Research Article

Inhibition of Experimental Autoimmune Encephalomyelitis in Human C-Reactive Protein Transgenic Mice Is FcγRIIB Dependent

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We showed earlier that experimental autoimmune encephalomyelitis (EAE) in human C-reactive protein (CRP) transgenic mice (CRPtg) has delayed onset and reduced severity compared to wild-type mice. Since human CRP is known to engage Fc receptors and Fc receptors are known to play a role in EAE in the mouse, we sought to determine if FcγRI, FcγRIIb, or FcγRIII was needed to manifest human CRP-mediated protection of CRPtg. We report here that in CRPtg lacking either of the two activating receptors, FcγRI and FcγRIII, the beneficial effect of human CRP are still observed. In contrast, if CRPtg lack expression of the inhibitory receptor FcγRIIB, then the beneficial effect of human CRP is abrogated. Also, subcutaneous administration of purified human CRP stalled progression of ongoing EAE in wild-type mice, but similar treatment failed to impede EAE progression in mice lacking FcγRIIB. The results reveal that a CRP → FcγRIIB axis is responsible for protection against EAE in the CRPtg model.

1. Introduction

C-reactive protein (CRP) is a widely used blood marker of inflammation [1], but it is increasingly apparent that the protein plays a causal role in host defense against microbial pathogens [2] and in cardiovascular disease [3]. Furthermore, in at least three different mouse models, human CRP has been shown to protect against autoimmune disease [4–6]. Importantly, we showed that human CRP transgenic mice (CRPtg) are resistant to experimental autoimmune encephalomyelitis (EAE) [6], an animal model of multiple sclerosis (MS). Thus in CRPtg compared to wild-type mice, EAE onset was delayed, its severity was attenuated, and infiltration of encephalitogenic T-cells and monocytes/macrophages into the CNS was prevented [6]. The encephalitogenic cells with which CRP interacts to manifest protection in EAE and the mode of action of human CRP on these cells were not identified. Since human CRP binds both human and mouse Fc receptors [7–10] and because there is growing evidence that Fc receptors play a major role in controlling the emergence of EAE and other autoimmune diseases [11–15], we sought to determine if FcyRs were required for human CRP-mediated protection against EAE in the mouse.

Here we show that for CRPtg mice lacking expression of the activating receptors FcyRI and FcyRIII, expression of human CRP delays onset and reduces severity of EAE as well as or better than it does in CRPtg with an intact FcyR repertoire. In contrast in CRPtg mice that lack expression of the inhibitory receptor FcyRIIB, no human CRP-mediated protection from EAE is observed. Likewise, administration of purified human CRP to wild-type mice with ongoing EAE prevented the disease from worsening, whereas the same treatment failed to halt worsening of EAE for mice lacking FcyRIIB. The combined data suggest that human CRP → mouse FcyRIIB interaction and its presumed
inhibitory consequences are essential for realizing human CRP-mediated protection against EAE in the CRPtg mouse model.

2. Materials and Methods

2.1. Animals. CRPtg mice have been described in detail elsewhere [16, 17]. The CRPtg strain (C57BL/6 background) carries a 31-kb human DNA fragment encoding the CRP gene, all the known cis-acting CRP regulatory elements (i.e., the entire human CRP promoter) and the CRP pseudogene [16]. Cis-acting regulatory elements within the transgene are responsible for both tissue specificity and acute phase inducibility of its expression, and the trans-acting factors required for its correct regulation are conserved from mouse to man [16, 17]. Human CRP is expressed as an acute phase reactant in CRPtg and reaches blood levels comparable to those observed in humans with inflammatory disease (up to 500 μg/mL) [17]. We showed earlier that human CRP level in mice lacking functional expression of the genes encoding the α-chains of FcγRI (FcγRI−/− mice) [12], FcγRIIB (FcγRIIB−/− mice) [18], and FcγRIII (FcγRIII−/− mice) [19]. FcγR-deficient versus sufficient and CRPtg versus non-CRPtg progeny were obtained in the expected Mendelian ratios each genotype appeared phenotypically normal, and none of the FcγR deficiencies significantly altered expression of human CRP (Figure 1). To identify the various genotypes, we used CRP transgene-specific and FcγR mutation-specific PCRs, as described [12, 16–19]. All mice were fed a standard chow diet (Ralston Purina Diet) and maintained at constant humidity (60 ± 5%) and temperature (24 ± 1°C) with a 12-hour light cycle (6 AM to 6 PM). All protocols were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham and were consistent with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 96-01, revised 1996).

