Immunology 1996 **89** 20–25

Diabetes-prone NOD mice are resistant to *Mycobacterium avium* and the infection prevents autoimmune disease

A. BRÁS* & A. P. ÁGUAS*† *Center for Experimental Cytology and †Department of Anatomy of the Abel Salazar Institute for the Biomedical Sciences, University of Porto, Porto, Portugal

SUMMARY

It was recently proposed that the diabetes genes of non-obese diabetic (NOD) mice are linked to the *Bcg* gene that is associated with resistance to infection by mycobacteria; however, it has not been established whether NOD mice are resistant or susceptible to the infection, although there are previous investigations on response of NOD mice to other intracellular parasites (e.g. Kaye *et al.*, *Eur. J. Immunol.* **22:** 357–364). We have investigated here this question, as well as the consequences of mycobacterial infection on the natural history of murine diabetes. Female NOD mice were intraperitoneally infected with 10^8 viable bacilli of *Mycobacterium avium* at 2 months of age, i.e. before the mice show diabetes; they were studied up to the sixth month of age (when more than half of untreated female NOD mice show glycosuria). To determine whether NOD mice were susceptible or resistant to *M. avium* infection, we have compared the kinetics of bacterial growths in liver and spleen of the mice with those determined in *M. avium*-susceptible (BALB/c) and resistant (C3H) strains of mice. NOD mice were able to control the *M. avium* infection, following a pattern similar to that observed in infected C3H mice. The mycobacterial infection prevented the expression of diabetes in all of the infected NOD mice and it also decreased the incidence of proteinuria in the treated mice. The infected NOD mice showed a marked enhancement in antibodies against the 65 000 mycobacterial antigen (heat-shock protein (hsp) 65) up to the second month of infection and these elevated titres slowly decreased in the following months; anti-hsp 65 antibodies were not detected in age-matched controls. This is the first demonstration that NOD mice are naturally resistant to mycobacterial infection, and we reinforce evidence on the role of immune response triggered by mycobacteria and its hsp 65 antigen in prevention of diabetes in NOD mice.

INTRODUCTION

Type I diabetes is one of the more frequent autoimmune diseases in humans.[1] Currently, there is still no adequate preventive treatment for this disorder. Numerous experimental studies on diabetes have been performed using a murine model of the disease, the so-called non-obese diabetic (NOD) mice.[2] Previous studies had shown that diabetes of NOD mice can be prevented by infection of the animals with *Mycobacterium bovis*, bacillus Calmette–Guérin (BCG)[3] or immunization of mice with complete Freund's adjuvant (which contains heat-killed *M. tuberculosis*);[4,5] further investigations have demonstrated that this effect can be obtained by immunization of NOD mice with the heat-shock protein of 65 000 of mycobacteria (hsp 65)[6] or even with a peptide of hsp 65 (the so-called p277 peptide).[7] Recently, the prevention of diabetes in NOD mice was also observed after immunization with glutamic acid decarboxylase.[8] Immunosuppressive therapy of autoimmune diseases in animal models, particularly with anti-CD3 and anti-CD4 monoclonal antibodies, as an alternative to the classic induction of immunosuppression by drug administration, is able to re-establish tolerance to self antigens.[9] All of these findings should be useful in the design of immunotherapies in humans susceptible to autoimmune disease. Two research groups have mapped a non-major histocompatibility complex (MHC) susceptibility locus for diabetes of NOD mice to chromosome 1.[10,11] This locus was found to be linked to interleukin-1 receptor and to the *Lsh/Ity/Bcg* genes.[11] Because expression of the *Bcg* gene in mice is associated with natural resistance to infection by intracellular parasites, it was postulated that NOD mice are likely to be resistant to these infectious agents, namely to mycobacteria. In spite of *Mycobacterium bovis*–BCG bacilli being used before to infect NOD mice,[3,12,13] no information is available on whether NOD mice are resistant to mycobacterial infection. In this study, we decided to determine whether NOD mice are resistant or not to an experimental infection with *Mycobacterium avium*, a mycobacterial species that plagues acquired immune deficiency syndrome (AIDS) patients.[14–16] In addition, we have

Received 28 February 1996; revised 16 May 1996; accepted 17 May 1996.

