Clin Exp Immunol 1996; **104**:228–235

Beneficial effect of *Salmonella typhimurium* infection and of immunoglobulins from *S. typhimurium*-infected mice on the autoimmune disease of (NZB × NZW)F_1 mice

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(Accepted for publication 16 January 1996)

SUMMARY

Various infections can precede or aggravate autoimmune diseases. Yet a beneficial effect of infection has also been described and various mechanisms have been postulated to explain this effect. The aim of this study was to examine the hypothesis that infection can have an immunoregulatory effect on the autoimmune process via the increased production of natural polyreactive antibodies. The effect of *Salmonella typhimurium* infection on the lupus-like disease of (NZB × NZW)F_1 (B/W) mice was therefore studied. The effect of IgM and IgG preparations isolated from the serum of *S. typhimurium*-infected C57Bl/6 and CBA mice on the autoimmune disease of B/W mice was also tested. C57Bl/6 and CBA mice were chosen because they are respectively genetically susceptible and resistant to *S. typhimurium* infection and they differ in their antibody response during the early phase of infection. CBA mice can mount a specific anti-bacterium antibody response, whereas C57Bl/6 mice present increased production of polyreactive antibodies. The infection effect was evaluated on several disease parameters, i.e. survival, incidence of high grade proteinuria and serum IgM and IgG antibody activity directed against a panel of autoantigens. Our main findings were: (i) infection of B/W mice with an attenuated strain of *S. typhimurium* delayed the course of the autoimmune disease when performed before the appearance of autoimmune symptoms; and (ii) IgM and IgG preparations from *S. typhimurium*-infected C57Bl/6 mice had a similar effect, whereas the IgM and IgG preparations from infected CBA mice, as well as from normal C57Bl/6 and CBA mice, were ineffective. These results suggest that *S. typhimurium* infection can beneficially influence the development of the autoimmune disease of B/W mice. The immunoregulatory effect of the infection seems to be related, at least partially, to the increase of a particular population of antibodies, the polyreactive antibodies.

Keywords (NZB × NZW)F_1 lupus prone mice *Salmonella typhimurium* infection effect of immunoglobulins

INTRODUCTION

Infectious agents can induce or accelerate the course of autoimmune diseases. For example, autoimmune manifestations normally present in NZB mice or their (NZB × NZW)F_1 (B/W) relatives are enormously enhanced by persistent infection with either a DNA or RNA virus; that is, autoantibodies form earlier and reach higher titres in the infected mice than in their uninfected counterparts [1]. A variety of mechanisms could be responsible, i.e. polyclonal B or T cell activation, cytokine release, molecular mimicry [2–4]. Yet a beneficial effect of infection on the course of autoimmune diseases has also been described. For instance, the lactic dehydrogenase virus and bacteria such as *Bordetella pertussis* and *Mycobacterium tuberculosis* have been shown to inhibit experimental autoimmune encephalomyelitis in mice [5,6]. Concerning systemic lupus erythematosus (SLE), it has been reported that the incidence of this autoimmune disease is low in regions with endemic malaria [7], and it has been found that *Plasmodium* infection produces long-lasting remission in lupus-prone B/W female mice [8]. More recently, we have provided results strongly suggesting that malaria infection of B/W mice exerted its protective effect by an increased production of IgM and IgG natural antibodies [9].

We postulated that the increased production of natural polyspecific antibodies is a general mechanism by which infection modifies the autoimmune process. For this reason, we examined in the present study the effect of another infection, the *Salmonella typhimurium* infection and the effect of polyclonal immunoglobulins isolated from the sera of *S. typhimurium*-infected

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C57Bl/6 and CBA mice on the autoimmune disease of B/W mice. The transfer of polyclonal immunoglobulin from these mice was performed because, as we have previously reported, the antibody response to *S. typhimurium* infection differs in genetically resistant CBA and susceptible C57Bl/6 mice. Resistant CBA mice, after a transient polyclonal activation, produce specific IgG anti-bacterium antibodies. In contrast, susceptible C57Bl/6 mice remain in the early stage of polyclonal activation, with the presence of high levels of IgM polyreactive antibodies [10]. The infection and immunoglobulin transfer effect were monitored by testing mice survival, the incidence of high grade proteinuria and serum IgM and IgG antibody activity directed against a panel of antigens.

MATERIALS AND METHODS

Mice

B/W female mice were bred in our animal facilities by mating NZB male with NZW female mice obtained from the Centre de Selection et d'Élevage d'Animaux de Laboratoire du CNRS (Orléans, France). After weaning, female mice were kept in cages of 10 and allowed free access to food (Extralabo M25; Pietrement, Longueville, France) and to sterile water. Control and experimental groups were age-matched and housed under identical conditions. Infected animals were kept isolated from uninfected ones.

