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Redundant and Antagonistic Functions of Galectin-1, -3, and -8 in the Elicitation of T Cell Responses

María Virginia Tribulatti,1 María Gabriela Figini,1 Julieta Carabelli, Valentina Cattaneo, and Oscar Campetella

Galectins, a family of mammalian lectins, have emerged as key regulators of the immune response. We previously demonstrated that galectin (Gal)-8, from the tandem-repeat subgroup, exerts two well-defined effects on mouse naive peripheral CD4 T cells: Ag-specific costimulation and Ag-independent proliferation. These stimulatory signals on naive T cells have not been described for any other Gal. Therefore, we investigated whether Gal-1 and Gal-3, two prominent members of the Gal family, share the stimulatory effects exerted by Gal-8 on naive T cells. We found that Gal-1 costimulated Ag-specific T cell responses similarly to Gal-8, as evaluated in the DO11.10 TCRova-transgenic mouse model, by acting simultaneously on APCs and target CD4 T cells. In contrast, Gal-3 failed to costimulate Ag-specific T cell responses; moreover, it antagonized both Gal-1 and Gal-8 signals. We observed that both Gal-1 and Gal-3 were unable to induce Ag-independent proliferation; however, when two Gal-1 molecules were covalently fused, the resulting chimeric protein efficiently promoted proliferation. This finding indicates that Gal-1 might eventually induce proliferation and, moreover, stresses the requirement of a tandem-repeat structure. Remarkably, a single dose of recombinant Gal-1 or Gal-8 administered together with a suboptimal Ag dose to DO11.10 mice strengthened weak responses in vivo. Taken together, these findings argue for the participation of Gals in the initiation of the immune response and allow the postulation of these lectins as enhancers of borderline Ag responses, thus representing potential adjuvants for vaccine formulations. The Journal of Immunology, 2012, 188: 2991–2999.

Galectins (Gals) constitute a family of secreted mammalian lectins with affinity for β-galactosides that bind glycoreceptors on target cells and induce multiple biological activities that include a broad spectrum of cellular responses, such as proliferation, apoptosis, differentiation, adhesion, migration, and cytokine secretion (1, 2). Gals are characterized by the presence of conserved carbohydrate-recognition domains (CRDs) and are structurally classified into three subgroups: prototype (one CRD, such as Gal-1, -2, and -7), tandem repeat (two linked CRDs, such as Gal-4, -8, and -9), and chimera (one CRD fused to a nonlectin domain; Gal-3). Prototypical and chimeric Gals can homodimerize or multimerize, respectively, thus participating in many cellular processes, such as cell–cell and cell–matrix interactions, by acting as bivalent or multivalent factors. Another remarkable feature of this family of proteins is the formation of ordered cell surface Gal-glycan structures termed lattices, which engage specific cell surface glycoconjugates by traditional ligand–receptor interactions. These structures have been involved in the control of receptor endocytosis, host–pathogen interactions, and immune system homeostasis (3).

Gals have arisen as important regulators of the immune response (2). Different members of this family have been found in primary and secondary lymphoid organs, as well as in circulating cells, and were shown to modulate several pathological processes, such as autoimmunity, chronic inflammation, infection, and tumor progression (4). Gal-1 and -3 are the most abundantly expressed and extensively studied members of the Gal family. With regard to T cell homeostasis, Gal-1 was shown to promote apoptosis of immature CD4+CD8− and CD4+CD8+ thymocytes, thus contributing to maintenance of self-tolerance (5). In the periphery, Gal-1 displayed anti-inflammatory effects by inducing apoptosis of activated T cells (6). Additionally, Gal-1 was shown to exert its immunoregulatory role by negatively modulating proinflammatory cytokine expression, skewing the balance from a Th1 toward a Th2 or T regulatory response (7).

Gal-3 is another key regulator of T cell homeostasis, with the peculiarity that it can induce T cell proliferation or apoptosis, depending on whether it acts intracellularly or extracellularly, respectively. When added exogenously, Gal-3 is able to induce apoptosis of several human T leukemia cell lines, as well as activated mouse cells (8). Moreover, this Gal also contributes to the maintenance of self-tolerance by triggering apoptosis of the CD4+CD8− thymocyte subset (9).