Correspondence: Dr Artur P. Águas, Center for Experimental Cytology, University of Porto, Rua do Campo Alegre 823, 4150 Porto, Portugal.

used this model to investigate the following questions: (i) does the *M. avium* infection have a significant effect on the natural history of diabetes in NOD mice?; (ii) what are the changes induced by the infection on the serum levels of antibodies against the antigen hsp 65? Portions of this work were previous presented in abstract form.[17]

MATERIALS AND METHODS

Mice

A breeding nucleus of NOD mice was established in this research centre from mice provided by Dr E. Leiter, Jackson Laboratory, Bar Harbour, USA. Female NOD mice were infected at 2 months of age and all of the animals were confirmed to be negative for glucose in the urine at the beginning of this age. BALB/c and C3H mice were purchased from a local breeder (Gulbenkian Institute of Science) and were used to comparatively define the resistance of NOD mice to *M. avium*; BALB/c is susceptible to mycobacteria (*Bcg*^{s} murine strain) and C3H is resistant to the infection (*Bcg*^{r} murine strain).[18–22]

Mycobacteria

Mycobacterium avium strain ATCC25291 (serotype 2) was grown in liquid culture at 37° in Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI) containing 0·04% Tween 80, as previously described.[23–25]

Infection with M. avium

Mycobacteria were harvested from liquid culture by centrifugation (6000 *g*) and washed three times in phosphate-buffered saline (PBS). Cells were suspended in saline containing 0·04% Tween 80 and diluted to a concentration of 2×10^8 colony-forming units (CFU) of *M. avium* per ml. NOD, BALB/c and C3H mice were injected intraperitoneally (i.p.) with 0·5 ml of this suspension and killed at different times of mycobacterial infection. The number of viable bacilli in liver was calculated by serial dilution and plating of the homogenates onto solid 7H10 agar medium (Difco Laboratories). Bacterial CFU were counted after incubation for 15 days at 37°.

Monitoring of glycosuria and proteinuria

The clinical onset of diabetes was ascertained by the presence of glucose in the urine. Urine was tested by using commercial test strips (Comber-Test, Boehringer, Mannheim, Germany). The strips were used according to manufacter's recommendations. Concentration of protein in the urine was also measured using commercial test strips (Combur-Test, Boehringer, Mannheim, Germany).

Histology

Pancreases and kidneys were excised at the time of killing of the mice. The tissue samples were fixed in buffered 10% formalin and processed for paraffin sectioning. Tissue sections were stained with haematoxylin and eosin. Sections of pancreas were examined by light microscopy for the presence of insulitis by using the following semiquantitative classification scheme: 0 = islet devoid of inflammation; 1 = infiltrating cells observed in periductal and/or perivascular locations; 2 = inflammatory cells observed at islet periphery; 3 = mild inflammation of the islet in which <25% of the islet area contained infiltrating cells; 4 = moderate to severe inflammation of the islet. Scoring of each animal was made using the predominant inflammatory lesion observed in the tissue section. Sections of kidney were analysed to look for lesions of glomeruli and of tubular components of the organ. All histologic specimens were interpreted without knowledge of the treatment.

Determinations of the serum levels of immunoglobulins against M. avium *sonicates and specific for the 65 000 heat-shock protein (hsp 65)*

Mice sera were collected after different timings of infection with *M. avium*. Sera obtained from non-infected mice of the same strain were used as controls. The titres of antibodies specific for whole-cell homogenates of *M. avium* or for the hsp 65 antigen were determined by a standard enzyme-linked immunosorbent assay (ELISA), as previously described.[26] Briefly, bacterial sonicates or hsp 65 were adjusted to the equivalent of 10 μg protein/ml, coated overnight at 4° to 5-mm polystyrene microtitre plates (Nunc, Roskilde, Dk-4000, Denmark) and then saturated during 1 hr with 1% bovine albumin in PBS at room temperature. Appropriate serial dilutions of the serum samples were then incubated in the plates for 2 hr at room temperature. After washing, the bound antibodies were revealed by the addition of peroxidase-labelled sheep antibodies anti-mouse total immunoglobulin (Amersham International, Amersham, UK). Orthophenylenediamine (Sigma) and H_2O_2 were used to develop the reaction and the colorimetric change was measured at 450 nm.

RESULTS

M. avium infection in NOD mice

In order to study the resistance/susceptibility of NOD mice to *M. avium* infection we have quantified the mycobacterial loadings of liver and spleen of infected animals, and compared them with those obtained in BALB/c (resistant to mycobacteria) and in C3H (susceptible to mycobacteria) mice. Liver and spleen organs were chosen because previous experiments in our laboratory showed that more than 80% of the viable bacilli will grow in the liver when mice are infected i.p. with *M. avium*,[27] and spleen was established before by work from Skamene's laboratory and by other workers as a reliable parameter to determine whether animals are either naturally resistant or naturally susceptible to mycobacteria.[18–22]

We found that NOD mice were able to control the *M. avium* infection, even though, as it is common in mycobacterial infections, there was not a complete elimination of the microorganisms during the 4-month period that the infection was followed (Fig. 1). In fact, the infection was produced by injecting the mice with 10^8 viable bacilli and, at all times of infection, the CFU numbers found in the liver and spleen were lower than 10^7. In the spleen of NOD mice a decrease in bacterial loads, typical of naturally resistant mice, was seen after the second month of infection, while in the liver there was a bacteriostactic effect throughout the 4 months of infection that we studied. Comparison of the kinetics of infection between NOD mice and BALB/c or C3H mice revealed that NOD mice are *M. avium*-resistant animals with kinetics of bacterial loads in liver and spleen that are similar to that observed in C3H mice.