CBA and C57Bl/6 female mice were obtained from Iffa Credo (L'Abresle, France) and were used between 6 and 8 weeks of age.

Salmonella typhimurium *infection*

Salmonella typhimurium C5TS, a temperature-sensitive avirulent mutant derived from the virulent strain C5 (kindly provided by C. E. Hormaeche, Cambridge, UK), was grown for 18 h at 30°C in tryptic soy broth. The virulent strain C5 was grown in the same medium at 37°C. *Salmonella typhimurium* infection of B/W mice was performed for two different purposes. The first purpose was to determine the susceptibility of B/W mice to *S. typhimurium* infection. B/W mice were therefore infected intravenously at different ages (4 and 8 months) with 10^3 colony forming units (CFU) of the virulent C5 strain of *S. typhimurium*. Viable bacteria were enumerated in the spleen at various time intervals by plating 10-fold serial dilutions in saline of spleen homogenates onto tryptic soy agar. Colonies were counted after overnight incubation at 37°C.

The second purpose was to examine the consequence of infection on the development of the lupus syndrome. For this reason, 2-month-old (early infection) or 6-month-old (late infection) B/W mice were inoculated intravenously with 10^6 CFU of the avirulent C5TS strain in 0·2 ml saline. Infected B/W mice were followed for disease progression until death.

Immunoglobulin preparation and B/W treatment

Sera were obtained from normal and infected CBA and C57Bl/6 mice. In order to obtain immunoglobulin preparations from infected mice, CBA and C57Bl/6 mice were infected by i.v. injection with 10^6 CFU of *S. typhimurium* C5TS. Twenty to 40 days after infection, CBA and C57Bl/6 mice were bled once a week. Sera were pooled and stored at −20°C. IgG- and IgM-enriched fractions were prepared from these pooled sera by using a protein A-Sepharose column according to a previously described procedure [11]. The IgM fractions were tested by enzyme immunoassay (EIA) for eventual IgG cross contamination; the 'IgM-enriched fraction' will be referred to as 'IgM fraction'. The IgM and IgG concentration was evaluated by radial immunodiffusion (Serotec, Oxford, UK) and antibody activity by an EIA as described below.

The IgG and IgM fractions were administered intraperitoneally to B/W female mice. The immunoglobulin treatment (100 μg/mouse in 0·2 ml PBS) was started at the age of 6 months and given thereafter every 10 days, until death. The control group received 0·2 ml PBS.

Monitoring of disease progression

Mice survival was recorded daily. Urine was monitored for proteinuria every 15 days. Serum samples for autoantibody analysis were collected every month by bleeding from the orbital plexus.

Enzyme immunoassays

The presence of IgM and IgG antibodies directed against DNA, *S. typhimurium* lipopolysaccharide (LPS; Difco Labs, Detroit, MI), actin, myosin, tubulin, myoglobin, trinitrophenyl-bovine serum albumin (TNP-BSA) was evaluated by EIA, as previously described [12]. Briefly, 96-well polystyrene flat-bottomed microtitre plates (CML, Nemours, France) were coated with the various antigens (1 h at 37°C and overnight at 4°C). The plates were washed extensively with PBS containing 0·1% Tween-20 and dilutions (1 : 100) of the serum samples were tested in duplicate (1 h incubation at 37°C). After extensive washing, 1 μg/ml of β-galactosidase-labelled sheep anti-mouse immunoglobulin (Biosys, Campiègne, France) was added. After another hour of incubation at 37°C, the plates were washed and enzyme activity revealed using the enzyme substrate (*o*-nitrophenyl-β-D-galactopyranoside). Optical density was measured at 414 nm. The antibody activity of the IgM and IgG preparations against the panel antigens was also tested using an EIA method as described above.

The isotypes of the IgG preparations were also determined using an EIA method. Plates were coated with sheep anti-mouse IgG (5 μg/ml) and serial dilutions of the IgG preparations from normal and infected CBA and C57Bl/6 mice were added in duplicate. Then, alkaline phosphatase-labelled anti-mouse IgG isotypes (Southern Biotechnology, Birmingham, AL) were added. After washing, enzyme activity was revealed using the enzyme substrate (*p*-nitrophenyl phosphate) and optical density was measured at 414 nm. The concentrations of the IgG isotypes were determined using IgG1, IgG2a, IgG2b and IgG3 standards (Southern Biotechnology). The IgM fractions were tested by a similar EIA method for possible cross-contamination with IgG isotypes.

