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Fungal β-Glucan, a Dectin-1 Ligand, Promotes Protection from Type 1 Diabetes by Inducing Regulatory Innate Immune Response

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β-Glucans are naturally occurring polysaccharides in cereal grains, mushrooms, algae, or microbes, including bacteria, fungi, and yeast. Immune cells recognize these β-glucans through a cell surface pathogen recognition receptor called Dectin-1. Studies using β-glucans and other Dectin-1 binding components have demonstrated the potential of these agents in activating the immune cells for cancer treatment and controlling infections. In this study, we show that the β-glucan from Saccharomyces cerevisiae induces the expression of immune regulatory cytokines (IL-10, TGF-β1, and IL-2) and a tolerogenic enzyme (IDO) in bone marrow–derived dendritic cells as well as spleen cells. These properties can be exploited to modulate autoimmunity in the NOD mouse model of type 1 diabetes (T1D). Treatment of prediabetic NOD mice with low-dose β-glucan resulted in a profound delay in hyperglycemia, and this protection was associated with increase in the frequencies of Foxp3+, LAP+, and GARP+ T cells. Upon Ag presentation, β-glucan–exposed dendritic cells induced a significant increase in Foxp3+ and LAP+ T cells in vitro cultures. Furthermore, systemic coadministration of β-glucan plus pancreatic β cell Ag resulted in an enhanced protection of NOD mice from T1D as compared with treatment with β-glucan alone. These observations demonstrate that the innate immune response induced by low-dose β-glucan is regulatory in nature and can be exploited to modulate T cell response to β cell Ag for inducing an effective protection from T1D. The Journal of Immunology, 2014, 193: 3308–3321.
The innate immune response is mediated through a number of PRRs, predominantly the TLRs and C-type lectin receptors (24). Dectin-1, a C-type lectin receptor, plays a crucial role in the recognition of fungi such as Candida and Aspergillus by binding to the β-glucan–like polysaccharides in their cell walls. This receptor has been shown to collaborate with TLR2 in inducing balanced pro- and anti-inflammatory innate immune responses by the APCs (5, 6, 25). Importantly, TLR2 deficiency in NOD mice leads to reduced susceptibility to T1D (26). Paradoxically, administration of TLR2 ligands results in enhanced regulatory T cell (Treg) function and protection of NOD mice from diabetes (27, 28). However, coengagement of TLR2 and Dectin-1 by zymosan, a fungal cell wall component, results in expression of the immune regulatory cytokines IL-10, IL-2, and TGF-β1 and suppression of the proinflammatory cytokines such as IL-12 (12–16, 25, 29, 30). However, independent engagement of TLR2 in immune cells results in the induction of IL-10, but not IL-2 or TGF-β1. This indicates that signaling through Dectin-1 may be critical for the expression of these immune regulatory cytokines in immune cells. In fact, previous reports have shown that IL-2 expression induced by zymosan in APCs requires Dectin-1 engagement (1, 31). Therefore, in this study, we examined the immunoregulatory properties of the Dectin-1 ligand β-glucan derived from Saccharomyces cerevisiae and its ability to modulate T1D in NOD mice.

Our observations show that β-glucan induces mixed pro- and anti-inflammatory responses and this mixed innate immune response promotes Treg and Th17 responses both in vitro and in vivo. Treatment of 12-wk-old NOD mice at the prediabetic stage with low-dose β-glucan resulted in the protection of pancreatic β cells from immune destruction and the mice from T1D. Importantly, coadministration of β cell Ag along with β-glucan resulted in better protection of NOD mice from diabetes as compared with treatment with β-glucan alone. These observations show that the Dectin-1–dependent innate immune response induced by β-glucan is regulatory in nature and it could be exploited to modulate the immune response to self-antigen for preventing Th1-dominated autoimmune conditions such as T1D.

Materials and Methods

Mice

Wild-type NOD/LtJ, NOD-BDC2.5 TCR-transgenic (Tg), NOD-Scid, and OT-II TCR-Tg mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Foxp3-GFP knockin (KI) (32) mice in the C57BL/6 (B6) background were provided by Dr. V.K. Kuchroo (Harvard Medical School, Boston, MA). Breeding colonies of these strains were established and maintained in the pathogen-free facility of the University of Illinois at Chicago or the Medical University of South Carolina. NOD (NOD/LtJ) mice from our breeding colony or freshly purchased from The Jackson Laboratory were used in this study. Dectin-1–deficient mice in the B6 background (29) were provided by Dr. Y. Iwakura (University of Tokyo, Tokyo, Japan). To generate OT-II TCR-Tg-Foxp3-GFP KI F1 generation background for 12 generations. To detect hyperglycemia in NOD mice, Th1-dominated autoimmune conditions such as T1D.

Peptide Ags, cell lines, and Abs

Immunodominant β cell Ag peptides used in this study—namely, 1) insulin B2-23, 2) GAD6526–220, 3) GAD65524–543, 4) IA-2β175–227, 5) IGRP23–31, 6) BDC2.5 TCR-reactive peptide (YVRPLWVRME; referred to as BDC peptide), and 7) OVA257–264 peptides—were custom synthesized (GenScript). Peptides 1–5 were pooled at an equal molar ratio and used as β cell Ag as described in our earlier studies (33–35). The MFB-F11 TGF-β1 activity reporter cell line was provided by Dr. T. Wyss-Coray (Stanford University, Stanford, CA).

Zymosan of S. cerevisiae origin was purchased from Sigma-Aldrich, boiled for 30 min, washed extensively, and suspended in PBS as described earlier (12, 13). β-Glucan (glucan from baker’s yeast, S. cerevisiae), ≥98% pure, was purchased from Sigma-Aldrich for this study and tested for purity using thioglycolate-activated macrophages as described before (25, 36). Unlike zymosan, β-glucan from Sigma-Aldrich failed to induce considerable amounts of IL-12 and IL-10 in thioglycolate-activated mouse peritoneal macrophages (Supplemental Fig. 1), indicating that this β-glucan preparation is of acceptable purity. Bacterial LPS, CpG oligodeoxynucleotide, polyinosinic-polycytidylic acid, l-tryptophan, IFN-γ, IL-7, IL-9, IL-13, IL-17, IL-4, IL-2, IFN-γ, IL-17, IL-2, IL-4, IL-17, IFN-γ, IL-12, IL-1β, and IL-6 were purchased from R&D Systems, BD Biosciences, Invitrogen, and eBioscience. β-Glucan Stock was suspended in PBS, incubated at 56°C for 30 min, and washed with sterile PBS before making aliquots and storing at −20°C. Normal rat serum, purified anti-mouse TGF-β1 (clone A75-2), anti-CD16/CD32 (Fc block), various fluorochrome-conjugated reagents, and anti-mouse CD11c, CD4, CD25, CD80, CD86, CD40, MHC class II, Foxp3, IFN-γ, IL-17, IL-4, IL-2, IFN-γ, IL-17, IFN-γ, IL-2, IL-4, IL-17, IFN-γ, IL-2, IL-1β, and IL-6 were purchased from R&D Systems, BD Biosciences, Invitrogen, and eBioscience.

