Vet. Res. 33 (2002) 55–69

DOI: 10.1051/vetres:2001006

Original article

Cross-protection of *Salmonella abortusovis*, *S. choleraesuis, S. dublin* and *S. gallinarum* in mice induced by *S. abortusovis* and *S. gallinarum*: bacteriology and humoral immune response

Serge BERNARD*, Roger BOIVIN, Pierrette MENANTEAU, Frédéric LANTIER

Pathologie Infectieuse et Immunologie, INRA, Centre de Tours, 37380 Nouzilly, France

(Received 8 February 2001; accepted 21 August 2001)

Abstract – Cross-protection induced by primary infection with Abortusovis and Gallinarum was examined against challenge injection with these Salmonella serotypes as well as with Dublin and Choleraesuis, the other virulent serotypes. Abortusovis induced efficient protection against the other Salmonella. Gallinarum was ineffective against Choleraesuis. Even with low multiplication in mice, the Gallinarum J91 strain induced a weak but significant protection against Dublin (same O group serotype). The antibodies in the blood of mice were tested with ELISA specific for the Salmonella antigens used to prime or to challenge animals. The Gallinarum J91 strain was detected to be more antigenic in ELISA than the other Salmonella antigens. It is difficult to conclude on a correlation between IgM or IgG antibodies and induction of protection, because of the variability in immune response according to the different serotype used. Nevertheless, the negative linkage between a number of bacteria in the spleen of mice challenged with Gallinarum and Dublin, and the level of IgM and IgG antibodies specific for the challenging serotype, showed that humoral immune response could be one element of cross-protection, mainly by the immune response against the same O serotype.

Salmonella / cross-protection / antibody / *Ity*^{r} mice

Résumé – Protection croisée contre *Salmonella abortusovis, S. choleraesuis, S. dublin* et *S. gallinarum* induite chez la souris par *S. abortusovis* et *S. gallinarum*: charge bactérienne et réponse immunitaire humorale. La protection croisée induite par Abortusovis et Gallinarum a été étudiée après une épreuve vis-à-vis de ces deux sérotypes de Salmonelles ainsi que de Dublin et Choleraesuis, autres sérotypes de Salmonelles. Abortusovis induit une protection croisée significative contre les autres sérotypes de salmonelles. Gallinarum n'induit pas de protection effective contre Choleraesuis. En

* Correspondence and reprints
Tel.: (33) 2 47 42 78 86; fax: (33) 2 47 42 77 79; e-mail: Serge.Bernard@tours.inra.fr

revanche même avec une multiplication faible chez la souris, Gallinarum J91 est capable d'induire une protection faible mais significative contre Dublin (même sérotype O). Les anticorps du sang ont été titrés par ELISA contre les antigènes de salmonelles spécifiques de la souche utilisée pour la sensibilisation ou l'épreuve. Gallinarum J91 a donné des réactions de reconnaissances antigéniques, bien supérieures à celles obtenues avec les autres sérotypes. Devant la variabilité de réponses des différentes souches vis-à-vis des différents antigènes, il est difficile de relier la présence d'anticorps IgM ou IgG à l'induction d'une protection. Toutefois, la liaison négative qui existe entre le taux de bactéries dans la rate de souris éprouvées par Gallinarum ou Dublin et le taux d'anticorps IgM et IgG dirigés contre la souche sensibilisante de Gallinarum, pourrait être expliquée par une réponse immunitaire contre le même sérotype du groupe O.

Salmonelle / protection croisée / anticorps / souris *Ity*^{r}

1. INTRODUCTION

Salmonella diseases cause severe and economically important diseases in all farm animals with impairments in production and consequential effects on the economy. Salmonellosis is also currently one of the most important zoonoses in Europe. The protection and welfare of animals against Salmonella disease require the use of effective live vaccines, more suitable to induce both humoral and cellular immune response [25]. Host-specific Salmonella serotypes are only able to infect one or a few animal species, because the infection is controlled in the other species. Since Salmonella bacteria are used more and more to carrying foreign genes, it is necessary to learn more about the possibility of immunisation or cross-protection of different farm animals with these host-specific Salmonella serotypes [36, 47]. *S. dublin* is the cause of systemic salmonellosis in cattle [41, 45]. It constitutes a considerable problem for the dairy industry in European countries, and for human pathology. *S. abortusovis* causes abortion and mortality in new-born lambs. This bacteria occurs mainly in the uterus and causes economic losses throughout the Mediterranean region [33]. *S. gallinarum* is associated with fowl typhoid, one of the most important diseases in reared chickens [1]. *S. choleraesuis* is associated with systemic salmonellosis in pigs. It is presently only found in a few European countries, including England. In the USA large outbreaks have occurred in very low prevalence areas. The presence of this bacteria, even at a low level, is a permanent threat to the European pig industry [15]. *S. dublin* and *choleraesuis* are zoonotic and account for large cases of non-typhoid systemic human salmonellosis in England and many other countries [12]. First experiments were undertaken in mice to study the host-immune response toward infections with host-specific Salmonella serotypes and to investigate if primed animals were protected against a challenge with other host-specific serotypes. Two different sets of Salmonella serotypes were compared using resistant *ity*^{r} (Nramp1 gene) congenic C.CB mice. Resistant *ity*^{r} mice were chosen in order not to kill all the animals injected. Through recent studies it has been demonstrated that this gene has pleiotropic effects on the humoral and cellular immune response [14, 32, 38]. Humoral IgM and IgG antibody immune response was assessed against bacterial antigens specific for the Salmonella used to prime or to challenge the animals. Even using resistant mice, it was not possible to prime animals with *S. dublin* and *choleraesuis*, since these serotypes were too virulent. The two *S. abortusovis* serotypes induced good protection against the other Salmonella serotypes, while protection with *S. gallinarum* was less efficient than that induced with

S. abortusovis and ineffective against *S. choleraesuis*. During the time course of infection, the level of antibody was related to the bacterial number in each animal. After challenge injection, while no relationship between IgM antibody level and protection was shown for *S. gallinarum*, a good level of protection was always linked with high IgG response against Salmonella antigens used for the challenge, whatever the serotype used to prime mice.

2. MATERIALS AND METHODS

2.1. Mice

The studies were conducted with *Ity*^{r} C.CB mice. These mice are congenic with *S. typhimurium*-susceptible BALB/c mice except for a small segment of chromosome 1 derived from *S. typhimurium*-resistant CBA mice. Mice were raised in filtered air, with free access to water and sterilised food. Mice were infected when 8–10 weeks old.