Gal-8, which belongs to the tandem-repeat group, is intrinsically a heterodimer, because it has an N-terminal CRD (N-CRD) and a C-terminal CRD (C-CRD) joined by a hinge linker peptide of variable length. It is expressed in different organs and tissues under physiological or pathological conditions, such as several human cancers (10, 11). We previously reported the expression of

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two Gal-8 isoforms in mouse thymus and spleen, which differed only in the length of the linker region. Both isoforms were able to induce apoptosis of the immature CD4<sup>high</sup>CD8<sup>high</sup> thymocyte subpopulation involving caspase pathway activation, suggesting that this Gal could also participate in central negative selection (12). At the periphery, we found that Gal-8 exerts two actions on naïve CD4 T cells: at high concentrations, it induces strong Ag-independent proliferation, whereas at low concentrations, it costimulates T cells in the presence of APCs and the corresponding Ag. These activities are mediated by interaction of the T cell surface glycoprotein CD45 and involve the activation of ZAP70- and ERK signaling pathways (13). The molecular requirements for both of Gal-8’s stimulatory effects were recently assessed; its tandem-repeat structure is only essential for proliferation and not for costimulation (14).

Interestingly, Gal-8’s proliferative and costimulatory signals on primary T cells have not been described for any other Gal and suggest a possible involvement of this lectin in inflammatory and autoimmune processes. Moreover, these stimulatory activities differentiate Gal-8 from other Gals, particularly Gal-1 and Gal-3, which are known to display immunoregulatory functions (2). As discussed above, the ability to kill activated peripheral T cells endowed Gal-1 and Gal-3 with anti-inflammatory properties, which were reflected by their therapeutic effects on experimental models of T cell-mediated autoimmune disorders and inflammatory diseases (15). In this regard, we recently showed that, although Gal-8 is able to induce strong proliferation of freshly isolated human PBMCs, it promoted cell death when these cells were preactivated with PHA or CD3/CD28 stimulators (14). Thus, the effector phase of ongoing responses could be limited, not only by Gal-1 and Gal-3, but also by Gal-8.

The analysis of the existence of redundant or antagonistic functions between Gals is a major concern, because these proteins can converge under normal or pathological conditions, such as inflammatory or certain tumors (16–19). The proapoptotic activity of Gal-1 and Gal-3 has been extensively characterized, although most experiments were performed using activated T cells or T cell lines. Thus, there is scarce or contradictory information regarding the effect of Gal-1 and Gal-3 on primary naïve T cells. Therefore, the aim of the present work was to contrast the effects of Gal-1 and Gal-3, but also by Gal-8.

Reagents
ERK inhibitor [1-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-ene] (LY 294002), protein kinase C (PKC) inhibitor 3-[1-(3-dimethylaminopropyl)-5-methoxy-1H-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrolo-2,5-dione (Go-6983), and p38 MAPK inhibitor [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyrdinyl)-1H-imidazole] (SB-203580) were from Enzo Life Sciences (Farmingdale, NY). The CD45 protein tyrosine phosphatase (PTPase) inhibitor N-[9,10-dioxo-9,10-dihydrophenanthren-2-yl]-2,2-dimethyl-propionamide was from Calbiochem (Darmstadt, Germany). Thiodigalactoside (TDG) was from Carbosynth (Berkshire, U.K.). Con A and Mitomycin C were from Sigma.

Materials and Methods
Mice, cell lines, and cell purification
C57Bl/6J, C.Cg-Tg(DO11.10)1Dio/J (DO11.10), and BALB/c breeding pairs were obtained from The Jackson Laboratory (Bar Harbor, ME) and bred in our facilities. DO11.10 T CD4 cell hybridoma was kindly provided by Dr. P. Marrack (Howard Hughes Medical Center, Denver, CO). For mouse splenocyte purification, spleens from 4–8-wk-old animals were removed and disrupted against a stainless steel mesh in RPMI 1640 medium (Invitrogen, Carlsbad, CA). The cell suspension was washed and incubated with RBC lysis buffer (Sigma, St. Louis, MO) and washed again with medium. MiniMACS columns and anti-CD4–coupled paramagnetic particles (Miltenyi Biotec, Bergisch Gladbach, Germany) were used for CD4 T cell purification and depletion, following the manufacturer’s instructions. Cell purity was checked by FACs. All assays involving animals were approved by the Ethical Committee Board of our Institute.