Proteinuria

Proteinuria was assessed with tetrabromphenol paper (Albustix; Bayer Diagnostics Ltd., Basingstoke, UK) in fresh urine samples. Proteinuria was considered to be high when graded at least 3+ (>3 g/*l*).

Statistical analysis

Autoantibody titres and proteinuria were compared between control and infected or immunoglobulin-treated animals by the Mann–Whitney *U*-test. For the survival studies, the Fisher exact test was employed and $P \leqslant 0{\cdot}05$ was considered significant.

RESULTS

Susceptibility of B/W mice to S. typhimurium *infection*

In order to determine their susceptibility to *S. typhimurium* infection, B/W mice were infected with the virulent strain of

Table 1. Susceptibility of B/W mice to *Salmonella typhimurium* infection

	Log_{10} CFU/spleen*	
Time of infection†	Day 4	Day 7
Four months	$4{\cdot}63 \pm 0{\cdot}47$	$5{\cdot}40 \pm 0{\cdot}37$
Eight months	$5{\cdot}46 \pm 0{\cdot}68$	$7{\cdot}83 \pm 0{\cdot}24$

* Colony-forming units (CFU) were enumerated in the spleens on days 4 and 7 post-infection.

† B/W mice ($n = 3$) were infected with 10^3 CFU of the virulent C5 strain of *S. typhimurium.*

S. typhimurium at 4 months of age (i.e. before the appearance of autoimmune symptoms), or at 8 months (i.e. after the appearance of autoimmune symptoms). Groups of B/W mice were killed at 4 days and 7 days after infection in order to determine the number of bacteria in their spleens. We found that 4-month-old B/W behave like genetically resistant mice [13], whereas 8-month-old B/W mice were more susceptible to the infection (Table 1).

Survival, proteinuria and serum antibody profiles in S. typhimurium*-infected B/W mice*

Infection of B/W mice (groups of 10) at 2 months, with the avirulent strain of *S. typhimurium*, resulted in a 2-month prolongation of the 50% survival (Fig. 1a), but this survival prolongation was not statistically significant. The early-infected B/W mice presented also a low incidence of high-grade proteinuria until the age of 11 months that was significantly different from that of the non-infected controls ($P \leqslant 0{\cdot}05$, Fig. 2a). These mice presented in their sera an antibody profile similar to that of the non-infected controls (Fig. 3a), with the exception of antibodies directed against LPS and DNA. Indeed, control B/W mice did not present LPS-specific IgM or IgG, while early-infected B/W mice produced, as early as 30 days after infection, high levels of LPS-specific antibodies. The maximal levels of LPS-specific IgM and IgG antibodies were observed by day 60 post-infection, after which anti-LPS antibody levels slowly declined (Table 2).

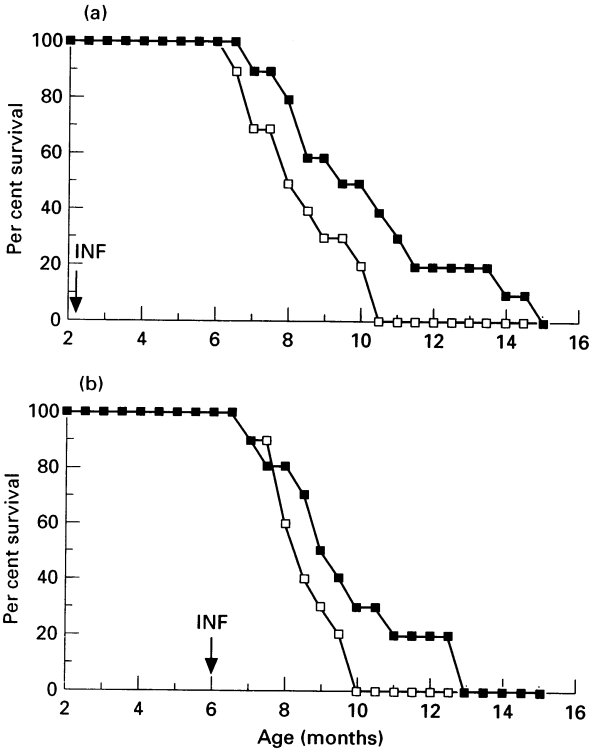

Fig. 1. Effect of *Salmonella typhimurium* infection on B/W survival. Groups of 10 B/W mice were infected at 2 months (a) or 6 months (b) with the avirulent strain C5TS of *S. typhimurium.* ■, Infected; □, controls.

