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Strain-Specific Induction of Experimental Autoimmune Prostatitis (EAP) in Mice

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Abstract

BACKGROUND—Prostatitis, a clinical syndrome characterized by pelvic pain and inflammation, is common in adult males. Although several induced and spontaneous murine models of prostatitis have been explored, the role of genetic background on induction has not been well-defined.

METHODS—Using a standard methodology for the induction of experimental autoimmune prostatitis (EAP), we investigated both acute and chronic inflammation on several murine genetic backgrounds.

RESULTS—In our colony, nonobese diabetic (NOD) mice evinced spontaneous prostatitis that was not augmented by immunization with rat prostate extract (RPE). In contrast, the standard laboratory strain Balb/c developed chronic inflammation in response to RPE immunization. Development of EAP in other strains was variable.

CONCLUSIONS—These data suggest that Balb/c mice injected with RPE may provide a useful model for chronic prostatic inflammation.

Keywords

prostatitis; autoimmunity; prostate; inflammation

INTRODUCTION

Prostatitis is a significant public health challenge—it has been estimated that 35–50% of men will be afflicted with prostatitis at some point in their lifetime and approximately 2 million office visits per year in the United States are attributable to prostatitis [1]. Prostatitis syndromes are classified into four categories: acute (I) and chronic (II) bacterial prostatitis,

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chronic pelvic pain syndrome (III), and asymptomatic inflammatory prostatitis (IV) [2]. Chronic pelvic pain syndrome is further classified into inflammatory (IIIA) and noninflammatory subtypes (IIIB) based on the presence of leucocytes in the semen [3]. A subset (6–30%) of patients with inflammatory prostatitis exhibits the hallmarks of active inflammation in the absence of infection, implicating an autoimmune etiology [3–6]. Relatively little is known about the pathogenesis and progression of the autoimmune subtype, and unfortunately, this patient population is the most likely to prove refractory to treatment [3]. Thus, novel insights into this disease process and an expanded therapeutic armamentarium would represent an important advance in the field.

Several animal models of autoimmune prostatitis (generally corresponding to Category III disease in humans) have proven helpful in exploring the immunological basis of this disease [6]. One of the earliest and best-characterized models of EAP involves auto-immunization of nonobese diabetic (NOD) mice with male accessory gland (MAG) extract derived from male Wistar rats [7]. As NOD mice develop spontaneous autoimmunity in several organs [8], this result was not totally unexpected. Nevertheless, this model has provided several important insights into the pathogenesis induced prostatic inflammation. For example, experimental autoimmune prostatitis (EAP) in NOD mice is mediated by T_H1 cellular and humoral immune responses [7]. Important follow-up studies showed that prostate steroid-binding protein (PSBP) is the primary autoantigen in the rat NOD mouse model [9]. In addition, induction of EAP is severely attenuated in mice lacking CD4 T cells, demonstrating an important role for adaptive immunity in its pathogenesis [9]. Evidence that prostatitis in NOD mice is autoimmune in nature is supported by more recent studies showing spontaneous PSBP responses in these animals [10]. Taken together, these studies provide important basic insights into the pathogenesis of prostatitis, but the more recent observation of disease in unmanipulated NOD mice begs the question as to the relative contribution of induced versus spontaneous inflammation.

To begin to address this issue, we examined several additional animal strains for evidence of either induced or spontaneous prostatic inflammation, using the well-characterized EAP model described above [7]. To better define a potential role for genetic background, we tested three additional auto-immune prone strains: [11] New Zealand Black (NZB), which spontaneously develop autoimmune hemolytic anemia, MRL which develop low-level lupus like disease, and Swiss Rockefeller (SWR) mice, which are susceptible to collagen-induced arthritis (CIA). In addition, we included two strains with standard laboratory backgrounds, Balb/c, and C57/Bl6. These strains are of particular interest since animals with the Balb/c background generally respond to parasitic infection with a T_H2 response characteristic of chronic inflammation [12], whereas C57/Bl6 mice respond to an identical infection with an immune response characterized by secretion of IFN-gamma and TNF-alpha (i.e., a T_H1 response). We attempted to control for allelic differences at the MHC locus to the extent possible: both NZB and Balb/c mice are H-2d and, for the C57/Bl6 background, we made use of the congenic B10.D2 line. These mice are H-2d at the MHC locus, but of the C57 background. All strains were initially examined in an acute inflammation model. Strains of particular interest were then tested for the induction of chronic inflammation in long-term follow-up studies.