Cell proliferation and costimulation assays
For T cell activation, splenocytes (5 × 10<sup>5</sup> cells) from C57Bl/6J mice were cultured at 37°C in 5% CO<sub>2</sub> for 48 h in flat-shaped, 96-well plates in 0.2 ml RPMI 1640 medium in the presence of 10% FBS (Invitrogen), 2 mM glutamine, and 5 μg/ml gentamicin (complete medium). For costimulatory assays, splenocytes (3 × 10<sup>5</sup> cells) from DO11.10 mice were cultured for 48 h in 0.2 ml complete medium in the presence of the cognate OVA<sub>323–339</sub> peptide from mouse Gal-8 (12). After the first step of purification, Gal-8 was alkylated in the presence of 0.1 M iodoacetamide (Sigma) overnight at 4°C. The use of alkylated Gal-8 prevents oxidation that leads to a loss of activity and, at the same time, overcomes the need to use reducing agents, such as DTT, which is known to introduce deleterious effects in cell cultures (20, 21). Gal-8 was biotinylated using EZ-Link Sulfo-NHS-Biotin (Pierce, Rockford, IL), following the manufacturer’s instructions. Lectin activity of these recombinant proteins was tested by hemagglutination assays.

Inhibition of cell proliferation
For T cell activation, splenocytes (5 × 10<sup>5</sup>) from C57BL/6J mice were cultured, as described in proliferation assays (see above), in the presence of 2.5 μg/ml Con A for 24 h. Then, preactivated cells were treated with the indicated amounts of Gals for an additional 24 h. Inhibition of cell proliferation was assessed by adding 1 μCi [<sup>3</sup>H]methyl-thymidine to each well 7 h before harvesting.

In vivo costimulation
Initially, to determine suboptimal Ag dose, 6–8-wk-old female DO11.10 mice were immunized i.p. with decreasing amounts (from 5 to 0.05 μg) of OVA (Sigma) in 0.2 ml PBS; spleens were collected 5 d after immunization. In vitro restimulation of splenocytes was performed basically as for costimulation assays (see above), using two doses of OVA<sub>323–339</sub> Cognate peptide.
peptide (0.1 and 0.25 μg/ml). For in vivo costimulation, mice received 0.5 μg OVA i.p., in combination with 10–25 μg Gal-8 (OVA + Gal-8) or 25 μg Gal-1 (OVA + Gal-1). Control groups consisted of mice that received Ag only (OVA), vehicle (PBS), or Gals (Gal-1 and Gal-8). In vitro restimulation was performed in the presence of 0.25 μg/ml OVA cognate peptide.

Flow cytometry
A FlowMax cytometer PASIII (Partec, Münster, Germany) and WinMdi 2.9 software were used.

Statistical analysis
The Student t test was used; p values < 0.05 were considered significant.

Results
Gal-1 costimulates Ag-specific T cell response
In our previous work, Gal-8 costimulatory activity on naive T cells became evident when splenocytes from DO11.10 mice were incubated in the presence of 0.1–0.2 μM of Gal-8, together with suboptimal doses of OVA cognate peptide (13). We first investigated whether Gal-1 was able to display a similar activity in this model. Remarkably, when Gal-1 was tested in costimulation assays, a dose-dependent effect similar to that of Gal-8 was observed (Fig. 1A). However, 100 times more Gal-1 was needed to equal the activity of Gal-8. Preincubation with TDG almost completely prevented Gal-1–induced costimulation, indicating that this effect relies upon lectin–glycan interaction (Fig. 1B). In the search for a synergistic effect in costimulatory activity, we tested the simultaneous addition of Gal-1 and Gal-8; a similar response was observed to that obtained with Gal-8 alone. Moreover, when lectins were added separately with a 1-hour delay, only a minor, if any, additive effect was observed (Fig. 1C). These findings suggest that these Gals probably recognize similar receptors at the T cell surface to induce the same activation pathways. In fact, we observed that the presence of Gal-1 reduced biotin-labeled Gal-8 binding to CD4 T cells, independently from the addition order, indicating that these lectins actually compete for their binding at the cell surface (Supplemental Fig. 1A).

