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Quantitation of Acute Phase Proteins and Protein Electrophoresis in Monitoring the Acute Inflammatory Process in Experimentally and Naturally Infected Mice

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Serologic screening for infectious disease in sentinel mice from rodent colonies is expensive and labor-intensive, often involving multiple assays for several different infectious agents. Previously, we established normal reference ranges for the protein fractions of several laboratory strains of mice by using a commercially available agarose system of protein electrophoresis. In the current study, we address protein fractionation and quantitation of acute phase proteins (APP) in mice experimentally infected with Sendai virus or mouse parvovirus. We further investigate this methodology by using samples from sentinel mice from colonies with endemic infection. All study groups showed significant increases in γ globulins. Various other protein fractions showed mild variable changes; significant differences were not detected for individual APP. These results contrast the significant changes observed in APP and protein electrophoresis by using the standard methods of inducing inflammatory responses through injection of complete Freund adjuvant or LPS. These present data suggest that although quantitation of individual APP may not be helpful, γ globulin levels may reflect infection in laboratory mice and provide a possible adjunct to traditional screening methods.

Abbreviations: APP, acute phase protein; CFA, complete Freund adjuvant; CRP, C-reactive protein; MPV, mouse parvovirus; SAA, serum amyloid A; SAP, serum amyloid P.

Sophisticated technologies including serology, culture, histology, and PCR are available to evaluate laboratory animals for the presence of infectious disease.[48] These analyses, albeit expensive and labor-intensive, are necessary to ensure that laboratory rodents are free from infectious agents that can interfere with research. In both human and veterinary medicine, the quantitation of acute phase proteins (APP) has been proposed to have diagnostic and prognostic utility to study disease and infection.[2,11,14,21,26,40] APP are blood proteins primarily synthesized by hepatocytes as part of the complex systemic response termed the acute phase response. The acute phase response is part of the early defense or innate immune system, which is triggered by various stimuli, including trauma, infection, stress, neoplasia, and inflammation. The acute phase response has been referred to as the 'molecular thermometer,' whereby quantitation of specific APP might reflect the response to the triggering event.[10,14,40,44] To this point, several studies have been conducted in companion, laboratory, and large animals profiling changes in APP after experimental and natural infection.[17,18,38,40,45,50]

Mice have several major APP that may reflect acute and chronic inflammatory processes including C-reactive protein (CRP), haptoglobin, serum amyloid P (SAP), and serum amyloid A (SAA).[20,47] ELISA assays for these proteins are commercially available. A broader view of the sum of APP changes and the overall acute phase response is obtained through the use of protein electrophoresis.[33] This technique uses an agarose gel to separate protein fractions into albumin, α1 globulins, α2 globulins, β globulins, and γ globulins. Protein electrophoresis does not quantitate single proteins but rather groups of proteins that are mediators of acute inflammatory process. α1 globulins include α1 antitrypsin and α1 acid glycoprotein; α2 globulins include α2 macroglobulin and haptoglobin; β globulins include transferrin, SAA, and CRP, and γ globulins are composed primarily of IgG.[33] Many diagnostic and prognostic uses of protein electrophoresis in veterinary medicine have been reported.[1,4,12,25,33] Although rarely diagnostic of a particular disease, protein electrophoresis is helpful for the detection of acute and chronic inflammatory processes and stimulation of humoral immunity.[12,15,33]

APP have been proposed to be valuable biochemical markers of stress, infection, and pain in laboratory animals.[14,42] Previously, we established normal reference ranges for the protein fractions of several laboratory strains of mice by using a commercially available agarose system of protein electrophoresis.[54] The primary goal of the current project was to study the potential changes in APP and protein fractions in laboratory mice after experimental infection with viral pathogens. These data were compared to those generated by using traditional means of inducing acute inflammation with the injection of LPS and complete Freund adjuvant (CFA). In addition, we addressed the

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possible application of protein fractionation and quantitation of APP by using samples from sentinel mice from colonies with endemic infection.

Materials and Methods

Animals and housing. All animals were maintained in accordance with the temperature and humidity recommendations of the *Guide for the Care and Use of Laboratory Animals* at the each of the facilities.[29] All facilities are AAALAC-accredited, and all experimental procedures were approved by the various universities' animal care and use committees.

For the initial study, sentinel mice maintained on dirty bedding were screened quarterly through serologic testing for mouse hepatitis virus, Sendai virus, *Mycoplasma pulmonis*, pneumonia virus of mice, minute virus of mice, Thieler murine encephalomyelitis virus, mouse parvovirus (MPV), mouse rotavirus, lymphocytic choriomeningitis virus, and parasitic infections. Once a year, the panel was extended to include murine norovirus, ectromelia virus, K virus, *Encephalitozoon cuniculi*, polyoma virus, mouse adenovirus, reovirus, murine cytomegalovirus, hantavirus, mouse thymic virus, *Clostridium piliforme*, and cilia-associated respiratory bacillus. *Helicobacter* testing by PCR was also conducted. All results were negative during the course of this study. Mice were maintained with autoclaved supplies and microisolation cage tops.