Treating mice with TLR ligands, β-glucans, and β cell Ag

In a trend-finding experiment, 10-wk-old female NOD mice were injected i.p. with zymosan (5 μg/mouse/d), Pam3Cys (0.5 μg/mouse/d), β-glucan (5 μg/mouse/d), or bacterial LPS (1 μg/mouse/d) for 3 alternate days and monitored for blood glucose levels. In a dose-determining pilot experiment, NOD mice were treated with varying amounts of β-glucan for 3 alternate days. (Supplemental Fig. 2). In some experiments, treated mice were euthanized 3 alternate days were euthanized 24 h postinjection and spleen cells were examined for innate immune response. Because in our previous study (12) zymosan was used at >25 μg/mouse/d, 5 μg zymosan or β-glucan per mouse per injection is considered a significantly low dose. In one set of experiments, 12-wk-old prediabetic stage mice were treated with β-glucan (i.p.; 5 μg/mouse/d) every other day for 30 d (total of 15 injections). Some of these treated mice were monitored for up to 30 wk posttreatment for hyperglycemia. The remaining mice were euthanized 30 d posttreatment and examined for insulitis and immune cell characteristics.

In another set of experiments, 12-wk-old prediabetic mice were treated with β-glucan (5 μg/mouse/d i.v. on days 1, 3, 5, 13, 15, and 17) and/or β cell Ag (0.5 μg/mouse/d i.v. on days 5 and 17). These mice were monitored weekly for hyperglycemia. Additional mice from parallel experiments were euthanized 30 d posttreatment and examined for insulitis and immune cell characteristics.

DCs and T cells

Peripheral CD11c+ DCs were enriched from spleen cells using magnetic bead–linked anti-CD11c Ab reagent and magnetic columns. The CD11c+ population was enriched to >90% using negative selection kits from Miltenyi Biotec or eBioscience. DC assays

Splenic CD11c+ DCs and BMDCs were incubated with β-glucan (25 μg/ml), zymosan (25 μg/ml), Pam3Cys (1.0 μg/ml), and IL-1 (2 μ/ml) for different time points. Initial experiments used varying amounts of these agents to optimize the long-term culture conditions for cytokine measurement and for Ag presentation assays. Optimum dose was determined by assessing the concentration of a specific agent required in the culture for inducing the highest amount of TNF-α without affecting the cell viability during the 48-h culture (Supplemental Fig. 3). For short-term cultures to induce IDO in vitro, β-glucan was used at 100 μg/ml concentration. Cells collected at 0 or 6 h time points were used for examining transcript levels.
of IDO and a housekeeping gene, β-actin, by qualitative or quantitative RT-PCR assays. In some assays, spleen cells were obtained from NOD mice that were injected with β-glucan or Pam3Cys for 24 h and processed for RT-PCR or cultured overnight for determining supernatants to determine in vivo–induced cytokines. RNA was prepared using TRIzol reagent (Invitrogen), first-strand cDNA was synthesized using a cDNA synthesis kit (Fermentas), and real-time PCR assays were performed using transcript-specific primer sets and SYBR Green PCR master mix ( ABI Prism or Bio-Rad). In some assays, cDNA samples were also used in a qualitative PCR analysis. DCs, obtained from the above-described overnight cultures, were pulsed with specific Ags and used in Ag presentation assays as described below. DCs from 36 h cultures were examined for the levels of activation markers on the surface, after staining with fluorochrome-labeled specific Abs. Spent media from these cultures were also tested for secreted cytokine levels by ELISA.

**T cell assays**

Purified total T cells from diabetic NOD mice (10^7 cells/well) were incubated with β-glucan-exposed (25 μg/ml) or LPS-exposed (2 μg/ml) or unexposed DCs (2 x 10^4 DCs/well) in the presence of anti-CD3 Ab (2 μg/ml). In some assays, DCs were incubated with β cell Ag (5 μg/ml) and β-glucan (25 μg/ml) or LPS (2 μg/ml) overnight and then washed and cultured with CD4+ T cells from diabetic NOD mice. β-glucan–exposed DCs were also cultured with FACS-sorted GFP+ and GFP – CD4+ T cells from NOD-Foxp3-GFP mice in anti-CD3 Ab–coated 96-well plates. Specific Ag peptide-pulsed and ligand-exposed B6 or NOD DCs (2 x 10^4 cells/well) were also cultured in 96-well round-bottom plates along with CFSE-labeled or unlabeled purified CD4+ T cells (1 x 10^5/well) from OT-II or B2C2.5 TCR-Tg mice. After 4 d of culture, cells were cultured using PE-linked CD4-specific Ab and examined for CFSE dilution by FACS. For some assays, in vitro–stimulated or freshly isolated T cells were restimulated using PMA (50 ng/ml) and ionomycin (500 μg/ml) in the presence of anti-CD3 Ab (2 μg/ml) or β cell Ag (5 μg/ml) for 48 h. Spent media from these cultures were tested for cytokines.

**FACS analysis**

Freshly isolated and ex vivo–cultured cells were washed using PBS supplemented with 2% FBS and 10 mM EDTA (pH 7.4) and blocked with anti-CD16/CD32 Fc block Ab or 5% rat serum on ice for 15 min. For surface staining, cells were incubated with FITC-, PE-, and PE-Cy5– or PE-Cy7–labeled antibodies (25 μl) from different combinations and washed three times before analysis. For intracellular staining, surface–stained cells were fixed and permeabilized using in-house reagents (2% paraformaldehyde and 0.1% saponin) or reagents from eBioscience, incubated with fluorochrome-labeled appropriate Abs, and washed three times before analysis. Stained cells were acquired using a FACSCalibur or LSR (BD Biosciences) or CyAn (DakoCytomation) flow cytometer, and the data were analyzed using WinMDI or Summit applications. Cells were also stained using isotype–matched control Abs for determining the background. Specific regions were marked and the gates and quadrants were set while analyzing the data based on the isotype control background staining. At least 10,000 cells were analyzed for each sample.