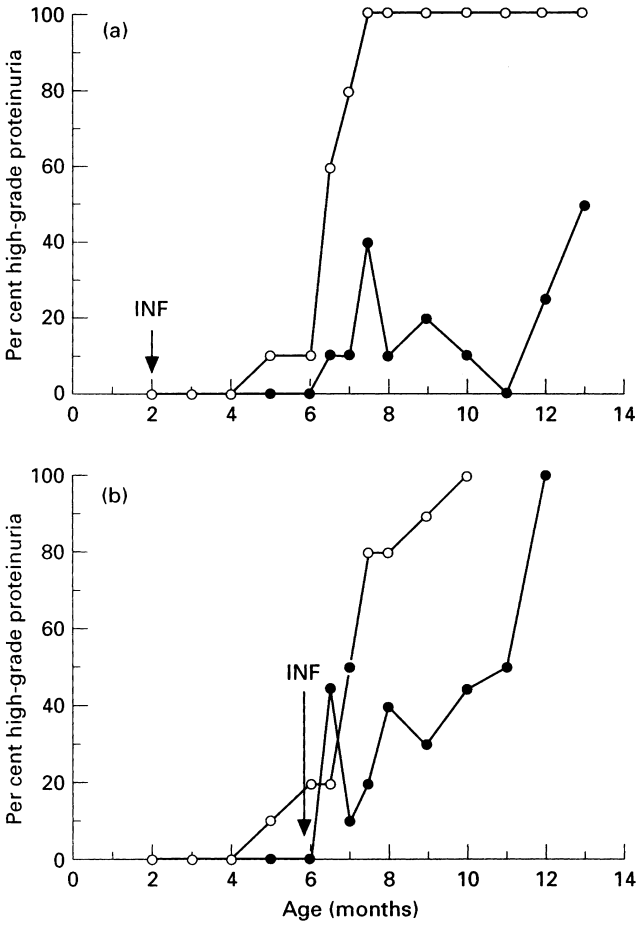

Fig. 2. The high grade proteinuria of B/W mice infected at 2 months (a) or at 6 months (b) was evaluated. Results are presented as per cent cumulative incidence of high-grade (3+) proteinuria. ●, Infected; ○, controls.

The IgM and IgG anti-DNA antibodies in the serum of early-infected B/W mice increased soon after infection. On day 45 post-infection, the early-infected B/W mice presented titres of serum anti-DNA antibodies significantly higher than the non-infected control B/W mice. After that time, serum anti-DNA antibody levels of the infected mice did not differ from control mice until 7·5 months of age. All infected B/W mice that survived after 8 months of age exhibited low anti-DNA titres, which were significantly lower than in controls (Fig. 3b).

B/W animals (groups of 10) infected by *S. typhimurium* at 6 months, at the onset of the autoimmune symptoms, presented a moderate (1 month) and not statistically significant prolongation of 50% survival (Fig. 1b). These late-infected mice presented, however, a significant fall in the incidence of high-grade proteinuria starting 1 month after infection and maintained until 12 months of age ($P \leqslant 0{\cdot}05$, Fig. 2b). Serum antibody activity, including

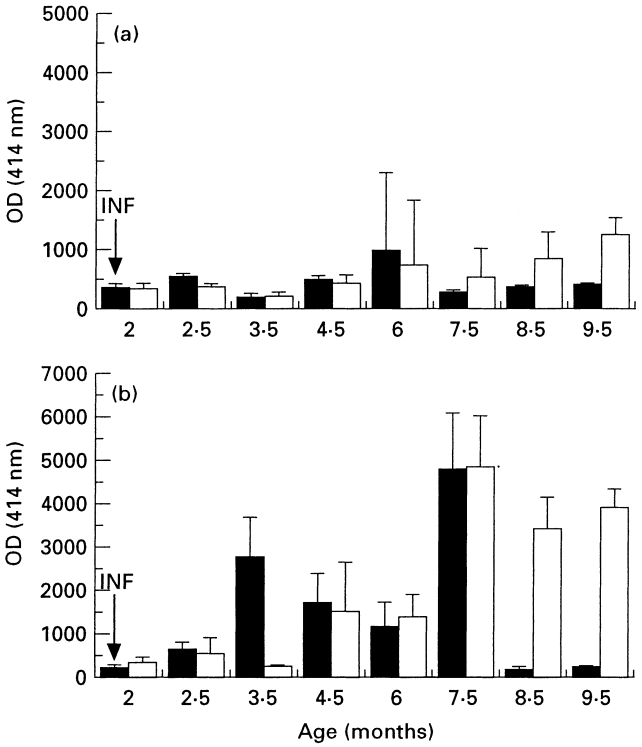

Fig. 3. Mean antibody titres against myosin (a) and dsDNA (b) in the sera from *Salmonella typhimurium*-infected ($n = 10$) and uninfected ($n = 10$) B/W mice. Results are the means ± s.d. of optical densities $\times 10^3$. ■, Infected; □, controls.

