Lupus (2014) 23, 407–411
http://lup.sagepub.com

CONCISE REPORT

Acceleration of SLE-like syndrome development in NZBxNZW F1 mice by beta-glucan

P Fagone[1], K Mangano[1], S Mammana[1], C Quattrocchi[1], G Magro[2], M Coco[1], S Imene[3], R Di Marco[4] and F Nicoletti[1]
[1]Department of Bio-medical Sciences, University of Catania, Catania, Italy; [2]Department GF Ingrassia, Azienda Ospedaliero-Universitaria 'Policlinico-Vittorio Emanuele', Anatomic Pathology, University of Catania, Catania, Italy; [3]Laboratory of Cellular and Molecular Biology, University of Bab-Ezzouar, Algiers, Algeria; and [4]Department of Medicine and Health Sciences, University of Molise, Campobasso, Italy

Beta-glucans are naturally occurring polysaccharides that exert important immunostimulatory activities. In the present study, we evaluated whether beta-glucans could modulate the development and the course of systemic lupus erythematosus (SLE). To this aim, we employed the classical model of SLE represented by the F1 hybrid between the NZB and NZW mouse strains which develop severe lupus-like phenotypes comparable to that of SLE patients. The administration of beta-glucan was associated to a more aggressive development of the disease and a worse prognosis, as observed from the clinical, biochemical and histopathological data. This finding implies that restraint should be practised in the possible use of beta-glucans as immunomodulators in human therapy in the context of SLE. *Lupus* (2014) **23,** 407–411.

Key words: Anti-DNA antibodies; nephritis; renal lupus

Introduction

Beta-glucans are naturally occurring polysaccharides that exert important immunostimulatory activity.[1] In fungi, beta-glucans are major structural components of the cell wall and occur as (1,3)-beta-linked glucose polymers with (1,6)-beta-glucose side chains of various lengths and distributions.[1] As major structural components of fungal cell wall, they represent key pathogen-associated molecular patterns (PAMPs) for the pattern recognition receptor (PRR)-mediated immune response to fungal infection.[1] Several receptors have been identified, including dectin-1, which mediate beta-glucan activation of phagocytosis and production of cytokines, a response co-ordinated by the toll-like receptor (TLR)-2.[2] The possible interference of beta-glucans with innate immune system and modulation of TLR pathways is consistent with their ability to exert protective effects in different rodent models of sepsis[3,4] and burn-induced oxidative damage in rats.[5]

These immunomodulatory properties of beta-glucan prompted us to test its effects on the development and the course of a mouse model of systemic lupus erythematosus (SLE). The NZB/W F1 is a classical model of SLE represented by the F1 hybrid between the NZB and NZW mouse strains (see Perry et al.[6] for a review). Both NZB and NZW display limited autoimmunity, while NZB/W F1 hybrids develop severe lupus-like phenotypes comparable to that of SLE patients. These lupus-like phenotypes include elevated serum antinuclear autoantibodies (ANA) and immune complex-mediated glomerulonephritis (GN) that becomes apparent at 5–6 months of age, leading to kidney failure and death.[6]

Our study demonstrates that the administration of beta-glucan was associated to a more aggressive development of the disease and a worse prognosis, as observed from the clinical, biochemical and histopathological data. This finding implies that restraint should be practised in the possible use of beta-glucans as immunomodulators in human therapy in the context of SLE and warns on the use of this drug in patients with SLE.

Correspondence to: F Nicoletti, Department of Bio-medical Sciences, University of Catania, Via Androne, 81 95124, Catania, Italy.
Email: ferdinic@unict.it
Received 7 March 2013; accepted 8 January 2014

10.1177/0961203314522333

Materials and methods

Animals and reagents

Female 15-week-old NZB/W F1 mice were purchased from Charles River (Milano, Italy). The animals were kept under standard laboratory conditions (non-specific pathogen free) with *ad libitum* food and water, and were allowed to adapt for one week to their environment before the study commenced. All procedures were done in compliance with the guidelines of the Institutional Animal Care and Use of the University of Catania, Italy.

Clinical grade highly purified soluble beta-glucans isolated from *Saccharomyces cerevisiae* were kindly provided by Biotec Pharmacon, Norway. Endotoxin levels were below 0.05 EU/ml.