For the MPV studies, weaned female BALB/cAnNHsd mice (Harlan Laboratories, Indianapolis, IN) were obtained. All mice were specified to be free of murine viruses, pathogenic bacteria and endo- and ectoparasites by the supplier. Mice were housed in static microisolation caging, and all animal manipulations were performed in a class IIA biological safety cabinet by using standard microisolation techniques.

For the Sendai studies, C57BL/6 mice originally purchased from Jackson Laboratories (Bar Harbor, ME) were rederived and maintained for experiments. Sentinel mice maintained on dirty bedding were screened quarterly for mouse hepatitis virus, Sendai virus, *Mycoplasma pulmonis*, pneumonia virus of mice, minute virus of mice, Thieler murine encephalomyelitis virus, mouse parvovirus, lymphocytic choriomeningitis virus, mouse norovirus, ectromelia virus, polyoma virus, mouse adenovirus, reovirus, and *Helicobacter* spp. All results were negative during the course of this study. Mice were housed in microisolation caging on a ventilated rack with autoclaved materials.

Injection of LPS and CFA. Sixty female BALB/c mice (age, 12 wk; Charles River Laboratories, Wilmington, MA) were injected intraperitoneally with endotoxin-free water (0.1 mL), LPS (50 µg *E. coli* serotype 0111.B4 in 0.1 mL endotoxin-free water), or CFA (0.05 mL endotoxin-free water emulsified in 0.05 mL CFA). All reagents were purchased from Sigma (St Louis, MO). All animals were examined daily to assess their clinical condition. On days 1, 2, 4, and 8 after injection, 5 mice per group (control, LPS, or CFA) were euthanized by CO_2 inhalation and terminally bled by cardiocentesis. Compressed CO_2 was administered to a euthanasia chamber at a flow sufficient to induce rapid loss of consciousness and death, according to the AVMA Guidelines on euthanasia.[3] The data presented are representative of 2 individual experiments. Serum was obtained by centrifugation, and samples were stored at −80 °C until analysis.

Experimental and natural infection. BALB/c mice (age, 4 wk) were designated as control mice (mock-inoculated; $n = 14$) or were inoculated orally with 100 ID_{50} MPV1e ($n = 18$) or 1000 ID_{50} MPV5 ($n = 17$). MPV1e and MPV5 were isolated from naturally infected mice, represent 2 distinct genotypes of MPV, and were kindly provided by Dr Ken Henderson (Charles River Laboratories). On day 2, 7, 14, and 28 after inoculation, at least 3 mice from each group were euthanized by CO_2 inhalation as described earlier. Blood samples were collected by cardiocentesis, and the resulting sera were stored at −80 °C until use. Results of multiplex fluorescent immunoassay or quantitative PCR (or both) were positive for MPV in all inoculated mice whereas mock-inoculated mice were uniformly negative for these assays (data not shown).

Twenty C57BL/6 mice (age, 8 to 12 wk) were anesthetized with 2,2,2-tribromomethanol (200 mg/kg) and infected intranasally with 250 egg ID_{50} Sendai virus (Enders strain). Five additional mice served as uninfected controls. On days 3, 5, 7, and 14 after inoculation, 5 mice per group were euthanized with an overdose of tribromoethanol, and blood was collected by cardiocentesis and collected in a vacuum phlebotomy tube with serum separator (BD Biosciences, San Jose, CA). Serum was obtained by centrifugation, and samples were stored at −70 °C until analysis.

Other samples were obtained from routine submissions to our diagnostic laboratory. These samples were obtained from sentinel mice from 2 conventionally maintained facilities with established histories of endemic viral and parasitic infection. From one facility, samples from 15 sentinels were examined after a 3-mo exposure period. From the other facility, samples from 8 sentinel mice were examined after a 6-mo exposure period.

Sample handling. Samples from The University of Arizona and the Trudeau Institute were shipped on dry ice to the University of Miami for analysis.

Protein electrophoresis. Serum samples were analyzed according to the manufacturer's procedure (Paragon SPEP-II Gel System, Beckman, Fullerton, CA). All reagents are prepackaged for use with this system. Briefly, all samples were diluted 1:4 in barbital running buffer, and 10 µL was applied to a preprepared 1% thin-layer agarose gel by using the plastic template provided. The gel was exposed to 100 V for 37 min. After being washed in water, the gel was fixed, dried, and stained in Beckman Blue Stain. Bands were quantitated by using a densitometer (Beckman). The results presented include the percentages of fractions according to the densitometric scan as well as absolute values, which were obtained by multiplying percentages by the total protein content as determined by the biuret method on an automated analyzer (Ortho Vitros 250, Rochester, NY). The albumin:globulin ratio was calculated by dividing albumin content by the sum of α, β, and γ globulins. Representative electrophoretograms can be found in our previous publication.[54]

ELISA for APP quantitation. Commercial ELISA kits for quantitation of SAA, CRP, and haptoglobin were purchased from Life Diagnostics (West Chester, PA). The SAP kit was purchased from Kamiya Biomedical Company (Seattle, WA). All species-specific assays were conducted according to the manufacturer's instructions.