2.2. Bacteria

Four different serotypes were used to immunise and challenge mice. Abortusovis (SAO) is a serotype specific for sheep (SAO 15-5; gift of P. Pardon, INRA, Nouzilly France; SS44; gift of S. Rubino, University of Sassary, Sassary, Italy). Gallinarum (SG) is a serotype specific for birds (SG J91 and 4223, gift of J.E. Olsen, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark). Dublin (SD) is a serotype specific for cattle, but can also infect humans (SD 2229 and SD 3246; gift of T. Wallis, Institute for Animal Health, Compton, United Kingdom). Choleraesuis (SCS) is a serotype specific for pigs, but also for humans (SCS A50 and SCS 14/74; gift of T. Wallis).

2.3. Bacterial inoculum and experimental infections

Bacteria were grown on tryptic soy agar (TSA, BioMérieux, Lyon, France). The strains were kept in a preservative agar (Pasteur, Paris, France). An isolated colony selected on a plate issued from the preserved culture was transferred onto TSA slopes. An overnight culture at 37 °C was used to prepare bacterial suspensions in phosphate-buffered saline (pH 7.4). The turbidity of each suspension was compared to a standard curve to estimate the bacterial concentration. The purity and the number of viable organisms were counted after plating serial dilution and culture (48 h).

2.4. Samples and bacterial counts in organs

Mice were killed by cervical dislocation and were exsanguinated at different times after inoculation. Spleens and livers were aseptically removed, weighed and stored for a short period at –20 °C. Blood samples from each mouse were centrifuged at 4 °C and sera were collected and stored at –20 °C.

Spleens were thawed at room temperature. Spleens were diluted 1/10 (W/V) in saline solution (0.9% NaCl) and serial 10-fold dilutions were plated. Colonies were counted after 48 h or 72 h incubation at 37 °C. Viable counts of salmonellae were expressed per organ as log10. The results of each experimental group were expressed as means and standard errors of the values. Organs without a colony were considered as infected by one bacterium and means were calculated from all mice of the experimental groups.

2.5. Serotypes and doses used to prime and challenge mice

For determination of serotypes and doses used to prime and challenge mice,

infections were undertaken by injection into the footpad of mice with 50 µL bacterial suspension. Primary infection and antibody responses were performed with 6 mice (3 males and 3 females), for each time point. Level of infection was assessed for 3 weeks, on Days +3, +7, +10, and +21. No significant difference was found in the level of bacteria in the spleen of males and females for the four serotypes studied (data not shown). Generally, maximum Salmonella colonisation in the spleen appeared between Days 7 and 10. Gallinarum was the exception, since no peak of bacteria was detected, and the number of Salmonella slightly decreased from Day +3 to Day +21 (data not shown). Dose-infection curves showed no significant difference between the two different strains for each Salmonella serotype used (data not shown). The level of bacteria in the spleen on Day 7 was chosen as a good estimator of protection after challenge injection.

Dublin (SD 2229) and Choleraesuis (SCS A50) were found to be extremely virulent, much more than Abortusovis (SAO 15-5) or Gallinarum (SG 4223). Even when mice genetically resistant to Salmonella, and with limited doses of bacteria (less than 50 Salmonella per mouse) were used, these serotypes killed most infected animals (data not shown). We concluded that Dublin and Choleraesuis were not suitable with a protocol elaborated for a period of 6 weeks.

Cross-protection experiments and antibody responses were performed with 12 mice (6 males and 6 females). Table I gives the doses used to prime and challenge mice with each strain of Abortusovis and Gallinarum. The first priming injection was done into the left footpad. The challenge injection was performed on Day +35 after the first injection, into the right footpad. Animals were sacrificed on Day +7 after the challenge. The doses for the challenge injection were chosen to obtain, in the spleen on Day +7, bacterial infection close to 10^6, except for Gallinarum where a higher non lethal dose was applied.

2.6. Dosage of immunoglobulin isotype antibodies in mouse sera

IgG and IgM antibodies in sera were measured by the ELISA technique. Plates were coated with 50 µL of heat-killed bacteria and dried at 37 °C overnight. For each serotype, an equivalent quantity of bacterial antigens, on the basis of turbidity (Optical

Table I. Serotypes and doses used to prime and challenge animals. Mice were primed in the left footpad and challenged in the right footpad.

	Abortusovis primed			Gallinarum primed		
	Serotype		Dose	Serotype		Dose
Priming	Abortusovis	15–5.	6.6×10^5	Gallinarum	J91	5.1×10^5
Challenge	Abortusovis	15–5.	1.1×10^6	Abortusovis	15–5.	1.1×10^6
	Gallinarum	J91	4.9×10^6	Gallinarum	J91	1.1×10^6
	Dublin	2229	4.9×10^5	Dublin	2229	5.1×10^5
	Choleraesuis	A50	3.8×10^5	Choleraesuis	A50	5.1×10^5
Priming	Abortusovis	SS44	5.5×10^4	Gallinarum	4223	4.5×10^5
Challenge	Abortusovis	SS44	1.0×10^6	Abortusovis	SS44	8.8×10^5
	Gallinarum	4223	4.4×10^6	Gallinarum	4223	4.4×10^6
	Dublin	3246	4.7×10^5	Dublin	3246	5.3×10^5
	Choleraesuis	14/74	3.2×10^5	Choleraesuis	14/74	3.2×10^5

Density at 600 nm) of the sample suspension, was deposited on wells. The number of bacteria coated per well was close to 2.5×10^7, except for Dublin, with 5×10^7 bacteria. After being dried, bacteria were fixed with 80% acetone (100 µL/well) at –20 °C for 30 min. Plates were washed twice with water, for 5 min, and 100 µL of 5% skimmed cow milk in PBS was added for 15 min at room temperature to block non-specific binding sites. Wells were then washed twice with 0.05% Tween 20 (Sigma) in PBS (PBS-Tween) for 5 min. Serum samples were two fold diluted in PBS, 0.05% Tween, 5% skimmed milk from a 1/50 initial dilution, and incubated (100 µL/well) at 37 °C for 2 h. Plates were then washed three times in water, and four times in PBS-Tween for 10 min, and incubated with conjugate peroxidase-labelled antibodies (Peroxidase-conjugated affinipure Goat anti-Mouse IgM, µ chain- specific and γ chain-specific, Jackson ImmunoResearch, USA) at 37 °C for 90 min (1/2000, 100 µL/well, in PBS- Tween, milk). Peroxidase activity was detected with 2,2-azino-3bis (3ethyl benthiaoline- 6-6-sulfonic acid) (ABTS, Boehringer- Mannheim, Germany) and H_2O_2 substrate solution (Sigma, Germany). Absorbance was read at 414 nm with an ELISA plate reader Camberra, (Titerteck, UK).