MATERIALS AND METHODS

Animals

All animals were purchased from the Jackson Laboratory. Specific strains studied included: Balb/c (H-2^d), B10.D2 (MHC = H-2^d on a C57/Bl6 background), NOD (nonobese diabetic, H-2^{g7}), SWR (Swiss Rockefeller, H-2^q), MRL (H-2^k), and NZB (New Zealand Black, H-2^d). Of note, the MRL mice used in these experiments were standard MRL animals,

without the *lpr/lpr* mutation in the *fas* locus that causes lymphoproliferation and accelerates spontaneous lupus-like disease. Animals were maintained in the Johns Hopkins Cancer Research Building, in specific pathogen-free conditions. All animal studies were performed in accordance with protocols approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine.

Preparation of Rat Prostatic Extract (RPE)

MAG homogenate was prepared from a pool Wister rat male tissues (prostate, seminal vesicle, and coagulating gland) as previously described [7]. Organs from three animals were collected and pooled. To prepare the immunogen (Rat Prostatic Extract, RPE) glands were homogenized in PBS with protease inhibitors, homogenate was centrifuged at 10,000g for 30 min and supernatant was collected. The supernatant was then aliquoted at 10 mg/mL and kept frozen at -80°C prior to use.

Immunization

RPE was thawed in a 37° water bath, then emulsified with an appropriate volume of complete Freund's adjuvant (CFA) using opposed glass syringes. For immunization, a total of 1 mg of RPE fully emulsified with CFA for a total volume of 150 μL was administered by subcutaneous injection into the flank. Control injections included CFA + PBS, and PBS alone. CFA + PBS groups were included since adjuvant alone is sufficient to induce autoimmunity in several animal murine models, that is, in adjuvant induced arthritis. All animals were studied at 4–6 weeks of age. In these studies, two immunizations were administered 2 weeks apart, in a typical prime-boost regimen.

Pathological Evaluation

Ventral and dorsal lobes of prostate glands were micro-dissected at the indicated times and fixed in 10% neutral buffered formalin solution (Richard Allen Scientific). Two surgical pathologists (C.M. and A.P.) evaluated H&E sections of the lobes for inflammation in a double-blinded manner. Prostate inflammation was scored on a scale of zero to four as previously described [13].

Statistics

P-values were calculated using two-tailed Student's *t*-test as implemented in the Prism 4.0 package (GraphPad Software).

RESULTS

NOD Mice Develop Spontaneous Prostatitis That Is not Augmented by Injection With RPE

To test whether the induction of EAP is strain-specific, 8-week-old mice were immunized with RPE (emulsified with CFA) as previously described [7]. For a negative control, animals were injected with vehicle alone (PBS). We also tested whether CFA alone could induce inflammation/autoimmunity, since strain-specific adjuvant-induced arthritis represents a reasonably well-accepted model of organ-specific autoimmunity [14]. In a typical prime-boost regimen, immunization was repeated 2 weeks later. Four weeks after the second immunization, animals were euthanized, and prostate glands harvested. As shown in Figure 1, mean inflammation score varied widely between groups. Interestingly, only a few strains developed inflammation significantly greater than background levels (those observed with PBS injection). As previously reported [7,10], NOD mice exhibited significant prostate-resident inflammation. However, in our colony this inflammation did not appear to be immunization-induced, as the median inflammation score was not significantly different between animals treated with vehicle, CFA, or RPE (+CFA). Interestingly, the only strain

that appeared to develop immunogen-specific inflammation was the standard laboratory strain Balb/c. The autoimmune prone strain NZB developed inflammation in response to CFA injection, but inflammation was markedly diminished in animals vaccinated with RPE. A second autoimmune strain (SWR) developed minor inflammation (mean score, 0.74), but this inflammation appeared to be nonspecific as it was observed in both the CFA and RPE groups. Micrographs of the dorsal lobe of the prostate from four RPE-treated strains are shown in Figure 1B. In Balb/c, NOD, and SWR animals, inflammation was predominantly lymphocytic in nature. However, NZB mice immunized with CFA displayed a pattern that was composed mainly of histiocytes. Taken together, these data show that a number of murine strains develop acute prostatic inflammation, but only in Balb/c mice does this inflammation develop specifically (i.e., as a result of immunization with xenogeneic prostatic proteins + CFA).