Statistical analysis. Mean and standard error were calculated for all tests. ANOVA analysis using the Dunnett multiple comparison test was used. A P value of 0.05 or less was considered statistically significant. All calculations were obtained by using Graph Pad Prism 4 software (La Jolla, CA).

Results

Changes in clinical condition. Animals were examined for changes in clinical condition after injection. Mice injected with CFA exhibited no noteworthy changes in coat condition or weight. Mice injected with LPS were lethargic and had a moderately ruffled coat on day 1 after injection but were visibly recovered by day 4 and exhibited a normal appearance by day 8. The mice infected with MPV and Sendai virus had no changes in clinical condition throughout the study.

Changes in protein fractions after injection of LPS and CFA. Protein electrophoresis was conducted on all samples at several time points. On day 1 after injection, the albumin:globulin ratio was significantly ($P < 0.05$) decreased in both groups compared with the control group. Concomitant significant increases were observed in α2 ($P < 0.05$) and γ globulins ($P < 0.05$) with a decrease in albumin ($P < 0.01$, Figure 1). These changes, including an increase in β globulins ($P < 0.05$), were observed on day 2 also. By day 4, the albumin:globulin ratio continued to be low ($P < 0.05$) and α2 globulins continued to be significantly ($P < 0.05$) increased in the CFA group only. Decreases ($P < 0.05$) in α1 globulins were observed on day 2 for LPS and CFA ($P < 0.05$) and day 4 for CFA only. The albumin:globulin ratio, albumin, and all globulins normalized for all groups by day 8 after injection, and no significant differences were present for experimental mice compared with control mice.

We also analyzed samples for SAA, CRP, SAP, and haptoglobin by ELISA. By day 1, moderate to marked significant increases were present in SAA, SAP, CRP, and haptoglobin ($P < 0.01$ to $P < 0.05$, Figure 2). This increase continued on day 2 for SAA, SAP, and haptoglobin ($P < 0.01$ to $P < 0.05$). CRP continued to be increased ($P < 0.01$) with CFA only. Day 4 SAA levels were higher ($P < 0.05$) in the CFA group (162.4×10^2 ng/mL) compared with the control group (0 ng/mL). Similar changes were observed with SAP ($P < 0.01$) and haptoglobin ($P < 0.01$) levels. Levels of all APP decreased and no longer differed from control values by day 8 after injection.

Changes in protein fractions during Sendai infection. Sendai-infected mice were bled on days 3, 5, 7, and 14 after inoculation. Serum albumin on day 3 was decreased significantly, concomitant with an overall decrease in total protein ($P < 0.01$, Table 1). This difference was reflected by decreases ($P < 0.05$) in α1 and α2 globulins.

A significant ($P < 0.05$) decrease in the albumin:globulin ratio was present on day 14, concurrent with a more than 2-fold increase ($P < 0.01$) in γ globulins. In addition, the same samples were analyzed for changes in CRP, haptoglobin, SAP, and SAA. No significant differences in APP were observed in the mice, with the exception of a transient mild increase ($P < 0.05$) in haptoglobin on day 5 ($52.0 \pm 1.5 \times 10^3$ ng/mL on day 0 compared with $85.0 \pm 11.2 \times 10^3$ ng/mL on day 5).

Changes in protein fractions during MPV infection. Mice were infected with either MPV1 or MPV5 and bled at multiple time points during infection including days 2, 7, 14, and 28. On day 7, the albumin fraction in the MPV1-infected group was slightly but significantly ($P < 0.05$) decreased compared with that in the control group (Figure 3). On day 14, samples from both infected groups exhibited significant increases in γ globulins (MPV1, $P < 0.01$; MPV5, $P < 0.05$); this change was also evident on day 28 ($P < 0.05$). On day 28, mild increases in α1 ($P < 0.05$), α2 ($P < 0.01$), and β globulins ($P < 0.01$) and a pronounced decrease ($P < 0.01$) in the albumin:globulin ratio were present in MPV5-infected mice. When we analyzed the same samples for changes in CRP, haptoglobin, SAP, and SAA, no significant differences were found at any time point (data not shown).

Changes in protein fractions in colony sentinel mice. BALB/c sentinel mice from 2 different mouse colonies were assessed by using routine serologic assays for infectious disease. Sentinels from one colony tested positive for both mouse hepatitis virus and MPV. Samples from these mice demonstrated significantly ($P < 0.05$) higher levels of α2 and γ globulins (Table 2). Mice from the other facility tested positive for mouse hepatitis virus, MPV, and murine norovirus as well as pinworms. Samples from these mice demonstrated significant ($P < 0.05$) increases in albumin and γ globulins. A subset of mice ($n = 4$) from the same facility were assessed several months after successful treatment for pinworms. The elevated γ globulin fraction continued to be observed (data not shown). In a separate experiment, sentinels were assessed for changes after transfer to a facility free from endemic infection. After being maintained for 3 mo by using a dirty bedding transfer system, no significant differences from baseline values in γ globulins were observed in samples for sentinel mice ($n = 20$, data not shown). No significant differences were found for any of the samples tested for APP, including CRP, haptoglobin, SAP, and SAA (data not shown).

