Immunomodulation Associated with Sustained-release Buprenorphine in Female CD1 Mice Challenged with Ovalbumin

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Opioid analgesics have immunomodulatory properties, which often result in immunosuppression. Sustained-release buprenorphine (SR-Bup) has recently become available as an analgesic for pain management in mice, and little is known regarding potential effects of SR-Bup on the murine immune response. To this end, we immunized female CD1 mice with ovalbumin in complete Freund adjuvant and then treated them with either saline, SR-Bup, Bup-HCl, or SR-vehicle (SR-Veh) for 18 d. Splenocytes were isolated for culture and stimulation to assess cytokine responses, and blood was collected to determine serum antibody responses to ovalbumin. In all treatment groups, levels of IL10, TNF α , and IFN γ increased in ovalbumin-stimulated splenocytes compared with unstimulated splenocytes. Cytokine responses after stimulation did not differ between treatment groups except for IL10, which was significantly higher in SR-Bup-treated mice compared with those given saline or Bup-HCl. The antibody response was significantly increased after immunization but did not differ across treatment groups, except that the response to SR-Veh was lower. These results suggest that the immunomodulatory effects of prolonged treatment with SR-Bup on innate and adaptive immunity are negligible.

Abbreviations: SR-Bup, sustained-released buprenorphine; SR-Veh, sustained-release vehicle

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Pain management of experimental animals is one of the central tenets in the practice of laboratory animal medicine. The *Guide for Care and Use of Laboratory Animals* requires that any animal experiencing more than momentary pain or distress due to experimental manipulation must be provided proper anesthesia, sedation and analgesia.¹⁹ Effective analgesia in experimental animals is not only an ethical matter, it is also a technical concern, given that analgesic use may impact experimental studies.^{34,35} Opioids are well known to modulate the immune response;^{2,26,32} therefore, it is important to make an informed decision when selecting an appropriate analgesic in experimental studies.

Opioids that act on the μ -opioid receptor, such as morphine and fentanyl, have an immunosuppressive effect. They reduce T and B cells proliferation; decrease natural killer cell activity; decrease IFN γ and IL2 production; reduce antibody responses; and decrease macrophage activity.^{2,26,32,36,37} The mechanism of this immune modulation is not completely known; but these analgesics suppress the immune response directly by binding to opioid receptors on macrophages and indirectly through activation of the HPA axis, which stimulates production of immunosuppressive glucocorticoids.^{6,7,10,20} Buprenorphine is a partial agonist of the μ -opioid receptor and an antagonist of the κ -opioid receptor.^{10,16} In addition, compared with morphine, buprenorphine has minimal immunomodulatory properties. For example, buprenorphine did not decrease primary or secondary antibody responses in ovalbumin-

Freund-adjuvant-immunized mice.²⁴ T-cell infiltrate in the CNS of mice experimentally infected with lymphocytic choriomeningitis virus were decreased in mice treated with buprenorphine.³⁰ Similarly, mice treated both acutely and chronically with high doses of buprenorphine showed no alterations in lymphoproliferation or IFN γ or IL2 responses compared with saline controls.²⁷ However, others have demonstrated changes associated with buprenorphine administration, including reduced lymphoproliferation, NK cell activity, and IFN γ production in rats treated with buprenorphine,⁴ and in mice, buprenorphine caused an increase in macrophage-produced IL6, TNF α , and TGF β .⁹

Although the effects of Bup on the immune response of mice have been widely studied with varied results, little is known about the immunomodulatory effects of sustained-released buprenorphine (SR-Bup). Sustained-release formulations of buprenorphine have been shown to provide prolonged blood plasma concentrations and efficacy in mice and rats, and SR-Bup offers the advantage of a single injection for 48 to 72 h of analgesia,^{3,11,15,22,23} with minimal side effects. The current study evaluated the possible immunomodulatory effects of SR-Bup by evaluating splenocyte cytokine responses and antibody production in ovalbumin-primed mice treated with SR-Bup compared with Bup-HCl and saline. Our hypothesis was that cytokine and antibody responses would not differ among treatment groups, similar to the immunomodulatory effects of Bup-HCl.

