Immune Serum-Mediated Effects on Brucellosis Evolution in Mice

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Immune serum injected into mice before a footpad challenge of virulent strain *Brucella abortus* 544 can prevent dissemination of infection to the spleen. Sera from mice infected with *Brucella* for at least 2 months or from mice vaccinated with a protein-bound cell wall peptidoglycan *Brucella* fraction completely stopped dissemination. *Brucella* lipopolysaccharide and polysaccharide cross-reacting *Yersinia* immune sera reduced dissemination. Both peptidoglycan and lipopolysaccharide immune sera injected simultaneously with an intravenous challenge caused a shift in *Brucella* from spleen to liver. When immune sera were injected simultaneously with an intravenous challenge, the kinetics of splenic infection showed two effects: an early one, optimally measured at day 7 postchallenge, showed reduced numbers in the spleen due to the shift of *Brucella* to the liver; a late effect, measured at day 21 postchallenge, showed reduced numbers in spleen and liver with nearly complete clearance by day 49 postchallenge. *Brucella* lipopolysaccharide and cross-reacting bacterial antisera induced the early effect only, whereas peptidoglycan and infected mouse sera induced both effects. When peptidoglycan immune serum was injected 2 or 7 days after intravenous challenge, the late effect was somewhat reduced. Hence, immune sera to protein and polysaccharide surface antigens can (i) prevent dissemination of systemic infection and (ii) help destroy intercellular bacteria (protein antigen only). These effects may represent a large part of vaccinal immunity.

Brucellosis, an infectious disease of certain animals and humans, evolves in successive pathogenic steps: passage through mucous membrane, lymph system drainage and colonization of nodes, and systemic dissemination through blood to main reticuloendothelial system organs and, on occasion, to certain target organs such as the placenta (22). The immunological response of the host can stop and reverse the invasive process, leading eventually to self-cure. However, visceral lesions may be established that hinder the cure, and from these foci the infectious process can start again. Fairly good immunity can be induced in laboratory and farm animals by either living attenuated vaccines, such as *Brucella abortus* strain 19, or killed oil adjuvant bacteria, such as *B. melitensis* H.38 vaccine. Two bacterial fractions are endowed with at least a part of the immunogenic activity for mice, lipopolysaccharide (LPS) and the protein-bound cell wall peptidoglycan (PG) (5, 11). Immune mechanisms triggered by these vaccines are not well understood. Since in *Brucella* infection bacteria are mainly localized intracellularly, cellular mechanisms have been

emphasized, leading to a dogmatic preference for living vaccines. Antibody-mediated phenomena may contribute, however, to the overall immunity, either directly by restricting dissemination of infection (25, 38, 39) or through cell-effector mechanisms (31, 32).

An impartial assessment of mechanisms of antibrucella immunity would help in devising better vaccines and more effective methods for potency assay of vaccines (28).

We prepared several types of immune sera in mice and tested their effects on the course of experimental murine brucellosis. Using the footpad route of inoculation, we showed a strong immune serum-mediated lymph node barrier effect, preventing systemic dissemination of infection. Using the intravenous (i.v.) route, we demonstrated (i) a partial transfer of the challenge from spleen to liver and (ii) an enhanced destruction of spleen-localized *Brucella*.

MATERIALS AND METHODS

Basic protocols. In the first protocol, the challenge was injected into the footpad to study the effect of immune serum on the passage of challenge through the

popliteal lymph node (25). In the second protocol, the challenge was injected by the i.v. route, which bypasses the first steps of infection, to study the systemic infection.