Because this was the first incidence, to our knowledge, of T cell costimulatory activity described for Gal-1, and it seemed to contradict its previously reported immunoregulatory function, we assessed whether both functions are mutually exclusive. For this purpose, we incubated splenocytes in the presence of the T cell mitogen Con A for 24 h, and the indicated concentrations of Gal-1 and Gal-8 were added. A dose-dependent effect similar to that of Gal-8 was observed (Fig. 1D). As shown in Fig. 1D, both Gals inhibited the proliferation of activated T cells, an effect prevented by the addition of TDG, thus supporting the involvement of Gals and cell–glycan interactions. Unexpectedly, this antiproliferative effect could not be ascribed to cell death induction, because no increment of apoptotic cells was observed after Gal-1 or Gal-8 treatment of activated splenocytes, as assessed by propidium iodide incorporation or caspase-3 activation (data not shown).

Taken together, these results support a dual role for Gal-1 and Gal-8 in the immune response by enhancing initial T cell responses but limiting those that become exacerbated.

Gals activate TCR downstream signaling pathways to induce costimulation
To better characterize Gal-1 and Gal-8 costimulation activities, we performed assays in the presence of Ick (Src)-, PI3K-, PKC-, p38 MAPK-, and ERK-specific inhibitors, as representative TCR downstream signal transducers. We also tested CD45 PTPase inhibitor, because we previously determined that Gal-8 interacts with CD45 and that its PTPase activity is involved in Gal-8–induced costimulation and proliferation (13). As expected, inhibition of TCR signaling pathways resulted in a decreased OVA-induced response (Fig. 2). The Gal-8 and Gal-1 costimulatory effect was strongly affected in the presence of these inhibitors, thus supporting that both Gals may reinforce the same intracellular pathways, activated by the corresponding Ag, to promote T cell costimulation.

Gals induce costimulation by simultaneously acting on APCs and CD4 T cells
To gain insight into the mechanism by which Gals mediate their costimulatory activity, we performed a set of experiments to determine whether these lectins are actually acting on APCs, T cells, or both. First, we designed costimulation assays in which Mito- mycin C-treated splenocytes from BALB/c mice were used as APCs, and highly purified CD4 T cells from TCR<sub>OVA</sub> DO11.10 mice were used as target. As observed in Fig. 3A, Gal-1 and Gal-8 readily stimulated CD4 T cells in the presence of OVA Ag, confirming that these cells are targets for the costimulatory effect.
Next, APCs and CD4 T cells were separately preincubated with Gal-8 or Gal-1 for 30 min on ice to allow Gal binding to the cell surface and then unbound lectins were washed out. Interestingly, when Gals were bound to only one of the populations involved, costimulation was no longer achieved. Furthermore, costimulation was also absent when APCs and T cells treated separately with Gals were mixed together. These findings indicate that to completely costimulate the Ag response, Gals need to bind on both APCs and T cells, at the very same time, during the Ag-presenting process. To confirm these observations, we repeated this assay using CD4 T cell-depleted splenocytes from DO11.10 mice as APCs (Fig. 3B). Again, Gal treatment on either APCs or T cells was ineffective in inducing costimulation.