Materials and Methods

Mice. Female CD1:Crl mice ($n = 20$; age, 6 to 8 wk) were purchased from Charles River Laboratories (Raleigh, NC) for use in this experiment. Mice were free from adventitious agents including Sendai virus, pneumonia virus of mice, mouse hepatitis virus, mouse parvovirus, mouse norovirus, reovirus,

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rotavirus, ectromelia virus, adenovirus, mouse cytomegalovirus, polyomavirus, Theiler murine encephalomyelitis virus, endoparasites, ectoparasites, and common bacterial agents of mice, except *Staphylococcus aureus*. All aspects of animal care were in accordance with the *Guide for Care and Use of Laboratory Animals*,¹⁹ and all procedures performed were approved by the IACUC. Mice were housed in IVC (Thoren, Hazelton, PA) on autoclaved aspen shavings (Envigo Teklad, Indianapolis, IN), were fed a standard pelleted diet (2918, Envigo Teklad), and were provided filter-sterilized water without restriction. Polycarbonate hideouts (BioServe, Flemington, NJ) were provided as a source of enrichment. Mice were kept on a 12:12-h light:dark cycle.

Treatment groups. Mice were divided into 4 treatment groups of 5 animals each. Mice in each treatment group were uniquely identified by ear punch (VWR International, Radnor, PA) and weighed at the start of the study. At this time, 200 μ L blood was collected from each mouse through tail venipuncture for baseline measurements of antiovalbumin antibody in serum.

Immunization. Before treatment administration began, mice were immunized subcutaneously with 25 μ L ovalbumin antigen (5.5 mg/mL) plus 25 μ L Freund complete adjuvant. Mice were given a booster of 25 μ L ovalbumin antigen (5.5 mg/mL) plus 25 μ L Freund incomplete adjuvant on day 18 of treatment. All of these reagents were purchased from Sigma Aldrich (St Louis, MO).

Analgesic administration. Mice were subcutaneously injected with 1 of the 4 possible treatments: 0.9% saline (0.15 mL), SR-Bup (0.6 mg/kg; ZooPharm, Windsor, CO), Bup-HCl (0.1 mg/kg; Rickett Benckiser Healthcare, London, England), or the SR vehicle (SR-Veh; 0.6 mg/kg; 50/50 DL PLC LMW Polymer Matrix, ZooPharm). Bup-HCl was diluted 1:10 in sterile water so that a feasible volume could be injected subcutaneously into each mouse. Bup-HCl was dosed every 24 h, whereas SR-Bup, SR-Veh, and saline were dosed every 72 h. Mice received treatments for 18 d.

Sample collection. Mice were euthanized in their home cages through CO₂ asphyxiation on day 21. Mice were exsanguinated by using cardiac puncture, to measure antiovalbumin antibody concentrations after treatment. Mice were necropsied immediately after blood collection, and spleens were collected for in vitro cytokine production.

Splenocyte isolation and stimulation. Spleens were removed by using aseptic technique, placed in 15-mL conical tubes containing 5 mL of HBBS containing 2% FBS, and processed accordingly.⁵ Individual samples were poured into a tissue grinder, and spleens were pulverized by using the plunger from a sterile 3-mL syringe. The tissue grinder was washed with additional media to maximize splenocyte recovery. This cellular suspension was transferred to a 50-mL conical tube, and additional media was added to the cell suspension to create a final volume of 15 mL. Samples were spun in the centrifuge for 10 min at 525 $\times$ g, and the supernatant was removed. Ammonium chloride–potassium lysis buffer (3 mL) was added to each sample for 5 min, to lyse any RBC in suspension. Samples were centrifuged again, and the supernatant removed. Samples were filtered through sterile gauze to decrease cellular clumping, and cells were centrifuged a final time. The supernatant was removed, and cells were resuspended in a final volume of 2 to 3 mL of cell culture media (DMEM, 5% FBS, and MEM nonessential amino acid solution [Gibco ThermoFisher Scientific, Waltham, MA]) at a concentration of 5×10^5 splenocytes per sample.

Samples were plated in triplicate onto 96-well round-bottomed plates in 2 groups. One group was stimulated with ovalbumin at 1 mg/mL; the other group was not stimulated. Plates were incubated for 72 h at 37 °C with 5% CO₂.