**Cytokine detection**

Culture supernatants were tested for pro- and anti-inflammatory cytokines by ELISA using paired Ab sets and kits from eBioscience, BD Biosciences, Invitrogen, and R&D Systems. Bioassay for TGF-β was performed using the MFB-F11 cell line, which secretes alkaline phosphatase upon stimulation with TGF-β1. Cells were cultured at 2 x 10^4/well in a 24-well plate overnight, and spent medium was replaced with fresh medium containing rTGF-β1 orontreated or with HCl/NaOH–treated (to release active TGF-β1) culture supernatants and cultured for an additional 24 h. Clarified supernatants (25 μl) from these cultures were also incubated with 225 μl β-nitrophenyl phosphate substrate for 2 h in a 96-well plate, and the OD values were measured at 405 nm using an ELISA reader. Blank values were subtracted from test values and fold induction was calculated against values from the untreated group.

**T cell transfer experiment**

Total T cells were enriched from spleen of control and treated mice using a magnetic bead-based pan T cell isolation (negative selection) kit. Enriched cells, which contained both CD4+ and CD8+ populations, were transferred into 8-wk-old NOD-scid mice or prediabetic female NOD mice. Recipient mice were tested for blood glucose every week. In some experiments, freshly isolated T cells from hyperglycemic mice (2 x 10^6 cells/well) were cultured ex vivo along with CD11c+ DCs (5 x 10^6 cells/well) in the presence of anti-CD3 Ab (2 μg/ml) and β-glucan (25 μg/ml). T cells were purified from these cultures and injected into 8-wk-old NOD-Ltj mice (Iv) and monitored as described above.

**Histological and immunofluorescence analysis of pancreatic tissues**

Pancreata were fixed in 10% formaldehyde, and μm paraffin sections were made and stained with H&E. Stained sections were analyzed using a grading system as follows: 0, no evidence of infiltration; 1, peri-islet infiltration (<5%); 2, 5–25% islet infiltration; 3, 25–50% islet infiltration; and 4, >50% islet infiltration as described in our earlier studies (33–35, 37). Areas that appeared to have completely lost islets were not included in this grading approach.

**Statistical analysis**

Mean, SD, and statistical significance (p value) were calculated using Microsoft Excel, GraphPad online, and/or other online statistical applications. A two-tailed t test was employed unless specified for values from in vitro and ex vivo assays. Log-rank analysis was performed to compare T1D incidence (hyperglycemia) of the test group with that of control group. A Fisher exact test was used for comparing the total number of infiltrated islets in test versus control groups. A p value ≤0.05 was considered significant.

**Results**

β-Glucan–exposed DCs express both immune regulatory and proinflammatory factors

We and others have reported that simultaneous engagement of TLR2 and Dectin-1 by zymosan from the fungal cell wall can induce both anti- and proinflammatory cytokines in APCs (12–16). Therefore, we examined whether engagement of these two receptors, independently, can produce tolerogenic factors in APCs. The BMDCs and splenic DCs were stimulated with optimized dose of β-glucan and Pam3Cys, as the primary Dectin-1– and TLR2–interacting ligands, along with other control agents (zymosan and bacterial LPS) for different durations and examined for the expression of cytokines. As observed in Fig. 1A and 1B, BMDCs that were exposed to β-glucan produced large amounts of IL-2, IL-10, and TGF-β, similar to DCs that were exposed to zymosan, as compared with the untreated control. However, Pam3Cys induced the expression of high levels of IL-10 and TNF-α, but not IL-2 or TGF-β. Importantly, β-glucan induced higher levels of IL-2 and TGF-β in BMDCs compared with zymosan. Importantly, although the ELISA and bioassay results suggest that upon treatment of BMDCs with either β-glucan or zymosan, only a portion of the secreted TGF-β1 is in its active form, but the total TGF-β1 produced by β-glucan–exposed BMDCs is relatively higher than that induced by zymosan (Fig. 1B). Bacterial LPS, a TLR4 ligand, failed to induce significant amounts of regulatory cytokines, suggesting a dominant and specific effect of Dectin-1 engagement on the expression of these cytokines. Splenic DCs that were exposed to β-glucan and zymosan also produced considerable amounts of IL-10 and TGF-β1, albeit much lower than that produced by BMDCs (not shown). Zymosan-, Pam3Cys–, β-glucan–, and LPS-exposed DCs induced comparable levels of the proinflammatory cytokine TNF-α (Fig. 1A). To realize the other properties of Dectin-1– and TLR2–engaged DCs, expression level of the tolerogenic enzyme IDO was also examined in similarly treated DCs. Fig. 1C and 1D show that only the β-glucan–exposed, but not Pam3Cys–, zymosan–, or LPS–exposed, BMDCs and splenic DCs expressed considerable levels of IDO. Overall, these observations show that Dectin-1, but not TLR2 or TLR4...
engagement, induces a combination of immune regulatory and proinflammatory factors in APCs.

**Regulatory innate immune response induced by β-glucan in DCs is Dectin-1–dependent**

To assess whether the β-glucan–induced regulatory innate immune response is primarily Dectin-1–dependent, DCs were generated from BM of wild-type (WT) and Dectin-1 knockout (KO) mice and activated using the Dectin-1 ligand β-glucan or TLR2 ligand Pam3Cys and coligands of both receptors, zymosan. Cytokine levels in the spent medium from these cultures were examined by ELISA and their IDO expression was determined by quantitative PCR (qPCR). Fig. 2 shows that β-glucan–treated Dectin-1 KO DCs secrete/express profoundly low levels of TGF-β1, IL-2, IL-10, and IDO, as well as the proinflammatory cytokine TNF-α compared with their WT counterparts, and the levels of these factors were comparable to those of untreated cells. Alternatively, WT and Dectin-1 KO DCs that were exposed to TLR2 ligand Pam3Cys showed a comparable upregulation of IL-10 and proinflammatory cytokine TNF-α. Furthermore, Pam3Cys failed to induce IDO in WT and Dectin-1 KO DCs. Importantly, zymosan induced the expression of considerable levels of IL-2 and TGF-β1 in WT DCs but at a significantly lower level in Dectin-1 KO DCs. These observations show that immunoregulatory and proinflammatory responses induced by β-glucan and zymosan in DCs are primarily Dectin-1–dependent.

