

Binding of *Toxoplasma gondii* Glycosylphosphatidylinositols to Galectin-3 Is Required for Their Recognition by Macrophages*

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We showed that the production of tumor necrosis factor (TNF) α by macrophages in response to *Toxoplasma gondii* glycosylphosphatidylinositols (GPIs) requires the expression of both Toll-like receptors TLR2 and TLR4, but not of their co-receptor CD14. Galectin-3 is a β -galactoside-binding protein with immune-regulatory effects, which associates with TLR2. We demonstrate here by using the surface plasmon resonance method that the GPIs of *T. gondii* bind to human galectin-3 with strong affinity and in a dose-dependent manner. The use of a synthetic glycan and of the lipid moiety cleaved from the GPIs shows that both parts are involved in the interaction with galectin-3. GPIs of *T. gondii* also bind to galectin-1 but with a lower affinity and only through the lipid moiety. At the cellular level, the production of TNF- α induced by *T. gondii* GPIs in macrophages depends on the expression of galectin-3 but not of galectin-1. This study is the first identification of a galectin-3 ligand of *T. gondii* origin, and galectin-3 might be a co-receptor presenting the GPIs to the TLRs on macrophages.

Glycosylphosphatidylinositol (GPI)⁴-anchored proteins dominate the surface of the *Toxoplasma gondii* tachyzoite (1, 2). We have shown that *T. gondii* GPIs, as well as their glycan and lipid moieties, induce the production of tumor necrosis factor (TNF)- α in macrophages through the activation of the transcription factor NF- κ B (3). Toll-like receptors (TLRs) rec-

ognize microbial components leading to cytokine production and antimicrobial responses through NF- κ B activation (4). We have shown that both TLR2 and TLR4 are involved in the NF- κ B-dependent signaling cascade leading to production of TNF- α by macrophages exposed to *T. gondii* GPIs (5). The membrane protein CD14 is a co-receptor that associates with TLR4 to form a receptor complex for bacterial lipopolysaccharide (LPS) (6). CD14 is involved in the recognition of lipoproteins from *Mycobacterium tuberculosis* by TLR2 (7). In addition, CD14 is necessary for the TLR2-dependent response of macrophages to GPIs of *Trypanosoma cruzi* (8). In contrast, CD14 is not required for the signaling leading to production of TNF- α by macrophages in response to the GPIs of *T. gondii* (5). The association of galectin-3 with TLR2 was demonstrated on PMA-differentiated THP-1 human macrophages infected with *Candida albicans* (9), and immunofluorescence microscopy showed the co-expression of galectin-3 with TLR4 on bone marrow-derived macrophages (10). Galectin-3 is a lectin specific for β -galactosides, composed of a C-terminal carbohydrate-recognition domain (CRD) and an N-terminal domain (ND) (11).

The galectin-3-dependent recruitment of effector cells may be an important mechanism of resistance to parasite infection. Indeed, high levels of galectin-3 were found in the granulomas surrounding eggs and worms during *Schistosoma mansoni* infection (12). In addition, galectin-3 co-localizes with GalNAc β 1-4GlcNAc expressed on the surface of eggs and can mediate GalNAc β 1-4GlcNAc recognition and phagocytosis by macrophages. This implicates GalNAc β 1-4GlcNAc as a molecular parasite pattern for galectin-3-mediated immune recognition. In galectin-3^{-/-} mice infected with *S. mansoni*, the size of the granulomas surrounding eggs was significantly decreased compared with infected wild type mice (13). Galectin-3 deficiency affects the number of splenic T and B cells in *S. mansoni*-infected mice, whereas it does not modulate the number of dendritic cells. *T. gondii* infection of wild type mice leads to an up-regulation of galectin-3 expression in various tissues (14). *T. gondii*-infected galectin-3^{-/-} mice develop reduced inflammatory responses in all organs, except for the lungs, and exhibit a higher parasite burden in the lungs and the brain (14).

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⁴ The abbreviations used are: GPI, glycosylphosphatidylinositol; CRD, carbohydrate recognition domain; ND, N-terminal domain; RU, resonance units; SPR, surface plasmon resonance; TLR, Toll-like receptor.

Comparable survival rates were observed in galectin-3^{-/-} and galectin-3^{+/+} mice orally infected with *T. gondii*. In contrast, higher mortality was observed in galectin-3^{-/-} mice after intraperitoneal infection with *T. gondii* associated with a deficient influx of neutrophils and macrophages into the peritoneal cavity. Furthermore, a recent study indicates that galectin-3 has an important modulator function by interfering in the life span and activation of neutrophils early after their infection with *T. gondii* (15). In the present work, we show that the GPIs of *T. gondii* are ligands of galectin-3 and that the production of TNF- α induced by GPIs in macrophages requires the expression of galectin-3.