After incubation, the cell suspension from each well was placed in a 1.5-mL Eppendorf tube and centrifuged for 10 min at 11,300 $\times$ g. The supernatant was collected from each sample and evaluated for cytokine concentrations by using commercial ELISA kits (Quantikine, R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Samples were assayed in triplicate. Optical densities of the colorimetric change associated with cytokine concentration were analyzed by using a microplate reader (Multiscan Spectrum, Thermo Fisher Scientific, Waltham, MA). The mean concentration of each sample was calculated. Concentrations of IL10, TNF α , and IFN γ from both unstimulated and ovalbumin-stimulated splenocytes were compared among the 4 (saline, SR-Bup, Bup-HCl, and SR-Veh) treatment groups.

Antiovalbumin antibody response. Blood samples were centrifuged for 10 min at 2100 $\times$ g, and serum was collected immediately after centrifugation and stored at –20 °C until analysis. Direct ELISA was used to measure antiovalbumin antibody levels in serum, as previously described.²⁵ Briefly, plates were coated with antigen prepared by dissolving 0.5 mg of ovalbumin in bicarbonate buffer (1.5 g Na₂CO₃, 3.0 g NaHCO₃, 500 mL deionized H₂O; pH 9.6); 100 μ L of antigen solution was added to each well of 96-well flat-bottomed plates, which were then kept overnight at 4 °C. Wells were washed 3 times with ELISA wash buffer solution, 200 μ L of blocking buffer (0.5% dried milk in PBS) was added to each well, and plates were incubated at room temperature for 2 h. Wells were washed 3 times with wash buffer solution. Serum samples were diluted 1:1000 in blocking buffer and assayed in duplicate; 100 μ L of diluted serum was added to each well, and plates were incubated for 90 min at 37 °C with 5% CO₂. Wells were washed 3 times with wash buffer solution. Antimouse antibody conjugated with horseradish peroxidase was diluted 1:500 in PBS; 100 μ L of the diluted antimouse antibody conjugate was added to each well, and plates were incubated for 90 min at 37 °C with 5% CO₂. Wells were washed a final 5 times with wash buffer solution, and 100 μ L of substrate solution was added to each well. After the plates incubated at room temperature for 30 min, 100 μ L of stopping solution was added to each well to terminate the reaction. Optical densities of the colorimetric changes were measured by using a microplate reader (Multiscan Spectrum, Thermo Fisher Scientific).

Statistical analysis. We chose to include 5 mice per group on the basis of a calculated power of 0.89, reflecting a difference in 10% of the mean value between unstimulated and stimulated cytokine responses, a 5% standard deviation, and $\alpha = 0.05$. This held true for the IL10 response, but the variation in the TNF α and IFN γ responses was greater than expected. Data are presented as mean $\pm$ SEM. Differences in cytokine response between the 4 treatment groups and stimulated and nonstimulated cells between the 4 treatment groups were evaluated by using one-way ANOVA with posthoc Tukey adjustments. Differences in antibody responses between the 4 treatment groups were evaluated by using repeated-measures ANOVA. A *P* value of less than 0.05 was considered statistically significant.

Results

Cytokine responses. Mice were euthanized on day 21, corresponding with a nadir in the buprenorphine concentration.²² Cytokine responses are shown in Figure 1. IL10 concentration

Figure 1. Cytokine responses from ovalbumin-stimulated cultured splenocytes after treatment with either saline, SR-Bup, Bup-HCl, or SR-Veh. The assay was completed in triplicate, with 5 mice per group. Significant (*, $P < 0.005$; x, $P < 0.05$) differences between the cytokine's stimulated splenocytes and unstimulated controls are indicated for each treatment group; +, significant ($P < 0.005$) difference compared with stimulated splenocytes from saline- and Bup-HCl-treated mice. Other than the increased IL10 response in SR-Bup-treated mice, there are no significant treatment-associated differences in cytokine response.

