Yeast-derived Beta-(1-3),(1-6)-D-glucan Induces Up-regulation of CD86 on Dectin-1^{positive} Human B-Lymphoma Cell Lines

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Abstract. *Background: The knowledge of direct effects of β-glucans on tumor cells is limited. This study evaluated the impact of a soluble yeast-derived beta-(1-3),(1-6)-D-glucan, containing a fraction of aggregated sugar polymers, on viability, proliferation and expression of CD86 of the human B-cell lymphoma cell lines Daudi and Raji. Materials and Methods: Proliferation of carboxyfluorescein diacetate succinimidyl ester (CFSE)-stained cell lines was determined by measuring depletion of the dye and cell death was quantified by staining with propidium iodide, both by flow cytometry. Surface expression of CD86 and the beta-glucan receptors dectin-1 and complement receptor 3 (CR3) was assessed by flow cytometry. Results: Exposure to the carbohydrate increased the expression of CD86 on both dectin-1$^+$CR3$^-$ cell lines, whereas proliferation and viability of the cells were not affected. Conclusion: Yeast-derived beta-glucan lacks cytotoxic effects towards B-lymphoma cells but up-regulation of CD86 suggests maturation of the cells via dectin-1 by the carbohydrate.*

Beta-(1-3),(1-6)-D-glucans are branched glucose polymers derived from the cell wall of a variety of microorganisms and plants. In numerous studies, beta-glucans were shown to exert tumor-inhibitory effects *in vivo* due to the activation of innate immune cells such as dendritic cells (DCs), macrophages and granulocytes (1-3). These carbohydrates mediate their activating effects on leukocytes, which comprised enhanced production of pro-inflammatory or immune-stimulatory cytokines, up-regulation of major histocompatibility complex (MHC) class-II and T-cell co-stimulatory molecules or tumoricidal activity, *via* binding to the C-type lectin receptor dectin-1, complement receptor (CR) 3 (Mac-1;CD11b/CD18) or toll-like receptors (TLR) (2-7). The major beta-glucan receptor dectin-1 is expressed on murine and human monocytes, macrophages, DCs, granulocytes and subsets of B- and T-cells and signals through the spleen-tyrosine kinase (SYK) or the RAF-1 pathway (3, 4, 7-12). CR3 is abundantly present on neutrophils, natural killer (NK) cells and macrophages (13, 14). Thus, the majority of cell lines tested for responsiveness to beta-glucans of different origin are of the monocytic lineage next to a variety of solid tumor cell lines (15-20). These carbohydrates showed only limited potential to induce maturation of dectin-1$^+$ monocytic leukemia cell lines and promoted or transiently inhibited their growth, whereas significant antiproliferative effects of these compounds were observed on sarcoma-180 and cervical cancer cell lines *in vitro* (15-17, 19, 20). The effects of β-glucans on lymphocytic tumor cells are not well characterized, despite constitutive expression of dectin-1 and SYK being reported from human B-cell lymphoma cell lines (21-23). This study evaluated the impact of a yeast-derived beta-(1-3),(1-6)-D-glucan on the viability, proliferation and expression of CD86 of human B-cell lymphoma cell lines.

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Key Words: Dectin-1, beta-glucan, B-cell lymphoma, CD86, proliferation, cell death.

Materials and Methods

Cell lines and culture. The human B-cell lymphoma cell lines Daudi and Raji were purchased from the ATCC (Manassas, VA, USA) and were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin (all from Biochrom AG, Berlin, Germany).

Reagent. Aqueous preparation of soluble underivatized beta-(1–3), (1–6)-D-glucan (20 mg/ml) derived from the cell wall of *Saccharomyces cerevisiae* was provided by Biotec Pharmacon (Tromso, Norway). The glucan preparation contained a fraction of aggregated glucan polymers and its endotoxin level was <0.05 EU/ml.

Detection of surface markers. Expression of dectin-1 was determined by flow cytometry using murine anti-human dectin-1 antibodies GE-2 (AbD Serotec, Oxon, UK) or 259931 (R&D,

Abingdon, UK) and as secondary antibody a polyclonal fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (clone: Poly4053; BioLegend, Fell, Germany) or allophycocyanin (APC)-conjugated donkey anti-mouse IgG (eBioscience, Frankfurt, Germany). Surface expression of CD86 was determined by flow cytometry using phycoerythrin (PE)-conjugated antibody IT2.2 (eBioscience). Appropiate isotype control antibodies were from eBioscience. Mean fluorescence intensity (MFI) of the surface marker was assessed after 72 h incubation with beta-glucan.