Each blood sample was tested on ELISA plates coated with the Salmonella serotypes used to prime mice (Abortusovis or Gallinarum) and the Salmonella homologue with a challenge injection (Abortusovis, Gallinarum, Dublin and Choleraesuis). Titre is the inverse of the dilution that corresponds to Logit OD = 0, from a plot of optical density (OD) logit transformation, versus twofold serum dilutions.

The level of sera antibodies was analysed after the primary and the challenge infections with the different Salmonella serotypes. ELISA was performed with specific Salmonella antigens homologous for the serotypes used for the infection, and also with the other heterologous Salmonella antigens.

2.7. Statistical analysis

The results were analysed by variance analysis and compared by the F and the Fisher protected LSD test, by linear regression with a degree of significance of $P < 0.05$ (SuperAnova, Abacus concept) or by the U test of Mann-Whitney and the Spearman Rank non-parametric test (Statview, Abacus concept).

3. RESULTS

3.1. Cross-protection

With Abortusovis serotype (SAO 15-5 and SAO SS44) (Figs. 1A and 1B), six weeks after the first contact with Salmonella, levels of bacteria in spleens of mice were close to the inferior limit of detection (between 70 and 100 bacteria per spleen). The comparison of mice, primed or unprimed, and challenged, showed significant protection for the 4 serotypes used. After challenge with the homologous Abortusovis or Gallinarum serotype, the level of bacteria in the spleen was not statistically different from mice without challenge. The level of bacteria in Abortusovis primed mice, was higher after Dublin challenge, but significantly different from non-immunised animals. Results obtained after Gallinarum priming injection (Figs. 1C and 1D) were similar for the two strains SG J41 and SG 4223; the cross-protection against the other serotypes was less important than with Abortusovis. No protection was found against Choleraesuis, and differences in bacteria counted in the spleen of Gallinarum primed and non-primed mice were no more than 1 or 2 log, compared to 3 and 4 in the other experiments with Abortusovis-primed animals. The group challenged with the same Salmonella

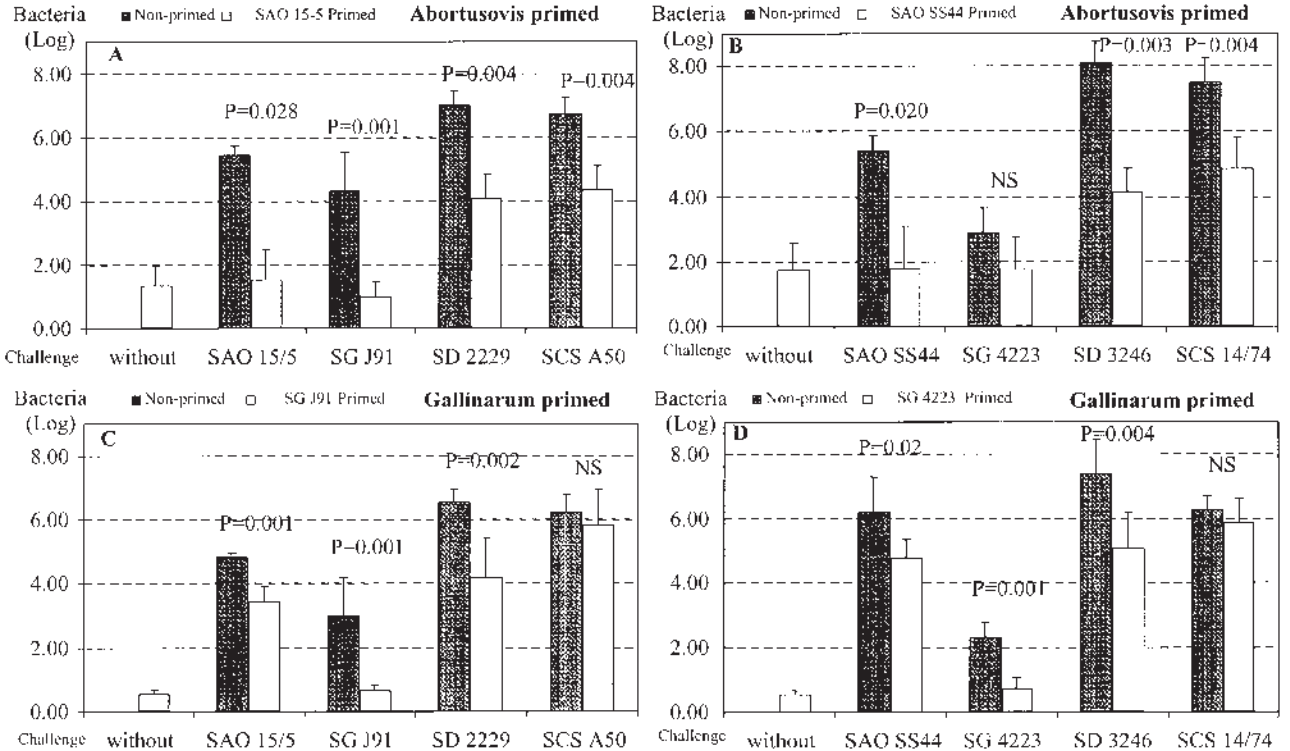

Figure 1. Cross-protection after Abortusovis (A, B) or Gallinarum immunisation (C, D): Mice were primed with SAO 15-5, SAO SS44, SG J91 or SG 4223 in the left footpad, and challenged 35 days later in the right footpad with the 4 other serotypes. Doses are given in Table I. Each value represents the mean (log10) number of bacteria in the spleen ± SE from initially 12 sex-matched mice (P = level of significant, NS: No-significant differences between primed and non-primed groups calculated with the U Mann-Whitney non-parametric test).

Gallinarum serotype was the only group with significant protection, and a level of bacteria close to non-challenged mice.

3.2. Antibody response

3.2.1. After a primary infection

Maximum IgM antibodies were found on Day +10. The level of IgM titres was not very high compared to the non-infected control mice (Fig. 2A). The IgM antibody titres were in relation to the level of bacteria injected (results not shown). The results obtained with the other Salmonella serotype antigens were equivalent, except with Gallinarum J91 antigens, for which titres were much higher than with the other antigens.

Antibody IgG titres were given on Day +21 (Fig. 2B). Antibodies titrated against Gallinarum J91 bacterial antigens were always higher even when compared with homologous serotype antigens. However, after a primary induction with Gallinarum, IgG antibody titre was low, similar to that induced by Choleraesuis A50, but in this case only one mouse stayed alive on Day +21 after infection and was highly infected (Fig. 2B).

3.2.2. After a challenge infection

Antibody titres showed were assessed on Salmonella antigens homologous to Salmonella used to challenge animals. The production of antibodies was also compared with non-primed, only challenged groups.