Discussion

In the present study we induced acute inflammation with LPS and CFA to examine effects on APP values and protein electrophoresis fractions and to provide positive controls for a study of infectious agents. LPS, the predominant outer cell wall constituent of gram-negative bacteria, and CFA were chosen for their potent inflammatory activity and to provide standards with which to compare other results from the experimental infection studies.[5,23,36,37,51] By ELISA analysis at day 1 after LPS injection, increases in CRP, SAA, SAP, and haptoglobin were observed. These changes persisted only through day 2 and are consistent with findings by others. For example, 2-dimensional gel analysis of serum from mice injected with LPS reflected 20-fold or more increases in haptoglobin, SAA, and SAP.[16] Five- to 10-fold increases in SAP were reported in many strains of mice 24 h after injection of LPS.[39] Another study demonstrated 10,000-fold increases in serum levels of SAA within 24 h of exposure by the intraperitoneal route.[23] Haptoglobin levels after LPS injection reportedly increased 4-fold during the first 48 h and normalized by 96 h.[52]

Our protein electrophoresis data are consistent with the current and previously published ELISA data, with an increase in the β globulin fraction, where CRP migrates, observed on days 1 and 2 in the LPS group (Figure 1).[33] Similarly, α2 globulin increases were also apparent on days 1 and 2, with a concomitant increase in haptoglobin. A marked decrease in haptoglobin was present on day 4 as alpha 2 globulins returned to normal levels. A decrease in α1 globulins was observed on day 2, perhaps indicating that this fraction contains APP that serve as negative acute phase proteins. The relative sensitivities of ELISA versus protein electrophoresis methods differ, with quantification of proteins at the ng/mL and mg/mL levels, respectively. Therefore, a 10,000-fold increase in expression of a single APP might alter protein electrophoresis results. Importantly, protein electrophoresis and ELISA are 2 independent methods with different sensitivities in addressing the course of the acute phase response, and protein electrophoresis