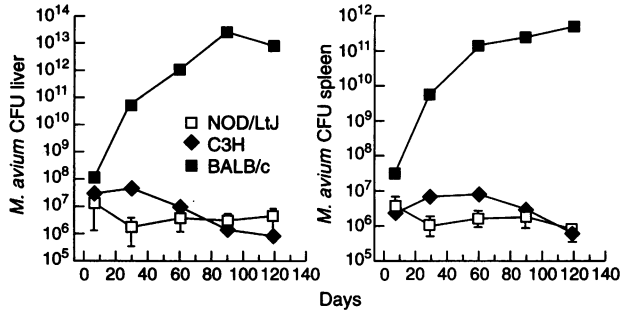

Figure 1. Comparison of mycobacterial loads in liver and spleen of NOD/Ltj mice with those quantitified in the same organs of BALB/c mice (a *Bcg*^{s}-strain that is susceptible to *M. avium* infection) and C3H mice (a *Bcg*^{r}-strain that is resistant to *M. avium* infection). All mice were intraperitoneally inoculated with 10^8 CFU of *M. avium* and the natural history of the infection was studied up to its fourth month. The data show that NOD/ltj mice were able control the infection, similarly to the *M. avium*-resistant C3H mice, whereas the bacilli were allowed to proliferate in both liver and spleen of the the mycobacteria-susceptible BALB/c mice.

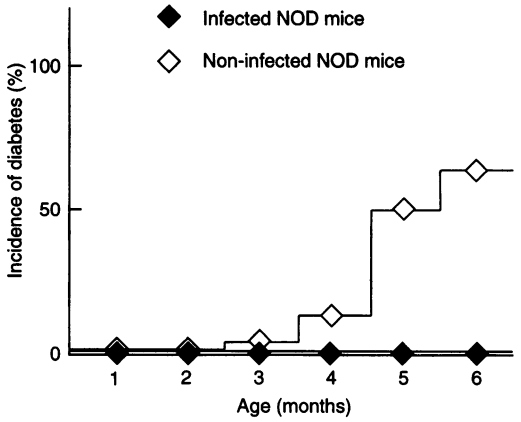

Figure 3. Comparison of incidence of diabetes between NOD mice that were infected at 2 months of age with 10^8 CFU of *M. avium* bacilli (black diamonds) and untreated age-matched NOD mice that were used as controls (white diamonds). Up to the sixth month of age of the NOD mice, the infection resulted in complete prevention of diabetes whereas more than half of untreated NOD mice presented diabetes by the same age.

Weight indices of liver and spleen of *M. avium*-infected NOD mice

As additional indicators of the effect of the *M. avium* infection in NOD mice we have calculated the weight indices of liver and spleen of animals. We found that the enlargement induced by the infection in the spleen of NOD mice was higher than the one caused in the liver of the same animals (Fig. 2). The organs' indices were higher at 3 and 4 months of infection. The increase in the spleen weight index was not accompanied, however, by enhancement in the mycobacterial loading of the organ (compare Fig. 2 with Fig. 1).

Effects of *M. avium* infection on the incidence of diabetes in NOD mice

We found that up to the sixth month of age none of the female NOD mice (n = 21) that were submitted to *M. avium* infection developed diabetes (Fig. 3). This observation was based on the absence of glucose in urine and also on the low incidence of histologic lesions of pancreatic islets (Table 1 and Fig. 4). In contrast, several of the non-infected age-matched female NOD mice presented with glycosuria by the third month of age, showing overt diabetes 2 weeks later. At 6 months of age, the

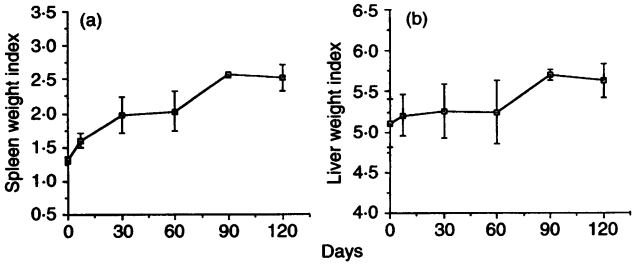

Figure 2. Kinetics of weight indeces of liver and spleen of NOD/Ltj mice infected with 10^8 CFU of *M. avium* bacilli; the organ bacterial loadings of these mice are shown in Fig. 1.