EXPERIMENTAL PROCEDURES

Extraction and Purification of GPIs—*T. gondii* tachyzoites (strain RH) were grown in Vero cells (free of *Mycoplasma*) and released from host cells with the help of glass beads in the Mixer Mill homogenizer (Retsch, Haan) and purified by glass wool filtration (16). GPIs were extracted from tachyzoites as described previously (17). Briefly, glycolipids were extracted with chloroform-methanol-water (10:10:3, by volume) and partitioned between water and water-saturated *n*-butyl alcohol. GPIs present in *n*-butyl alcohol were precipitated under a stream of nitrogen. The six different *T. gondii* GPIs (18) were then separated by thin layer chromatography (TLC), with [³H]glucosamine-labeled *T. gondii* GPIs used as tracers. For this, a part of the tachyzoite culture was metabolically labeled in glucose-free DMEM containing 20 mM sodium pyruvate and 0.5 mCi of [³H]glucosamine hydrochloride (Hartmann Analytic GmbH, Braunschweig) for 6 h at 37 °C in 5% CO₂ atmosphere. Labeled GPIs were extracted as above. TLC was performed with the solvent *n*-hexane-chloroform-methanol-water-acetic acid (3:10:10:2:1, by volume). Chromatograms were scanned for radioactivity (Berthold LB 2842 linear analyzer), and areas corresponding to individual *T. gondii* GPIs were scraped off and reextracted with chloroform-methanol-water (10:10:3, by volume) by sonication. GPIs were separated from residual silica gel and recovered in the *n*-butyl alcohol phase after *n*-butyl alcohol/water partition. GPI quantification was based on monosaccharide composition of each GPI, determined by gas chromatography (GC) on a GC Trace gas chromatograph (Thermo Fisher Scientific) equipped with a 25-m \times 0.25-mm ECT^{M-1} capillary column, 0.25- μ m film thickness (Alltech, Templemars, France) after methanolysis (0.5 N HCl-methanol for 24 h at 80 °C), *N*-reacetylation, and trimethylsilylation. Diacylglycerols were obtained by the cleavage of TLC-purified GPIs using 1 unit of *Bacillus cereus* phosphatidylinositol phospholipase C (Sigma) in 100 μ l of 0.1 M Tris-HCl (pH 7.4), 0.1% sodium deoxycholate overnight at 37 °C. The enzyme was inactivated by treatment for 3 min at 100 °C. Diacylglycerols were recovered in the butanol phase after partition between water and water-saturated *n*-butyl alcohol. The absence of endotoxin in purified *T. gondii* GPIs was checked with the *Limulus* Amebocyte Lysate kit QCL-100 (Bio-Whittaker, Walkersville, MD). GPIa was chemically synthesized according to Pekari *et al.* (19). This molecule has no lipid moiety and corresponds to the glycan part of GPI III of *T. gondii*.

Direct Binding Assays by SPR—Binding analyses were performed on a BIAcore 3000 instrument (BIAcore Ab; Uppsala, Sweden). Human recombinant galectin-3 and galectin-1 (carrier-free; R&D Systems, Minneapolis, MN) were covalently coupled to a CM5 (carboxymethylated) sensor chip following the manufacturer's instructions (standard amine immobilization kit; BIAcore). Immobilized bovine serum albumin (BSA) was used as control for nonspecific binding. Amounts of 9,000–12,000 resonance units (RU) and 2,500–4,000 RU of galectin-3 and galectin-1, respectively, were immobilized under these conditions, where 1 RU corresponds to a concentration of 1 pg/mm² of immobilized protein (20). *T. gondii* GPIs, GPIa, or diacylglycerols diluted in PBS by sonication were injected using the multichannel mode. In some experiments, the GPI molecules were injected with 15 mM or 30 mM lactose. After injection of 65- μ l samples at 10 μ l/min and at 25 °C, the formed complexes were allowed to dissociate in PBS. Regeneration of the sensor chip surface was achieved by repeated pulses of 90% ethylene glycol and 10 mM NaOH, at a flow rate of 30 μ l/min. Laminin, a well known substrate, was injected to check that the regeneration process did not modify the structure and the activity of the galectins. Control flow-cells were obtained by injection of the respective analyte solutions on a chip submitted to the activation-deactivation coupling protocol and on a chip with immobilized BSA. Data were analyzed using the BIAcore 3.0 evaluation software. Results represented as sensorgrams are expressed in RU, which are proportional to the quantity of analytes bound to immobilized galectin-3 or galectin-1. Because binding equilibrium was not reached during the time of injection, the equilibrium response values (R_{equ}) were calculated from the association phases obtained with increasing concentrations of analytes. The measured association (k_{on}) and dissociation (k_{off}) rate constants allowed the determination of the equilibrium dissociation constant K_D , through the ratio $k_{\text{off}}/k_{\text{on}}$. K_D was also determined from Scatchard plot analysis by linearization of the saturation binding curve $R_{\text{equ}}/[GPI]$ versus $[GPI]$, with R_{equ} the response units at equilibrium. The slope corresponds to $-1/K_D$.