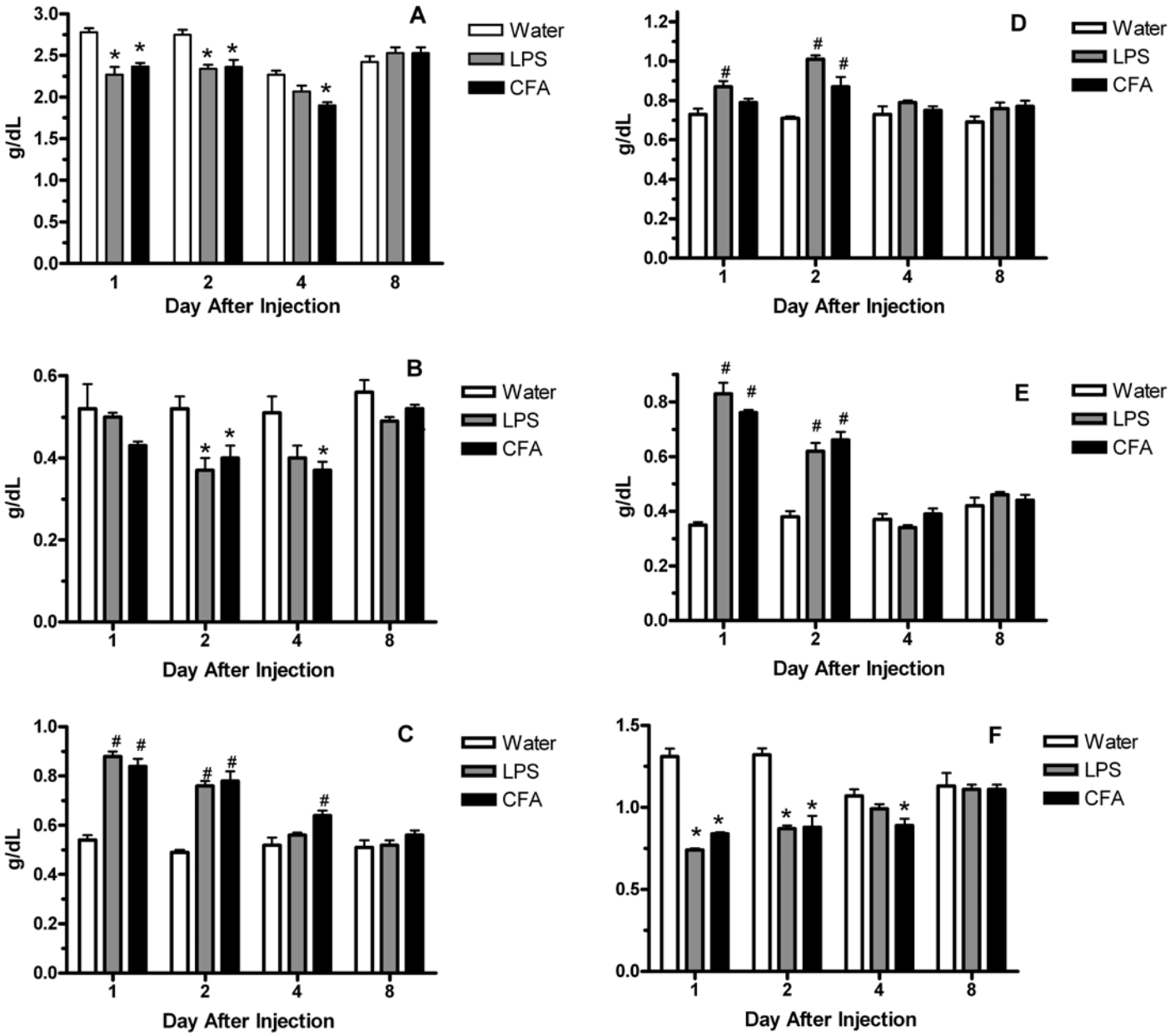

Figure 1. Comparison of fraction concentrations and the albumin:globulin ratio in the experimental groups (water, lipopolysaccharide [LPS], CFA) over days 1, 2, 4, and 8 after injection. Mean and standard error are shown. *, Value significantly ($P \leq 0.05$) decreased from control; #, value significantly ($P \leq 0.05$) increased from control. (A) Albumin. (B) α1 Globulins. (C) α2 Globulins. (D) β Globulins. (E) γ Globulins. (F) Albumin:globulin ratio.

does not reflect changes of individual APP. Interestingly, similar to the ELISA data, protein electrophoresis revealed a significant change in the acute phase response as indicated by the decrease in the albumin:globulin ratio found as early as day 1. This decrease resulted from a decrease in albumin, a negative APP, and increases in α2 globulins.[33] Notably, the time frame of these changes paralleled the ELISA data as well as the clinical appearance of the LPS-injected mice. Similar changes were observed in both the APP and protein electrophoresis results of CFA-injected mice.

In contrast to ELISA, which is specific for a single APP, data gained from protein electrophoresis provide a view of the overall acute phase response. Others have made similar comparisons of protein electrophoresis and specific APP quantitation previously. After venom injection, increases in CRP were observed, with an increase in α globulins; increases in β globulin were observed when CRP levels had returned to normal.[43] In a rat model using CFA injection, CRP and haptoglobin were observed at 36 h, when α2 and beta globulins were increased.[22]

Both protein fraction and APP changes have been used to characterize experimental models of infection. Previously, mice infected with *Staphylococcus aureus* were found to have a decreased albumin:globulin ratio according to protein electrophoresis techniques from 2 to 3 wk after inoculation.[35] In models of experimental infection by *Trypanosoma* spp. which result in significant changes in clinical condition, marked increases in SAP and haptoglobin were observed.[18,41] Increases in α and β