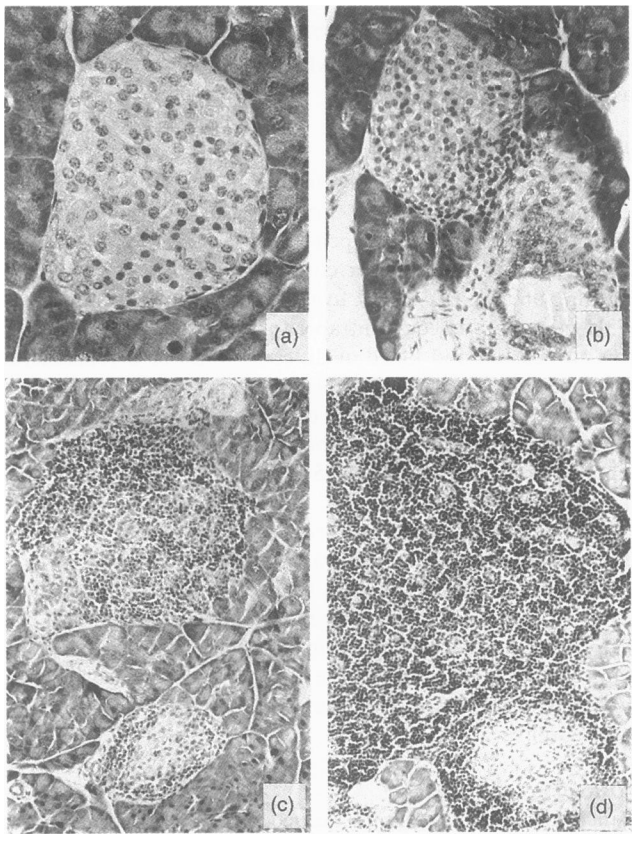

Figure 4. Light micrographs of paraffin sections of pancreatic tissue of NOD mice submitted (a and b) or not (c and d) to *M. avium* infection. The micrographs show the morphology of endocrine islets of 5–6 month old mice that were either non-diabetic (a and b) or diabetic animals (c and d). (a) Islet with normal histology (grade 0). (b) Inflammatory infiltrate at the islet periphery (grade 2). (c) Invasion of the islet by infiltrate of mononuclear cells with a just minor area of endocrine cells remaining (grade 4). (d) Complete destruction and substitution of islet by inflammatory cells (grade 4).

Table 1. Grade of insulitis in female NOD mice after 4 months *M. avium* infection

Group	No. of mice	Severity of insulitis	
		Sum of individual scores*	Percentage of insulitis score†
1 month-infected	5	5 (20)	25·0%
2 month-infected	4	8 (16)	50·0%
3 month-infected	4	9 (16)	56·2%
4 month-infected (6 months of age)	4	8 (16)	50·0%
Non-infected (6 months of age)	6	21 (24)	87·5%

* Individual mice were scored (0–4) according to the severity of the histological lesions of the pancreas: the values depicted in the table correspond to the sum of these individual scores. In parentheses is the maximum possible numerical sum of the scores for each group.

† This value represents the ratio between the sum of scores observed for each group and the maximum possible score for the same group.