Production of TNF- α by Primary Macrophages from Galectin-deficient Mice—The experimental procedures using mice were performed according to the institutional animal care and use guidelines from the Monod Institute, Paris Diderot University. Thioglycollate-elicited peritoneal macrophages were obtained from wild type (129Sv), galectin-1^{-/-}, galectin-3^{-/-}, and galectin-1/3^{-/-} mice by peritoneal washing with serum-free medium (Panserin 401; PAN Biotech GmbH, Aidenbach). Macrophages were incubated for 24 h with the medium alone or with the different GPIs of *T. gondii* at 2 μ M resuspended in the medium by sonication. The amount of TNF- α in culture supernatants was determined by using a specific sandwich ELISA (BD Biosciences).

Statistics—The unpaired Student's *t* test was adopted for statistical evaluation. $p < 0.05$ was considered significant.

RESULTS

***T. gondii* GPIs, the Glycan GPIa, and Diacylglycerols Bind to Human Galectin-3**—SPR was used to test whether human galectin-3 may interact with GPIs, the main glycolipids of *T.*

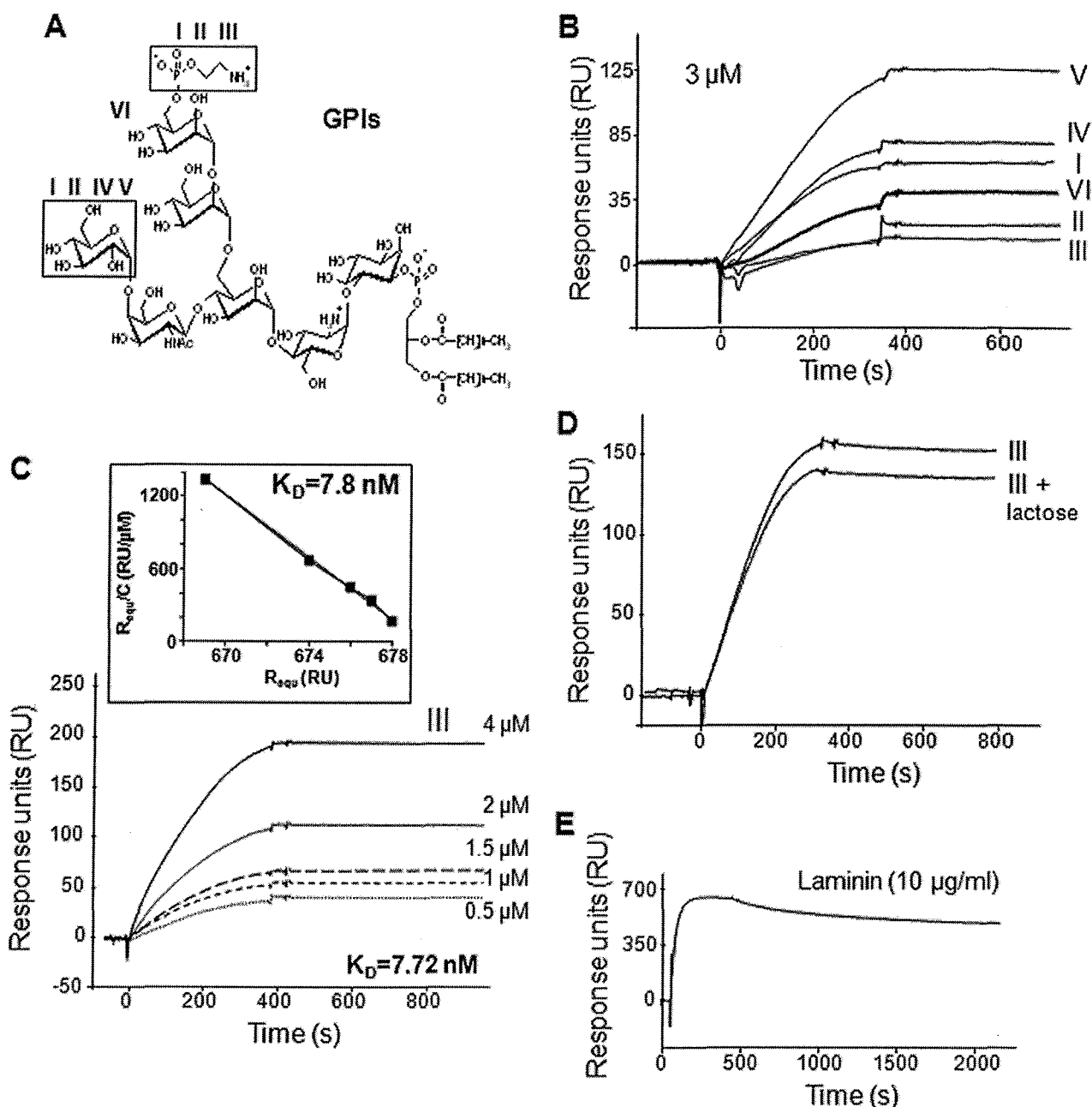


FIGURE 1. GPIs of *T. gondii* associate with galectin-3. **A**, structures of *T. gondii* GPIs: GPIs I and II, (EtN-P)-Man α 1-2Man α 1-6(Glc α 1-4GalNAc β 1-4)Man α 1-4GlcN α -Ino-P-DAG; GPI III, (EtN-P)-Man α 1-2Man α 1-6(GalNAc β 1-4)Man α 1-4GlcN α -Ino-P-DAG; GPIs IV and V, Man α 1-2Man α 1-6(Glc α 1-4GalNAc β 1-4)Man α 1-4GlcN α -Ino-P-DAG; and GPI VI, Man α 1-2Man α 1-6(GalNAc β 1-4)Man α 1-4GlcN α -Ino-P-DAG. GPIs I and II, and GPIs IV and V differ in their diacylglycerol composition with palmitic and stearic acids as predominant fatty