Balb/c Mice Develop Chronic Prostatitis Induced by RPE Injection

To determine whether the RPE-induced inflammatory response observed in the prostate gland of Balb/c mice led to a chronic process, we repeated the experiments above with a second cohort of animals. For these chronic studies, we limited the groups to the two that showed a specific response, that is, Balb/c, which developed inflammation in response to RPE and NZB, which developed a histiocytic inflammation in response to CFA (but not RPE) injection. A cohort of animals was harvested at 4 weeks, and a second, larger cohort was harvested at 8 weeks. These data (Fig. 2A) confirmed those above—Balb/c mice developed inflammation specifically mediated by RPE + CFA. Interestingly, this inflammation did not appear to resolve with time, but rather to progress. By 8 weeks, the mean inflammation score in Balb/c mice treated with RPE/CFA had increased to 2.6. Anatomical examination of these animals was impressive—in several mice inflammation had progressed to the point of focal adhesions. The data suggesting that NZB mice develop acute inflammation in response to CFA injection was not confirmed by this second set of experiments. In these studies, NZB mice examined at 4 weeks showed only minor inflammation. This inflammation was observed in the RPE group, and not in the CFA only group as shown above. By 8 weeks, inflammation in the autoimmune-prone NZB animals had largely resolved. Histological data are presented in Figure 2B; these data confirm the marked inflammation in Balb/c mice treated with RPE + CFA.

DISCUSSION

Laboratory mice provide a useful and clinically relevant tool to model many aspects of the immune system. It should be understood that individual strains vary widely in their response to infection as well as in their propensity to develop autoimmunity. Based on these well-established observations, it is reasonable to consider the strain-specific characteristics of inflammatory models when attempting to model human disease. Here, we studied a well-established model of experimental prostatitis, immunization of mice with RPE + CFE, in several normal and autoimmune-prone strains mouse strains. Our results were somewhat different from those previously reported—instead of the more widely reported induced disease; we found that NOD mice show a consistent background level of intra-prostatic inflammation regardless of treatment (RPE + CFA, vs. CFA alone, vs. PBS). This apparent discrepancy is not particularly troubling; several autoimmune models in mice seem to show a dependence on the colony studied or on the origin of the animals in question. Instead, our results in NOD mice confirm the importance of including appropriate untreated controls in any given study of spontaneous or induced inflammation.

Perhaps more interesting, our data showed that NOD mice were not unique their ability to manifest prostate-resident inflammation. Indeed, both NZB mice (which develop sporadic spontaneous autoimmune hemolytic anemia), and SWR mice (notable for their susceptibility

to collagen induced arthritis) showed greater prostatic inflammation than background. The patterns of prostatitis development in these strains was notable. NZB mice developed baseline inflammation that was augmented with nonspecific immunization (CFA alone), while SWR mice developed only induced prostatitis. Both of these models may be worthy of additional investigation regarding the specific protein(s) involved, the relative contribution of CD4 versus CD8 T cells, and other important facets of EAP that have been explored in the NOD model [6]. Of the two “normal” strains evaluated, Balb/c mice, which generally develop a T_H2 response to pathogen challenge, showed strong evidence for RPE-induced prostatitis. Given the notion that chronic inflammation may be mediated by a T_H2 response [15], this result is consistent with previous data. In fact, the observation that acute inflammation was not observed in the MHC identical, but T_H1 prone B10.D2 strain suggests that EAP might be controlled some extent by the T_H1/T_H2 balance, a result consistent with that observed in several human studies of benign prostatic hypertrophy (BPH) [16].

Perhaps the most interesting result of our study involves the long-term results. Surprisingly, the acute inflammation observed in the NZB strain resolved over time. These data suggest that the NZB background could potentially provide a system in which to study mechanisms involved in the resolution of inflammation [17], a relatively underappreciated topic. We also found that induced prostatitis on the Balb/c background appeared to be progressive, with dramatic lymphocytic infiltration observed 2 months post-immunization. These data suggest that the RPE/ Balb/c model might prove to be similarly useful for studying the mechanisms by which chronic prostatic inflammation is maintained. While our overall results clearly require independent confirmation, they demonstrate that EAP in mice is likely strain-dependent, a factor to be accounted for in future studies.

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Fig. 1. Strain-specific development of acute prostatic inflammation. **A:** Mean inflammation score. No Treatment = PBS alone, CFA = Complete Freund's Adjuvant, RPE = rat prostatic extract + CFA. N = 5 animals per group. **B:** RPE induced inflammation in normal and autoimmune strains. Representative H&E sections at the indicated magnification are shown.

Fig. 2. Strain-specific development of chronic prostatic inflammation. **A:** Mean inflammation score. No Treatment = PBS alone, CFA = Complete Freund's Adjuvant, RPE = rat prostatic extract + CFA. N = 15 animals per group, with five animals harvested at week 4 and 10 animals harvested at week 8. **B:** Chronic (8-week) RPE induced inflammation in Balb/c and NZB mice. Representative H&E sections at the indicated magnification are shown.