No significant difference was found in the level of specific IgM antibodies, between animals primed or not with Abortusovis, except for those challenged with the homologous strains (Figs. 3A and 3B). With the exception of Dublin and Choleraesuis with primed with Gallinarum J91, no difference was found in the level of IgM for animals primed with Gallinarum, whatever the serotypes used for the challenge (Figs. 4A and 4B). Patterns of salmonella IgM antibodies specific for Abortusovis or Gallinarum antigens used to prime mice (results not shown) were very similar to those described with antigens specific for the challenge. In contrast to the results obtained with IgM, it is possible to see a significant difference ($P < 0.01$ or less) in the IgG antibody level (Figs. 3C and 3D) between primed and non-primed groups, except for animals primed with Gallinarum and challenged with Choleraesuis (Figs. 4C and 4D). As for IgM, IgG antibody profiles specific for Salmonella antigens used to prime animals were similar whatever the serotype used to challenge the mice (data not shown).

3.3. Relationship between antibody response and protection

Statistical analyses with a non-parametric Spearman rank test of the relationship between the amount of antibodies and number of Salmonella in the spleen are given in Table II. For this test, the two strains of each serotype were grouped, in order to observe a global effect. When Abortusovis was used to prime mice, no correlation was detected between the level of IgM or IgG antibodies and the level of bacteria, whatever the antigens used, except when Gallinarum was used to challenge the animals. In this case a negative correlation was observed between the level of IgM and IgG antibodies specific for the challenge and protection. In Gallinarum-primed mice, A strong negative correlation was observed between the level of IgM or IgG and the level of bacteria (positive correlation for protection) when the animals were challenged with Gallinarum or Dublin (same O group serotype). However, a positive correlation exists in groups primed with Gallinarum and challenged with Abortusovis, between the level of IgG antibodies and the level of Abortusovis. In this case, antibodies could be the reaction of mice against the bacteria multiplication.

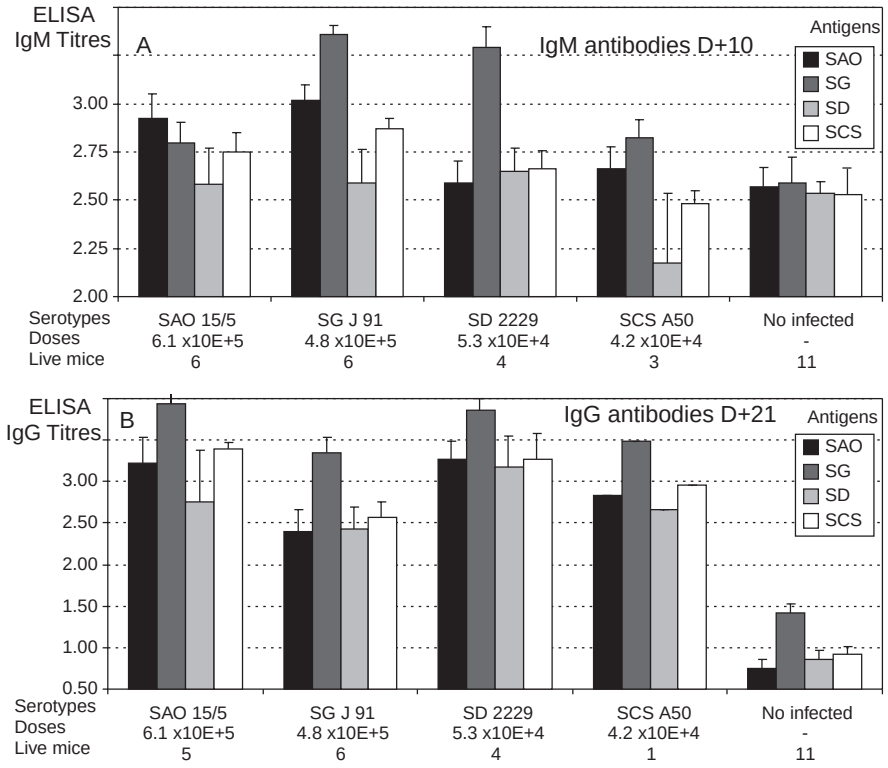

Figure 2. IgM and IgG antibody titres against the 4 serotype antigens. IgM antibody titres of serum were assessed on Day +10 after infection (A) and for IgG on Day +21 (B). Antigens of *S. abortusovis* (SAO black), *S. gallinarum* (SG heavy grey), *S. dublin* (SD light grey), and *S. choleraesuis* (SCS white), were coated on microplates. ELISA titres are given as the mean of log10 values ± SE. Serotype, number of bacteria used for inoculation and number of mice still alive at the time of sampling are noted under the bars.

4. DISCUSSION

One objective of this work, in the framework of a European contract, was to study the host-immune response towards infections with host-specific Salmonella serotypes. This knowledge is important since a variety of antigens from a number of different other organisms have been expressed in Salmonella carriers [36] with great variation in the immune response [38]. One factor that might have a great effect on the efficacy of the salmonella carrier is the existence of pre-existing immunity to the carrier strain. Many contradictory publications concerning immunity have claimed the involvement of cellular mechanisms or humoral antibodies. The reality is more complex; natural and acquired resistance to Salmonella depend upon combined humoral and cellular immune responses of the infected host [21].

Using live attenuated strains mainly with Typhimurium, Dublin or Choleraesuis results depended on the attenuated strains