Experimental study plan

To evaluate the effect of beta-glucan on the course of SLE-like syndrome in NZB/W F1 mice, two groups of mice ($n = 12$ per group) were created that were treated respectively *per os* (p.o.) by gavage administration either with a soluble beta-glucan formulation in water at the dose of 20 mg/kg or with vehicle (water for injection) five times a week for 25 consecutive weeks starting when the mice reached 16 weeks of age. This dose of beta-glucans has been chosen on the basis of a previous study that showed that when administered orally at a dose of approximately 400 μg for 29 days to mice, beta-glucans enhanced the anti-tumour efficacy of chemotherapeutic monoclonal antibody.[7] As our experimental design implied treatment of the mice for a prolonged time, we chose a dose equal to 600 μg, five times a week.

Mice were checked twice a week for body weight variation and followed for the development of renal disease as measured by proteinuria and for survival. Proteinuria was measured using commercially available semiquantitative strips (Albustix, Miles Laboratories, Elkhart, IN) graded as: trace (+/−) = 10 mg/dl; (+) = 30 mg/dl; (++) = 100 mg/dl; (+++) = 300 mg/dl; (++++) = 1000 mg/dl. For statistical analysis, the intensity of the colorimetrical reaction of each mouse was reported numerically (10 mg/dl = 0.5; 30 mg/dl = 1; 100 mg/dl = 2; 300 mg/dl = 3; 1000 mg/dl = 4) and the mean value from each experimental group calculated by dividing the total score by the number of mice in that group, as described elsewhere.[8]

In a parallel study, to evaluate the effects of beta-glucan treatment on autoantibody production and development of nephritis, another two groups of mice were created that were treated as above with either beta-glucan or vehicle for 10 consecutive weeks, starting from the age of 16 weeks, after which they were sacrificed by CO_2 asphyxiation. The mice were bled for measurement of autoantibody at the beginning of the study and, by cardiac puncture, at sacrifice at week 26. Additional measurement of autoantibody was carried out in the surviving mice used for the survival study at the 36th week of age. Kidneys from mice sacrificed after 10 weeks of beta-glucan treatment were used for histological analysis of nephritis and for PAS staining.

Autoantibody measurement

Anti-double-stranded DNA autoantibodies (anti-dsDNA Abs) in the serum were measured by an in-house-developed custom ELISA assay. Briefly, deoxyribonucleic acid from calf thymus (Sigma, Milan, Italy) was used as coating Ag. NZB/W F1 mouse serum was incubated overnight and goat anti-mouse pAb-HRP (Abcam, Cambridge, UK) used as detection Ab. Plates were developed by using tetramethylbenzidine/H_2O_2 and reaction stopped using 1.8 M ortho-phosphoric acid. Plates were read at 450 nm by spectrophotometry.

Glomerulonephritis and PAS staining

Kidneys from mice sacrificed after 10 weeks of treatment were collected and weighed and the left kidney fixed in 10% buffered formalin for subsequent H&E and PAS staining histopathological analysis. All histological scoring was performed by an independent medical pathologist. The pathological lesions were graded from 0 to 4 as follows: 0 = normal; 1 = a small increase of cells in the glomerular mesangium; 2 = a larger number of cells in the mesangium; 3 = glomerular lobular formation and thickened basement membrane; 4 = glomerular crescent formation sclerosis, tubular atrophy and casts. The score for each animal was calculated by dividing the total score for the number of glomeruli observed (at least five sections per animal), as described elsewhere.[8]

Statistical analysis

Data are presented as the mean ± SD. Statistical analysis for significant differences was performed according to Student's t test for unpaired data or Mann–Whitney U test. Mantel–Cox log-rank test was used to compare the survival curves of beta-glucan-treated versus vehicle-treated groups.

A value of $p < 0.05$ was considered to be statistically significant.

Results

Effects of beta-glucan treatment on SLE development

Vehicle-treated mice started to die at 23 weeks of age and reached the median survival by the 44th week of age. In the group of mice treated with beta-glucan the mice started to die at week 31 but median survival was reached earlier than the vehicle controls, by the 34th week of age. The difference in survival between treated and control mice was statistically significant ($p = 0.0175$ by Mantel–Cox test) at the end of the study (Figure 1(a)). The worse prognosis of beta-glucan-treated mice was also evident by the reduced body weight increase compared with vehicle-treated mice, the AUC for beta-glucan-treated mice being 6798 *vs.* 7559 for control mice (Figure 1(b)) ($p = 0.0086$ by Mann–Whitney *U* test). However, proteinuria levels in the soluble beta-glucan-treated mice were not significantly different compared with those from the vehicle-treated mice throughout the study period (Figure 1(c)).