acids. EtN, ethanolamine, P, phosphate; Man, mannose; Glc, glucose; GalNAc, acetylgalactosamine; GlcN, glucosamine; Ino, inositol; DAG, diacylglycerol. **B-E**, SPR analysis of the interaction of immobilized galectin-3 with the six different *T. gondii* GPIs (I-VI) at 3 μM (**B**); different concentrations of *T. gondii* GPI III with Scatchard plot analysis of the sensorgram data shown in the inset (slope = -127.4) (**C**); GPI III at 3 μM in the presence of 30 mM lactose (**D**); or laminin at 10 μg/ml (**E**). Results are expressed as RU (proportional to the quantity of molecules) after the subtraction of the nonspecific binding. Sensorgrams are representative of independent experiments with similar results.

gondii. The six different GPIs of *T. gondii* (structures in Fig. 1A) were injected separately over immobilized galectin-3. The molecular association and dissociation phases were illustrated by sensorgrams obtained after subtraction of the nonspecific binding to a sensor chip control cell. As shown in Fig. 1B, all *T. gondii* GPIs bound to galectin-3, and the very slow dissociation that occurred in PBS suggests the formation of highly stable complexes. The results presented here show that the different

GPIs bound to galectin-3 with variable amounts (RU). However, repeated experiments with other galectin-3-immobilized sensorchips indicated that these differences between GPIs were not significant, suggesting that the binding is independent of the variation between the six GPI structures. Injection of increasing concentrations of GPI permitted us to calculate the association and dissociation constants. The binding of GPI to galectin-3 is dose-dependent as illustrated with GPI III in Fig.