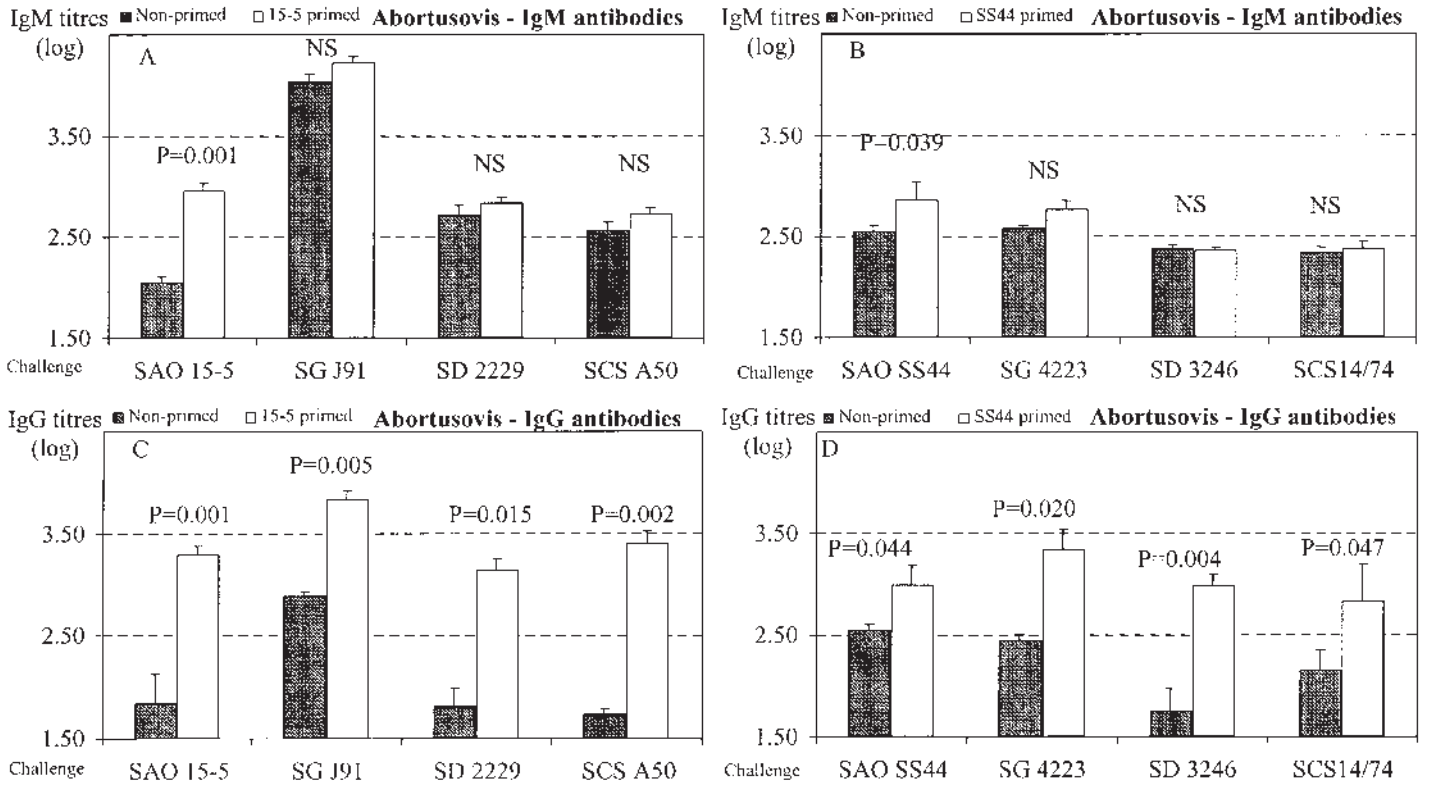

Figure 3. A comparison of IgM and IgG antibody titres in groups of mice primed or not with 15-5 (A, C) or SS44 (B, D) Abortusovis serotype , and challenged with Abortusovis, Gallinarum, Dublin and Choleraesuis serotypes. Levels of IgM (A, B) and IgG (C, D) antibodies specific for Salmonella antigens used for the challenge were compared between non primed animals (closed) and Abortusovis primed animals (open). (P = level of significant, NS: No-significant differences between primed and non-primed groups calculated with the U Mann-Whitney non-parametric test).

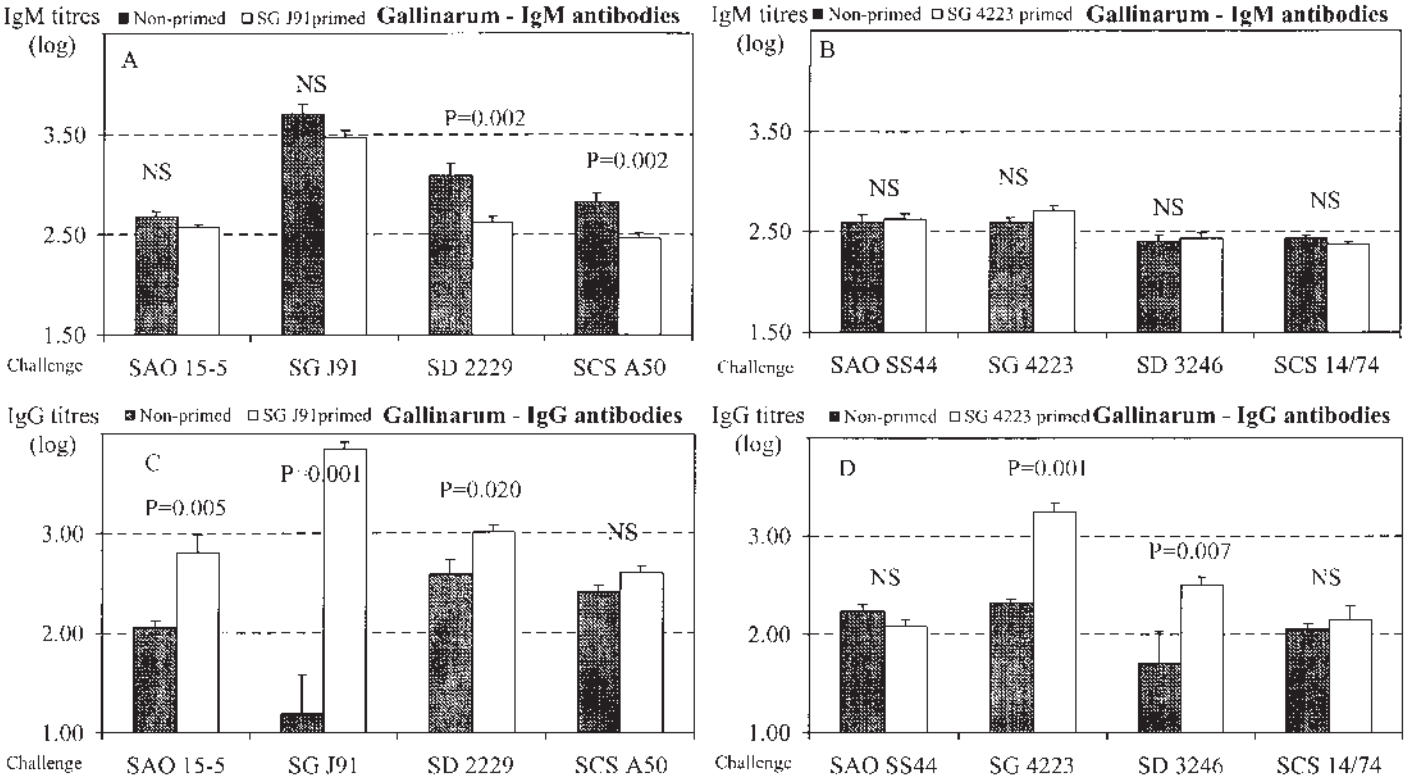

Figure 4. A comparison of IgM and IgG antibody titres in a group of non-primed animals (closed) and primed animals (open) with J91(A, C) or 4223 (B, D) Gallinarum serotype and challenged with Abortusovis, Gallinarum, Dublin and Choleraesuis serotypes (see comments in Fig. 3)

Table II. Statistical analyses with the non-parametric Spearman rank test of the relationship between the amount of IgM and IgG antibodies specific of antigens used for challenge and number of Salmonella in the spleen. Limit of significance < 0.05.

