However, we did not compare the incidence of active *B. burgdorferi* infections in dogs and people, but, rather, the prevalence of antibody in these two species. We found that the seropositivity rate (an index of exposure), was greater in dogs than in people in this population.

THOMAS R. ENG
Viral and Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, Georgia

References

1. Greene RT. Lameness and asymptomatic *Borrelia burgdorferi* seropositivity in dogs. J Infect Dis **1989**;160:346
2. Eng TR, Wilson ML, Spielman A, Lastavica CC. Greater risk of *Borrelia burgdorferi* infection in dogs than in people. J Infect Dis **1988**;158:1410–1411

THE JOURNAL OF INFECTIOUS DISEASES • VOL. 160, NO. 2 • AUGUST 1989
 0022-1899/89/6002-0031$01.00

Interpretation of Data Regarding the Protection Afforded by Serum, IgG, or IgM Antibodies after Immunization with the Rough Mutant R595 of *Salmonella minnesota*

TO THE EDITOR – Several crucial problems in the field of protective antibody to endotoxin core were summarized by Ziegler in an editorial in the August 1988 issue [1]. One is the controversy regarding the protection observed with core antibodies in experimental animals; some investigators who have been unable to reproduce the results published by others have raised the issue of proper controls [2, 3]. Another important problem is that the epitope specificity and the immunoglobulin class of the core antibodies responsible for protection are debated. Two papers in the same issue addressed these problems experimentally [4, 5].

McCabe et al. [4] fractionated sera of both rabbits and humans immunized with the Re mutant of *Salmonella minnesota* on Sephadex G200 columns (Pharmacia LKB Biotechnology, Piscataway, NJ), which divide the serum proteins into three fractions. They found that the first fraction, which contains IgM and many other proteins, afforded a degree of protection similar to that of unfractionated serum in animals challenged with two gram-negative bacteria (*Klebsiella pneumoniae* and *Morganella morganii*) or with *Salmonella typhi* lipopolysaccharide (LPS). They concluded that protection in postimmune rabbit or human sera was mediated by IgM antibody to core LPS. We fear that no definitive proof was provided for this conclusion. First, the inference that IgM was the protective factor is not supported by the data, because no IgM purification was attempted. Second, the conclusion that core LPS antibodies were the protective factor is also not supported by the data, since neither immunopurification of core LPS antibodies nor absorption experiments were reported.

DeMaria et al. [5] immunized 122 healthy subjects with the rough mutant Re of *S. minnesota* and tested the protective power of pre- and postimmunization sera in mice challenged with one inoculum of the *K. pneumoniae* or *M. morganii* strains or with one dose of *S. typhi* LPS. They concluded that an enhanced protective activity was demonstrated after immunization in most subjects. We believe that this strong conclusion is not fully supported by the data. First, the results obtained in more than half of the volunteers immunized with the rough mutant were not reported. The criteria for excluding them was that their preimmune sera were already "protective." Although the term "protection" was not defined, one may wonder whether these exclusions were made "a posteriori," since the reason given for exclusion was that a preexisting protection precluded the demonstration of any additional protection after immunization. If "protection" is defined as having high survival rate, excluding the volunteers with protective preimmune sera would reduce the data to only those with low survivals in the preimmune sera.

If one supposes that immunization does not enhance the protective activity at all, the sampling distribution of survivals for the entire population would be the same for both pre- and postimmune sera. With the design of excluding protective preimmune sera, the sampling distribution of survivals in preimmune sera is artifically skewed toward low values, whereas the sampling distribution remains unskewed for postimmune sera. In other words, the results are biased toward showing increased survival rates for postimmune sera, which might lead to significant statistical differences even if the populations of pre- and postimmune sera were actually similar. Therefore, the exclusion of the results observed with the sera of more than half of the preimmune volunteers represents a crucial problem. Moreover, in experiments in which the survival of 8–10 mice injected with preimmune sera is compared with the survival of a similar small number of mice injected with postimmune sera, the experimental variability could account for important differences in terms of observed percentage survivals. Thus, the authors' conclusions cannot be accepted unless they report the sampling distribution of the entire population of preimmune sera and the experimental variability in their model.

Although the studies by McCabe et al. [4] and by DeMaria et al. [5] represent an enormous amount of work, we do not believe that the data reported support in a definitive way the conclusions drawn.

J. D. BAUMGARTNER, M. M. WU, AND M. P. GLAUSER
Division of Infectious Diseases, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

References

1. Ziegler EJ. Protective antibody to endotoxin core: the emperor's new clothes? J Infect Dis **1988**;158:286–290
2. Greisman SE, DuBuy JB, Woodward CL. Experimental gram-negative bacterial sepsis: reevaluation of the ability of rough mutant antisera to protect mice (40231). Proc Soc Exp Biol Med **1978**;158:482–490

Please address requests for reprints to Dr. J. D. Baumgartner, Division of Infectious Diseases, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland.