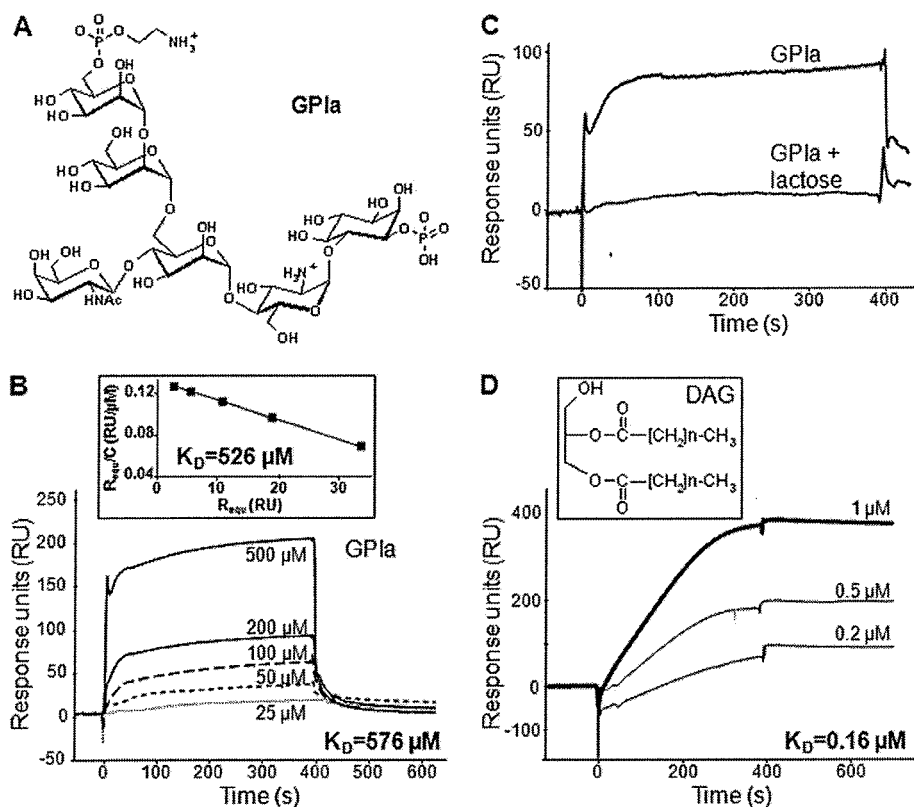


FIGURE 2. Both glycan and lipid moieties participate in the association of *T. gondii* GPIs with galectin-3. A, structure of GPIa, the chemically synthesized glycan of *T. gondii* GPI III. B–D, SPR analysis of the interaction of immobilized galectin-3 with GPIa at different concentrations (B) (Scatchard plot shown in the inset, slope = -0.0017); GPIa at $200 \mu\text{M}$ in the presence of 15 mM lactose (C); or diacylglycerols (DAG, general structure in the inset) isolated from GPI III at different concentrations (D). Results are expressed as RU (proportional to the quantity of molecules) after the subtraction of the nonspecific binding. Sensorgrams are representative of independent experiments with similar results.

1C. The equilibrium dissociation constant K_D calculated from the ratio of the kinetic rate constants (dissociation rate constant $k_{\text{off}} = 1.75 \times 10^{-6} \text{ s}^{-1}$ /association rate constant $k_{\text{on}} = 227 \text{ M}^{-1}\text{s}^{-1}$) was 7.72 nM . The K_D determined by Scatchard plot analysis was consistent with the kinetically determined value ($K_D = 7.8 \text{ nM}$; Fig. 1C, inset). To understand which part of galectin-3 is involved in its association with *T. gondii* GPIs, an excess of lactose that specifically blocks the CRD of galectin-3, was added. However, 30 mM lactose induced a very low reduction (12%) in the binding of GPI III at $3 \mu\text{M}$ (Fig. 1D). This suggests that the ND is also involved in the association of galectin-3 with *T. gondii* GPIs. A saline buffer (pH 7.2) containing 1 M NaCl was inefficient to regenerate the sensor chip, and alternated pulses of ethylene glycol (90%) and NaOH (10 mM) were required to release bound GPIs, suggesting the involvement of hydrophobic interactions. This treatment did not alter the structure of galectin-3 because its well known ligand laminin (at $10 \mu\text{g/ml}$) was able to associate with galectin-3 after several regeneration processes (Fig. 1E).

All GPIs are constituted by a glycan moiety and a lipid moiety, and we then investigated which part is involved in the interaction with galectin-3. To this end, a chemically synthesized molecule, GPIa, corresponding to the glycan moiety of GPI III and lacking the lipid moiety (structure in Fig. 2A) was tested in

SPR assay. This molecule was also able to interact with galectin-3 but with a much lower affinity than the whole GPI ($K_D = 576 \mu\text{M}$; see also the Scatchard plot analysis in the inset $K_D = 526 \mu\text{M}$), illustrated by a complete dissociation at the end of the GPIa injection (Fig. 2B). Because of its glycan structure GPIa might interact with the CRD of galectin-3. This hypothesis was confirmed by the addition of an excess of lactose (15 mM) that strongly reduced (80%) the binding of GPIa at $200 \mu\text{M}$ to galectin-3 (Fig. 2C). This inhibition assay gives good evidence that GPIa interacts with the CRD of galectin-3. The discrepancy between the binding of the whole GPI and the binding of GPIa suggests that the lipid moiety of the entire GPI also participates in the interaction with galectin-3. To check this hypothesis, the diacylglycerol was isolated by the cleavage of the *T. gondii* GPI III with a phosphatidylinositol-specific phospholipase C and tested in SPR assay. The diacylglycerol alone was able to bind to galectin-3 (Fig. 2D) with an intermediate affinity between those of GPIa and of the entire GPI ($K_D = 0.16 \mu\text{M}$). These results suggest that the glycan moiety and the lipid moiety act in synergy in the interaction with galectin-3. Because lactose only partially inhibited the binding of the whole GPIs, the ND is certainly involved in the interaction with the lipid moiety.

T. gondii GPIs and Their Diacylglycerols, but Not GPIa, Bind to Human Galectin-1—To understand further the specificity and the characteristics of the GPI binding to human galectin-3, we have tested the interaction of GPIs to human galectin-1, a β -galactoside-binding protein related to galectin-3 but lacking a ND. As for galectin-3, galectin-1 interacted with the GPIs of *T. gondii* without significant difference between the six structures, but the dissociation was more rapid, suggesting that GPI association with galectin-1 is less stable than with galectin-3 (Fig. 3A). A lower affinity for galectin-1 than for galectin-3 was obtained with the injection of increased concentrations of the GPI III (Fig. 3B), with a calculated K_D of $0.565 \mu\text{M}$ ($k_{\text{off}} = 3.24 \times 10^{-4} \text{ s}^{-1}/k_{\text{on}} = 573 \text{ M}^{-1}\text{s}^{-1}$), correlated with the value of $0.550 \mu\text{M}$ obtained with the Scatchard plot analysis (Fig. 3B, inset). Because galectin-1 is only constituted by a CRD, the synthetic glycan GPIa should bind to galectin-1 to same extent as the entire GPIs. Surprisingly, GPIa was unable to interact with galectin-1 (Fig. 3C). In contrast, the GPI diacylglycerols suspected to be recognized by the ND of galectin-3 interacted with