In the footpad protocol, 5- to 6-week old Swiss female mice (two strains of mice giving similar results [29] were randomly used, CD-1 from Charles River, Elbeuf, France, or OF1 from Iffa-Credo, St. Germain, France) were injected i.v. with 0.1 ml of normal or immune serum; then about 16 h later, about 10^6 CFU of *B. abortus* in 0.05 ml were injected into the hind footpad. The exact CFU number was determined for each experiment. Groups of five mice, unless otherwise stated, were killed by cervical dislocation at indicated times after challenge for enumeration of *Brucella* in spleen. The spleen was removed aseptically, weighed and homogenized with a glass grinder in a buffered saline solution, and then appropriately diluted and seeded onto two plates of Trypcase soy agar (Biomérieux, Marcy l'Etoile, France). After 5 days of incubation in a 10% CO₂ atmosphere, CFU were counted and expressed by the log₁₀ per spleen value. When no colony was found, we considered that there was 1 CFU on one of the two plates to compute means and standard error.

In the i.v. protocol, the mice were injected i.v. with the challenge (2×10^5 CFU in 0.1 ml) and the serum (0.1 ml). Serum and challenge were either (i) mixed, incubated at 37°C for 30 min with gentle shaking, and then injected; or (ii) injected successively, the serum being injected either 16 h before or 2 or 7 days after the challenge. Groups of five or six mice, as indicated, were killed for enumeration of *Brucella* in spleen at the stated times after challenge, usually at 7 and 21 days. In some experiments, liver and both lungs and popliteal lymph nodes were also examined with appropriate dilution for enumeration.

Bacterial strains and vaccinal fractions. Challenge strain *B. abortus* 544, originally obtained from Central Veterinary Laboratories (Weybridge, U.K.), is a virulent strain long used in experimental bovine and murine brucellosis. A batch of standard challenge was prepared and lyophilized as indicated elsewhere (28). For each assay, a new ampoule was taken and properly diluted in buffered saline solution without subculture.

Strains used for preparation of vaccines were as follows: *B. abortus* 19, an avirulent vaccinal strain (National Animal Diseases Laboratory, Ames, Iowa); *B. melitensis* 16M, a virulent strain (originally obtained from Central Veterinary Laboratories), used for extraction of LPS; *Yersinia enterocolitica* serotype VIII, strain PY 636, and serotype IX, strain MY 79 (Y₈ and Y₉, originally obtained from P. Ahvonen through R. Diaz) (Y₉ is known to bear an LPS that crosses strongly with *Brucella* LPS [1, 10, 16], and Y₈ was used as a negative control); *Salmonella urbana* (Urb) (Pasteur Institute Collection), which reacts slightly with *Brucella* LPS via a small analogy in the polysaccharide structure (15).

Three types of vaccines were used. (i) Formalin-killed microbial cells (MC) were prepared as indicated elsewhere (5) from *B. abortus* 19 (MC.B19), Y₈ (MC.Y₈), Y₉ (MC.Y₉), and Urb (MC.Urb). (ii) Vaccinal fractions have been described elsewhere (5, 11). (iii) For PG, briefly, cells of *B. abortus* were inactivated by heating at 65°C for 1 h and broken with glass

beads in a Braun MSK homogenizer. The cell walls were recovered by centrifugation, and then the PG was extracted by boiling sodium dodecyl sulfate at 100°C. Residual cell walls obtained by centrifugation were then washed in distilled water and lyophilized. (iv) LPS is the LPS (endotoxin) fraction extracted by the phenol-water method from either the phenol phase (*B. melitensis* 16M and Y₉) or the aqueous phase (Y₈ and Urb) (16, 18). After extraction from the appropriate phase, LPS were purified further by methanol precipitation, washed, and lyophilized.