**Treatment with low-dose β-glucan promotes relatively better protection of NOD mice from T1D than by zymosan and Pam3Cys**

Previously, we showed that treatment with zymosan, which is known to interact with TLR2 and Dectin-1, induces a regulatory innate immune response and prevents T1D in NOD mice (12–16). Additionally, other studies have shown that the innate immune...
response induced by Pam3Cys, which targets TLR2, can suppress autoimmunity even in the mouse model of T1D (27, 28). Because Dectin-1 signaling is necessary for zymosan-induced TGF-β1 and IL-2 responses in APCs, the effect of β-glucan–induced innate immune response on T1D incidence in prediabetic NOD mice was compared with that of LPS, Pam3Cys, and zymosan. Ten-week-old NOD mice were treated with the above agents for 3 alternate days and monitored for hyperglycemia. As observed in Fig. 3, mice that received only β-glucan, but not LPS, Pam3Cys, or zymosan, showed a statistically significant delay in diabetes compared with control group mice during the 15-wk monitoring period. Whereas treatment with low-dose zymosan also caused considerable delay in hyperglycemia, albeit not significant statistically, Pam3Cys and LPS treatment showed little or no protective effect in 10-wk-old prediabetic NOD mice. These observations confirm that the Dectin-1 ligand β-glucan has a better immune regulatory property in the T1D model as compared with TLR2 and TLR4 ligands.

β-Glucan induces regulatory immune response in vivo

Results presented in Fig. 3, in association with earlier reports, including ours, on the therapeutic effects of zymosan in NOD mice (12, 14) indicate that Dectin-1–, but not TLR2–, dependent innate immune response is the primary player in zymosan-induced prevention of T1D. Additionally, Figs. 1 and 3 show that β-glucan, but not Pam3Cys, induces immune regulatory factors such as IL-2 and TGF-β1 in DCs, in a similar manner to zymosan. Interestingly, only β-glucan, but not zymosan or Pam3Cys, induced IDO expression in DCs. Therefore, we compared β-glucan and Pam3Cys for their abilities to induce immune regulatory cytokines and IDO in vivo. Prediabetic NOD mice were left untreated or treated with β-glucan and Pam3Cys for 3 alternate days as de-
described for Fig. 3. Spleen cells were harvested 24 h posttreatment and subjected to qPCR or cultured to examine the levels of spontaneously released cytokines. As observed in Fig. 4, cells from β-glucan−, but not Pam3Cys−, treated mice showed significant induction of IL-2, TGF-β1, and IDO. Alternatively, spleen cells from both β-glucan− and Pam3Cys-treated mice produced significant similar amounts of IL-10 and TNF-α as compared with cells from untreated control mice. These observations, in association with the results of Figs. 1–3, show that the Dectin-1 ligand β-glucan has better immune regulatory properties than does the TLR2 ligand Pam3Cys.

**Treatment with β-glucan protects prediabetic NOD mice from T1D**

Our trend-finding study using 10-wk-old NOD mice (Fig. 3) showed that treatment with a low dose of β-glucan, but not the TLR2 and TLR4 ligands (Pam3Cys and LPS respectively), for 3 d delayed hyperglycemia in NOD mice. Furthermore, as compared with zymosan, low-dose β-glucan showed a superior ability to delay hyperglycemia when treated at the prehyperglycemic stage. Therefore, the effect of prolonged treatment with this Dectin-1 ligand was examined in prediabetic stage NOD mice. As observed in Fig. 5A, prolonged treatment of 12-wk-old prediabetic NOD mice with a low dose of β-glucan could delay hyperglycemia in NOD mice for a significant duration and prevented the disease in 50% of the mice for at least 30 wk posttreatment as compared with noninjected controls, whereas 100% of the untreated control mice developed diabetes by 26 wk of age (within 14 wk after treatment initiation).

The pancreatic islets of prediabetic mice that received β-glucan showed significantly less severe immune cell infiltration and insulitis compared with untreated control mice 30 d posttreatment (Fig. 5B). Whereas ~70% of the islets in mice that received β-glucan showed an insulitis grade of ≤1, at least 80% of the islets in untreated mice showed an insulitis severity grade of >2. Importantly, whereas ~30% of the islets in β-glucan recipient mice were found to be free from immune cell infiltration, <5% of islets of untreated mice were insulitis-free. These results suggest that the β-glucan-induced innate immune response in vivo is regulatory in nature and protects NOD mice from T1D.

**T cells from β-glucan–treated mice produce IL-10, TGF-β1, and IL-17**

Because mice that were treated with β-glucan showed significant protection from hyperglycemia, T cells from these mice were examined for their functional properties in comparison with their counterparts from control mice. Spleen and PnLN cells obtained from mice that were euthanized 30 d posttreatment were examined for their cytokine secretion profiles upon activation using anti-CD3 Ab. The cytokine profiles of cells from β-glucan recipient groups were significantly different as compared with control mice (Fig. 6A). Whereas cells from the β-glucan recipient and control mice produced comparable levels of IFN-γ in both the spleen and PnLN, cells from β-glucan–treated mice, PnLN cells in particular, produced significantly higher amounts of IL-10, IL-17, and TGF-β1 as compared with T cells from control mice. These observations show that the Dectin-1-dependent innate immune response induced by β-glucan modulates T cell function in vivo, leading to the protection of NOD mice from T1D.