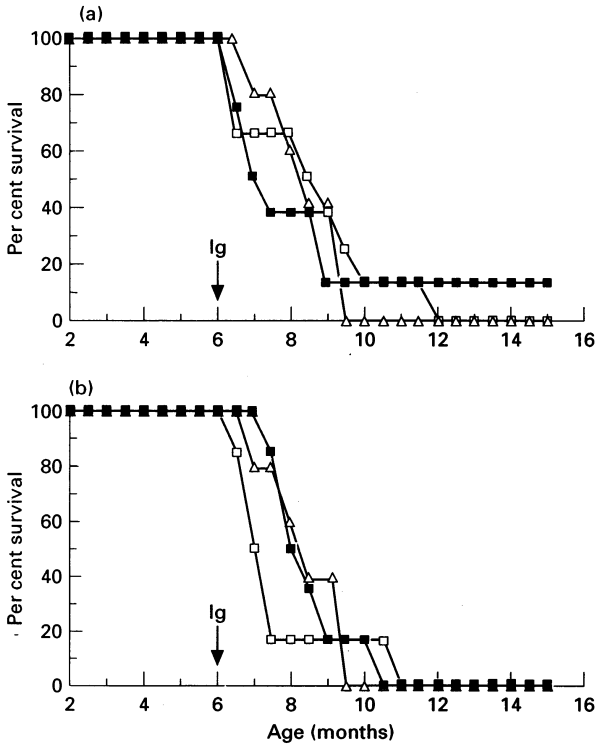

Fig. 4. Per cent survival of B/W mice ($n = 8$) injected at the age of 6 months with various immunoglobulin preparations from the sera of *Salmonella typhimurium*-infected (a) and uninfected (b) CBA mice. Immunoglobulin treatment was started at the age of 6 months and thereafter given every 10 days, until death. □, IgM preparations; ■, IgG preparations; △, PBS.

anti-DNA IgG of late-infected B/W mice, was not significantly different from that of non-infected controls (data not shown). We must notice, however, that these late-infected animals already exhibited high anti-DNA IgG titres when the infection was performed. These late-infected B/W mice were still capable of mounting IgM and IgG anti-LPS antibodies similar to those of early-infected mice (Table 2).

Immunoglobulin treatment

B/W mice (groups of eight) were treated with preparations of IgM and IgG obtained from normal and *S. typhimurium*-infected CBA and C57Bl/6 mice (respectively resistant and susceptible to *S. typhimurium* infection). Treatment was started at 6 months, i.e. at the appearance of the autoimmune symptoms, and given thereafter every 10 days, until death. When the IgM preparations were tested by EIA for eventual IgG cross-contamination, it was found that IgM fractions from normal and infected C57Bl/6 mice contained also low amounts (0·4%) of IgG3 immunoglobulins. IgM fractions from normal CBA mice contained also very low amounts (0·1%) of IgG3 immunoglobulins, while IgM from infected CBA mice contained 0·2% of IgG1 and 0·5% of IgG2a. Concerning the predominant IgG isotype among the IgG preparations, we found that in IgG preparations from normal C57Bl/6 mice the predominant isotype was IgG2b, while in the preparations from infected C57Bl/6 mice the predominant isotype was IgG3. In IgG preparations from normal and infected CBA mice, the predominant isotype was IgG2a.

The IgM and IgG preparations from normal CBA and C57Bl/6 mice recognized all the antigens of the panel (DNA, actin, myosin, tubulin, myoglobin, TNP-BSA), except LPS. The IgM and IgG preparations from infected CBA mice recognized all the antigens tested, but also exhibited a pronounced anti-LPS antibody activity. The immunoglobulin preparations from infected C57BL/6 mice also recognized all the antigens tested, but presented low IgG anti-LPS antibody activity (Table 3).

We found that the injection of IgM and IgG preparations from *S. typhimurium*-infected CBA mice into B/W mice modified their survival only moderately, compared with controls treated with PBS. Such a moderate and non-significant difference in survival

Table 2. Anti-lipopolysaccharide (LPS) antibody activity in the sera of non-infected, early-infected (2 months), and late-infected B/W (6 months) mice

Infection	Immunoglobulin isotype	Days post-infection 0	30	60	90	120
None	IgM	12	21	18	32	15
	IgG	13	17	21	27	26
Early	IgM	14	127	92	110	127
	IgG	17	231	687	546	450
Late	IgM	23	134	120	110	35
	IgG	29	915	870	760	420

Anti-LPS antibody activity was determined by an enzyme immunoassay (EIA) method and expressed as mean OD $\times 10^3$.