Immune sera. Immune sera were prepared in groups of 30 to 120 mice. Mice were (i) infected with strain *B. abortus* 544 and the serum was obtained by cardiac puncture after 3 or 4 months of infection (or at different times after infection), or (ii) they were vaccinated. The vaccines, either killed microbial cells or lyophilized fractions, were suspended or solubilized in buffered saline solution and then mixed by vigorous shaking with an equal volume of the following oil adjuvant: Arlacel A, 5 g (Serva, Heidelberg; West Germany); Tween 20, 3.3 g (Serva); Bayol F or Marcol 52 (Esso, Paris, France), up to 100 ml; sterilization at 120°C for 20 min. The vaccines were injected subcutaneously twice 5 weeks apart, at doses of either 10⁸ cells or 100-μg fractions. Sera were usually taken 3 weeks after the second vaccination or at different times after the first and the second vaccinations and then pooled and frozen at -20°C until use. Sera from normal mice were also obtained for control groups. None of the sera were heat treated at any time.

Statistics. Variance analysis (F test) was used to compare mean responses of groups by either single comparisons or orthogonal analysis according to the experimental design (37). F values or probabilities of occurrence or both are given.

RESULTS

Antibody-mediated lymph node barrier effect. *Brucella* injected into the footpad can reach target organs, such as liver and spleen, via the lymphatic system and general circulation (22). The passage through the lymph node system, as shown in preliminary experiments, is a very fast phenomenon: 1 h after challenge, the splenic colonization was already very high and reached a plateau after 6 h. Later on this plateau was very stable, as seen in Fig. 1, where levels at 2 and 7 days postchallenge were about equal. Subsequently, we measured the colonization at day 2 only.

Immune serum injected before challenge can stop the invasive process at the popliteal lymph node (25), where we recovered *Brucella* in large amounts in either control or serum-treated mice. Several immune sera were first tested in the system (Fig. 1). They can be classified into three groups. (i) The first group included sera inducing a strong barrier effect that kept splenic colonization below or about 1 on the log scale. These were from mice vaccinated with *Brucella* MC or PG and from *Brucella*-infected mice. When such a serum was strong enough to prevent any systemic dissemination beyond the node at day

2, its effect lasted at least 1 month. (ii) The second group included sera having an intermediate effect, with the response being between 1 and 2. These were obtained by vaccination with *Brucella* LPS and with Y₉ fraction MC or LPS (not shown). Urb antisera, either MC or LPS (not shown), had slight but reproducible effects. (iii) Control and MC.Y₈ sera had no effect at all.

This observation suggested that both LPS and PG antigens induce antibodies that help trap bacteria in the node, but PG is more efficient. To extend this observation, we compared the effect of dilution of serum on trapping or blocking activity of three sera (Fig. 1). PG serum had, in addition to a higher activity, a steeper slope to dilution than LPS or Urb sera. We also determined when, after vaccination or infection, the serum activity developed (Fig. 1). Although

FIG. 1. Splenic colonization 2 days and (upper) 7 days (spotted bar) after footpad challenge in mice injected 1 day before with immune serum (0.1 ml). (Upper) Sera from control (C) mice, 3-month infected mice (Inf), or mice vaccinated twice 5 weeks apart with MC or fractions (PG and LPS) from *Brucella*, *Y. enterocolitica* VIII (Y₈) and IX (Y₉), or *S. urbana* (Urb). Five mice per point. Challenge: 1.1×10^6 CFU. (Middle) Sera as above (*Brucella* PG and LPS), diluted. Three mice per point. Challenge: 1.4×10^6 CFU. (Lower) Anti-*Brucella* sera taken at different times after primary vaccination (second vaccination at 5 weeks) or after infection. Five mice per point. Mice found infected in spleen are given as a fraction. Challenge: 0.9×10^6 CFU.

some activity was already present at 1 week, it did not increase very much thereafter in LPS-vaccinated mice even after booster, whereas it increased in infected and PG-vaccinated mice. A total or near total barrier effect was attained, respectively, after 2 months or after the booster.

Effect of immune sera in transferring i.v. injected *Brucella* from spleen to liver. A challenge injected i.v. localized mainly in liver and spleen (9, 20, 26). An immune serum injected with the challenge can reduce the number of bacteria in the spleen at 2 and 7 days (2) or at 5 days (21), but the effect on liver colonization was not known.