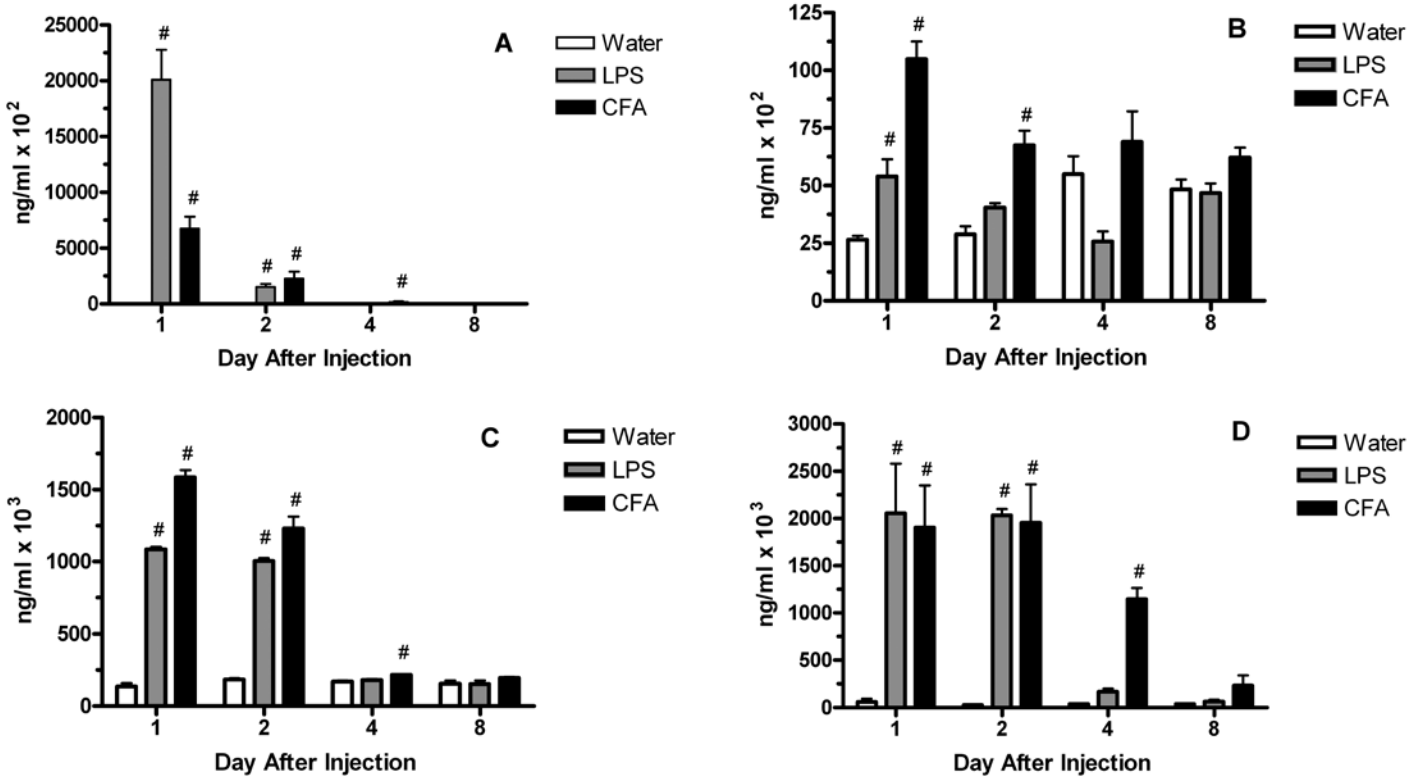

Figure 2. Comparison of (A) SAA, (B) CRP, (C) SAP, and (D) haptoglobin (HP) levels as determined by ELISA in the experimental groups (water, LPS, CFA) over days 1, 2, 4, and 8 after injection. Mean and standard error are shown. #, Value significantly ($P \leq 0.05$) increased from control.

Table 1. Protein electrophoresis fractions (g/dL; mean ± SE) and albumin:globulin (A/G) ratio from control mice and those infected with Sendai virus

		Sendai-infected mice: day after inoculation			
	Control Mice	Day 3	Day 5	Day 7	Day 14
Albumin	2.82 ± 0.48	1.09 ± 0.35[a]	2.05 ± 0.11	2.10 ± 0.17	2.37 ± 0.07
α1 Globulin	0.57 ± 0.15	0.21 ± 0.19[a]	0.33 ± 0.02	0.43 ± 0.04	0.61 ± 0.04
α2 Globulin	0.88 ± 0.20	0.36 ± 0.14[a]	0.75 ± 0.05	0.79 ± 0.10	0.97 ± 0.03
β Globulin	0.93 ± 0.16	0.51 ± 0.23	0.74 ± 0.04	1.16 ± 0.20	1.04 ± 0.05
γ Globulin	0.12 ± 0.02	0.07 ± 0.03	0.18 ± 0.01	0.17 ± 0.03	0.38 ± 0.03[b]
Albumin:globulin ratio	1.17 ± 0.06	1.20 ± 0.17	1.03 ± 0.03	0.86 ± 0.08	0.80 ± 0.05[a]

[a]Significantly ($P \leq 0.05$) decreased from control (day 0) value
[b]Significantly ($P \leq 0.05$) increased from control (day 0) value

globulins were observed by paper electrophoresis of serum from mice terminally infected with *Toxoplasma gondii*.[45] In the current study, we used experimental models of Sendai and MPV infection. Sendai virus, a type I parainfluenza virus, causes infection in the respiratory epithelium and appears to be closely related to human parainfluenza virus, which can cause severe respiratory tract infection in children.[8,19,24] Infections in laboratory animal colonies are usually highly contagious but, according to recent reports,[8,9,19] are no longer found frequently in rodent colonies. In the current model, viral titers peak by day 4 to 6 and are cleared by day 10.[28] Seroconversion of the IgG isotype, assessed by routine commercially available ELISA, was found by day 14, as previously reported.[46] In contrast to Sendai virus, rodent parvoviruses, especially MPV, are common contaminants of rodent colonies.[7,9,30] Several studies have suggested that MPV may interact with the immune system and result in dysfunction.[30,31] Infection occurs in the intestine, and viral replication appears to be decreased with seroconversion, although it may persist in lymphoid tissues.[6,30,31] Interestingly, we observed increased α and β globulin fractions at day 28 after inoculation. The correlation of these changes with viral persistence remains to be determined.

In the current study, the animals seroconverted by day 14 after inoculation. Therefore, in both experimental models