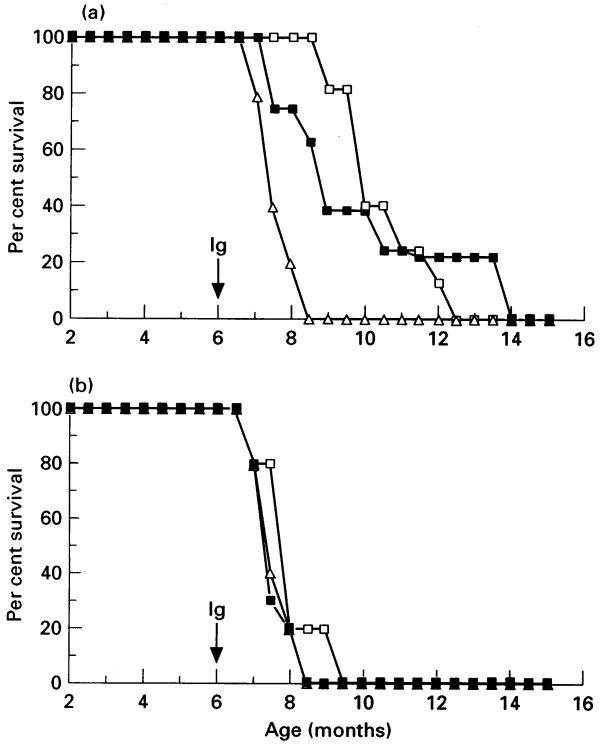

Fig. 5. Per cent survival of B/W mice ($n = 8$) injected with various immunoglobulin preparations from the sera of *Salmonella typhimurium*-infected (a) and uninfected (b) C57Bl/6 mice. Immunoglobulin treatment was started at the age of 6 months and thereafter given every 10 days, until death. □, IgM preparations; ■, IgG preparations; △, PBS.

was also observed with B/W mice that received IgM and IgG preparations from normal CBA mice (Fig. 4a,b). Concerning the high grade proteinuria, the B/W mice that received the immunoglobulin preparations from normal and infected CBA mice did not differ from control B/W mice receiving PBS (Fig. 6c,d).

In contrast, treatment with IgM and IgG preparations from infected C57Bl/6 mice resulted in a significant increase ($P \leqslant 0{\cdot}05$) of B/W survival compared with mice that received either IgM or IgG preparations from normal C57Bl/6 mice or PBS (Fig. 5a,b). In addition, B/W mice that received either IgM or IgG preparations from infected C57Bl/6 mice presented high grade proteinuria less frequently than mice treated with preparations obtained from normal C57Bl/6 mice or PBS ($P \leqslant 0{\cdot}05$ for both IgM and IgG preparations; Fig. 6a,b). The serum antibody profile from B/W mice that received immunoglobulin preparations either from infected CBA mice or from infected C57Bl/6 mice did not differ from the serum antibody profile of B/W mice that received immunoglobulin preparations from normal CBA and C57Bl/6 mice or PBS (data not shown).

DISCUSSION

Our main findings are: (i) *S. typhimurium* infection can modify the course of the autoimmune disease of B/W mice when performed before the appearance of the autoimmune symptoms; and (ii) IgM and IgG preparations from *S. typhimurium*-infected C57Bl/6 mice seem to have a pronounced immunomodulatory activity on the autoimmune disease of B/W mice.

There exist a number of possible mechanisms by which infection can improve the course of an autoimmune disease, the most obvious being immunosuppression. Indeed, immunosuppression occurs during *S. typhimurium* infection, especially in genetically susceptible mice [14,15] and seems to be mediated by macrophages and dysregulation of cytokine production [16,17]. It has been described that antigen-dependent immunoglobulin gene hypermutation occurs in the germinal centre environment [18] and that germinal centres may play a role in the maintenance of self tolerance [19]. Another possible mechanism therefore for explaining the beneficial role of infection in the autoimmune process is competition between self- and exogenous (bacterial)-antigens for localization in germinal centres of secondary lymphoid organs. Such competition may result in the disruption of maturation of the autoimmune response in B/W mice because of the reduction in germinal centre selection of autoreactive B cells. Since immune dysregulation associated with autoimmune disease may also be related to a Th1–Th2 imbalance [20], cytokine production during infection constitutes another possible mechanism by which infection modifies the autoimmune process. However, our results

Table 3. Antibody activity of the IgM and IgG preparations from normal (n) or *Salmonella typhimurium*-infected (i) CBA and C57Bl/6 mice

	LPS	DNA	Actin	Myosin	Tubulin	Myoglobin	TNP-BSA
CBA (n)							
IgM	35	295	260	185	235	209	418
IgG	17	190	115	113	180	121	300
CBA (i)							
IgM	1615	498	415	387	415	422	660
IgG	1800	437	350	330	360	380	580
C57Bl/6 (n)							
IgM	27	247	150	105	159	137	368
IgG	9	160	105	95	121	101	206
C57Bl/6 (i)							
IgM	1900	750	492	454	615	409	932
IgG	387	386	318	290	357	350	476

Antibody titres were determined by an enzyme immunoassay (EIA) method and expressed as mean OD $\times 10^3$.