Groups of mice were challenged i.v., the challenge being incubated in, and injected with, a normal serum or PG or LPS immune sera. Then, the *Brucella* were enumerated in liver, spleen, and lungs at successive times. Two successive experiments were pooled in Fig. 2. The second one was enlarged to 10 mice per group to obtain a better estimate of early distribution between liver and spleen. Lungs contained only a small fraction of the challenge and were deleted from the second experiment.

Table 1 analyzes the results in terms of fraction of challenge recovered from both organs. In control mice, 24 h after challenge, only a fraction of the challenge (62%) was recovered, with 73% of recovery from liver and 26% from spleen. After 48 h, bacterial growth had increased the total count 2.7 times, with most of it in the liver (96%). Immune sera drastically altered this picture by directing most of the bacteria after 24 h toward the liver (96%). Because the bacterial growth is faster in liver than in spleen, the switch led to a higher total bacterial count at day 7, mainly in liver (99%). Conversely, the spleen was less colonized after 24 h and, being less prone to support growth, was less infected in immune serum-treated mice. The early difference between control and serum-treated mice increased with time, when the bacterial killing

TABLE 1. Distribution of i.v. challenge between liver and spleen, and growth of *B. abortus* in both organs, expressed as percentage of the challenge dose in relation to time and injection of immune serum with the challenge

Serum	% of challenge at time after challenge:					
	24 h			48 h		
	Recovered in liver plus spleen	Distributed (%) between:		Recovered in liver plus spleen	Distributed (%) between:	
		Liver	Spleen		Liver	Spleen
Control	62	73	26	273	96	4
PG	110	96	4	266	99	1
LPS	135	97	3	367	98	2

FIG. 2. Distribution of the challenge among liver, spleen, and lungs and subsequent evolution of infection after i.v. inoculation of a *B. abortus* challenge inoculated i.v. and simultaneously injected with immune sera (control, ○; PG, ●; LPS, ▲). Data from 2, 7, and 21 days were from an experiment with a challenge of 2.05×10^5 and six mice per group. Day 1 was from an experiment with a challenge of 1.9×10^5 and 10 mice per group. Differences of the control versus PG + LPS (diagram, 1 df), tested by orthogonal analysis of variance, were significant when the control point was numbered. F values and probabilities of occurrence (in parentheses) were as follows: 1, 75 ($<10^{-9}$); 2, 8.83 (0.012); 3, 8.44 (0.014); 4, 61 ($<10^{-8}$); 5, 13.40 (0.003); 6, 24.61 ($<10^{-4}$); 7, 27.51 ($<10^{-4}$). Comparison of PG versus LPS is significant at day 21 only ($P = 0.008$).

phase occurred some time after day 7. At this phase, the liver was able to destroy most of the bacteria by itself, but it was strongly helped by immune sera. At day 7, for example, livers of serum-treated mice were more infected than the

control ones, but at day 21 they were significantly less infected (Fig. 2). In contrast, spleens of serum-treated mice were regularly less infected than the control ones.

In conclusion, in the i.v. model, immune sera have at least two effects. The first one transfers a higher fraction of the challenge toward the liver. Since this organ is more prone to both support growth and then to destroy the bacteria, the effect is beneficial in the long run. The second one facilitates the killing of bacteria in either liver or spleen. To study this second effect, we should avoid or minimize the first effect by measuring after early colonization has occurred, that is, 2 or 7 days postchallenge and later.

Effect of immune sera injected with i.v. challenge on long-term evolution of infection. A preliminary experiment had shown that very small differences only were observed when the serum was injected before or simultaneously with the i.v. challenge. So, we used this simultaneous protocol only in the following experiments.

In the first experiment (Fig. 3), we compared

FIG. 3. Effect of PG serum (two batches, PG1 and PG2) injected i.v. simultaneously with the challenge (*B. abortus*, 2.2×10^5) after incubation on long-term evolution of splenic infection. Comparison with two batches of normal serum and placebo (saline). Six mice per point. Vertical bar, standard error (given only at day 49, when maximum).