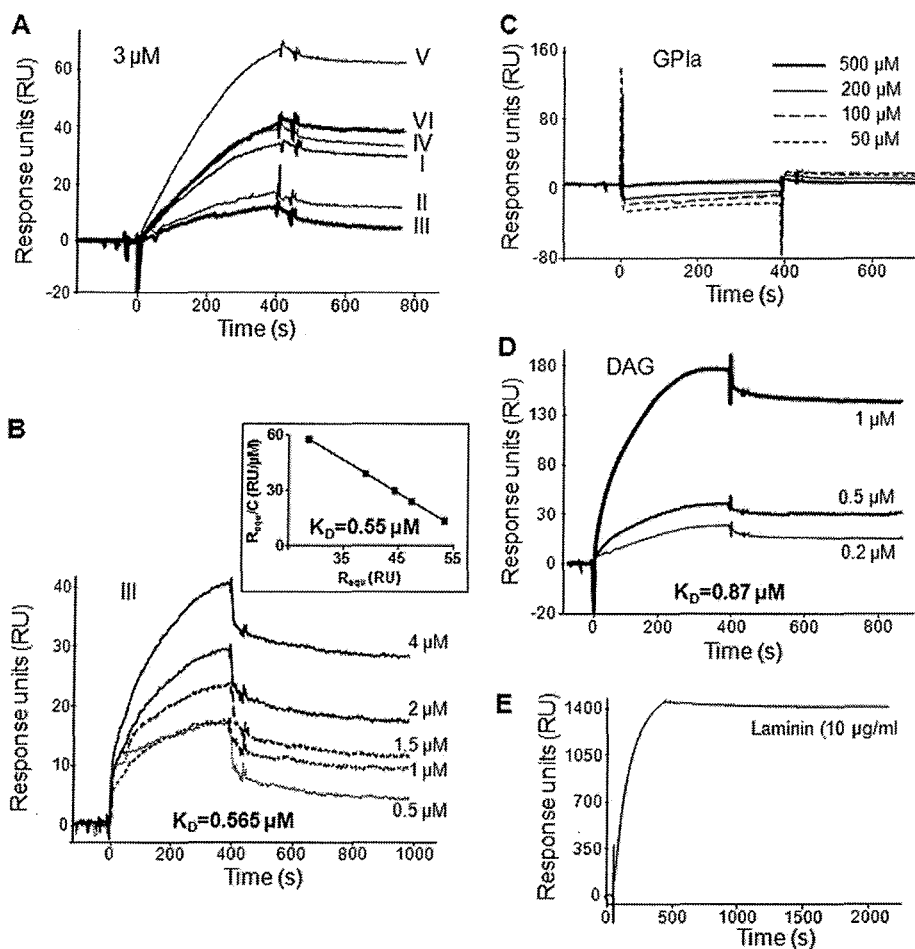


FIGURE 3. GPIs of *T. gondii* associate with galectin-1 through their lipid moiety. SPR analysis of the interaction of immobilized galectin-1 with (A) the six different *T. gondii* GPIs (I-VI) at 3 μ M; (B) GPI III at different concentrations (Scatchard plot shown in the inset, slope = -1.77); (C) GPIa at different concentrations; (D) diacylglycerols isolated from GPI III at different concentrations; or (E) laminin at 10 μ g/ml. Results are expressed as RU (proportional to the quantity of molecules) after the subtraction of the nonspecific binding. Sensorgrams are representative of independent experiments with similar results.

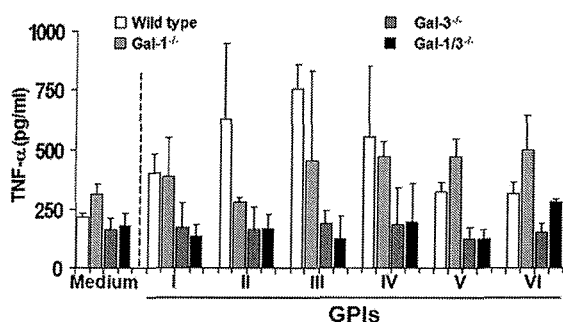


FIGURE 4. *T. gondii* GPIs require galectin-3 but not galectin-1 to induce TNF- α production in macrophages. Peritoneal macrophages from wild type (129Sv), galectin-1 $^{-/-}$, galectin-3 $^{-/-}$, and galectin-1/3 $^{-/-}$ mice were incubated for 24 h with medium alone or with the six different GPIs (I-VI) at 2 μ M. Supernatants were assayed for TNF- α production using a sandwich ELISA. Values represent the means \pm S.D. of triplicate samples.