from unstimulated splenocytes did not differ significantly between the 4 treatment groups (saline, 81.1 ± 5.9 pg/mL; SR-Bup, 86.5 ± 8.3 pg/mL; Bup-HCl, 70.8 ± 4.7 pg/mL; SR-Veh, 95.8 ± 12.0 pg/mL; $F_{3,6} = 1.61$, $P = 0.226$). Similarly, neither TNF α nor IFN γ concentrations from unstimulated splenocytes differed significantly between the treatment groups (TNF α : saline, 82.0 ± 9.9 pg/mL; SR-Bup, 140.1 ± 39.2 pg/mL; Bup-HCl, 65.7 ± 2.4 pg/mL; SR-Veh, 140.3 ± 42.3 pg/mL; $F_{3,16} = 1.77$, $P = 0.193$; IFN γ : saline, 14.9 ± 0.6 pg/mL; SR-Bup, 18.5 ± 2.1 pg/mL; Bup-HCl, 17.7 ± 2.0 pg/mL; SR-Veh, 18.7 ± 1.3 pg/mL; $F_{3,16} = 1.21$, $P = 0.339$). All stimulated splenocytes demonstrated an increase in cytokine production compared with unstimulated splenocytes. The concentration of IL10 from splenocytes stimulated with ovalbumin was significantly higher in the SR-Bup-treated mice (478.5 ± 79.8 pg/mL; $P = 0.04$) compared with mice treated with saline (170 ± 9.7 pg/mL) and Bup-HCl (139 ± 14.5 pg/mL; $F_{3,15} = 4.73$, $P = 0.016$). The IL10 concentration from the Bup-HCl and SR-Veh (314.5 ± 109.3 pg/mL) groups were not significantly different than saline-treated mice. The concentrations of TNF α (saline, 454.6 ± 59.0 pg/mL; SR-Bup, 447.5 ± 90.2 pg/mL; Bup-HCl, 132.9 ± 50.8 pg/mL; SR-Veh, 342.5 ± 128.7 pg/mL; $F_{7,31} = 2.9$, $P = 0.065$) and IFN γ (saline, 314.4 ± 79.6 pg/mL; SR-Bup, 693.3 ± 378.2 pg/mL; Bup-HCl, 46.9 ± 28.1 pg/mL; SR-Veh, 628.1 ± 499.7 pg/mL; $F_{3,14} = 1.40$, $P = 0.284$) from splenocytes stimulated with ovalbumin in culture did not differ significantly between control and treatment groups.

Antibody response. Antibody responses are shown in Figure 2. Antibody levels in mice as estimated by optical density measurements at 405 nm at time 0 before treatment administration did not differ significantly between groups (saline, 0.73 ± 0.10 ; SR-Bup, 0.57 ± 0.04 ; Bup-HCl, 0.50 ± 0.02 ; SR-Veh, 0.52 ± 0.03 ; $F_{3,15} = 3.12$, $P = 0.057$). As compared with baseline values, antibody levels were significantly higher after immunization in all treatment groups ($F_{1,31} = 160.1$, $P < 0.0003$). However, antibody levels after treatment did not differ significantly across treatment groups (saline, 3.61 ± 0.22 ; SR-Bup, 3.82 ± 0.12 ; Bup-HCl, 3.36 ± 0.41 ; SR-Veh, 2.47 ± 0.68 ; $F_{3,16} = 2.04$, $P = 0.149$), with the exception of the SR-Veh group, which was significantly ($P = 0.035$) lower than the saline group.

Discussion

Buprenorphine is a commonly used analgesic for pain management in laboratory mice and rats. Compared with morphine and fentanyl (pure μ -opioid agonists), buprenorphine is a partial μ -opioid receptor agonist, which results in a higher margin of safety. Pure μ -agonist opioids produce immunomodulatory effects by either directly binding to opioid receptors on the surface of host leukocytes or indirectly by binding opioid receptors in the CNS and thereby stimulating HPA axis.³⁶ For example, morphine inhibits adaptive immunity and reduces NK cell and macrophage function through the μ -opioid receptor.³³ Indirectly, morphine binding to CNS

Figure 2. Antibody response after ovalbumin immunization in mice treated with saline, SR-Bup, Bup-HCl, or SR-Veh. Assays were completed in duplicate, with 5 mice per group. In each treatment group, responses after immunization differ significantly (*, $P < 0.005$; x, $P < 0.05$) compared with those before immunization. In addition, the antibody response after immunization is significantly (+, $P < 0.03$) decreased in the SR-Veh-treated group compared with the saline, SR-Bup, and Bup-HCl groups. Responses after immunization did not differ between the saline, SR-Bup, and Bup-HCl treatment groups.