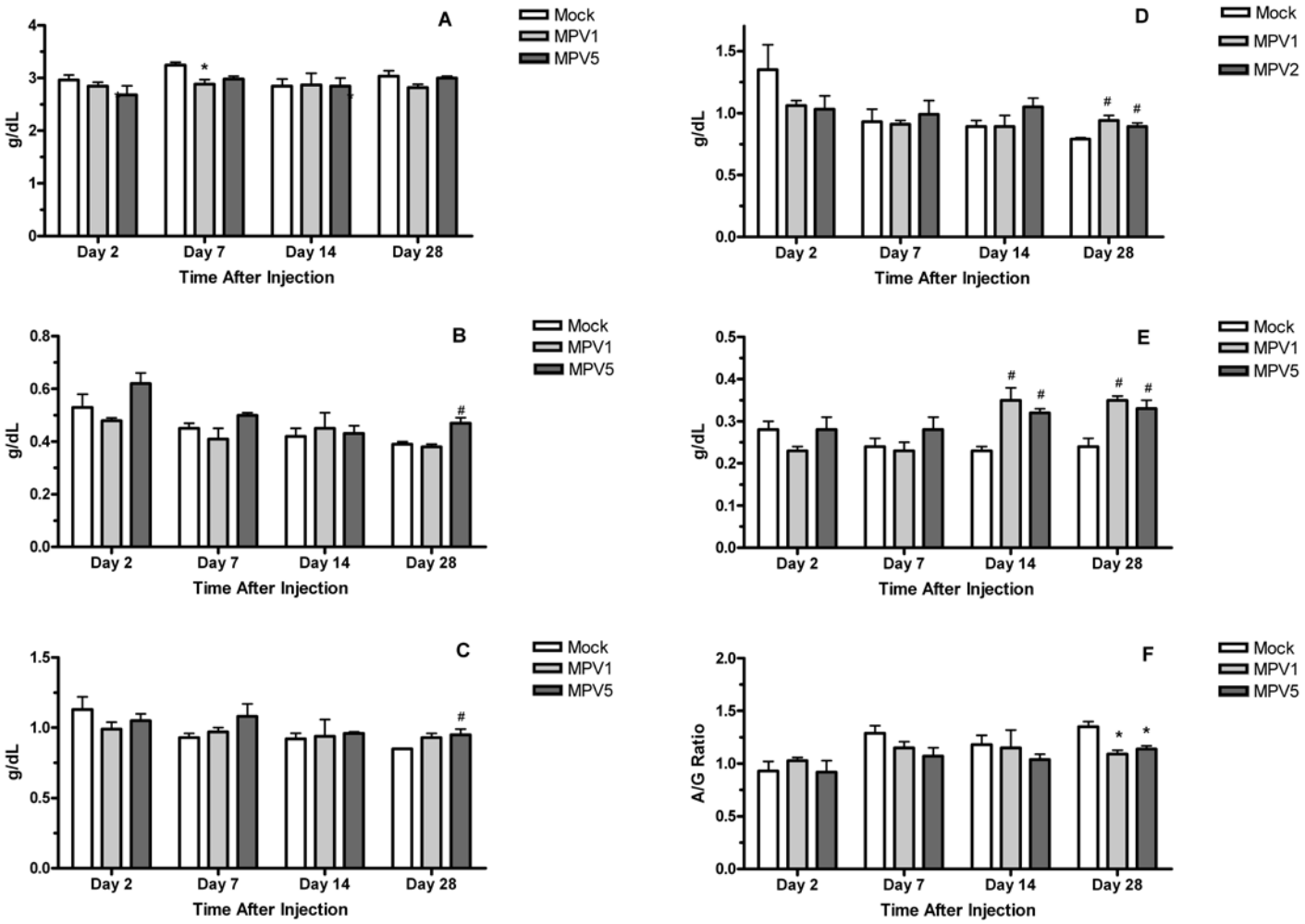

Figure 3. Comparison of fraction concentrations and the albumin:globulin of the mice infected with MPV1 or MPV5 over days 2, 7, 14, and 28 after injection. Mean and standard error are shown. *, Value significantly ($P \leq 0.05$) decreased from control; #, value significantly ($P \leq 0.05$) increased from control. (A) Albumin. (B) α1 Globulins. (C) α2 Globulins. (D) β Globulins. (E) γ Globulins. (F) Albumin:globulin ratio.

(Sendai and MPV), seroconversion occurred with the observed increase in γ globulins. Both conventional rodent colonies that were assessed have had long-term (more than 5 y) endemic infection with numerous agents. Sentinels were assessed 1 to 2 times annually by routine health screening techniques including serology by ELISA and standard parasitology exams. It is likely the positive antibody status for some, if not all, the agents represented exposure and clearance of the agents, although the possibility of persistent infection was not assessed. Notably, samples from these sentinels exhibited increased levels of γ globulins. Importantly, sentinels maintained with the same husbandry protocols but in a facility free of endemic infection exhibited no changes in gamma globulins. Given that all the specimens examined in the current study also had seroconversion to all the infectious agents, gauging whether the change in γ globulins was due to one of the agents (that is, mouse hepatitis virus) rather than the group of agents is difficult. Pursuing the use of protein electrophoresis as an adjunct tool would require gauging the relative sensitivity and specificity by using specimens from colonies with individual or unique groupings of infectious agents.