galectin-1 with a K_D value similar to that of the whole GPIs (Fig. 3D). As with galectin-3, the regeneration of the sensor chip was achieved only after using of ethylene glycol and NaOH. However, this treatment did not degrade or modify the conformation of galectin-1 because laminin (10 μ g/ml) associated with

galectin-1 after several regeneration processes (Fig. 3E). Altogether, these results indicate that the recognition of *T. gondii* GPIs is different and specific for galectin-3 and galectin-1.

Galectin-3 Is Required for the Production of TNF- α by GPI-stimulated Macrophages—Because the affinity of *T. gondii* GPIs is higher for galectin-3 than for galectin-1, galectin-3 may be specifically involved in the TNF- α production by macrophages in response to *T. gondii* GPIs. To study this point, peritoneal macrophages from galectin-3 $^{-/-}$, galectin-1 $^{-/-}$, galectin-1/3 $^{-/-}$, and control (129Sv) mice were incubated with individual GPIs at 2 μ M, and TNF- α secretion was measured in supernatants. According to our previous work (5), wild type macrophages were stimulated by the six *T. gondii* GPIs to produce higher amounts of TNF- α than in the presence of medium alone (Fig. 4). This induction of TNF- α by *T. gondii* GPIs was not inhibited in the absence of galectin-1 (galectin-1 $^{-/-}$). The reduction observed for the GPI II in the experiment shown here is not significant ($p = 0.069$). On the contrary, macrophages lacking galectin-3 (galectin-3 $^{-/-}$) were not stimulated by the GPIs, and the levels of TNF- α

were similar to those measured in the supernatant of cultures with medium alone. Lack of TNF- α induction was also obtained with double null mutant macrophages (galectin-1/3 $^{-/-}$) and it was certainly due to the absence of galectin-3 and not of galectin-1. These data suggest that galectin-3 is involved in the recognition of *T. gondii* GPIs at the surface of the macrophages.

DISCUSSION

Galectin-3 has more extended carbohydrate-recognition sites than galectin-1. This structural difference allows galectin-3 to bind to a wider range of oligosaccharide structures, including structures containing mannose (21). We identified the GPIs of *T. gondii* as ligands of galectin-3. K_D values of galectin-3 for diacylglycerols and entire GPIs at the μ M level and the nM level, respectively, were in the range of K_D values of galectin-3 for other ligands, for example 3.6–6.1 μ M for (Gal β 1–3) n lactose, and 1.4 μ M for biantennary *N*-linked oligosaccharide (22–24). Six different GPIs are synthesized by the parasite. GPIs III and VI present a GalNAc β 1–4 branched on the first mannose of the evolutionary conserved core glycan, whereas GPIs I,

II, IV, and V have an additional glucose residue linked with an $\alpha 1-4$ bond to the GalNAc. This glucose residue does not influence the recognition of the GPIs by galectin-3, because no difference was observed in the amounts of GPIs interacting with galectin-3 in the SPR assay. Binding of GalNAc $\beta 1-4$ -containing glycans is not common to all galectin family members. Indeed, galectin-3 but not galectin-1 binds GalNAc $\beta 1-4$ GlcNAc-glycans of *S. mansoni* (12). Galectin-3 can accommodate the O-2 N-acetyl moiety of the GalNAc residue, whereas a bulky histidine (at position 52) in galectin-1 may prevent GalNAc binding. Binding of galectin-3 to GalNAc $\beta 1-4$ GlcNAc was completely prevented in the presence of lactose, supporting involvement of the CRD in this interaction (12). These results are in agreement with our observation that the synthetic glycan GPIa does not bind to galectin-1 but binds to the CRD of galectin-3, as evidenced by the lactose inhibitory effect. Galectin-3 recognizes the polygalactose structure, (Gal $\beta 1-3$) $_n$, found on lipophosphoglycan of *Leishmania major* (25). Galectin-1 was not shown to have significant affinity for *L. major*, suggesting that the polygalactose epitope binds to the galectin-3-unique extended binding pocket, which is not present in galectin-1 (25). The polygalactose is not expressed by *Leishmania donovani*. Thus, galectin-3 can distinguish between the two related parasites. In contrast, GPIs are expressed on both virulent (RH) and nonvirulent (PTG) strains of *T. gondii*, and the slight differences we have found in their structure⁵ did not affect the interaction with galectin-3 ($K_D = 0.2$ nM, not shown).