**β-Glucan–treated mice show increased frequency of T cells with regulatory phenotype**

To realize whether β-glucan treatment induced Tregs, spleen and PnLN cells from mice that were euthanized 15 d posttreatment were examined for Foxp3+, LAP, and GARP T cells by FACS. As observed in Fig. 6B, significantly higher numbers of Foxp3+ CD4+ T cells were found in the PnLN of mice treated with β-glucan as compared with the control mice. However, the splenic Foxp3+CD4+ T cell frequencies in β-glucan–treated and control mice were comparable. Interestingly, significantly higher numbers of splenic CD4+Foxp3− T cells from mice that were treated with β-glucan were positive for another Treg marker, GARP. However, PnLN cells from β-glucan–treated and control mice showed no

![FIGURE 4. β-glucan, but not Pam3Cys, induced IL-10, IL-2, and IDO in vivo. (A) Ten-week-old prediabetic female NOD mice (three per group) were injected with β-glucan and Pam3Cys for 3 alternate days or left untreated (control) as described in Fig. 3 and euthanized after 24 h. Spleen cells were cultured for 48 h and supernatants were tested for spontaneously secreted cytokines by ELISA. (B) cDNA prepared using spleen cells was subjected to real-time qPCR for detecting IDO. Expression levels were calculated relative to actin expression, and the values of ligand-treated mice were compared against the value of the untreated (none) group, which was considered as 1. This experiment was repeated twice. Statistical significance of the treated group was calculated against the untreated (none) group. **p < 0.01, ***p < 0.001.](http://www.jimmunol.org/doi/abs/10.4049/jimmunol.1701707)

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FIGURE 5. Activation of innate immune response using β-glucan prevents hyperglycemia in NOD mice. (A) Twelve-week-old prediabetic female NOD mice were treated i.p. with β-glucan (5 μg/mouse/d; every other day for 30 d; total of 15 injections) or left untreated (control). Mice were checked every week for hyperglycemia, and a glucose level of 250 mg/dl for 2 consecutive weeks was considered diabetic. A log-rank test was performed to compare the hyperglycemia incidence in treated and control groups of mice and the p value is shown. (B) Euglycemic β-glucan–treated and control mice from parallel experiments were euthanized 30 d after the last injection. Pancreatic tissues were processed for H&E staining to evaluate insulitis. Representative islets (left panel) and percentages of islets with different grades of insulitis plotted as a bar diagram (right panel) are shown. Images were acquired under original magnification ×20. Sections of pancreatic tissues from at least five mice per group were examined for insulitis, and at least 180 islets per group were examined.

A significant difference in GARP expression (not shown). Furthermore, similar to Foxp3, the expression of another natural Treg marker, Helios, was not significantly different in spleen cells from β-glucan–treated and control mice. Splenic and PnLN cells from β-glucan–treated and control mice were also examined for surface-bound LAP by FACS. Significantly higher frequencies of CD4+CD25+ T cells from the PnLNs and spleen of β-glucan–treated mice were positive for surface LAP.

Because spleen cells of β-glucan–treated mice showed Foxp3+ and CD25+ T cells with Treg frequencies, CD4+CD25+ and CD4+CD25− T cells were enriched from spleens of β-glucan–treated and control mice, activated using anti-CD3 Ab, and examined for secreted cytokines. Fig. 6C shows that splenic CD4+CD25− T cells from β-glucan–treated mice produced profoundly higher amounts of TGF-β1 and IL-10 as compared with their counterparts from control mice. Interestingly, only IL-10, but not TGF-β1, secretion by CD4+CD25+ T cells from β-glucan–treated mice was significantly higher compared with these cells from control mice. As indicated by the p values, the difference in IL-10 production between T cells from β-glucan–treated and control mice was more pronounced with CD4+CD25− than CD4+CD25+ T cells. These observations along with the cytokine profile described in Fig. 6A indicate that treatment with β-glucan induces both Foxp3+ and Foxp3− Treg populations, and the Foxp3+ and LAP+ populations appear to be trafficking selectively to the pancreatic microenvironment and enhance suppression of insulitis.

T cells from β-glucan–treated mice are less diabeticogenic and suppress hyperglycemia in NOD mice

T cells isolated from mice that were euthanized 30 d posttreatment with β-glucan were adoptively transferred into 10-wk-old female NOD mice to examine their ability to modulate ongoing autoimmunity and prevent hyperglycemia. Our initial experiments showed that the CD4+CD25+ T cell populations isolated from the spleen of treated and untreated mice did not have a noticeable difference in their ability to suppress autoimmunity when transferred alone to WT female mice or along with diabeticogenic T cells into NOD-Scid mice (not shown), perhaps due to the lack of difference in Foxp3+ cell frequencies in the spleen. As observed in Fig. 6B, CD25−Foxp3− Treg frequency was significantly higher in the PnLN, but not in the spleen, of mice that were treated with β-glucan as compared with controls. Because it is extremely difficult to obtain sufficient numbers of CD25+ (Foxp3+) T cells from the PnLN for adoptive transfer studies, and considering the ability of splenic T cells from β-glucan–treated mice to produce large amounts of IL-10 and TGF-β1 (Fig. 6), total T cells from the spleens of β-glucan–treated and control mice were used in adoptive transfer experiments. As observed in Fig. 7A, albeit not statistically significant, NOD-Scid mice that received T cells from β-glucan–treated mice developed hyperglycemia, relatively slowly, compared with mice that received T cells from control mice. Similarly, WT NOD mice that received T cells from β-glucan–treated mice also developed hyperglycemia relatively slower than did those that received control T cells (Fig. 7B). These observations, in conjunction with Fig. 6, suggest that considerable numbers of T cells with regulatory properties are induced upon treatment with β-glucan, and these cells contribute to the prevention of T1D in β-glucan–treated mice.

β-Glucan- and LPS-exposed DCs induce similar levels of T cell proliferation, despite phenotypic differences

To understand the Ag-presenting properties of β-glucan–exposed splenic DCs, surface levels of activation markers on them, in comparison with untreated and LPS-treated DCs, were examined. A considerable increase in the expression of CD80 and CD40 was observed on β-glucan–activated DCs as compared with untreated DCs (Supplemental Fig. 4A). However, LPS-exposed DCs showed an upregulation of CD40, CD86, MHC class II, and PD-L2, but not CD80. Expression of high CD80 on β-glucan−, but not LPS−, exposed DCs suggests the preferential engagement of CTLA-4 on T cells by these DCs upon Ag presentation, leading to a better immune regulation. Our previous studies have shown that B7.1 is a preferential ligand for CTLA-4, and dominant engagement of CTLA-4 by B7.1 or CTLA-4 agonists upon Ag presentation leads to the induction and expansion of Tregs (33, 34).