In vitro treatment with β-glucan. Cells (at 1× or 2×10^4) were seeded in 1 ml complete medium in duplicates or triplicates of a 24-well plate and were incubated with the indicated concentrations of beta-glucan or remained untreated. In single experiments, medium without cells was incubated with beta-glucan to control for interference of the flow cytometric signals of cells and glucan aggregates. Dead cells were quantified by flow cytometry after 24 h and 72 h by staining with propidium iodide (2 μg/ml) immediately prior to the measurement. To determine the effect of the beta-glucan on cell proliferation lymphoma cells were stained with 0.5 μM solution of carboxyfluorescein diacetate succinimidyl ester (CFSE) (Sigma, Taufkirchen, Germany). The MFI of the dye was assessed as described above.

Statistical analysis. Data are presented as the mean±standard error of the mean (SEM) of duplicate or triplicate determinations. Statistical significance was determined using the two-sided, paired Student's *t*-test. A *p*-value <0.05 was considered to indicate a statistically significant difference.

Results

Expression of dectin-1 on human B-cell lymphoma cell lines. Surface expression of dectin-1 was detected on both cell lines with low to moderate intensity (Figure 1). In contrast, the B-lymphoma cell lines did not express CR3 (data not shown).

Effect of β-glucan on the expression of CD86 on B-cell lymphoma cell lines. Expression of CD86 was significantly up-regulated on both cell lines by exposure to a broad dose-range of beta-glucan within 72 h (Figure 2A). Modulation of CD86 expression was seen as early as 48 h of incubation with the carbohydrate (data not shown) and peaked by treatment of Raji cells with 1 μg/ml and Daudi cells with 0.1 μg/ml of the polymeric sugar. It was possible to separate aggregates of the beta-glucan preparation from lymphoma cells even at the highest dose of the agent by gating during flow cytometric data analysis (Figure 2B).

Effect of beta-glucan on viability of human B-cell lymphoma cell lines. Treatment of both cell lines with a broad dose-range of beta-glucan exhibited no notable effect on the viability of the cells after 24 h and 48 h *in vitro* (Figures 3A and B). Only a weak tendency for an increased percentage of dead cells was observed in Daudi cells incubated with 0.1 μg/ml and 1.0 μg/ml beta-glucan (Figure 3B).

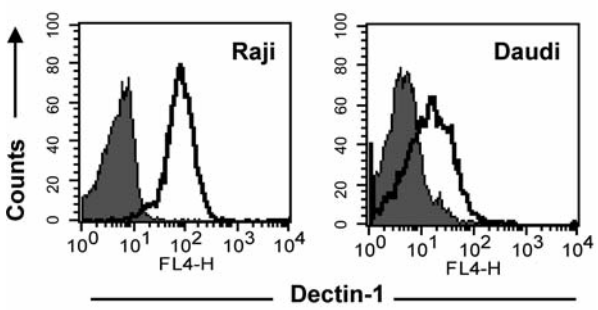

Figure 1. *Expression of dectin-1 on human B-cell lymphoma cell lines. Raji cells (left) and Daudi cells (right) were stained with a monoclonal antibody against dectin-1 and an allophycocyanin (APC)-labeled secondary antibody before analyzed by flow cytometry. The thick line represents expression of dectin-1 and the thin line (grey area) denotes the isotype control. Data shown are representative of three independently performed experiments.*

Effect of beta-glucan on proliferation of human B-cell lymphoma cell lines. Treatment with different concentrations of beta-glucan also showed no effect on the proliferation of Raji and Daudi cells after 72 h (Figure 4).

Discussion

In this study, we evaluated the effect of a beta-(1,3),(1-6)-D-glucan derived from the cell wall of *Saccharomyces cerevisiae* on surface expression of CD86, and the viability and proliferation of the human Burkitt lymphoma cell lines Raji and Daudi. We found that Daudi cells express the major beta-glucan receptor dectin-1 and confirm expression of this receptor by Raji cells as a prerequisite for a direct susceptibility of the cells to beta-glucan activity (21). CR3 was not detectable on lymphoblastoid cell lines as reported for Raji cells, whereas Daudi cells were shown to carry the receptor on 25% of the cells (13, 24). In recent studies, beta-glucans of different origin, including the preparation used in the present study, induced significant increase of the expression of co-stimulatory molecules such as CD86 and CD40 on macrophages or DCs *in vitro* and *in vivo* (3-7, 25-27). Accordingly, up-regulation of CD86 on both B-cell lymphoma cell lines indicates further maturation of the cells and was likely mediated by binding of glucan aggregates to dectin-1 (26, 28). Recently, the dectin-1/SYK-pathway was demonstrated to mediate the strong up-regulation of co-stimulatory surface molecules by beta-(1,3),(1-6)-D-glucans on macrophages and DCs (3, 26, 29, 30). In primary B-lymphoma and B-lymphoma cell lines, SYK was shown to be constitutively activated (22, 23), and expression of CD86 was found to be reduced on peritoneal B-1a cells upon inhibition of this kinase (31). Thus, the increased CD86 expression on Raji and Daudi cells could have been mediated by a further