FIG. 4. Effect of PG serum injected i.v. simultaneously with three challenge doses on long-term evolution of splenic infection. (Challenge doses: Δ , 1.09×10^6 ; $\square$, 2.18×10^5 ; $\circ$, 4.3×10^4 .) At 49 days, some serum-treated mice escaped the progressive control of the infection, elevating the mean and widening the standard error (bar). Six mice per point.

the effects of two batches of PG serum with three controls (two normal mouse sera and one saline) on long-term evolution of splenic infection. No significant decrease of infection was observed in control groups, even after day 21. In contrast, splenic infection decreased very quickly after day 7, down to about log 2.0 at day 49 in immune serum-treated mice. At this time, however, all spleens were still infected, even if only 1 or 2 CFU were found, but 2 livers and 8 of 12 lungs were free of infection.

We showed previously in this model of experimental brucellosis (29) that the evolution of infection in control mice was dependent on challenge dose, with degree of splenic infection and challenge dose being roughly parallel. This suggested a well-regulated immune response that injection of immune serum may modify. In the present study we compared the evolution of infection in groups of mice inoculated with three challenge doses incubated in normal or PG immune serum (Fig. 4). Clearly, immune serum

had about the same efficiency in each group. The lower challenge group was almost totally cured at day 49: one mouse had only 1 CFU in spleen; four livers and two of six lungs were free. Contrary to the previous experiment, however, some mice at day 49 escaped the progressive evolution toward cure and evidenced a strong increase of infection; i.e., five mice had a level higher than 5.4.

In two other analogous experiments (not shown) which compared PG serum to serum of 3-month infected mice, the same escape phenomenon was observed in 2 of 24 mice.

Comparison of different immune sera and of dilutions of PG and LPS immune sera. Twelve immune sera were compared with two controls by enumerating the *Brucella* in the spleen at the two times we considered the most significant, 7 and 21 days (Fig. 5). Three immune sera from Y_8 or Urb were without any significant activity,

FIG. 5. Comparisons of different immune sera injected i.v. simultaneously with the challenge after incubation. Two assays with respective challenge: A, 2.2×10^5 ; and B, 2.0×10^5 *B. abortus*. Six mice per point. Symbols for sera are as indicated in the legend to Fig. 1: i.e., fraction plus bacterial species (when other than *Brucella* are given (e.g., LPS Y_9), or from 3- or 4-month infected mice (Inf 3 m). Three groups of serum can be recognized according to their effects: no effect (similar to control); early effect, at day 7; late effect, at day 21.

FIG. 6. Comparisons of PG and LPS immune sera at successive dilutions ($3 = \frac{1}{3}$). The two early and late effects are well illustrated: the early effect, shown at day 7, is common to both sera, but with a different response to dilution. The late effect, shown at day 21 by PG serum, is strongly dilution dependent. Challenge: 2.1×10^5 *B. abortus*. Six mice per point.

similar to controls. Four sera, two batches of *Brucella* LPS serum and two from Y₉ (MC and LPS) sera, had a strong activity as shown at day 7, but the difference between control and serum-treated mice at 21 days did not widen or did so only slightly. Five sera, two from infected mice, one from MC, and two from PG *Brucella* vaccines, evidenced a strong early (day 7) and a strong late (day 21) effect.

Early and late effects were more clearly illustrated by dilutions of PG and LPS immune sera (Fig. 6). Both sera exhibited a strong early effect, but the activity of LPS serum decreased with dilution, whereas dilution of PG serum had no effect up to a dilution of 1/81 at 7 days. On the contrary, PG serum was strongly dose dependent at day 21.