The presence of anti-dsDNA Abs is commonly used as a biomarker associated with poor prognosis of SLE and is strongly associated with developing lupus nephritis. The effect of beta-glucan on anti-dsDNA autoantibody production was evaluated after 10 weeks of treatment and at the end of the experimental period. Only slightly increased levels of anti-dsDNA antibodies were found at 26 weeks of age in both vehicle and beta-glucan-treated animals. In contrast, at 20 weeks of the study, when mice were 36 weeks old, beta-glucan-treated mice showed significantly higher levels of autoantibodies in the serum ($p < 0.0001$ by Mann–Whitney *U* test) (Figure 1(d)).

Beta-glucan exacerbates nephritis

Nephritis was evaluated by histopathology and scored by an independent pathologist for the assessment of renal damage. Vehicle-treated NZB/W F1 mice at the 26th week of age showed glomeruli with increased mesangial cellularity and matrix

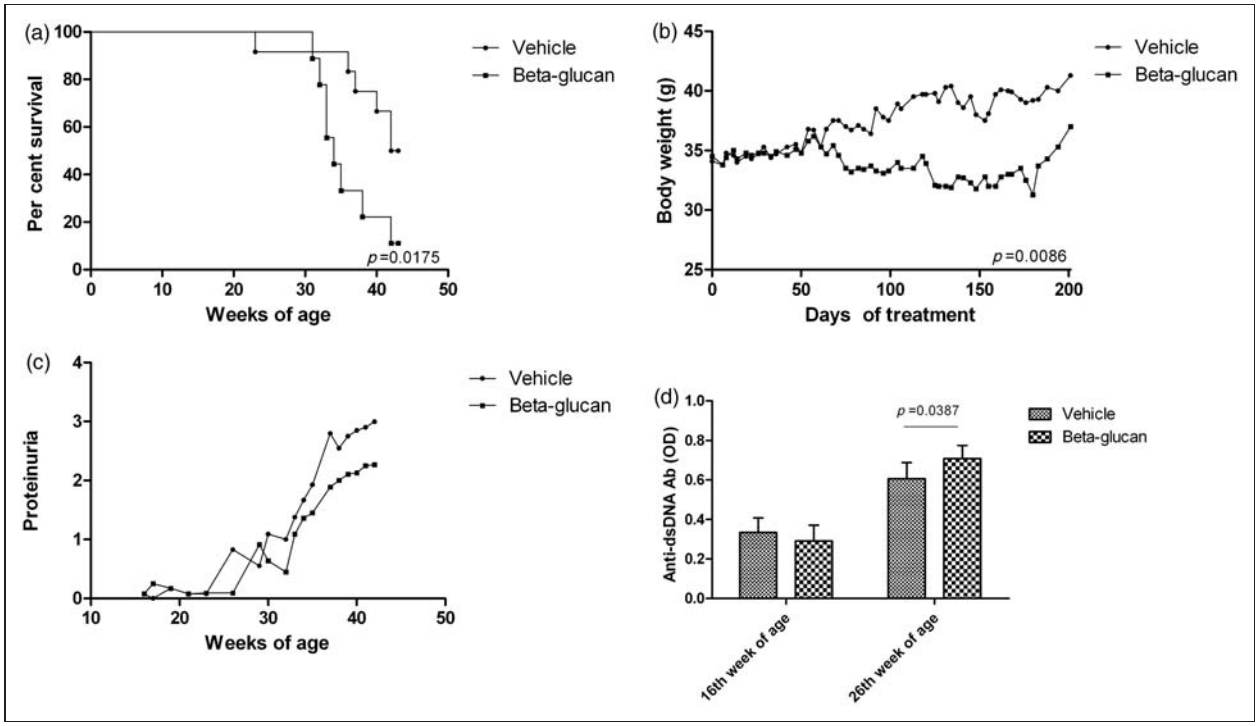

Figure 1 NZB/W F1 mice ($n = 12$ per group) were treated *per os* with a soluble beta-glucan formulation in water at the dose of 20 mg/kg or with vehicle, five times a week when the mice reached 16 weeks of age. Survival was monitored up to the 50th week of age (a) and mice were checked twice a week for body weight variation (b). Proteinuria was measured weekly using commercially available semiquantitative strips (c) and serum anti-dsDNA antibodies (anti-dsDNA Ab) measured by semiquantitative ELISA assay (d).
OD: optical density