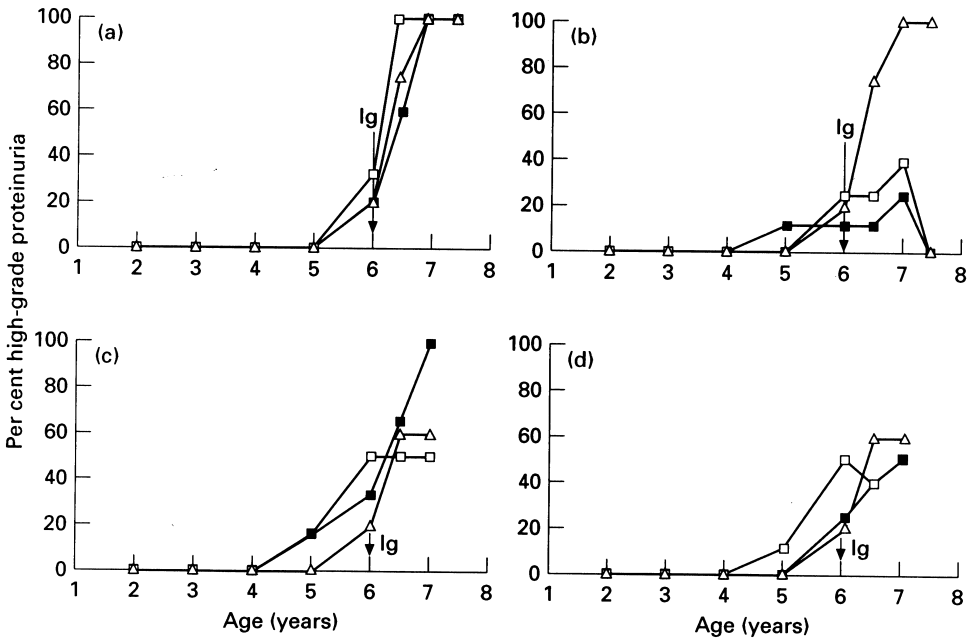

Fig. 6. The high grade proteinuria of B/W mice treated at 6 months with IgM and IgG preparations from normal C57Bl/6 (a), infected C57Bl/6 (b) or normal CBA (c) and infected CBA (d) mice. Results are presented as per cent cumulative incidence of high-grade (3+) proteinuria. □, IgM preparations; ■, IgG preparations; △, PBS.

suggest that, at least partially, the effect of *S. typhimurium* infection is related to the production of antibodies, since the transfer of immunoglobulins was effective in improving transitorily the autoimmune disease in B/W mice. We have previously studied the production of *S. typhimurium*-specific antibodies *versus* polyreactive antibodies in the sera of CBA and C57Bl/6 infected mice by EIA and immunoblotting [10]. We observed that CBA mice, which are resistant to *S. typhimurium* infection, were able to mount a specific anti-bacterium response after a transient increase of serum natural polyreactive antibody levels. In contrast, susceptible C57Bl/6 mice mount an increased production of polyreactive antibodies during the first month of *S. typhimurium* infection, but are unable to mount a specific anti-bacterium response. As we found that only immunoglobulin preparations from C57Bl/6, but not from CBA, infected mice were able to modify significantly the course of the autoimmune disease in B/W mice, we can hypothesize that the immunoregulatory effect of these preparations was related to the presence of high levels of natural polyreactive antibodies. We have previously reported that another infection, the *P. chambaudii* infection of mice, induced the synthesis of natural antibodies with immunoregulatory properties [9,21]. It would thus appear that mice can produce high levels of natural antibodies during the early phase of infection, as the consequence of polyclonal activation. In addition, we have previously reported that hypergammaglobulinaemia during HIV infection is associated with enhanced titres of polyreactive natural antibodies. These polyreactive antibodies recognize autoantigens, HIV antigens and other antigens such as the TNP hapten and can interfere with the HIV *in vitro* infection [22,23]. Concerning their role during infection, polyreactive natural antibodies have been described to interfere with infection by the recognition of the microorganism and/or by the recognition of cellular receptors [24]. The results described here suggest that polyreactive natural antibodies produced during the early phase of infection can also have an immunoregulatory role. This effect seems not to be specifically related to the presence of antibodies directed against particular bacterial components, since such antibodies are produced during the early phase of various infections. However, the mouse genetic background and the type of infection can determine the levels and other characteristics of natural antibodies, such as idiotypes, affinity, etc., and therefore their immunoregulatory properties. Whether all populations of these natural polyreactive anti-bodies have an immunoregulatory effect remains to be elucidated.