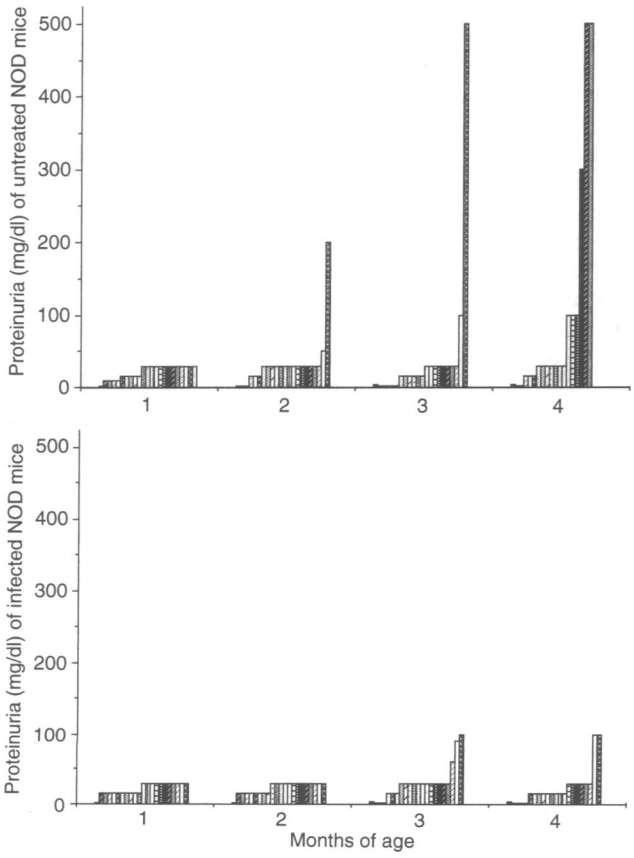

Figure 5. Example of comparison of values of protein in urine among age-matched NOD mice that were kept untreated (top graph) or were infected with *M. avium* (bottom graph). The infection decreased the frequency of proteinuiria in NOD mice.

Table 2. Comparison between the serum levels of immunoglobulin anti-*M. avium* sonicates and anti-hsp 65 found in NOD/LtJ female mice infected i.p. with 10^8 viable bacilli of *M. avium*

Time of infection	Serum immunoglobulin antibodies	
	Anti-*M. avium* sonicates	Anti-hsp65
1 month	18 453 ± 7673	1860 ± 1784
2 months	12 985 ± 3963	11 100 ± 2882
3 months	18 533 ± 1398	5366 ± 1528
4 months	11 948 ± 7334	4388 ± 2416

Results are means of four samples and are expressed in titres of antibodies (as the reciprocal of serum dilution). Control values in both cases were found to be 30.

number of mice that had clearly developed diabetes was 14 out of 22 mice (Fig. 3 and Table 1): that is 64% of untreated NOD mice developed diabetes, whereas the autoimmune disease did not affect any of the age-matched NOD mice that were infected with mycobacteria. Body weights of *M. avium*-infected NOD mice were also significantly higher than those of non-infected mice (25·1 g ± 1·22 versus 21·2 g ± 3·01, $P < 0·01$).

We have used a semiquantitative method to compare the severity of insulitis, as observed by histological examination of pancreas, between the infected and non-infected NOD mice. The most severe tissue lesions of pancreatic islets were restricted to mice that were not submitted to infection, as illustrated in Fig. 4. The data is shown in Table 1 where it is clear that the infection had a beneficial effect on the expression of diabetic lesion by pancreas of NOD mice.

Protein concentration in the urine of NOD mice

Interestingly, we found that a complement of untreated NOD mice spontaneously showed protein in urine which was observed only after the fifth month of age (Fig. 5, top graph). At 6 months of age, seven out of 22 untreated mice showed high values of proteinuria (above 300 mg/dl). In infected NOD mice only two out of 21 mice had protein in urine, and even these two mice at lower concentrations (100 mg/dl, Fig. 5).

Serum levels of specific antibodies against hsp 65 and *M. avium* sonicates in *M. avium*-infected NOD mice

We found that serum levels of specific immunoglobulin against hsp 65 were markedly increased at the first and at the second month of infection, slowly decreasing hereafter (Table 2). These values contrasted with the narrower variations observed in the titres of antibodies against *M. avium* sonicates observed during the infection (Table 2). The controls, i.e. age-matched non-infected NOD mice, showed low titres of anti-hsp 65 antibodies.

DISCUSSION

A non-MHC locus of susceptibility for diabetes of NOD mice has been mapped to chromosome 1;[10] it was proposed that this locus was linked to the *Bcg* gene.[11] This gene is associated with natural resistance of mice to mycobacteria.[18–22] It was considered that NOD mice would likely be naturally resistant

to mycobacteria, and that the host response to intracellular parasites might have an important immunomodulator role on the natural history of this autoimmune disease.[11] Here, we have investigated both of these two postulates.

This study offers the first demonstration that NOD mice are indeed resistant to mycobacterial infection, and we also show that infection with *M. avium* prevents diabetes in NOD mice. This vaccination effect was found here to be associated with elevated titres of anti-hsp 65 antibodies. Recent work from this laboratory suggests that this *M. avium*-induced protection of NOD mice from diabetes is mediated by helper T cells that are produced in response to the infection.[28]

Our conclusion that NOD mice are naturally resistant to *M. avium* infection is based on the changes in bacterial loads that we measured in the spleen and liver of the mice up to the fourth month of infection. In fact, we found that the variation in the liver and spleen loads of *M. avium* in NOD mice was similar to those that we have measured in C3H mice, which are known to be a *Bcg*^{r} mycobacteria-resistant animals.[19]