3. Griesman SE, Johnston CA. Failure of antisera to J5 and R595 rough mutants to reduce endotoxemic lethality. J Infect Dis **1987**;157:54-64
4. McCabe WR, DeMaria A Jr, Berberich H, Johns MA. Immunization with rough mutants of *Salmonella minnesota*: protective activity of IgM and IgG antibody to the R595 (Re chemotype) mutant. J Infect Dis **1988**;158:291-300
5. DeMaria A Jr, Johns MA, Berberich H, McCabe WR. Immunization with rough mutants of *Salmonella minnesota*: initial studies in human subjects. J Infect Dis **1988**;158: 301-311

THE JOURNAL OF INFECTIOUS DISEASES • VOL. 160, NO. 2 • AUGUST 1989
 0022-1899/89/6002-0032$01.00

Reply

To THE EDITOR – The letter by Baumgartner et al. [1] challenges the findings and conclusions in our articles in the August 1988 issue [2, 3].

Although they do not challenge development of enhanced resistance after immunization, they question whether the protective activity of lapine and human post-Re immunization antisera was clearly established as due to Re IgM antibody. Also, Sephadex G-200 immunoglobulin fractionation is criticized because the fraction 1 (IgM) contains "many other proteins"; however, these putative contaminants of our preparation are not identified.

Sephadex G-200 immunoglobulin fractionation is a standard technique [4]. Although other methods may afford more complete purification, Sephadex G-200 is particularly useful for IgM isolation because it excludes proteins ≥700,000-800,000 M_r [5]. Few other serum proteins are this large (only α_2 macroglobulin immediately comes to mind). It would have been helpful had Baumgartner et al. [1] identified the alleged contaminating proteins, other than immunoglobulin, that are induced by immunization with rough (R) but not smooth gram-negative bacilli, persist for weeks, and clearly protect against challenge with heterologous gram-negative bacilli.

The suggestion that absorption experiments would support antibody as the protective modality indicates unawareness that these are described in two previous publications [6, 7]. Also, protective activity of whole Re and J5 antisera and their IgM fractions is completely removed by absorption on Re and J5 lipopolysaccharide-Sepharose columns. More recent studies (unpublished data) of a component vaccine of modified Re or J5 LPS coupled to a protein "carrier" (converting a T-independent to a T-dependent antigen) demonstrated protection in the IgM fraction after primary immunization, but that "booster" immunization induced protective activity localized almost exclusively to IgG. These studies with component vaccines further confirm the validity of our conclusions.

Regarding the comments in paragraph 3, "They concluded that an enhanced protective activity was demonstrated after immunization in most subjects" and "this strong conclusion is not fully supported by the data," we believe that Baumgartner et al. [1] misinterpreted our statements. We concluded that postimmunization protection was demonstrated in almost all *whose preimmunization sera were not protective* (see pp. 303, 307, and 308 [2]).

The postulation in paragraph 3 is not clear. If it is implied that those with protective preimmunization serum samples should be classified as vaccine failures, this is an erroneous assumption. These represent volunteers with preexisting high titers of specific protective antibody to the test strains, which precluded testing with these strains. Exclusion does not indicate that development of protective activity would not be demonstrable if other assay strains were available. This was illustrated in numerous subjects with preexisting protection against one assay strain but in whom postimmunization protection was demonstrated against the other test strains. Ideally, we would have preferred to screen all volunteers initially and select only those with nonprotective sera for immunization, but this adversely affected volunteer recruitment. To obtain adequate numbers of volunteers, all healthy subjects were accepted for immunization. We have previously demonstrated that type-specific antibody exerts much greater protective activity than the lesser but significant protection afforded by antibody to R mutants [6] as to mask protection induced by the latter. Therefore, an a priori decision was made to assay postimmunization protection only in those whose preimmunization sera were not protective. Because there would be no justification for attempting to demonstrate development of protective activity in subjects in whom such activity is present preimmunization, those with preexisting protective activity were used only to assess vaccine toxicity and antibody response.

Finally, the questions concerning the meaning and significance of protective activity are best illustrated by review of tables 2, 4, and 5 [2]. We hope this information clarifies our work. We believe the available data clearly support the conclusions expressed in these publications.

WILLIAM R. MCCABE, ALFRED DEMARIA, JR., MARGARET A. JOHNS, HILDA BERBERICH
Maxwell Finland Laboratory for Infectious Diseases, Boston City Hospital, Boston, Massachusetts

References

1. Baumgartner JD, Wu MM, Glauser MP. Interpretation of data regarding the protection afforded by serum IgG or IgM antibodies after immunization with the rough mutant R595 of *Salmonella minnesota*. J Infect Dis **1989**;160:347-348
2. McCabe WR, DeMaria A Jr, Berberich H, Johns MA. Immunization with rough mutants of *Salmonella minnesota*: protective activity of IgM and IgG antibody to the R595 (Re chemotype) mutant. J Infect Dis **1988**;158:291-300
3. DeMaria A Jr, Johns MA, Berberich H, McCabe WR. Immunization with rough mutants of *Salmonella minnesota*: initial studies in human subjects. J Infect Dis **1988**;158: 301-311