, 30]. Except in hamsters, sex hormone control of SAP is rare, although its dimorphic expression as seen in the IRW mouse represents a rather modest (3-fold) sex difference in concentration, compared with the 100-fold sex differences of SAP levels in the Syrian hamster [31]. It is not known whether induction of SAA amyloid is sex linked in the IRW mouse, as it is in the hamster, or whether the accumulation of PrP amyloid is different in male and female IRW scrapie-affected mice.

At present, antemortem diagnosis of TSE requires biopsy and processing of affected tissue because there is no serological test or known plasma change associated with these so-called noninflammatory diseases. The increase of plasma SAP in mouse scrapie does suggest, for the first time, that there is a systemic inflammatory response in this disease. Even though the APR is a nonspecific response, if it is a common component of TSE, a sensitive APR element such as human CRP could provide a serological tool that would be helpful in the diagnosis of, or screening for, TSE.

References