Galectin-1 binds to Davanat (composed of $\beta 1-4$ -linked D-mannopyranosyl units and D-galactopyranosyl residues attached via $\alpha 1-6$ linkage) with an apparent equilibrium dissociation constant of 10 μ M, compared with 260 μ M for lactose (26). The β -galactoside-binding domain remains accessible in the galectin-1·Davanat complex because lactose can still bind with no apparent loss in affinity. Galectin-1 acts as a receptor for a protein, the tissue plasminogen activator. The interaction of tissue plasminogen activator with galectin-1 is higher than with galectin-3 (K_D 9.1 μ M and 17 nM, respectively) (27). This illustrates that galectins interact not only with β -galactosides. Here, we show that the diacylglycerols of *T. gondii* GPIs interact strongly with galectin-1, which lacks a ND. Because the CRD of both galectins has affinities for different structures, further investigations are needed to conclude whether the lipid moiety of GPIs interacts with the CRD or the ND of galectin-3. We have shown previously that the diacylglycerols cleaved from the *T. gondii* GPIs are able to stimulate macrophages to produce TNF- α (3) via signaling of both TLR2 and TLR4, whereas only the TLR2 signaling seems to be triggered by GPIa or the whole GPIs (5). The three-dimensional conformation of the complexes galectin-3·diacylglycerol and galectin-3·GPI (or GPIa) might determine their recognition or not by TLR4. Free GPIs are present in the extracellular medium in their entire form, probably secreted by the parasites.⁶ We assume that macro-

phage enzymes like phospholipases, cleave the GPI lipid moiety, allowing its interaction with galectin-3 and the TLR signaling.

Galectin-3 may play a major role in controlling cytokine production. LPS markedly increased amounts of inflammatory cytokines in galectin-3^{-/-} cells (10). *S. mansoni*- and *T. gondii*-infected galectin-3^{-/-} mice mount a higher Th1-polarized immune response and a decreased inflammation (14). Because its absence was associated with the production of higher levels of Th1 cytokines when these cells are stimulated, it suggests that galectin-3 may act in the regulatory loops controlling Th1 cytokine production. It was shown that galectin-3^{-/-} macrophages produce lower amounts of TNF- α after incubation with *Candida albicans*, compared with galectin-3^{+/+} macrophages (9). We showed in the present study that galectin-3^{-/-} macrophages are unable to produce TNF- α in response to *T. gondii* GPIs. Cells other than macrophages might be responsible for the higher production of inflammatory Th1 cytokines observed in galectin-3-deficient animals. Our previous study indicates that the *T. gondii* GPI-induced TNF- α production involves both TLR2 and TLR4 in macrophages (5). CD14 is the well known associated molecule for the recognition of LPS, the historical ligand of TLR4. On the contrary, CD14 is not required for the production of TNF- α by mouse macrophages in response to the GPIs of *T. gondii* (5). We hypothesize that galectin-3 is a TLR-associated protein involved in the cell response to the GPIs of *T. gondii*. It is possible that extracellular galectin-3 molecules interact with N-glycans present in TLRs (28). It has been demonstrated that the surface expression of TLR2 is enhanced on galectin-3^{-/-} macrophages (29). This up-regulation cannot lead to an increased production of TNF- α in response to the GPIs because the expression of galectin-3 is absolutely needed. Our results show that galectin-1 is not involved in the TNF- α production by macrophages in response to GPI stimulation. Exogenous galectin-1 was found to inhibit LPS-induced NF- κ B activation macrophages (30). We suggest that galectin-1 blocks α -2,3-sialyl residues linked to β -galactoside glycans on TLR4 receptors, and so Neu1 sialidase can no longer hydrolyze these sialic acids. In contrast, the GPI-induced TNF- α production was sometimes higher in galectin-1^{-/-} compared with wild type macrophages (Fig. 4), suggesting that galectin-1 may partially neutralize the TLR4 signaling in response to *T. gondii* GPIs.

Different studies indicate that galectin-1 is involved in the recognition of protozoan parasites. Endogenous galectin-1 expression is up-regulated *in vitro* and *in vivo* by *T. cruzi*, and the expression of galectin-1 is up-regulated in cardiac tissue from patients with chronic Chagas disease (31). Because the adhesion of *T. cruzi* to heart muscle cells is mediated by protein-carbohydrate interactions, an overexpression of galectin-1 on heart tissue could facilitate parasite invasion toward this vulnerable infection site (32). Galectin-1 acts as a receptor for the protozoa *Trichomonas vaginalis* by interacting with parasite lipophosphoglycans at the surface of human cervical epithelial cells (32). Accordingly, galectin-1 may be involved in the attachment of *T. gondii* to the host cell, supporting by this way the invasion process.

⁵ S. Niehus, T. K. Smith, N. Azzouz, M. A. Compos, J. F. Dubremetz, D. Sibley, R. T. Schwarz, R. T. Gazzinelli, and F. Debierre-Grockieo, unpublished observations.

⁶ F. Debierre-Grockieo and R. T. Schwarz, unpublished observations.

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