As described in Fig. 1, β-glucan−exposed DCs produced a relatively superior regulatory innate immune response as compared with other ligand-exposed DCs. Therefore, to further understand β-glucan−induced pro- and anti-inflammatory cytokine profiles of DCs, splenic CD11c+ cells were treated with β-glucan for 48 h and the levels of secreted cytokines were compared with those of LPS-treated DCs. Whereas β-glucan exposure induced both regulatory (IL-10 and TGF-β1) and proinflammatory (IL-1β and TNF-α) cytokines in these DCs, LPS treatment led to an innate immune response involving primarily proinflammatory cytokines (IL-6, IL-1β, and TNF-α) (Supplemental Fig. 4B). These obser-
Observations suggest that the Ag-presenting potential of peripheral DCs is enhanced upon exposure to β-glucan through higher expression of costimulatory molecules as well as cytokines. To validate this notion, a T cell proliferation assay was carried out using Ag-pulsed, β-glucan– or LPS-treated DCs and OT-II TCR-Tg T cells. As observed in Supplemental Fig. 4C, no significant dif-

**FIGURE 6.** T cells from β-glucan–treated mice produce TGF-β1 and IL-10 and show regulatory phenotype. (A) Spleen and pancreatic PnLN cells obtained from control and β-glucan–treated 12-wk-old NOD mice (treated as described above for Fig. 5; cells were obtained 15 d after the final dose of β-glucan) were stimulated using anti-CD3 Ab (1 μg/ml) for 48 h in a 96-well round-bottom plate (1 × 10^5 cells/well) and supernatants were tested for IL-10, TGF-β1, IL-17, and IFN-γ by ELISA. Background cytokine values of supernatants from nonstimulated cultures were subtracted from respective anti-CD3 Ab–stimulated cultures to exclude cytokines released by APCs spontaneously and plotted as bar diagrams. Means ± SD of values from a total of eight to nine individual mice tested in triplicate are shown. (B) Fresh cells were also examined for the expression of Foxp3, GARP, and Helios, and surface LAP by FACS. Representative FACS graphs (upper panels) and means ± SD of cells from at least four mice of a representative experiment tested in duplicate (lower panels) are shown. (C) Splenic CD4^+CD25^+ and CD4^+CD25^- T cells from control and β-glucan–treated mice were examined for cytokine profiles. Enriched T cell populations were cultured with anti-CD3 and anti-CD28 Abs (2 μg/ml) in a 96-well round-bottom plate (1 × 10^5 cells/well) for 72 h and the spent medium was tested for TGF-β1 and IL-10. Each bar represents mean ± SD of cells from three to four mice of a representative experiment tested in triplicate. Treated and control groups of mice were compared, and the p value is shown.
ference in proliferation was observed in the OT-II T cells upon activation using either β-glucan– or LPS-treated DCs. Overall, these observations indicate that although untreated and β-glucan–treated DCs show comparable abilities to induce T cell proliferation, they may influence T cell differentiation differently.

Ag presentation by β-glucan–exposed DCs induces Foxp3+ and IL-10-secreting T cells

Because β-glucan– and LPS-exposed splenic DCs, despite their phenotypic differences, induced similar levels of T cell proliferation, these DCs were further tested for their ability to promote T cell differentiation using OT-II and/or OT-II-Foxp3-GFP KI T cells. The DCs that were exposed to β-glucan could induce significantly higher frequencies of Foxp3+ and LAP+ T cells in cultures, compared with unexposed- or LPS-exposed DCs. Although β-glucan–exposed DCs could promote higher GARP expression in T cells as compared with untreated DCs, LPS-exposed DCs also promoted a significant increase in the expression of GARP on CD4+ T cells (Fig. 8A). Importantly, culture supernatants from similar primary cultures, where β-glucan–exposed DCs were used, showed significantly high levels of IL-10, IL-4, TGF-β1, and IL-17 as compared with supernatants from cultures where LPS-treated or untreated DCs were used (Fig. 8B). OT-II T cells from similar primary cultures were also examined for intracellular cytokines after restimulation using PMA/ionomycin. Fig. 8C shows that β-glucan–treated and untreated DCs induced IFN-γ in a comparable percentage of T cells, but at relatively lower frequencies than by LPS-exposed DCs. However, β-glucan–exposed DCs also induced significantly higher numbers of IL-10– and IL-4–producing T cells in these in vitro cultures as compared with untreated or LPS-treated DCs.

Because β-glucan–exposed DCs produced significant amounts of TGF-β1 along with proinflammatory cytokines and they induced Foxp3, TGF-β1, and LAP expression in T cells upon Ag presentation, whether addition of IL-6 can result in Th17 cell differentiation in these cultures was examined. Fig. 8D shows that addition of IL-6 alone did not help β-glucan–exposed DCs or untreated as well as LPS-exposed DCs to skew the OT-II T cell response to Th17 type. However, addition of exogenous TGF-β1 along with IL-6 skewed almost all IFN-γ-producing cells to IL-17–producing cells in cultures where untreated DCs were used. Similarly, β-glucan–exposed DCs also skewed the T cell response to Th17 type in the presence of exogenous IL-6 and TGF-β1. A considerable number of IL-17+ T cells also expressed IFN-γ in these cultures. Importantly, LPS-exposed DCs appeared to be more resistant in their ability to induce Th17 cells, even in the presence of exogenous IL-6 and TGF-β1. About 70% of cytokine-producing cells in these cultures were either IFN-γ+ or IFN-γ− and IL-17+. Alternatively, >70% of the cytokine-producing T cells that were activated using β-glucan–exposed DCs, in the presence of IL-6 and TGF-β1, expressed only IL-17. Overall, these observations suggest that β-glucan–exposed APCs can promote a combination of Tregs and IL-17–producing T cells upon Ag presentation under appropriate conditions. Alternatively, LPS-exposed APCs mainly promote differentiation of the IFN-γ–producing T cells. Importantly, although Tregs can promote protection from autoimmunity, IL-17– and IL-4–producing cells are considered less pathogenic than IFN-γ–producing cells in T1D.

Coadministration of β-glucan and β cell Ag produces enhanced protection of NOD mice from T1D as compared with β-glucan treatment

Because β-glucan–exposed DCs could induce T cells that have a regulatory phenotype as well as express IL-17 in vitro, and β-glucan treatment resulted in an increase in Foxp3+, LAP+, and GARP+ populations, we examined whether coadministration of β-glucan and β cell Ag produces better protection of NOD mice from T1D. It is assumed that if T cell response against β cell Ag is modulated, then long-lasting protection from T1D will be achieved without the need for long-term treatment with β-glucan alone. Prediabetic mice were treated with β-glucan plus β cell Ag alone or in combination for a shorter duration than that described for Fig. 5. As observed in Fig. 9A, mice that received β-glucan plus β cell Ag showed significantly delayed hyperglycemia as compared with β-glucan recipients. Alternatively, most mice that received β cell Ag alone developed hyperglycemia as rapidly as did noninjected controls.