Serotypes		Spearman Rank Correlation Coefficient (probability)*			
Primed	Challenged	Bacteria/IgM		Bacteria/IgG	
Abortusovis 15–5 - SS44	Abortusovis	0.09		0.11	
	Gallinarum	0.03	(–)	0.05	(–)
	Dublin	0.61		0.97	
	Choleraesuis	0.52		0.38	
Gallinarum J91-4223	Abortusovis	0.64		< 0.01	(+)
	Gallinarum	< 0.01	(–)	< 0.01	(–)
	Dublin	0.03	(–)	0.10	
	Choleraesuis	0.81		0.80	

* Statistical probability to reject the null hypothesis, positive (+) and negative (–) correlation.

used. Generally, homologous protection is better than heterologous protection [8, 23] but sometimes heterologous challenge with Typhimurium gives better protection than against homologous Choleraesuis [31]. Typhimurium live attenuated strains are also recommended to protect hens against Enteritidis [17, 18], or sheep against Abortusovis [24]. The host specificity of Salmonella infection in chickens and mice has been examined at the level of the reticuloendothelial system, with Gallinarum, Pullorum, Choleraesuis and Dublin [2]. To date, no studies have been conducted in mice on heterologous protection against four serotypes, Gallinarum, Abortusovis, Choleraesuis and Dublin specific of four different animal species, chicken, sheep, pigs and cows. Possible modifications of the pathogeny or tropism of modified attenuated strains justified the use of virulent strains to prime animals.

Genetic and immune status of the host are now included in the concept of bacterial virulence [5, 11]. In mice, early bacterial replication following infection with Salmonella is regulated by at least the *Nramp1* gene (formerly *Ity* locus) on chromosome 1, encoding for the natural resistance of mice to Salmonella, Leishmania, and BCG [35, 39]. The *Nramp 1* gene (for Natural Resistance-Associated Macrophage Protein) identified by positional cloning [43], encodes for a polytopic membrane-associated protein similar to eukaryote nitrate transporters. The NRAMP1 protein directly or indirectly regulates many events in macrophage cells [44, 48]. Recent studies have demonstrated that the expression of this gene influences not only bacterial multiplication but also humoral [14] and cellular [6] immune response, from the interaction with the bacterial lipopolysaccharide (LPS) [26] to the cascade of cytokines, [4, 9, 38], through Ag processing [22]. Thus, host genetics might also have an influence on the ability to induce a protective immune response to recombinant vaccine. The choice of wild virulent Salmonella strains for the different serotypes of Salmonella conducted us to use congenic resistant C.CB mice to restrict mortality in the injected animal. C.CB mice were selected and bred in our lab. Since numerous works have been carried out on mice which are susceptible and resistant to Typhimurium, few have been conducted on Abortusovis [3, 14, 32], Dublin [9, 23, 42, 46], Choleraesuis [10] and Gallinarum [34]. It is clear that during the first phase of infection three possibilities arise: (a) a continuous increase of bacteria in the spleen of infected

mice, (b) an increase, plateau phase and decrease of bacteria and (c) a continuous decrease of bacteria. The first pattern was observed upon infection with Dublin and Choleraesuis, and even with low doses of inoculum (less than 50 bacteria); no regulation of the infection occurred. These resistant mice ended up dying three weeks after inoculation. This very high susceptibility of mice to wild serotypes of Salmonella has also been described [2]. This was also the case with high doses of Abortusovis; but more generally during time course infection with Abortusovis, maximum infection in the spleen appeared around Day +7, followed either by the death of the animals or by a remission phase in relation to the initial dose used. The last pattern was found for mice infected with Gallinarum. Following oral inoculation, this bacterium appears to be incapable of entering the murine Peyer's patch epithelium [34].

Bacteria from each serotype were heat-killed and coated on ELISA microplates. Whatever the doses used, the best response was on Day +10 for IgM, and on Day +21 for IgG. The highest antibody titres were obtained when Gallinarum J91 antigens were used to coat the plates, in particular for IgM when mice were inoculated with Gallinarum and Dublin. The fact that these two serotypes belong to the same O group (O 1, 9, 12) could explain this observation. It is interesting to note that IgG titres were also more effective against Gallinarum J91 antigens whatever the serotype used for the injection. Since an equivalent quantity of bacteria was used to prepare the antigens, a greater common antigenic determinant seems more accessible with Gallinarum J91 compared to the others.

In cross-protection experiments, the priming injections were done only with Abortusovis and Gallinarum. The use of Dublin and Choleraesuis was not possible considering the high virulence of these serotypes and the low inoculum required to keep enough mice alive for the experiments. The doses applied to primed mice were determined to induce high levels of bacteria in the spleen, but low levels of mortality, and low residual bacteria in the spleen of mice primed 5 weeks before injection. It was not possible to inject more than 5×10^5 Gallinarum, because even with a low replication level, this bacteria induced a rapid death in mice, most certainly the result of lipopolysaccharide (LPS) toxicity. Challenges were performed to obtain similar spleen infection. Six weeks after the priming injection, levels of bacteria in the spleen of non-challenge animals were less than 100 for Abortusovis and less than 10 for Gallinarum. The use of Abortusovis 15-5 or SS44 induced an efficient significant protection (between 2 and 3 log10 bacteria reduction). The exception for Gallinarum 4223 can be easily explained by the low level of the positive control compared to the inferior limit of bacterial detection in the spleen. When Gallinarum J91 or 4223 were used to prime animals, no protection was observed against Choleraesuis.

It is essential to note that even with a low level of infection, Gallinarum is able to induce protection against the extremely virulent Dublin serotype. A good correlation exists between IgM and IgG antibody responses and protection (Tab. II). These two serotypes belong to the same O group (O 1, 9, 12), but immunity against the LPS was described to be necessary though not sufficient for mice protection. In a murine model, anti-lipid A monoclonal [7] or anti-idiotype antibodies [13] protected mice against a lethal challenge with rough LPS. However, it was demonstrated that by using direct passive transfer of anti-LPS antibodies [20, 21, 28, 29, 37], LPS alone cannot fully account for the specificity of protection by antibodies. The specificity of cross-protection was previously studied [19], using Typhimurium, Enteritidis and Dublin for vaccination and challenge, with variants that differed in the main LPS antigen (0-4 or 0-9). It was found that

other factors than the O antigens could be involved and the nature of the antigen(s) responsible for protection would not appear to be the main 0-specific antigen [19]. Many other results have demonstrated that both antibodies and T-cells are required for an immunity booster to oral challenge with virulent salmonella [16, 21, 25, 27, 30, 40].