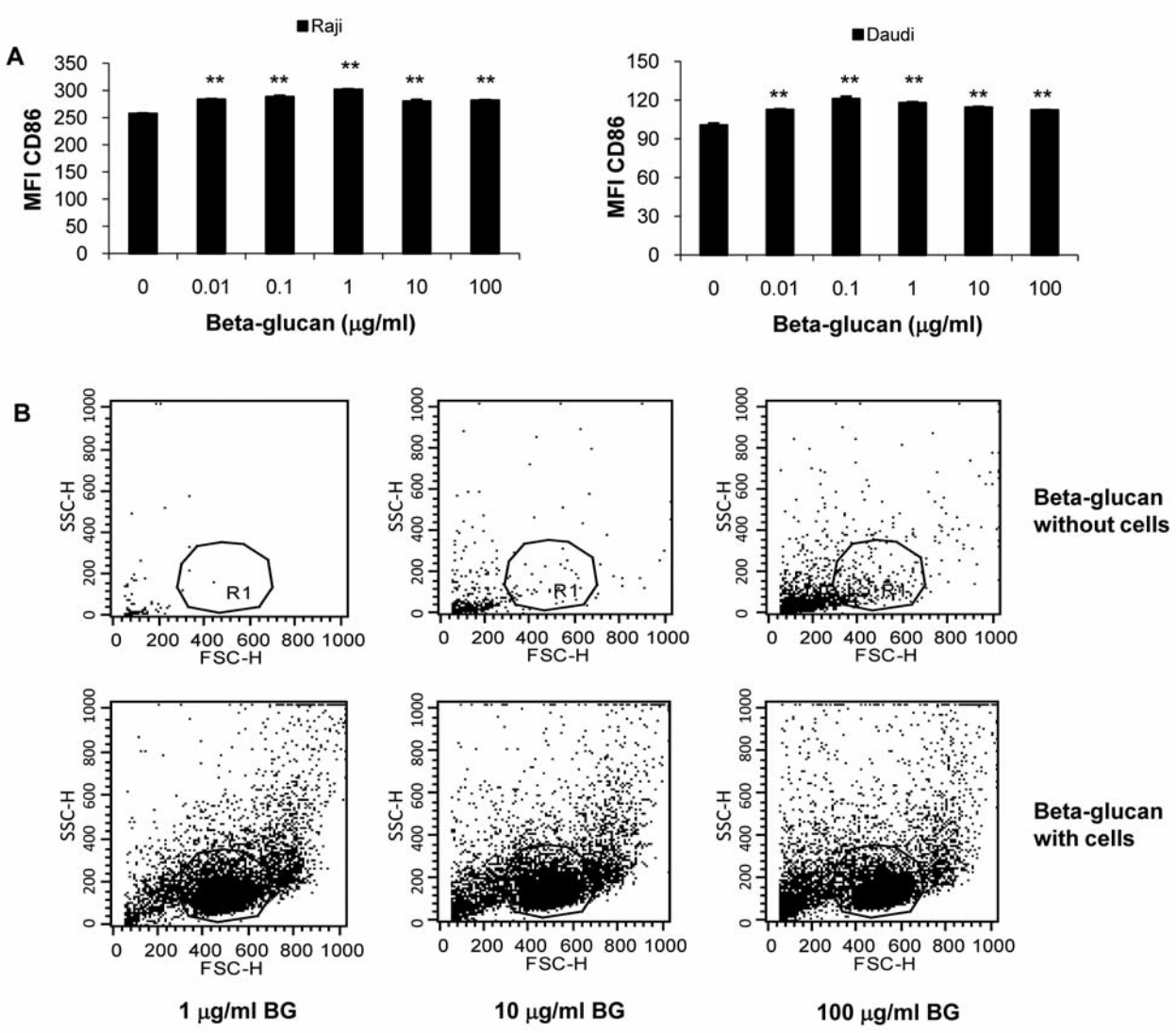

Figure 2. *Effect of beta-glucan on expression of CD86 on human B-cell lymphoma cell lines. A: Raji cells (left) and Daudi cells (right) were incubated with the indicated concentrations of beta-glucan or remained untreated for 72 h before expression of CD86 was determined by flow cytometry. B: To localize aggregates of beta-glucan (BG) polymers in the FSC/SSC-plot, different concentrations of the carbohydrate were measured by flow cytometry after 48 h incubation in medium without Daudi cells (upper row) or with cells (lower row). Data show the mean±SEM of triplicate determinations of one experiment representative for three independently performed experiments (CD86) and dot plots from duplicate determinations representative for two independently performed experiments (BG with/without cells). */**p<0.05/0.005 of treated cells vs. control cells.*