Work done before by Kaye and coworkers[29] has shown that NOD mice are resistant to leishmaniasis and that they become susceptible to the infection after transgenic introduction of an I-E molecule (NOD-E-3 mice). The expression of the transgene was found to decrease the capacity of $CD8^+$ T cells to produce interferon-γ (IFN-γ), thus indicating the important role that class II gene products have on the host response of NOD mice to leishmaniasis.[29]

The herein report of prevention of diabetes of NOD mice by *M. avium* infection are consistent with previous investigations that have demonstrated that it is possible to vaccinate NOD mice against diabetes by infecting the animals with *M. bovis*–BCG[3] or by immunization with complete Freund's adjuvant (which contains heat-killed *M. tuberculosis*).[4,5] Investigations by Cohen's group[6,7,30] have shown that NOD diabetes can be altered by immunization of the mice with an autoantigen of 60 000 (hsp 60) which belongs to the heat-shock protein family. The homologue of this protein in mycobacteria is hsp 65. hsp 65 shows more than 40% amino acid sequence homology with its mouse and human homologues.[31] Elias *et al.*[7] reported that the diabetogenic epitope of hsp 60 was located in a conserved fragment of the protein which is, therefore, shared with the mycobacterial hsp 65. For these reasons we decided to monitor the variation in the serum levels of antibodies specific for the hsp 65 mycobacterial antigen in the *M. avium*-infected and control NOD mice.

Our results revealed that *M. avium* infection induced a early and marked elevation in antibodies against hsp 65. This finding is consistent with the view that protection of NOD mice against autoimmune diabetes, herein induced by the *M. avium* infection, may be associated to an early and strong production of antibodies against hsp 65. In our study, we infected NOD mice at 2 months of age and this treatment resulted in increase of anti-hsp 65 antibodies. This timing of hsp 65 antibodies in NOD mice preceeds the timing reported for both hsp 60 antigen and anti-hsp 60 autoantibodies to occur in NOD mice that later develop diabetes.[6] It can be speculated that the anti-hsp 65 antibodies induced by infection in our experiments may (because of the hsp 60–hsp 65 homology) neutralize the hsp 60 antigen. It is possible that the *M. avium* infection that we have performed will cause the same down-regulatory effect on spontaneous immunity to hsp 60 family of proteins, similar to what has been found by Elias *et al.*[6,7,30] after injection of the mycobacterial hsp 65 in a non-immunogenic form in NOD mice.

Previous investigations using BCG infection or immunization with complete Freund's adjuvant have indicated that in NOD mice immune reactivities to antigens other than hsp 65 may also participate in the mycobacteria-induced prevention of diabetes.[3–5] The hsp 65 is also an important antigen in a mycobacteria triggered autoimmune disease, the adjuvant arthritis of the Lewis rat.[32,33] In adjuvant arthritis it is now clear that other immune reactivities participate in aetiopathogenesis of the autoimmune process.[34–38] Recent studies have shown that the modulatory effects of mycobacterial heat shock proteins on adjuvant arthritis of Lewis rats are not restricted to the hsp 65 but can also be observed after immunization of the rats with the 10 000 and 71 000 heat-shock elements.[37,38] At least for the 1000 antigen, its modulatory effect on arthritis appears to be associated with changes in the reactivity of both 10 000 protein and hsp 65.[38] It is thus conceivable that the prevention of autoimmunity triggered by mycobacteria in NOD is not due solely to host response to hsp 65.

Intravenous injection of heat-killed *M. bovis*–BCG bacilli to NOD mice was shown to cause a lupus-like disease that involved the expression of proteinuria by the mice.[39] This was interpreted as mycobacteria-induced redirectioning of autoimmune propensity of NOD mice from an organ-specific disease (diabetes) to a systemic disorder (lupus). In our experiments, we found an opposite effect of infectious mycobacteria: more than half of untreated NOD mice spontaneoulsy showed proteinuria at 6 months of age, whereas this abnormality was unfrequent among the *M. avium*-infected NOD mice. Since we used viable mycobacteria, and Baxter and coworkers[39] injected the animals with dead bacilli, the difference between our findings may indicate that the immune system of NOD mice responds differently to proliferating mycobacteria and to antigens from heat-killed bacteria. The fact that mycobacteria are able to alter the frequency of rheumatic disease in NOD mice can be learned from both studies.

In conclusion, we have demonstrated that NOD mice are naturally resistant to mycobacteria and that the infection protects the mice from diabetes and rheumatic disease expressed by proteinuria. This vaccination effect of the infection was associated with the induction of circulating antibodies specific for hsp 65, thus strenghtening previous investigations that have indicated that heat shock proteins of the 60 000 family are important autoantigens in the aetiopathogenesis of diabetes of NOD mice.