2.2. Induction of EAE. An immunodominant myelin oligodendrocyte protein (MOG) peptide was used to immunize 10–12-week-old mice, as described in [6]. On days 0 and 7, mice received subcutaneously an injection of 150 μg MOG peptide emulsified in complete Freund’s adjuvant containing 500 μg heat-killed Mycobacterium tuberculosis (Difco, Detroit, MI). On days 0 and 2, mice received an intraperitoneal injection of pertussis toxin (500 ng) (List Biological Laboratories, Campbell, CA). Development of EAE symptoms was monitored daily using a clinical scale ranging from 0 to 6 as follows: 0, asymptomatic; 1, loss of tail tone; 2, flaccid tail; 3, incomplete paralysis of one or two hind limbs; 4, complete hind limb paralysis; 5, moribund (in which case animals were humanely euthanized); 6, dead. Mice were observed for at least 30 days, and those with a score of at least 2 for more than 2 consecutive days were deemed to have developed EAE. The maximum clinical score achieved by each animal during the 30-day observation period was used to calculate average maximum clinical score (severity) for each experimental group. To study the time-course of disease, average clinical scores were calculated daily for each group of mice, and cumulative disease index was calculated by area under the curve. When determining the average day of onset of EAE, animals that did not develop any symptoms of EAE during the 30-day period were assigned a day of onset of 31.

2.3. Administration of Human CRP to Mice with EAE. EAE was induced as described above, and the development of symptoms was monitored. On the day their disease symptoms achieved or eclipsed a score of 2 (flaccid tail), each mouse received subcutaneously an injection of 50 μg of highly purified (95%–98%) human CRP (US Biological, Swampscott, MA). Development of EAE symptoms was then followed for an additional 10 days. The CRP preparation was sodium azide-free, contained <0.4 ng endotoxin/mg protein by Limulus amebocyte assay, and had pentameric integrity as judged by overloaded native polyacrylamide gel electrophoresis (data not shown). Control animals received 200 μg of heat-denatured (boiled for 5 minutes) human CRP.

2.4. Statistical Analyses. Among genotype differences in EAE, day of onset and maximum clinical score (mean ± sem)
Figure 2: CRP-mediated protection from EAE requires FcγRIIB. CRPtg versus littermate wildtype (a) or their respective counterparts lacking expression of FcγRI (b), FcγRIII (c), or FcγRIIB (d) were injected with MOG peptide, and EAE symptoms were monitored. Presence of the CRP transgene (closed circles in each panel) delayed onset of EAE in mice with intact FcγRs (a) and delayed onset and reduced severity of EAE in mice lacking FcγRI (b) or FcγRIII (c). In contrast in mice lacking FcγRIIB (d), expression of human CRP had no beneficial effect. See Table 1 for sample sizes and statistical analyses.
were evaluated by one-way ANOVA and posthoc Neuman-Keul’s multiple comparison tests. A P value less than .05 was considered significant.

3. Results and Discussion

As we reported previously in [6], onset of EAE was delayed by ~1 week for CRPtg compared to wild type mice (Figure 2(a) and Table 1; P < .001, t-test), and this delay led to reduced cumulative disease index (Table 1; 32.8 versus 46.55) even though average disease severity was not significantly lowered (Table 1 and Figure 2(a)). In comparison, for CRPtg that lacked expression of either FcγRI or FcγRIII (Figures 2(b) and 2(c), resp.), human CRP-mediated protection included not only a delay in EAE onset and a reduced cumulative disease index but also a significant reduction in disease severity (Table 1). In contrast, for CRPtg mice lacking the inhibitory receptor FcγRIIB, expression of human CRP conferred no protective benefit (Table 1 and Figure 2(d)).