Effect of immune serum injected 2 or 7 days after challenge. When *Brucella* are injected i.v., they colonize the spleen almost immediately and disappear from blood in a few hours (2, 21). In a preliminary experiment, we observed that spleen counts at 4, 8, and 24 h postchallenge did not differ significantly. Multiplication started at around 32 to 48 h. Most of the bacteria are considered to be in an intracellular location at day 2 and even more at day 7 postchallenge. Thus, one wonders how immune sera can modify the outcome of infection so drastically as late

as 21 and even 49 days after injection. To answer this question, groups of mice were first challenged; then, after a delay of 2 or 7 days, they were injected with either normal or PG, LPS, or infected immune serum (Fig. 7). They were killed 7 or 21 days later (that is, respectively, 2 or 7 days later than in previous experiments relative to challenge).

Seven days after injection of serum, all treated groups had somewhat lower spleen counts than controls, and at day 21 the differences were significant.

The overall aspect of responses was very similar to those of previous experiments. Absolute difference between control and serum-treated groups, however, decreased with delay in serum treatment. For example, 21 days after challenge, the differences between control and serum-treated groups were on average 2.5 (log) when serum and challenge were mixed, about 1.5 when the delay was 2 days (Fig. 7A), and <1 when delay was 7 days (Fig. 7B).

This may simply mean that the bacteria were progressively less accessible to antibodies as the lesions evolved.

DISCUSSION

Immune serum can alter drastically the evolution of experimental brucellosis in mice by at least three effects: lymph node barrier, spleen to liver transfer, and reduction of splenic infection.

FIG. 7. Effect on splenic infection of immune sera injected 2 or 7 days after i.v. challenge, 2.2×10^5 . Six mice per point. Because of the similarities of response of the three sera, significance was tested by orthogonal analysis of variance (control versus PG + LPS + infected, 1 df). The differences were not significant at day 7 after injection of serum and (A) highly significant ($F = 21.2$; $P < 10^{-4}$) and (B) significant ($F = 7.42$; $P = 0.013$) 21 days after injection of serum.

The lymph node barrier effect first demonstrated by Pardon (25) from studies by Sulitzeanu (39) stops the footpad-injected bacteria at the popliteal lymph node. Depending upon the type and titer of serum, the effect may be total or partial. *Brucella* LPS and anti-LPS cross-reacting bacteria (Y_9 , Urb) antisera had only a partial effect which appeared as soon as 1 week after vaccination. In contrast, *Brucella* PG, MC, and infected mice sera slowly developed a total or near total effect.

The spleen to liver transfer effect results from a higher affinity of "opsonized" challenge bacteria for the liver than for the spleen. This effect could have been expected from experimental salmonellosis studies (3) and from in vitro studies in which immune serum enhanced attachment of *B. abortus* to Kupffer cells (17). Both our PG and LPS sera gave very similar results in that respect. Since the liver is a better place than the spleen for *Brucella* to multiply at the beginning of infection (Fig. 2), the total body load of bacteria was first (at day 7) higher in serum-treated mice than in controls. Thereafter, the liver being more able to destroy a greater load of *Brucella* than the spleen (Fig. 2) (9, 20, 26), it may be free of infection in serum-treated mice at day 49, when spleens were still infected even if minimally.

Reduction of the splenic infection effect can be divided in two. The early effect, seen at day 2 to 7 postchallenge, clearly results from the lower initial colonization, due to the transfer effect. Since all of our sera are able to induce this effect, notably LPS and PG as well as Y_9 sera, the most likely candidates are the protein cell wall and the polysaccharide LPS surface antigens. Two groups have recently studied the effect of immune serum and immunoglobulin 7S on brucellosis in mice. They showed a "positive" effect on splenic colonization at days 7 and 5, respectively, after i.v. challenge (2, 21). Our early or our spleen to liver transfer effect is similar to this positive effect.