ACKNOWLEDGMENTS

We thank Professor Manuel Teixeira da Silva for support. This work was funded by grants from the JNICT (Portuguese Research Council) and the European Commission (Biomedicine and Health).

REFERENCES

1. Bach J.F. & Lesavre P. (1989) Immunologie: de la biologie à la clinique. *Médecine-Sciences Flammarion.*
2. Makino S., Kunimoto K., Muraoka Y. *et al.* (1980) Breeding of a non-obese, diabetic strain of mice. *Exp Anim* **29,** 1.
3. Harada M., Kishimoto Y. & Makino S. (1990) Prevention of overt

diabetes and insulitis in NOD mice by a single BCG vaccination. *Diabetes Res Clin Pract* **8,** 85.

4. SADELAIN M.W.J., QIN H.Y., LAUZON J. & SINGH B. (1990) Prevention of type 1 diabetes in NOD mice by adjuvant immunotherapy. *Diabetes* **39,** 583.
5. QIN H.Y., SADELAIN M.W.J., HITCHON C., LAUZON J. & SINGH B (1993) Complete Freund's adjuvant-induced T-cells prevents the development and adoptive transfer of diabetes in nonobese diabetic mice. *J Immunol* **150,** 2072.
6. ELIAS D., MARKOVITS D., RESHEF T., VAN DER ZEE R. & COHEN I.R. (1990) Induction and therapy of autoimmune diabetes in non-obese diabetic (NOD/Lt) mouse by a 65 KDa heat shock protein. *Proc Natl Acad Sci USA* **87,** 1576.
7. ELIAS D., RESHEF T., BIRK O.S., VAN DER ZEE R., WALKER M.D. & COHEN I.R. (1991) Vaccination against autoimmune mouse diabetes with a T cell epitope of the human 65 KDa heat shock protein. *Proc Natl Acad Sci USA* **88,** 3088.
8. TISCH R., YANG X.D., SINGER S.M., LIBLAU R.S., FUGGER L. & MCDEVITT H.O. (1993) Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. *Nature* **366,** 72.
9. BACH J.-F. (1993) Immunosuppressive therapy of autoimmune diseases. *Immunol Today* **14,** 322.
10. GARCHON H.J., BEDOSSA P., ELOY L. & BACH J.-F. (1991) Identification and mapping to chromosome 1 of a susceptibility locus for periinsulitis in non-obese diabetic mice. *Nature* **353,** 260.
11. CORNALL R.J., PRINS J.B., TODD J.A. *et al.* (1991) Type 1 diabetes in mice is linked to the interleukin 1 receptor and *Lsh/Ity/Bcg* genes on chromosome 1. *Nature* **353,** 262.
12. YAGI H., MATSUMOTO M., SUZUKI S. *et al.* (1991) Possible mechanisms of the preventive effect of BCG against diabetes mellitus in NOD mouse. I. Generation of suppressor macrophages in spleen cells of BCG-vaccinated mice. *Cell Immunol* **138,** 130.
13. YAGI H., MATSUMOTO M., KISHIMOTO Y. *et al.* (1991) Possible mechanisms of the preventive effect of BCG against diabetes mellitus in NOD mouse. II. Suppression of pathogenesis by macrophage transfer from BCG-vaccinated mice. *Cell Immunol* **138,** 142.
14. GRANGE J.M. & YATES M.D. (1986) Infections caused by opportunist mycobacteria: a review. *J R Soc Med* **79,** 226.
15. NIGHTINGALE S.D., BYRD L.T. & SOUTHERN P.M. *et al.* (1992) Incidence of *Mycobacterium avium-intracellulare* complex bacteremia in human immunodeficiency virus-positive patients. *J Infect Dis* **165,** 1082.
16. JACOBSON M.A., HOPEWELL P.C., YAJKO M. *et al.* (1991) Natural history of disseminated *Mycobacterium avium* complex infection in AIDS. *J Infect Dis* **164,** 994.
17. BRÁS A. & ÁGUAS (1994) Protection against autoimmune diabetes by *Mycobacterium avium* infection in NOD mice. *Abstract Book of the 12th European Meeting of Immunology*, Barcelona, pp. 99, abstr. no. W12-59.
18. GROS P., SKAMENE E. & FORGET A. (1981) Genetic control of natural resistance to *Mycobacterium bovis* (BCG) in mice. *J Immunol* **127,** 2417.
19. SKAMENE E., GROS P., FORGET A. *et al.* (1982) Genetic regulation of resistance to intracellular pathogens. *Nature* **297,** 506.
20. ORME I.M., STOKES R.W. & COLLINS F.M. (1986) Genetic control of natural resistance to nontuberculous mycobacterial infections in mice. *Infect Immun* **54,** 56.