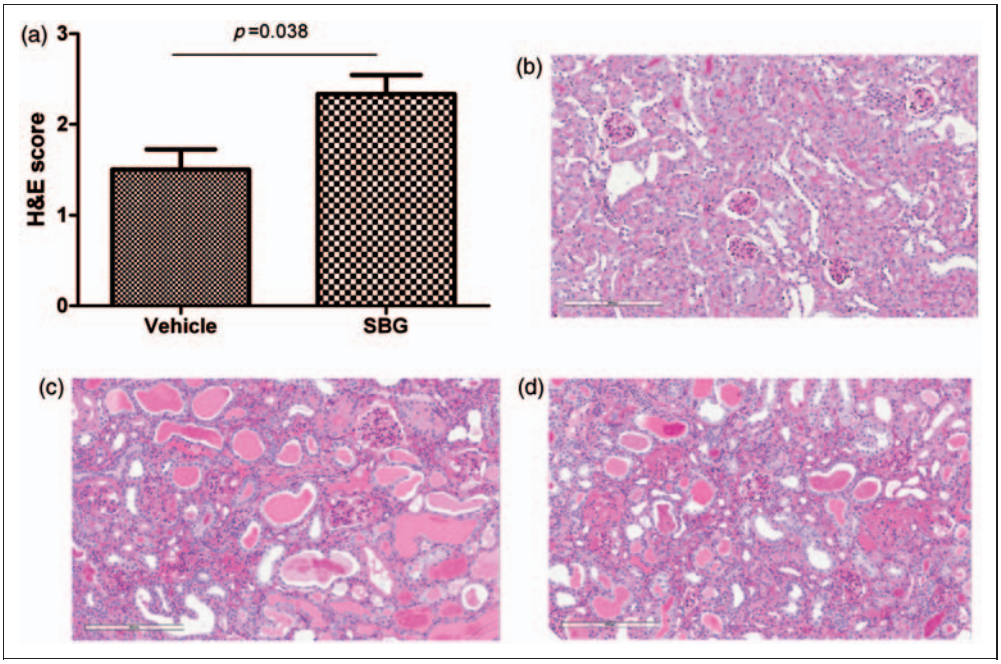

Figure 2 NZB/W F1 mice ($n = 6$) were treated *per os* with a soluble beta-glucan (SBG) formulation in water at a dose of 20 mg/kg or with vehicle, five times a week for 10 consecutive weeks starting when the mice reached 16 weeks of age. At the end of the treatment, the left kidney was fixed in 10% buffered formalin for histopathological analysis. (a) Histogram showing the H&E histopathological score of kidneys from vehicle- and SBG-treated mice. (b) Representative PAS-stained section of kidneys from a healthy mouse showing normal glomeruli architecture. (c) Representative PAS-stained section of kidneys from a vehicle-treated mouse showing increased mesangial cellularity and matrix deposition. (d) Representative PAS-stained section of kidneys from a SBG-treated mouse showing severe glomerulosclerosis.

deposition as compared with healthy mice. Increased mesangial cellularity and matrix deposition was observed in the beta-glucan-treated group when compared with the vehicle-treated group (Figure 2). Mean scores were 1.6 ± 0.4 and 2.6 ± 0.7 for the vehicle and the beta-glucan groups, respectively ($p = 0.038$ by Mann–Whitney U test) (Figure 2(a)). No differences in the weight of spleen, thymus and kidneys were observed (data not shown).

Discussion

The present study demonstrates that prolonged treatment with beta-glucans accelerates SLE-like mortality in the NZB/W F1 mice. Simultaneously beta-glucan-treated mice exhibited progressive increase of the autoantibody titres and more severe nephritis than vehicle-treated controls. It is, however, difficult to define precisely to what extent the enhancement of autoreactivity and nephritogenesis induced by the treatment has been responsible for the reduced life-span of the NZB/W F1 mice. In fact, no significant differences in the extent and severity of proteinuria were observed between the mice treated with either beta-glucans or vehicle throughout the study period. It is possible that the nephritogenic effects of beta-glucans were of a sufficient magnitude to be observed histologically without modifying a relatively insensitive laboratoristic parameter of kidney function such as proteinuria.

The results of our study confirm and extend an old study from Harima and coworkers, who found that prolonged subcutaneous treatment of NZB/W F1 mice with glucan for three months induced early death, with significant differences in accumulated mortality rates over 33–37 weeks, when compared with controls.[9] However, glucan treatment was found to protect NZB/W F1 mice from septic shock induced by *Klebsiella pneumoniae*. This empirically implied that the protective mechanism promoted by glucan against septic shock induced by *K. pneumoniae* may turn into pathogenetic effectors of lupus development in these mice. It is interesting to note that beta-glucans seem to specifically exacerbate autoimmunity in NZB/W F1 mice. In fact, we found no signs of nephritis or lethality in 'normal' non-autoimmune prone six-week-old female Balb/c and C57Bl6 mice treated for four consecutive months with the same dosing of

beta-glucans that exacerbated SLE-like syndrome in NZW mice (data not shown). In addition, beta-glucans do not seem to accelerate all forms of rodent autoimmune diseases as we did not find exacerbation of autoimmune diabetes in female NOD mice and proteolipid protein-induced experimental autoimmune encephalomyelitis in SJL mice (data not shown).