The late effect results in progressive reduction of splenic infection. At day 7, the differences observed between control and serum-treated mice evidence the initial splenic colonization. These differences may stay about constant thereafter (early effect only) or may increase with time due to the late effect. We measured it conveniently at day 21, but it may be observed earlier and may last up to the end of the observation (Fig. 3 and 4). At that time (day 49), most of the serum-treated mice were minimally infected; some, however, escaped the progressive control of infection by the late effect as if, when the serum was exhausted, no relaying immunity had developed. The late effect was regularly obtained with PG, MC, and infected mice sera, but

usually not with LPS serum. Late effect activity developed slowly after inoculation or vaccination of donor mice, being at its maximum 1 to 2 months after injection, and then declined. We have studied the late effect mostly with PG serum, in three models: challenge incubated with the serum; serum administered before the challenge; and serum administered 2 or 7 days after the challenge, when the *Brucella* are supposed to be mostly in an intracellular location (2, 21). The effect was observed in these three instances, even if it was considerably lower in the third.

When PG or infected sera were administered before, or simultaneously with, the challenge, the late effect was evidenced by an accelerated decreasing phase of splenic infection, very similar to what was observed in vaccinated mice (26). Because macrophage nonspecific activation is usually considered as the main effector of this decreasing phase (9, 13, 19, 36), we looked at macrophage activation against a *Listeria* challenge in control and in *Brucella* serum-treated mice: no extra activation was observed (M. Plommet and A. M. Plommet, International Symposium on Brucellosis, Algiers, 18–20 April 1983, p. 74). We may conclude that immune sera have a direct effect on the macrophage-bacteria system, making virulent bacteria behave like avirulent ones. The role of immune sera in killing intracellular *Brucella* was first considered as nil (14, 30) even if some data may suggest a certain effect (8). Then, Ralston and Elberg (31–35), in in vitro experiments; demonstrated two factors: a macrophage-stimulating factor that increases attachment and engulfment of *Brucella* by macrophages, and a *Brucella*-inhibiting factor that restricts intracellular growth of bacteria. The factors endowed with our early (or transfer) effect and late effect may be identical to macrophage-stimulating factor and *Brucella*-inhibiting factor, respectively.

We have not tried in this paper to fully identify the serum factors or antigens or both involved in the three effects: the most interesting PG fraction, which strongly induced the three effects and is a good vaccinal fraction for mice (5, 12) and guinea pigs (6), contains three major polypeptides but also a small proportion of contaminating LPS (11; G. Dubray and C. Charriaut, Ann. Rech. Vet., in press). This precludes any clear conclusion, which may be made more difficult by the following facts: PG and LPS are both polyclonal activators (23, 24, 40), which may modify the type and avidity of immunoglobulins elicited; PG and LPS used as vaccines in mice interact negatively on immunity (5); in murine brucellosis, macrophages do have an elevated general activity with, in particular, an enhanced immunoglobulin G Fc receptor activi-

ty, which may facilitate attachment and lysis of bacteria (4). However, before research in progress using purer fractions, antibody titrations of each immunoglobulin class, and complement is completed, it is clear from the use of polysaccharide cross-reacting bacterial antisera that polysaccharide antibodies were involved in lymph node barrier and transfer (early) effects, but that only PG (and infected) sera exhibited a total barrier and a regularly strong late effect.

We do not know whether the three effects described here have equivalents in large animals and humans. We only know that the barrier effect was shown in guinea pigs after vaccination or transfer of immune serum (27; P. Pardon, personal communication). But if so, the humoral arm of vaccinal immunity would be of paramount importance. It could trap the bacteria in the regional lymph node on their way from the portal of entry to systemic dissemination. It could divert a bigger part of invading bacteria toward the main scavenger organ, the liver. It could help splenic phagocytic cells to kill intracellular bacteria. Brucellosis, however, is a slowly evolving disease: immune response to infection itself may add to prechallenge immunity in protection and cure. We need better knowledge of regulation and expression of these immune mechanisms to devise better vaccines.

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