**A tandem-repeat Gal-1 chimera displays Ag-independent proliferative activity**

In addition to the costimulatory activity, Gal-8 induces strong proliferation of mouse resting CD4 T cells in the absence of an antigenic stimulus, when used at relatively high concentrations (13). To test whether Gal-1 also shares this effect, we incubated C57BL/6J mouse splenocytes in the presence of increasing amounts of Gal-1. Even at the highest concentrations used (20 μM), Gal-1 was unable to induce the strong cell proliferation observed with 2 μM of Gal-8 (Fig. 4A). It is important to point out that, although it homodimerizes under certain conditions, Gal-1 contains only one CRD, whereas Gal-8 is a tandem of two CRDs fused by a linker peptide, thus being intrinsically a dimer. This difference could explain why Gal-1 can only display costimulatory, but not proliferative, activity on T cells (14). In fact, we recently demonstrated that both Gal-8 single N-CRDs and C-CRDs
are sufficient to costimulate T cell response to cognate peptide but, in strong contrast, they are unable to trigger Ag-independent proliferation. In contrast, chimeric homodimers N-CRD–N-CRD and C-CRD–C-CRD triggered proliferation, indicating that Gal-8 tandem-repeat structure is essential for Ag-independent proliferation but not for costimulation induction (14). This prompted us to investigate whether the stable dimerization of Gal-1 could overcome its inability to trigger T cell proliferation. For this purpose, we designed a chimeric protein in which two Gal-1 CRDs are covalently fused by the Gal-8L “long” linker peptide (12), Gal-1-8-1 (Fig. 4B). Gal-1-8-1 retained lectin activity and exhibited 2-fold greater hemagglutinating activity than did Gal-1 (6.25 and 12.5 μg/ml, respectively). Remarkably, Gal-1-8-1 displayed a significant proliferative effect from 10 μM, which was inhibited by preincubation with the Gal inhibitor, TDG (Fig. 4C). Of note, a 10-fold greater amount of chimeric Gal-1-8-1 was needed to equal Gal-8’s proliferative activity (20 and 2 μM, respectively). Moreover, Gal-1-8-1 induced costimulation at a similar rate as did Gal-1 (Fig. 4D), in agreement with the fact that Gal-8 monomeric CRDs are sufficient to trigger this effect (14). Taken together, these results indicate that stable linkage of two CRDs provides Gal-1 with a proliferative ability, although it does not necessarily imply an increase in its costimulatory activity. Alternatively, differences in Gal-1 and Gal-8 potency might be explained by a fine distinction among CRDs’ specificity rather than Gal molecular requirements. In this regard, the induced cellular phenotype of cultured splenocytes was completely different in the presence of Gal-1 or Gal-8; although Gal-8 induced an adhesive and spread phenotype, both Gal-1 and Gal-1-8-1 promoted strong cell agglutination, without adhesive or spread phenotype (Fig. 4E). This is in agreement with the fact that Gal-8 contains two CRDs with different glycan specificities that enables it to promote cell adhesion by linking the extracellular matrix and the cell surface (22). The strikingly different cellular phenotypes induced by Gal-1-8-1 and Gal-8 suggest that they might involve distinct mechanisms of cellular activation to induce the proliferation of T cells.

**Gal-3 antagonizes Ag-specific T cell response**

Next, we investigated whether Gal-3, another prominent member of the Gal family, was also able to exert costimulation of naive T cells. In contrast to Gal-1 and Gal-8, Gal-3 was unable to costimulate Ag-specific responses at any of the concentrations tested (5–10 μM, data not shown), and it inhibited the OVA response when the cognate peptide dose was ≤1 μg/ml (Fig. 5A). It was reported that the lattice formed by Gal-3 negatively regulates the TCR response by restricting receptor lateral motility (23–25). These reports support the lack of costimulation in the presence of Gal-3, as well as its inhibitory effect on the Ag-specific T cell response. Subsequently, we investigated the addition of Gal-3 on Gal-1 and Gal-8 costimulatory activity using low (1 μg/ml) and high (2 μg/ml) OVA peptide concentrations. Under low-Ag conditions, at which Gal-3 inhibits the OVA response per se, it also prevented Gal-1 and Gal-8 costimulatory effects (Fig. 5B, 5C). Gal-3 inhibited Gal-1’s effect, despite whether the lectins were added simultaneously or were separated by 1 hour and independently of the addition order. The costimulatory effect of Gal-8 was inhibited when Gal-3 was added simultaneously or 1 h before, but not when added 1 h after (Fig. 5B). Under high-Ag conditions, even when Gal-3 was unable to affect the OVA response per se, it was able to prevent either Gal-1 or Gal-8 costimulation when added simultaneously. However, Gal-3 was unable to affect either Gal-1– or Gal-8–induced costimulation when added separately (Fig. 5D, 5E). These results suggest that Gal-3 negatively reg-
from C57BL/6J mice were incubated for 48 h in the presence of 10 μg/ml of increasing amounts of the cognate OVA peptide (OVA), together with Gal-1 and Gal-8 (Fig. 6B). Splenocytes from mice that received only Gal-1 or Gal-8 displayed an increased response after in vitro restimulation compared with those derived from mice that received Ag alone (Fig. 6B). Splenocytes from mice that received only Gal-1 or Gal-8 recombinant proteins (without Ag) responded similarly to those from mice that received PBS (data not shown). Remarkably, when larger doses of Ag were used to immunize animals, Gal-induced costimulation was no longer evident (data not shown), stressing its ability to costimulate otherwise borderline responses. These results correlate with Gal-1 and Gal-8 costimulatory properties observed in vitro (Fig. 1A) and strongly suggest that both Gal-1 and Gal-8 have the potential to costimulate T cell specific-responses in vivo.