The mechanisms of the immunoregulatory action of IgM and IgG preparations in the present work could be similar to those reported for normal human immunoglobulins, used for the treatment of several autoimmune diseases: non-specific Fc blockade, inhibition of antibody synthesis, anti-idiotypic modulation or effects on various cell subpopulations [25–30]. The results obtained in this study suggest a modification in the formation and/or composition of immune complexes, since B/W mice that survived presented also a decreased proteinuria. However, with the panel of antigens used, we did not observe marked differences in serum antibody levels between groups of B/W mice that survived and groups of animals that did not. In addition, since the predominant IgG isotype within the IgG preparations was found to vary, we cannot exclude the possibility that the IgG preparations bind to different members of the Fc receptor family. It is well known that the heterogeneity of the Fc receptor is evident at the level of specificity for a wide variety of immunoglobulin classes and subclasses, but also at the level of receptor function [31]. It remains possible that the different IgG preparations bind to different Fc receptors and may therefore mediate different activities that interfere with the autoimmune process in various ways.

The panel of antigens used to study antibody activity in the serum of infected B/W mice consisted mainly of autoantigens, in order to determine the effect of infection on autoantibody

production during the lupus-like disease of B/W mice. In contrast, the specific anti-bacterial response in the serum of infected B/W mice was tested only against LPS because the extensive study of the capacity of B/W mice to mount specific anti-bacterial response was not within the scope of this study. We do not believe that anti-LPS antibodies play a significant role, because only the transfer of immunoglobulin from C57Bl/6 infected mice had an effect on the B/W autoimmune disease, and C57Bl/6 mice are not able to mount specific anti-LPS antibodies during the early phase of *S. typhimurium* infection. Furthermore, early and late-infected B/W mice were both able to mount an anti-LPS antibody response. The prolongation of their survival, however, differed. Concerning autoantibody production, only early-infected B/W animals, but not IgM- and IgG-treated B/W mice, presented different anti-DNA antibody profiles compared with the other groups (data not shown). We must note, however, that the relevance of anti-DNA autoantibodies to the development of nephritis is controversial [32]. In addition, we do not know whether the anti-DNA antibodies that arose during the infection are identical to the anti-DNA population that rises during autoimmune disease.

IgM and IgG preparations obtained from the sera of infected CBA and C57Bl/6 mice were injected separately because we have previously reported that IgM from the serum of normal mice can inhibit IgG binding to autoantigens [33]. The administration of IgM preparation from infected C57Bl/6 mice was the one possessing the more pronounced immunoregulatory effect. The IgM preparation displayed higher titres of natural antibodies and relatively lower anti-LPS antibody titres than the IgG preparation.

Finally, our results suggest that the autoimmune process can also influence resistance to infection. Natural resistance of mice to infection depends on genetic background. Resistance to *S. typhimurium* infection in the early phase is controlled by the *Ity* gene [34,35]. B/W mice were resistant to *S. typhimurium* infection before the onset of autoimmune disease. This is in agreement with previous results suggesting that inheritance of SLE susceptibility by New Zealand mice is associated with natural resistance to mycobacteria infection [36]. We note that the *Lsh*, *Ity*, and *Bcg* genes that respectively control natural resistance to *Leishmania donovani*, *S. typhimurium* and mycobacteria infection are identical and now named *Nramp* [37]. *Salmonella typhimurium* is a facultative intracellular pathogen, and cellular immunity plays an important role in bacterial clearance from organs. The relative susceptibility of B/W mice to infection after the appearance of autoimmune symptoms may be related to a progressive deficit of the cellular response and impaired cytokine production. B/W mice and other lupus-prone mice have been described to present impaired production of different cytokines, and administration of various cytokines or antibodies with anti-cytokine activities has been reported to prolong animal survival [38–41]. B/W mice produce exceptionally low levels of tumour necrosis factor-alpha (TNF-α) [42], and replacement therapy with recombinant TNF-α significantly delays development of nephritis in these mice [43]. TNF-α is produced during infection, and has been shown to participate in the resistance of normal mice to *S. typhimurium* infection [44]. It is likely that B/W autoimmune mice produce TNF when infected by *S. typhimurium*. Whether the TNF production during *S. typhimurium* infection participates also in the regulation of severity of the autoimmune disease remains to be elucidated.

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