To summarise these experiments, by using different serotypes of salmonella in a mouse model, we found that Abortusovis induced good protection against other farm animal Salmonella infections, whatever the O antigen. Gallinarum less efficient in terms of protection, seemed to be more linked with the presence of specific LPS antibodies and significant individual variations were observed between strains within a same serotype. It is clear that SG J91 was much more antigenic than all the other serotypes.

ACKNOWLEDGEMENTS

We would like to thank Mr H. Le Roux and Mrs N. Sommerhalter for taking care of the mice in the experimental unit of the lab. This work was supported by the European Grant "Mechanisms behind host-specificity of bacteria, investigated by using host-specific Salmonella serotypes." Contract FAIR nº 96-1743.

REFERENCES

[1] Allan D., Duffus W.P., The immunopathology in fowls (*Gallus domesticus*) of acute and subacute *Salmonella gallinarum* infection, Res. Vet. Sci. 12 (1971) 140-151.

[2] Barrow P.A., Huggins M.B., Lovell M.A., Host specificity of Salmonella infection in chickens and mice is expressed in vivo primarily at the level of the reticuloendothelial system, Infect. Immun. 62 (1994) 4602-4610.

[3] Bernard S., Buzoni-Gatel D., Carnaud C., Resistance of NOD mice to salmonellosis, C.R. Acad. Sci. III 316 (1993) 780-783.

[4] Blackwell J.M., Black G.F., Sharples C., Soo S.S., Peacock C.S., Miller N., Roles of Nramp1, HLA, and a gene(s) in allelic association with IL-4, in determining T helper subset differentiation, Microbes Infect. 1 (1999) 95-102.

[5] Casadevall A., Pirofski L.A., Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity, Infect. Immun. 67 (1999) 3703-3713.

[6] Chen Z.M., Jenkins M.K., Clonal expansion of antigen-specific CD4 T cells following infection with *Salmonella typhimurium* is similar in susceptible (Itys) and resistant (Ityr) BALB/c mice, Infect. Immun. 67 (1999) 2025-2029.

[7] Cornelissen J.J., Makel I., Algra A., Benaissa-Trouw B.J., Schellekens J.F., Kraaijeveld C.A., Verhoef J., Protection against lethal endotoxemia by anti-lipid A murine monoclonal antibodies: comparison of efficacy with that of human anti-lipid A monoclonal antibody HA-1A, J. Infect. Dis. 167 (1993) 876-881.

[8] Coynault C., Norel F., Comparison of the abilities of *Salmonella typhimurium* rpoS, aroA and rpoS aroA strains to elicit humoral immune responses in BALB/c mice and to cause lethal infection in athymic BALB/c mice, Microb. Pathog. 26 (1999) 299-305.

[9] Eckmann L., Fierer J., Kagnoff M.F., Genetically resistant (Ityr) and susceptible (Itys) congenic mouse strains show similar cytokine responses following infection with *Salmonella dublin*, J. Immunol. 156 (1996) 2894-2900.

[10] Emoto M., Naito T., Nakamura R., Yoshikai Y., Different appearance of gamma delta T cells during salmonellosis between Ityr and Itys mice, J. Immunol. 150 (1993) 3411-3420.

[11] Falkow S., Perspectives series: host/pathogen interactions. Invasion and intracellular sorting of bacteria: searching for bacterial genes expressed during host/pathogen interactions, J. Clin. Invest. 100 (1997) 239-243.

[12] Fang F.C., Fierer J., Human infection with *Salmonella dublin*, Medicine (Baltimore) 70 (1991) 198-207.

[13] Field S.K., Morrison D.C., An anti-idiotype antibody which mimics the inner-core region of lipopolysaccharide protects mice against a lethal challenge with endotoxin, Infect. Immun. 62 (1994) 3994-3999.

[14] Gautier A.V., Lantier I., Lantier F., Mouse susceptibility to infection by the *Salmonella abortusovis* vaccine strain Rv6 is controlled by the Ity/Nramp 1 gene and influences the antibody but not the complement responses, Microb. Pathog. 24 (1998) 47-55.

[15] Gray J.T., Fedorka-Cray P.J., Stabel T.J., Kramer T.T., Natural transmission of *Salmonella choleraesuis* in swine, Appl. Environ. Microbiol. 62 (1996) 141-146.

[16] Guilloteau L., Buzoni-Gatel D., Bernard F., Lantier I., Lantier F., *Salmonella abortusovis* infection in susceptible BALB/cby mice: importance of Lyt-2+ and L3T4+ T cells in acquired

immunity and granuloma formation, Microb. Pathog. 14 (1993) 45-55.

[17] Hassan J.O., Curtiss R.R., Effect of vaccination of hens with an avirulent strain of *Salmonella typhimurium* on immunity of progeny challenged with wild-Type Salmonella strains, Infect. Immun. 64 (1996) 938-944.

[18] Hassan J.O., Curtiss R.R., Efficacy of a live avirulent *Salmonella typhimurium* vaccine in preventing colonization and invasion of laying hens by *Salmonella typhimurium* and *Salmonella enteritidis*, Avian Dis. 41 (1997) 783-791.

[19] Hormaeche C.E., Joysey H.S., Desilva L., Izhar M., Stocker B.A., Immunity induced by live attenuated Salmonella vaccines, Res. Microbiol. 141 (1990) 757-764.

[20] Hormaeche C.E., Joysey H.S., Desilva L., Izhar M., Stocker B.A., Immunity conferred by Aro-Salmonella live vaccines, Microb. Pathog. 10 (1991) 149-158.

[21] Hormaeche C.E., Mastroeni P., Harrison J.A., Demarco de Hormaeche R., Svenson S., Stocker B.A., Protection against oral challenge three months after i.v. immunization of BALB/c mice with live Aro *Salmonella typhimurium* and *Salmonella enteritidis* vaccines is serotype (species)-dependent and only partially determined by the main LPS O antigen, Vaccine 14 (1996) 251-259.

[22] Lang T., Prina E., Sibthorpe D., Blackwell J.M., Nramp1 transfection transfers Ity/Lsh/Bcg-related pleiotropic effects on macrophage activation: influence on antigen processing and presentation, Infect. Immun. 65 (1997) 380-386.

[23] Lindberg A.A., Segall T., Weintraub A., Stocker B.A., Antibody response and protection against challenge in mice vaccinated intraperitoneally with a live aroA O4-O9 hybrid *Salmonella dublin* strain, Infect. Immun. 61 (1993) 1211-1221.