Discussion

Gals have arisen as key mediators of the innate and adaptive immune response that participate in several processes, such as host–pathogen interaction, inflammatory and autoimmune disorders, host-versus-graft disease, fetal–maternal tolerance, and self-tolerance maintenance (2). Therefore, how Gals control immune cell homeostasis and the underlying mechanisms are crucial. Given that some Gals can be simultaneously present in the same microenvironment, the cross-talk among these lectins is another key aspect that is now emerging. In the present work, we demonstrated that Gal-1, as well as Gal-8, can costimulate borderline Ag-specific T cell responses. Blockade of established TCR signaling pathways (Lck, PI3K, PKC, p38 MAPK, and ERK) prevented Gal-1- and Gal-8-induced costimulation, indicating that these Gals actually enhance weak signals from borderline Ag-specific T cell responses. These results correlate with Gal-1 and Gal-8 costimulatory properties observed in vitro (Fig. 1A) and strongly suggest that both Gal-1 and Gal-8 have the potential to costimulate T cell specific-responses in vivo.

Gal-8 and Gal-1 increase T cell-specific responses in vivo

We found that both Gal-8 and Gal-1 display costimulatory activity in T cell Ag-specific responses. Next, we investigated whether these Gals could costimulate an in vivo-induced immune response. For this purpose, DO11.10 mice were immunized with a single dose of OVA whole protein alone or mixed with recombinant Gals. After 5 d, splenocytes were restimulated in vitro with the cognate OVA peptide. The Ag dose used to immunize animals (0.5 μg) was pinpointed in preliminary assays to generate a slight response upon in vitro restimulation with 0.25 μg/ml of cognate peptide (Fig. 6A). Remarkably, splenocytes derived from mice that were immunized with a combination of Ag and 25 μg of Gal-1 or Gal-8 displayed an increased response after in vitro restimulation compared with those derived from mice that received Ag alone (Fig. 6B). Splenocytes from mice that received only Gal-1 or Gal-8 recombinant proteins (without Ag) responded similarly to those from mice that received PBS (data not shown). Remarkably, when larger doses of Ag were used to immunize animals, Gal-induced costimulation was no longer evident (data not shown), stressing its ability to costimulate otherwise borderline responses. These results correlate with Gal-1 and Gal-8 costimulatory properties observed in vitro (Fig. 1A) and strongly suggest that both Gal-1 and Gal-8 have the potential to costimulate T cell specific-responses in vivo.
FIGURE 6. Gal-1 and Gal-8 increased Ag-specific response in vivo. (A) Determination of Ag dose for immunization. DO11.10 mice were immunized i.p. with the indicated amounts of OVA whole protein dissolved in PBS. After 5 d, spleens were removed, and splenocytes were restimulated in vitro with two concentrations of cognate OVA peptide (0.1 and 0.25 μg/ml) for 48 h. Stimulation was assessed by [3H]thymidine incorporation. Doses of 0.5 μg/ml of OVA whole protein and 0.25 μg/ml of OVA cognate peptide were chosen for further immunization and restimulation, respectively. (B) Mice were immunized as in (A): PBS (n = 4), OVA (n = 6), OVA + Gal-8 (n = 5), and OVA + Gal-1 (n = 6). *p < 0.02, versus OVA, **p < 0.001, versus OVA.

its participation in Gal-1–induced apoptosis remains controversial. Recently, Earl et al. (26) reported that Gal-1 binding to CD45 and subsequent T cell death signaling were controlled by developmentally regulated glycosylation, as well as expression of specific CD45 glycoforms. Nevertheless, how Gal-1 and Gal-8 modulate CD45 PTPase activity to induce T cell costimulation needs further investigation.

Despite the fact that CD4 T cells were identified as target cells of the costimulatory effect, Gal-1 and Gal-8 have to bind APCs and CD4 T cells simultaneously to completely achieve TCR activation in the presence of Ag (Fig. 3). Possibly, binding of Gal-8 and Gal-1 stabilizes the immune synapses during Ag presentation, thus strengthening TCR-downstream signaling, which results in an increased cell-activation response, particularly when Ag signals are weak.