One set of euglycemic mice from a similar experiment was euthanized 30 d posttreatment and pancreatic tissues were examined for insulitis. The pancreatic islets of mice that received β-glucan plus β cell Ag showed significantly less severe immune cell infiltration and insulitis as compared with β cell Ag–treated and untreated control mice (Fig. 9B). Whereas ~70% of the islets in mice that received β-glucan plus β cell Ag showed an insulitis grade of ≥2, at least 80% of the islets in untreated mice and β cell Ag recipient mice showed an insulitis severity grade of ≥2. Importantly, mice that received short-term treatment (compared with that shown in Fig. 5) with β-glucan alone also showed relatively less severe insulitis, albeit not statistically significant. However, mice that were treated with β-glucan plus β cell Ag showed profoundly less severe insulitis as compared with mice that re-
These results suggest that β-glucan–induced innate immune response promotes β cell Ag–specific immunomodulation leading to better protection of NOD mice from T1D.

β-Glucan plus β cell Ag treatment causes Th cell skewing and an increase in the frequency of T cells with regulatory phenotype

Because mice that were treated with β-glucan plus β cell Ag showed significant protection from hyperglycemia, T cells from these mice were examined for their phenotypic and functional properties, in comparison with their counterparts from control groups. PnLN cells obtained from mice that were euthanized 30 d posttreatment were examined for their ability to respond to ex vivo challenge with β cell Ag. CD4+ T cells from mice that received β cell Ag or β-glucan plus β cell Ag showed a comparable extent of CFSE dilution upon ex vivo challenge, which was significantly higher than their counterparts from untreated control mice (Fig. 10A). PnLN cells from β-glucan– and/or β cell Ag–treated mice were examined for Foxp3+ T cells by FACS. Significantly
FIGURE 9. Treatment using β-glucan and β cell Ag resulted in better protection of NOD mice from hyperglycemia as compared with treatment with β-glucan or β cell Ag alone. Twelve-week-old euglycemic female NOD mice were left untreated (control) or treated with β-glucan i.v.; on days 1, 3, 5, 13, 15, and 17 with 5 μg/mouse/d. Some groups of animals received β cell Ag on days 5 and 17. (A) Mice were checked every week for hyperglycemia, and a blood glucose level of 250 mg/dl for 2 consecutive weeks was considered diabetic. A log-rank test was performed to compare hyperglycemia incidence in β-glucan– and β-glucan plus β cell Ag–treated groups with control, and the p value is shown on each graph. The group that received β cell Ag was also compared with control mice. (B) One set of treated and control mice from parallel experiments were euthanized 4 wk after the last injection. Pancreatic tissues were processed for H&E staining to evaluate insulitis as described above for Fig. 2. The islets with representative insulitis grades and percentages of islets with different grades of insulitis plotted as bar diagram are shown. Sections of pancreatic tissues from at least five mice per group were examined for insulitis, and insulitis scores of at least 150 islets/group were plotted as a bar diagram.

higher numbers of Foxp3+CD4+ T cells were found in the PnLNs of mice treated with β-glucan alone or along with β cell Ag as compared with β cell Ag–treated mice. To examine the functionality of T cells, freshly prepared PnLN cells from treated and untreated mice were stimulated with PMA and ionomycin and stained for intracellular cytokines. Fig. 10A shows that whereas the CD4+IFN-γ+ T cell frequencies were significantly low in β-glucan and β-glucan plus β cell Ag recipient mice, a higher percentage of PnLN cells from the latter group showed IL-10, IL-17, and IL-4 expression compared with their counterparts from β-glucan– or β cell Ag–treated mice. Importantly, levels of cytokines secreted by PnLN cells from β-glucan plus β cell Ag recipient groups, when challenged with β cell Ag ex vivo, were also significantly different from those of β-glucan or β cell Ag recipients (Fig. 10B). Whereas all β cell Ag recipient groups produced comparable levels of IFN-γ, which is significantly higher than that of control and β-glucan treated mice, β-glucan plus β cell Ag recipients produced significantly higher amounts of IL-10 and IL-17 as compared with mice that received β cell Ag alone. These results suggest that exposure of APCs to β-glucan in vivo leads to the skewing of T cell response from a pathogenic to protective/less pathogenic type and that these T cells with altered function may eliminate the need for continuous long-term treatment with a nonspecific immune modulator.

Discussion

The immunostimulatory properties of β-glucans of fungal, bacterial, and plant origin have been recognized for many decades (3). Primary sources of β-glucans include baker’s yeast (S. cerevisiae), mushrooms, bacteria (Alcaligenes faecalis), barley, and seaweed (Laminaria digitata) (3, 4). β-glucans from these and other sources have the ability to stimulate immune cells. Although several PRRs have been described for their ability to interact with β-glucans and β-glucan–containing microbial products, Dectin-1 is thought to be the primary receptor for fungal β-glucans (1, 4). With the exception of a few reports that show Dectin-1–dependent IL-10 and IL-2 responses by innate immune cells (31, 38), previous studies were primarily focused on the proinflammatory and host defense aspects of β-glucan– and Dectin-1–associated innate immune response. Furthermore, β-glucan and β-glucan–containing fungal cell wall preparations such as zymosans have been used for inducing autoimmune arthritis in a genetically susceptible mouse model (18–21), suggesting that Dectin-1–dependent innate immune response involves proinflammatory factors. In the present study, we show that the β-glucan–induced innate immune response comprises both proinflammatory cytokines and immune regulatory factors that can prevent T1D in NOD mice. We also show that the innate immune response induced by β-glucan promotes the generation and/or expansion of T cells with regulatory phenotype and could be exploited for achieving pancreatic β cell Ag–specific modulation of autoimmunity and lasting protection from T1D.