μ -opioid receptors results in glucocorticoid release from the adrenal gland.³² Similarly μ -opioid receptor binding can activate the sympathetic nervous system, leading to catecholamine release. Both of these actions have an immunosuppressive effect.³² However, buprenorphine does not have the same immunosuppressive effects as morphine.^{12,13}

Even though buprenorphine is known to cause minimal to no immune perturbations in mice and rats,^{4,24,27,30} the use of a sustained-release formulation may be preferable in a laboratory setting. For example, Bup-HCl must be dosed at least every 12 h, whereas SR-Bup can be dosed less frequently (that is, every 48 to 72 h).^{3,11,15,22,23} Although there is evidence supporting that buprenorphine has minimal modulatory effects on the immune response, little information regarding potential effects of SR-Bup on the immune system is available. In our current study, cytokine and antibody responses were evaluated to assess immune perturbations in mice treated with either Bup-HCl, SR-buprenorphine, SR-vehicle, or saline.

TNF α and IFN γ represent proinflammatory and Th1 cytokine responses, respectively, and IL10 represents regulatory T-cell cytokine responses. In all treatment groups, concentrations of all 3 cytokines from activated splenocytes were increased compared with unstimulated splenocytes. Mice treated with Bup-HCl or SR-Veh did not have any differences in cytokine concentration compared with the saline control, suggesting these treatments did not cause any additional immune alterations in treated mice. However, mice treated with Bup-SR had a more robust IL10 response compared with the saline- and Bup-HCl-treated groups.

Whereas all treatment groups had an increase IL10, TNF α , and IFN γ responses after stimulation of splenocytes, mice treated with SR-Bup had significantly increased IL10 levels compared with the saline and Bup-HCl groups. However even though some of these cytokine responses were not statistically significant, they might still exert biologic effects. Elevated TNF α concentrations result in macrophage activation and enhanced inflammatory response, which can lead to a systemic fever.³¹ Increased IFN γ enhances antibody production, antiviral activity, and NK cell activity.³¹ In contrast to activating the immune response, IL10 has immunosuppressive and antiinflammatory effects.³¹ These changes could have an impact on research outcomes. For example, mouse models of autoimmune arthritis have exacerbated disease associated with deficiencies in IL10;¹⁴ perhaps treatment with buprenorphine increases the IL10 response, thus attenuating the autoimmune induced arthritis. The biologic activity of these cytokine changes requires further research to determine their effects in various studies, such as those of autoimmune arthritis. Given that the effects of buprenorphine on cytokine production are similar to those in saline-treated mice in both unstimulated and ovalbumin stimulated splenocytes, this influence likely is minimal.

The plasma concentration of buprenorphine at the time of euthanasia and splenocyte stimulation may have an effect on the immune response. According to previously published pharmacokinetics,²² plasma levels of buprenorphine were expected to be at the nadir, and samples collected at the peak concentration may have yielded different results. However, similar to our results, chronic administration of buprenorphine for 7 d through an im-

planted osmotic minipump resulted in IFN γ and IL2 production from stimulated splenocytes,²⁷ with concentrations similar to those in saline-treated mice. Another study evaluated the effects of SR-Bup in mice after cecal ligation and perforation surgery.¹⁷ Mice were dosed with either a single dose of SR-Bup at 1 mg/kg SC, which did not alter levels of monocyte chemoattractant protein 1 or IL6 compared with those in mice given Bup-HCl at 0.1 mg/kg every 6 h for 6 doses. However, the absence of a no-treatment control group precluded evaluation of the effect of buprenorphine on the immune response.¹⁷ In another study involving a cecal ligation–puncture model, BALB/c mice treated with Bup-HCl at 0.1 mg/kg SC every 6 h for 4 doses had elevated mean IL10 and TNF α responses from peritoneal macrophages.¹⁸ Similar cytokine perturbations have been seen in other species after Bup treatment. In rats that received a surgical catheter implant, Bup-HCl resulted in an increase in serum IL10 and TNF α ,⁸ and in dogs, Bup-HCl treatment resulted in IL10 and TNF α responses from stimulated peripheral blood.²⁸

One important control in our study is SR-Veh, which is a proprietary biodegradable matrix. It is a viscous material that forms a palpable depot after injection. The presence of the vehicle in the mixture enhanced the cytokine response, compared with Bup-HCl alone. Although the chemical composition of the proprietary matrix is unavailable, presumably the matrix incites an antigen response, similar to depots that are formed when using oil-based adjuvants.²¹ The mechanism of action of the immune response in the presence of the matrix requires further evaluation, but the SR-Veh clearly incites a cytokine response, although one not significantly different than after saline treatment.