activation of SYK after glucan-binding to dectin-1 but could have been limited due to the existing activation level of SYK, which correlates with the relatively mature phenotype of these B-cell lymphomas (32). Accordingly, up-regulation by beta-glucan from *Ganoderma lucidum* of CD86 on the monocytic leukemia cell line THP-1, which shows constitutive activation of SYK but expresses dectin-1 at much lower levels compared to the B-cell lymphoma cell lines, was reportedly low and required a 100-fold dose of the carbohydrate as used in this study (15, 16, 33, 34). The demonstrated maturation of THP-1 cells to a DC-like phenotype by the combination of beta-glucan and interleukin-4 (IL-4)/granulocyte-macrophage colony-stimulating factor (GM-CSF) but not by the toll-like receptor (TLR)-4 agonist lipopolysaccharide (LPS) or the TLR-2/dectin-1 ligand zymosan plus cytokines provides further evidence that dectin-1 solely mediates maturation signals of the carbohydrates into leukemic cells (15). The lack of a cytotoxic effect of the beta-glucan on the viability of the lymphoma cell lines may also be attributed to activation of SYK which was shown to mediate pro-survial and growth-

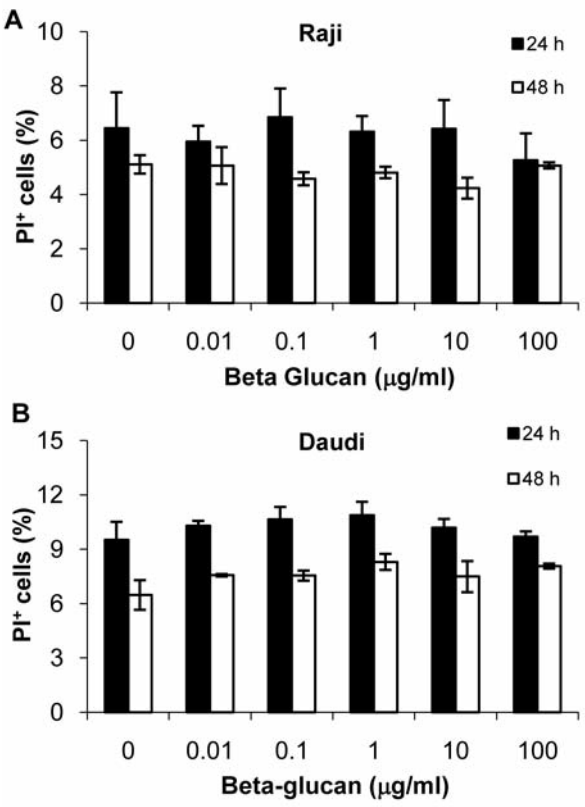

Figure 3. *Effect of beta-glucan on viability of B-cell lymphoma cell lines. A and B: Raji cells and Daudi cells were incubated with beta-glucan or remained untreated for 24 h and 48 h before dead cells were quantified by PI staining and flow cytometry. Data show the mean±SEM of duplicate or triplicate determinations and are representative of three independently performed experiments.*

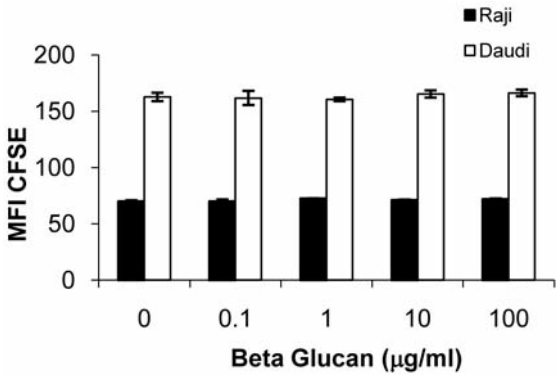

Figure 4. *Effect of beta-glucan on proliferation of B-cell lymphoma cell lines. Raji cells and Daudi cells were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) and incubated with beta-glucan or remained untreated for 72 h before the mean fluorescence intensity of the dye was measured by flow cytometry. Data show the mean±SEM of duplicate or triplicate determinations and are representative of three independently performed experiments.*

promoting signals in B-cell lymphomas (22, 23). Similarly, in THP-1 cells, beta-glucan from *Ganoderma lucidum* was shown to fail to induce cell cycle arrest (15). However, a stimulatory effect of the beta-glucan on cell proliferation which was demonstrated in monocytic leukemia cell lines would have been difficult to detect in the B-cell lymphoma cell lines due to their higher cell division rates (15, 32). Taken together, we provide evidence that a yeast-derived beta-glucan induces maturation of human B-cell lymphoma cell lines, likely *via* binding to dectin-1, without affecting their proliferation and viability. Our results may have implications for the usage of beta-glucans in the tumor therapy of patients with B-cell lymphoma.

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Received October 4, 2011
Revised November 17, 2011
Accepted November 18, 2011