Previously, we and others have reported that inducing regulatory innate immune response through TLR2 and Dectin-1 using a fungal cell wall agent, zymosan, could suppress autoimmunity and prevent T1D in NOD mice (12, 14). However, zymosan is also known to bind to many other receptors, including TLR2, and the IL-10, TGF-β1, and IL-2 responses induced by this agent are thought to be the result of co-operative signaling through TLR2 and Dectin-1 receptors (13, 16, 38). Additionally, a tolerogenic enzyme, Raldh1A2, induced in DCs by zymosan was reported to be TLR2-dependent (16). Yeast β-glucan does not induce signaling through TLR2 and can trigger the expression of IL-10, TGF-β1, and IL-2 along with the tryptophan-catabolizing tolerogenic enzyme IDO as well as proinflammatory cytokines, including TNF-α in DCs. Our observations highlight the regulatory nature of Dectin-1–dependent innate immune responses. In fact, unlike zymosan (15, 16), β-glucan failed to induce Raldh1A2 expression in peripheral DCs (not shown), confirming that TLR2 engagement may be necessary for Raldh1A2 induction. Alternatively, previous studies have shown that engagement of TLR2 by its ligands induces, along with proinflammatory cytokines, IL-10, but not IL-2 or TGF-β1 in APCs (16, 27, 39), further substantiating the notion that Dectin-1 engagement is responsible for zymosan-
induced IL-2 and TGF-β1 production. Importantly, the observation that β-glucan, but not zymosan, induces IDO in DCs suggests that interaction of zymosan with other receptors may have counteractive effects on Dectin-1–dependent IDO expression.

Previous studies have shown that interaction with fungi, Candida in particular, can induce IDO expression in innate immune cells (40, 41). However, the specific ligand/receptor interaction responsible for IDO expression was not known. Our observation that purified β-glucan can induce IDO expression in peripheral DCs shows that engagement of Dectin-1 may be responsible for fungal recognition-associated IDO expression. IDO-dependent immune tolerance involves at least two major mechanisms: 1) depletion of tryptophan by IDO in the immune microenvironment can dampen T cell proliferation and their effector function (42), and 2) IDO-induced kynurenines, which are l-tryptophan–derived metabolites, can drive the conversion of naïve T cells to Tregs in the presence of TGF-β1 (42, 43). Our in vitro and in vivo studies show that the β-glucan–induced innate immune response promotes the induction and/or expansion of Foxp3+ and LAP+ T cells, suggesting the involvement of TGF-β1, IL-2, and/or IDO in protection of β-glucan–treated NOD mice from T1D.

Treatment of NOD mice at young ages with TLR2, TLR3, TLR4, and TLR9 ligands resulted in a considerable delay in T1D by activating both innate and adaptive immune responses (27, 28, 44–46). However, these ligands were not known to be effective in inducing protection from the disease at later stages. Although it

FIGURE 10. T cells from β-glucan– and β cell Ag–treated mice produce higher IL-17, IL-10, and IL-4 as compared with control groups. PnLN cells were obtained from control and treated 12-wk-old NOD mice (treated as described above for Fig. 9; cells were obtained 30 d after the final injection with agents). (A) Cells were examined for peptide-specific proliferation by CFSE dilution assay. Percentage values of CFSElow cells at 96 h time point are shown. Cells were also examined for intracellular Foxp3 expression by FACS. Fresh PnLN cells were stimulated using PMA and ionomycin for 4 h in the presence of brefeldin A and stained for intracellular cytokines for FACS analysis. Percentage values of CD4+ T cells with cytokine expression are shown. Representative FACS graphs (left panels) and means ± SD of cells from three to four mice of a representative experiment tested in duplicate (right panels) are shown. (B) Fresh cells were also stimulated using β cell Ag for 48 h in 96-well round-bottom plates (2 × 10⁵ cells/well), and the supernatants were tested for cytokines by ELISA. Means ± SD of values from a total of three to four individual mice tested at least in duplicate are shown. *p < 0.05, **p < 0.01, ***p < 0.001.
has been shown that treatment with β-glucan, starting at the preinsulitis stage, caused a significant delay in diabetes in BB rats (17), the effect of innate immune response induced by this Dectin-1 ligand at later stages of disease progression in this rat model or NOD mice was not studied before. Our current study shows that treatment of 12-wk-old prediabetic NOD mice with a low dose of β-glucan results in protection from T1D, and this effect appears to be associated with anti-inflammatory cytokines (IL-10, TGF-β1, and IL-2) and IDO- mediated enhanced immune regulation. The immune regulatory cytokine- (IL-10, TGF-β1, and IL-2) and IDO- induced metabolic products such as kynurenines are known to play a role in the induction, expansion, survival, and/or functioning of both natural and adaptive Tregs (47, 48). Therefore, we anticipated that Ag presentation by β-glucan–exposed DCs can modulate the T cell response from proinflammatory to regulatory type. Our observation that β-glucan–exposed DCs can promote Foxp3 and LAP expression in OT-II TCR-Tg T cells upon Ova peptide presentation support this notion.

Interestingly, T cells from β-glucan–treated mice also produced higher amounts of IL-17, a cytokine that is known to play a protective, or less pathogenic, role in T1D. However, this cytokine is not produced in large amounts of IL-17, a cytokine that is known to play a pro-inflammatory role in arthritis (49–52). It appears that TGF-β1, along with some of the proinflammatory cytokines such as IL-6, IL-1β, TNF-α, and/or IL-23 induced upon Dectin-1 engagement on innate immune cells, could be responsible for the IL-17 response in β-glucan–treated NOD mice. Whereas purified splenic CD11c+ DCs do not produce considerable amounts of IL-6, BMDCs produce IL-6 when exposed to β-glucan (not shown).

Therefore, it is possible that IL-6 is produced by other innate immune cells in vivo upon treatment with β-glucan. Furthermore, Dectin-1–dependent production of IL-23, a cytokine responsible for the maintenance of Th17 cells, has been reported in the past (25). Importantly, our in vitro Ag presentation assays show that although secreted IL-17 levels were high in the primary T cell cultures, β-glucan–exposed DCs did not induce a large number of IL-17+ T cells without exogenous IL-6 and TGF-β1. Additionally, this cytokine is not produced in large amounts of IL-17, a cytokine that is known to play a pro-inflammatory role in arthritis (49–52).

In conclusion, our observations show that the Dectin-1–dependent innate immune response induced by β-glucan is significantly different from that induced by other PRRs. Furthermore, Dectin-1 signaling not only appears to be the primary contributor of previously reported zymosan-induced regulatory innate immune response (12–16), but it also induces IDO expression in DCs. The complex pro- and anti-inflammatory responses triggered by β-glucan can promote both Treg and Th17 responses, leading to suppressed Th1-dominated β cell Ag–specific autoimmune protection and protection of NOD mice from T1D. Most importantly, Dectin-1–induced innate immune response can be exploited to modulate the T cell response against pancreatic β cell Ag for achieving long-term protection from T1D.

Disclosures

The authors have no financial conflicts of interest.

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