21. APPELBERG R. & SARMENTO A.M. (1990) The role of macrophage avtivation and of *Bcg*-encoded macrophage function(s) in the control of *Mycobacterium avium* infection in mice. *Clin Exp Immunol* **80,** 324.
22. MALO D., VOGAN K., VIDAL S. *et al.* (1994) Haplotype mapping and sequence analysis of the mouse Nramp gene predict susceptibility to infection with intracellular parasites. *Genomics* **23,** 51.
23. SILVA M.T. & MACEDO P.M. (1983) The interpretation of the ultrastructure of *Mycobacterium* cells in transmission electron microscopy of ultrathin sections. *Int J Lepr* **51,** 225.
24. SILVA M.T., APPELBERG R., SILVA M.N.T. & MACEDO P.M. (1987) *In vivo* killing and degradation of *Mycobacterium avium* within mouse peritoneal macrophages. *Infect Immun* **55,** 2006.
25. ÁGUAS A.P., ESAGUY N., SUNKEL C.E. & SILVA M.T. (1990) Crossreactivity and sequence homology between the 65-kDa mycobacterial heat shock protein and human lactoferrin, transferrin and DR subsets of MHC class II molecules. *Infect Immun* **58,** 1461.
26. LUNDKVIST I., HOLMBERG D., IVARS F. & COUTINHO A. (1986) The immune response to bacterial dextrans. III. Ontogenic development and strain distribution of specific clonal precursors. *Eur J Immunol* **16,** 357.
27. ESAGUY N., MACEDO P.M., CASTRO A.P. & ÁGUAS A.P. (1992) Acquisition of autoimmunity genes by New Zealand mice is associated with natural resistance to infection by mycobacteria. *J Autoimm* **5,** 641.
28. MARTINS T.C. & ÁGUAS A.P. (1996) Changes in B and T lymphocytes associated with mycobacteria-induced protection of NOD mice from diabetes. *J Autoimm* (in press).
29. KAYE P.M., COOKE A., LUND T., WATTIE M. & BLACKWELL J.M. (1992) Altered course of visceral leishmaniasis in mice expressing transgenic I-E molecules. *Eur J Immunol* **22,** 357.
30. BIRK O.S. & COHEN I.R. (1993) T-cell autoimmunity in type 1 diabetes mellitus. *Curr Opin Immunol* **5,** 903.
31. JINDAL S., DUDANI A.K., HARLEY C.D., SINGH B. & GUPTA R.S. (1989) Primary structure of a human mitochondrial protein homologous to the bacterial and plant chaperonins and to the 65-kilodalton mycobacterial antigen. *Mol Cell Biol* **9,** 2279.
32. VAN EDEN W., HOLOSHITZ J. & COHEN I.R. (1987) Antigenic mimicry between mycobacteria and cartilage proteoglycans: the model of adjuvant arthritis. *Concepts Immunopathol* **4,** 144.
33. COHEN I.R. (1991) Autoimmunity to chaperonins in the pathogenesis of arthritis and diabetes. *Annu Rev Immunol* **9,** 567.
34. ÁGUAS A.P., ESAGUY N., VAN EMBDEN J.D.A. & SILVA M.T. (1991) Autoimmune disease and leprosy: role of molecular mimicry between host and Mycobacterium leprae proteins. *Health Coop Papers (Italy)* **12,** 87.
35. ESAGUY N., FREIRE O., VAN EMBDEN J.D.A. & ÁGUAS A.P. (1993) Lactoferrin triggers *in vitro* proliferation of T cells of Lewis submitted to mycobacteria-induced adjuvant arthritis. *Scand J Immunol* **38,** 147.
36. ESAGUY N. & ÁGUAS A.P. (1996) Prevention of adjuvant arthritis in Lewis rats by neonatal bacille Calmette–Guérin (BCG) infection. *Clin Exp Immunol* **104,** 103.
37. KINGSTON A.E., HICKS C.A., COLSTON M.J. & BILLINGHAM M.E.J. (1996) A 71-kD heat shock protein (hsp) from *Mycobacterium tuberculosis* has modulatory effects on experimental rat arthritis. *Clin Exp Immunol* **103,** 77.
38. RAGNO S., WINROW V.R., MASCAGNI P. *et al.* (1996) A synthetic 10-kD heat shock protein (hsp 10) from *Mycobacterium tuberculosis* modulates adjuvant arthritis. *Clin Exp Immunol* **103,** 384.
39. BAXTER A.G., HEALEY D. & COOKE A. (1994) Mycobacteria precipitate autoimmune rheumatic disease in NOD mice via an adjuvant-like activity. *Scand J Immunol* **39,** 602.