In the current study, changes in major acute phase proteins were mild, when present. This finding was unexpected given the numerous publications in the veterinary literature indicating the sensitivity of these assays in the detection of inflammation and infection.[17,18,20,26,32,38,40,41,45,47] We therefore had hypothesized that the induction of an experimental infection would be adequate to result in significant increases in major APP, especially during the acute stage of infection. However, many of the other studies documented changes in the acute phase response or APP of mice with acute infection that was often fatal or with infectious agents that were highly pathogenic.[18,35,41,45] These types of experimental conditions were not reproduced in the current study. To this point, MPV-and Sendai-infected animals were in good clinical condition throughout all time points, and no mortality occurred. As with the control agents of CFA and LPS used in the current study, many studies of rodent APP have been limited to the use of acute self-limiting inflammatory agents. CRP, SAA, and SAP are markers of acute inflammation which often decrease rapidly (24 to 72 h) in the absence of continued stimulation, whereas haptoglobin and other markers have been proposed to be more sensitive of possible chronic changes in some species.[17,18,20,26,32,38,40,41,45,47]

Table 2. Protein electrophoresis fractions (g/dL; mean ± SE) and albumin:globulin (A/G) ratio from control seronegative mice and sentinels that tested positive for various agents

	Normal BALB/c mice	Mice from colony 1 (n = 15)	Mice from colony 2 (n = 8)
Albumin	2.00 ± 0.07	1.97 ± 0.12	2.64 ± 0.67[a]
α1 Globulin	0.41 ± 0.02	0.38 ± 0.05	0.39 ± 0.02
α2 Globulin	0.62 ± 0.02	0.90 ± 0.08[a]	0.62 ± 0.03
β Globulin	0.84 ± 0.13	0.93 ± 0.12	0.84 ± 0.04
γ Globulin	0.11 ± 0.11	0.47 ± 0.10[a]	0.37 ± 0.03[a]
Albumin:globulin ratio	1.09 ± 0.10	0.83 ± 0.08	1.20 ± 0.03

Mice from colony 1 were positive for mouse hepatitis virus and MPV; mice from colony 2 were positive for mouse hepatitis virus, MPV, murine norovirus, and pinworms.

[a]Significantly ($P \leq 0.05$) increased from value for normal mice

In mice, intermediate increases in haptoglobin were observed with persistent infection with *Trypanosoma* spp.[41] This APP may not have been modulated in the current study due to effective seroconversion and viral clearance. Haptoglobin and other APP may be increased in rodents with persistent viral infection; this possibility should be addressed in further studies.

A consistent finding among the experimentally and naturally infected mice was an increase in γ globulins. Whereas protein electrophoresis can quantitate changes in this protein fraction, quantitation of total protein and albumin by a clinical chemistry analyzer may also be sufficiently sensitive to reveal changes in γ globulin concentration and likely is more expedient to perform. Furthermore, although IgG levels can be quantitated by commercially available ELISA kits, γ globulin analysis by protein electrophoresis or chemistry analyzer would be preferred for its automation and low expense. However, in many species, the results obtained by clinical analyzers may not be accurate as albumin quantitation, given that most clinical analyzers have been optimized for determination of human albumin.[34,49,53] These findings have often led to the statement that protein electrophoresis should be used as a 'gold standard' for albumin and globulin quantitation.[27,33] To our knowledge, no such study has been conducted using rodent samples, but this hypothesis should be pursued.

Protein electrophoresis analysis may provide a primary test of sentinel specimens, prior to in-depth, specific ELISA testing. That is, samples with increased γ globulins are tested for virus-specific antibodies. Although the associated start-up costs, including the purchase of a protein electrophoresis system, may prevent its use onsite in many institutions, this type of analysis is commonly available at many commercial veterinary laboratories. According to our 15-y experience with this method, the reagent costs are less than USD$2 per sample and hold a very favorable position in comparison to the costs associated with extensive serologic panels. The system is highly automated and requires little technical time. In many of the protein electrophoresis platforms, 60 samples can be analyzed within a 2-h period. APP have previously been described to be stable at −20 °C but, in the event of prolonged storage, −70 °C is recommended.[10] Prolonged storage at −70 °C also does not affect protein electrophoresis results.[13] Specimen volume requirements are also very low (10 to 20 µL) and may provide flexibility in using nonterminal sentinel assessments (that is, live bleeds) conducted at an increased frequency or at a testing points between normally scheduled full assessments. This type of testing strategy may be advantageous in colonies that are at increased risk for infection or in which an outbreak would be particularly deleterious. Additional studies are required to assess the utility of these approaches. Sentinel strain differences and the type of sentinel program including the minimal and maximal periods for optimal sentinel housing prior to testing should be addressed. Successful implementation of this new procedure would be dependent on the presence of a sustained increase in γ globulins even with clearance of infection.

Because increases in γ globulin and changes in the albumin:globulin ratio may occur with infection, neoplasia, and stress, protein electrophoresis cannot be proposed as a replacement for standard diagnostic procedures such as PCR and ELISA. Furthermore, the diagnostic application of APP in rodents for health screening appears limited in the current study. Perhaps γ globulins, acute phase proteins, and other biomarkers may provide additional information on the status of rodent colonies. APP have been proposed as indicators of 'herd health' in large animals and are described as excellent markers of acute and chronic inflammation and stress.[14,40,44] Further studies should address other possible biomarkers as well as extend the APP and protein electrophoresis studies in a wider screening of other rodent colonies and infectious agents. In addition, these diagnostic procedures may have value in the assessment and characterization of animal models of disease, stress, and drug safety testing.

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