Other groups showed that human CRP administered subcutaneously to mice can reverse autoimmune- and antibody-induced inflammation [5, 20], a beneficial effect that reportedly requires certain FcyRs [20]. To test if human CRP administration might likewise protect mice from EAE and to test if FcγRIIB was required, we administered purified human CRP to wildtype versus FcγRIIB−/− mice with ongoing disease. The results are summarized in Figure 3.

We observed that for wildtype mice (Figure 3(a)) treatment with human CRP, but not treatment with heat-denatured CRP, halted progression of EAE. In contrast, no protective influence of CRP therapy was observed for FcγRIIB−/− mice (Figure 3(b)).

It has been documented that some of the in vivo activities of human CRP likely result (directly or indirectly) from the protein's ability to bind FcyRs [20, 21]. FcyRs are a family of receptors of which most mammals express four main types: FcγRI, FcγRII, FcγRIII, and FcγRIV [14, 22, 23]. Each of FcγRI, FcγRIII, and FcγRIV is comprised of a ligand binding α-chain paired with a common γ-chain (FcγRγ) that encodes an immunoreceptor tyrosine-based activation motif (ITAM) essential to propagate cell activating signals. FcγRIIB on the other hand is comprised of a single α-chain and it carries a cytoplasmic tyrosine-based inhibitory motif (ITIM) that propagates cell inhibiting signals. Various investigators have reported that human CRP binds to one or more isoforms of FcγRI, FcγRII, and FcγRIII in both mouse and man [7–10], and FcγRs reportedly influence EAE in the mouse [14, 24]. Thus in CRPtg, human CRP potentially could either exacerbate EAE by binding one of the inflammation-promoting FcyRs on encephalitogenic cells or dampen EAE by binding FcγRIIB. Using CRPtg mice with selective deletion of FcγRs, we were able to investigate if either capacity is realized in vivo.

Compelling evidence was obtained that the beneficial action of human CRP in mouse EAE depends mainly on
expression of the inhibitory receptor FcγRIIB. Thus in FcγRIIB−/− mice, EAE is neither delayed nor dampened by transgenic expression of human CRP. In fact the tempo and severity of EAE in CRPtg/FcγRIIB−/− was not significantly different from that seen in wild type mice. In contrast, the human CRP-associated delay in EAE onset and attenuation of EAE symptoms were fully expressed in mice that lacked the activating receptors: FcγRI or FcγRIII. We did not formally rule out the possibility that FcγRIV might play a role, as FcγRIIV−/− mice are not available to us, but we did perform experiments with mice that lack the FcR common gamma chain FcγRy, which are predicted to lack expression of FcγRI, FcγRIII, and FcγRIV [14]. Human CRP transgenic FcγRy−/− were obviously more resistant than wildtype mice (data not shown), nevertheless the contribution of FcγRy (and thus FcγRIV) to human CRP-mediated resistance to EAE remains unclear because FcγRy−/− mice per se are intrinsically very resistant to EAE [24]. Thus in their sum the data suggest that the EAE-protective effect of human CRP in the CRPtg mouse depends largely on the availability/expression of FcγRIIB. Presumably by binding FcγRIIB, human CRP expressed endogenously during the course of disease dampens the activation state of encephalitogenic (FcγRIIB-expressing) cells in CRPtg. Likewise, in nontransgenic mice, exogenously administered human CRP has the same effect as long as FcγRIIB is present.

4. Conclusions

For CRPtg mice, transgene-expressed human CRP inhibits EAE, and this beneficial action requires FcγRIIB. If as in CRPtg with EAE, a protective CRP → FcγRIIB axis exists in humans with MS, then CRP administration might be beneficial in the clinical treatment of patients with MS. Ongoing efforts in our laboratory are aimed at identifying the CRP-responsive FcγRIIB-expressing encephalitogenic cell(s) involved in this action, which we posit to be dendritic cells [25].

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