The pharmacological profile of glucans suggests that activation of TLRs might have been responsible for the exacerbation of SLE-like syndrome in NZB/W mice. This concurs with the increasing evidence that dysregulated activity of TLRs, in particular TLRs 2, 7 and 9, may play a key pathogenetic role both in murine and in human SLE.[10-12] It has been suggested that most TLRs can also recognize self-ligands and that mechanisms are required to discriminate between self- and non-self-ligands. Inappropriate activation of TLRs by self-components can result in sterile inflammation or autoimmunity. Known inhibitors of TLR7 and 9 such as antimalarial agents are known to exert beneficial effects in human SLE.[13]

Further highlighting a possible role of glucans in SLE exacerbation, Salazar-Aldrete and colleagues[14] have found that monocytes from SLE patients show an abnormal calcium flux response induced by dectin-1 ligands, that are expressed on glucans, as well as an enhanced release of interleukin (IL)-1β, IL-6 and TNF-α, but not IL-23, upon dectin-1 engagement. Thus drugs that potentially stimulate monocytes could possibly also lead to increased production of antibodies in SLE.

The results from this study indicate that beta-glucans, and possibly other immunomodulators that stimulate TLR function, should be avoided in SLE patients and in individuals with increased risk of its development.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors have no conflicts of interest to declare.

References

1 Kankkunen P, Teirilä L, Rintahaka J, *et al*. (1,3)-beta-glucans activate both dectin-1 and NLRP3 inflammasome in human macrophages. *J Immunol* 2010; 184: 6335–6342.
2 Gantner BN, Simmons RM, Canavera SJ, *et al*. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J Exp Med* 2003; 197: 1107–1117.
3 Sandvik A, Wang YY, Morton HC, *et al*. Oral and systemic administration of beta-glucan protects against lipopolysaccharide-induced shock and organ injury in rats. *Clin Exp Immunol* 2007; 148: 168–177.
4 Sener G, Toklu H, Ercan F, *et al*. Protective effect of beta glucan against oxidative organ injury in a rat model of sepsis. *Int Immunopharmacol* 2005; 5: 1387–1396.
5 Toklu HZ, Sener G, Jahovic N, *et al*. Beta glucan protects against burn-induced oxidative organ damage in rats. *Int Immunopharmacol* 2006; 6: 156–169.
6 Perry D, Sang A, Yin Y, *et al*. Murine models of systemic lupus erythematosus. *J Biomed Biotechnol* 2011; 2011: 271694.
7 Cheung NK, Modak S, Vickers A, *et al*. Orally administered beta-glucans enhance anti-tumor effects of monoclonal antibodies. *Cancer Immunol Immunother* 2002; 51: 557–564.
8 Nicoletti F, Di Marco R, Zaccone P, *et al*. Dichotomic effects of IFN-gamma on the development of systemic lupus erythematosus-like syndrome in MRL-lpr/lpr mice. *Eur J Immunol* 2000; 30: 438–447.
9 Harima HA, Mendes NF, Mamizuka EM, *et al*. Effect of glucan on murine lupus evolution and on host resistance to Klebsiella pneumoniae. *J Clin Lab Anal* 1997; 11: 175–178.
10 Anders HJ. A Toll for lupus. *Lupus* 2005; 14: 417–422.
11 Kirchner M, Sonnenschein A, Schoofs S, *et al*. Surface expression and genotypes of Toll-like receptors 2 and 4 in patients with juvenile idiopathic arthritis and systemic lupus erythematosus. *Pediatr Rheumatol Online J* 2013; 11: 9.
12 Choi J, Kim ST, Craft J. The pathogenesis of systemic lupus erythematosus – an update. *Curr Opin Immunol* 2012; 24: 651–657.
13 Meinão IM, Sato EI, Andrade LE, *et al*. Controlled trial with chloroquine diphosphate in systemic lupus erythematosus. *Lupus* 1996; 5: 237–241.
14 Salazar-Aldrete C, Galán-Díez M, Fernández-Ruiz E, *et al*. Expression and function of dectin-1 is defective in monocytes from patients with systemic lupus erythematosus and rheumatoid arthritis. *J Clin Immunol* 2013; 33: 368–377.