[24] Linde K., Bondarenko V., Sviridenko V., Prophylaxis of Salmonella abortus ovis-induced abortion of sheep by a Salmonella typhimurium live vaccine, Vaccine 10 (1992) 337-340.

[25] Mastroeni P., Villarreal-Ramos B., Hormaeche C.E., Adoptive transfer of immunity to oral challenge with virulent salmonellae in innately susceptible BALB/c mice requires both immune serum and T cells, Infect. Immun. 61 (1993) 3981-3984.

[26] Mattsby-Baltzer I., Ahlstrom B., Edebo L., de Man P., Susceptibility of lipopolysaccharide-responsive and -hyporesponsive ItyS mice to infection with rough mutants of *Salmonella typhimurium*, Infect. Immun. 64 (1996) 1321-1327.

[27] Mitov I., Denchev V., Linde K., Humoral and cell-mediated immunity in mice after immunization with live oral vaccines of *Salmonella typhimurium*: auxotrophic mutants with two attenuating markers, Vaccine 10 (1992) 61-66.

[28] Mukkur T.K., Walker K.H., Jones D., Wronski E., Love D.N., Immunizing efficacy of aromatic-dependent *Salmonella dublin* in mice and calves, Comp. Immunol. Microbiol. Infect. Dis. 14 (1991) 243-256.

[29] Muthukkumar S., Muthukkaruppan V.R., Mechanism of protective immunity induced by porin-lipopolysaccharide against murine salmonellosis, Infect. Immun. 61 (1993) 3017-3025.

[30] Nishikawa F., Kita E., Matsui N., Kashiba S., Transfer of protection to murine typhoid conferred by L-form *Salmonella typhimurium* in dependence of cooperation between L form-adopted macrophages and L form-induced Lyt-2+ T cells, Microbiol. Immunol. 38 (1994) 201-207.

[31] Nnalue N.A., Mice vaccinated with a non-virulent, aromatic-dependent mutant of *Salmonella choleraesuis* die from challenge with its virulent parent but survive challenge with *Salmonella typhimurium*, J. Med. Microbiol. 31 (1990) 225-233.

[32] Oswald I.P., Lantier F., Moutier R., Bertrand M.F., Skamene E., Intraperitoneal infection with *Salmonella abortusovis* is partially controlled by a gene closely linked with the Ity gene, Clin. Exp. Immunol. 87 (1992) 373-378.

[33] Pardon P., Sanchis R., Marly J., Lantier F., Guilloteau L., Buzoni-Gatel D., Oswald I.P., Pepin M., Kaeffer B., Berthon P., et al, Experimental ovine salmonellosis (*Salmonella abortusovis*): pathogenesis and vaccination, Res. Microbiol. 141 (1990) 945-953.

[34] Pascopella L., Raupach B., Ghori N., Monack D., Falkow S., Small P.L., Host restriction phenotypes of *Salmonella typhi* and *Salmonella gallinarum*, Infect. Immun. 63 (1995) 4329-4335.

[35] Plant J.E., Glynn A.A., Genetic control of resistance to *Salmonella typhimurium* infection in high and low antibody responder mice, Clin. Exp. Immunol. 50 (1982) 283-290.

[36] Roberts M., Bacon A., Li J., Chatfield S., Prior immunity to homologous and heterologous Salmonella serotypes suppresses local and systemic anti-fragment C antibody responses and protection from tetanus toxin in mice immunized with Salmonella strains expressing fragment C, Infect. Immun. 67 (1999) 3810-3815.

[37] Singh S.P., Williams Y.U., Benjamin W.H., Klebba P.E., Boyd D., Immunoprotection by monoclonal antibodies to the porins and lipopolysaccharide of *Salmonella typhimurium*, Microb. Pathog. 21 (1996) 249-263.

[38] Soo S.S., Villarreal-Ramos B., Anjam Khan C.M., Hormaeche C.E., Blackwell J.M., Genetic control of immune response to recombinant antigens carried by an attenuated *Salmonella typhimurium* vaccine strain: Nramp1 influences T-helper subset responses and protection against

leishmanial challenge, Infect. Immun. 66 (1998) 1910-1917.

[39] Taylor B.A., O'Brien A.D., Position on mouse chromosome 1 of a gene that controls resistance to *Salmonella typhimurium*, Infect. Immun. 36 (1982) 1257-1260.

[40] Thygesen P., Brandt L., Jorgensen T., Christensen H.B., Hougen H.P., Jensen E.T., Rygaard J., Immunity to experimental *Salmonella typhimurium* infections in rats. Transfer of immunity with primed CD4+CD25high and CD4+CD25low T lymphocytes, APMIS 102 (1994) 489-494.

[41] Vaessen M.A., Veling J., Frankena K., Graat E.A., Klunder T., Risk factors for *Salmonella dublin* infection on dairy farms, Vet. Q. 20 (1998) 97-99.

[42] Vassiloyanakopoulos A.P., Okamoto S., Fierer J., The crucial role of polymorphonuclear leukocytes in resistance to *Salmonella dublin* infections in genetically susceptible and resistant mice, Proc. Natl. Acad. Sci. USA 95 (1998) 7676-7681.

[43] Vidal S.M., Malo D., Vogan K., Skamene E., Gros P., Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg, Cell 73 (1993) 469-485.

[44] Vidal S.M., Pinner E., Lepage P., Gauthier S., Gros P., Natural resistance to intracellular infections: Nramp1 encodes a membrane phosphoglycoprotein absent in macrophages from susceptible (Nramp1 D169) mouse strains, J. Immunol. 157 (1996) 3559-3568.

[45] Wallis T.S., Paulin S.M., Plested J.S., Watson P.R., Jones P.W., The Salmonella dublin virulence plasmid mediates systemic but not enteric phases of salmonellosis in cattle, Infect. Immun. 63 (1995) 2755-2761.

[46] Weintraub B.C., Eckmann L., Okamoto S., Hense M., Hedrick S.M., Fierer J., Role of alphabeta and gammadelta T cells in the host response to Salmonella infection as demonstrated in T-cell-receptor-deficient mice of defined Ity genotypes [published erratum appears in Infect. Immun. 66 (1998) 882], Infect. Immun. 65 (1997) 2306-2312.

[47] Whittle B.L., Verma N.K., The immune response to a B-cell epitope delivered by Salmonella is enhanced by prior immunological experience, Vaccine 15 (1997) 1737-1740.

[48] Yoshida T., Kishi F., Expression of NRAMP1 molecule in human peripheral blood leukocytes, Immunol. Lett. 55 (1997) 105-108.