Splenocytes represent a mixed population of immune cells that includes macrophages, T cells, and B cells. Previous studies have demonstrated that Bup-HCl has a direct effect on macrophage function.⁹ Peritoneal macrophages were collected from mice treated with Bup-HCl for 7 d and stimulated *ex vivo*. The stimulated macrophages had an increased cytokine response (IL6, IL10 and TNF α) compared with unstimulated cells but a similar response to untreated controls and lower concentrations than morphine-treated mice. The origin of the cytokine response in our current study is unknown, and the specific cell population activated in response to SR-Bup requires further investigation.

Antibody responses involve a complex interplay of multiple immune cells, including macrophages, T cells, and B cells.²⁶ A reduced antibody response suggests immunosuppression; however, one would not be able to determine the exact mechanism from this information only, and it may not reflect a dysfunction of B cells alone. In contrast, an adequate antibody response suggests that there is no impairment of the cellular interplay. Our mice showed a robust antibody response, suggesting that SR-Bup lacks immunomodulatory effects. Mice in our current study were immunized with ovalbumin and adjuvant, similar to previous studies,²⁴ which likewise found no effect on antibody responses in Bup-HCl-treated mice. However, in another study, Bup-HCl treatment at 2 mg/kg for 7 d resulted in an increased antibody response to sheep RBC.⁹ Rather than through vaccinating, this antibody response was induced by using macrophages collected from buprenorphine-treated mice, pulsed *in vitro* with sheep RBC, and transferred to recipient mice. Freund adjuvant is a potent immunostimulant, and it is possible that the adjuvant effect overpowered any immunomodulatory effects of Bup-HCl or SR-Bup. Nonetheless, our studies corroborate previous work demonstrating that Bup-HCl and SR-Bup have no effect on the antibody response.

One possible explanation for the less pronounced cytokine production in the Bup-HCl treatment group is the increased number of handling and dosing occurrences compared with the SR-Bup, SR-Veh, and saline groups. Bup-HCl-treated mice were dosed every 24 h, with 18 total injections, compared with the 72-h dosing interval and thus only 6 injections in the SR-Bup, SR-Veh, and saline mice. This situation might have resulted in increased handling stress,¹ which could have caused stress-induced immunosuppression. Previous studies²⁹ have shown that handling mice for 2 min each day for 2 wk resulted in reduced T-cell and antibody responses compared with unhandled controls. In both cases, while there was a reduced response, it was not eliminated.²⁹ That is, although cytokine responses were less in the Bup-HCl group, which received additional handling compared with the other treatment groups, it does not appear as though this exposure affected the antibody response in our current study. However, if this study were to be repeated, mice in all treatment groups should be handled and injected the same numbers of times, to reduce the possible confounding factor of handling stress on cytokine production.

The mice in our study did not undergo any previous surgical procedures prior to analgesia treatment. In practice, these analgesics are typically used to reduce postoperative pain, which can significantly suppress the immune response through stress-induced glucocorticoid production.^{32,36} In our study, mice were chronically dosed with buprenorphine, rather than the more acute, short-term treatments associated with postoperative analgesia. We have 2 reasons for choosing the chronic approach. The first was that chronic administration of opioids has been shown to be immunosuppressive;² therefore we used a chronic dose to determine whether SR-Bup has a similar effect. The second reason was to avoid immunotolerance due to low-dose exposure to SR-Bup. If tolerance were induced, it would be difficult to assess any immunomodulatory effects. The dosing regimen could affect the immune response, and assessing the immune response to various doses of Bup or SR-Bup should be experimentally validated. Although not conclusive, it would be reasonable to assume that lower exposures would have less of an effect on the immune response.

The purpose of the current study was to evaluate the immunologic effects of SR-Bup on mice. Our results demonstrate that any potential immunomodulatory effects of SR-Bup on innate or adaptive immunity are negligible. Other than IL10, the cytokine and antibody responses to SR-Bup were no different than those in saline-treated mice. These findings are similar to previous studies evaluating the immunomodulatory properties of Bup-HCl. Given SR-Bup's lack of immunomodulatory effects with chronic treatment, whether it should be withheld for pain management should be thoughtfully considered.

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