The fact that Gal-3 affected the Ag-specific T cell response agrees with previous reports demonstrating that the lattice built by extracellular Gal-3 with surface glycoproteins compartmentalizes TCR complexes in such a way that inhibits downstream-signaling activation (24, 25). Apoptosis was discounted as a mechanism responsible for the antagonistic effects of Gal-3, because this Gal was shown to induce apoptosis of activated, but not resting, T cells (data not shown). In agreement, Gal-3 failed to induce phosphatidyserine exposure in resting T cells, indicating that T cell activation is required to sensitize cells to Gal-3–induced apoptosis (21). Although the negative effect of Gal-3 on TCR activation was absent in the presence of a higher dose of Ag, Gal-3 was still able to inhibit Gal-1– and Gal-8–induced costimulation only when they were added simultaneously. Given that it was previously shown that Gal-3 partially inhibits Gal-1 binding to the T cell surface (9), we reasoned that Gal-3 might displace Gal-1 or Gal-8 more efficiently when present simultaneously. However, we observed that Gal-3 partially reduced Gal-8 binding on T cells independently of the addition order, not fully explaining our findings. Alternatively, differences in the quaternary structures and cross-linking activities might explain the inhibitory effects of Gal-3 on the activities of Gal-1 and Gal-8. In this regard, the observation that exogenously added Gal-3 antagonizes the apoptotic effects of Gal-1 in susceptible T cells was previously related to a greater avidity of the Gal-3 pentamer compared with the Gal-1 homodimer for glycoprotein receptors on the cell surface (27).

The fact that 100-fold more Gal-1 is required to equal Gal-8 costimulatory activity could be explained by the observation that tandem-repeat Gals, which have two CRDs stably fused by a linker peptide, are more potent effectors than monomeric Gals. In fact, it has been demonstrated that two covalently linked Gal-1 CRDs induce apoptosis more efficiently than the monomeric form (28–30). However, chimeric Gal-1-8-1 did not induce greater costimulation than native Gal-1, indicating that structure is not the only important factor; rather, a fine distinction among CRDs’ specificity may account for the differences observed.

We previously established several molecular requirements for Gal-8 to induce its costimulation and T cell-proliferation properties: although the tandem-repeat structure is essential for Ag-independent proliferation, both single N-CRDs and C-CRDs are able to induce Ag-specific costimulation (14). We also determined that Gal-8’s single N-CRD is sufficient to trigger platelet activation (31). In agreement, in this study we showed that Gal-1 costimulated T cells in the presence of the cognate peptide, but it was unable to trigger proliferation of naive splenocytes, even when tested at a high concentration (20 μM). Notably, Gal-1 stable dimerization stabilized with Gal-8 peptide linker (Gal-1-8-1) overcame its inability to trigger proliferation in the absence of Ag, although 10-fold more Gal-1-8-1 was still needed to equal Gal-8 activity. Again, differences in potency could be related to distinct CRD specificity among these Gals, because we observed that proliferating splenocytes displayed an adhesive and spread phenotype in the presence of Gal-8, whereas cells were only agglutinated in the presence of Gal-1-8-1.

In the present work, Gal-1–stimulating activity on resting T cells is supported by demonstration of Gal-1–induced costimulation and chimeric Gal-1-8-1–induced proliferation. This constitutes a novel role for this Gal, because the majority of previous reports focused on its proapoptotic role on immature or activated, but not naive, T cells. In agreement with our results, Perillo et al. (6) demonstrated in an early report that Gal-1 induces apoptosis on PHA-activated PBMCs, but it is unable to exert the same effect on naive peripheral human T cells. In addition, it was shown that, although Gal-1 induces apoptosis of 2-d Con A-activated T cells, it promotes the survival of resting T cells without inducing proliferation (32). In contrast to our observations and those from other investigators, Matarese et al. (33) observed that Gal-1 sensitized human resting T lymphocytes to Fas-mediated cell death and that, at high doses, it was capable of inducing apoptosis in these primary cells. Discrepancies might reflect the different species used (human versus mouse), different protein preparations, or the method used to assess cell death, because phosphati-
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The authors have no financial conflicts of interest.

Disclosures

The authors have no financial conflicts of interest.

References


