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A phenotype survey of 36 mutant mouse strains with gene-targeted defects in glycosyltransferases or glycan-binding proteins

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The consortium for functional glycomics (CFG) was a large research initiative providing networking and resources for investigators studying the role of glycans and glycan-binding proteins in health and disease. Starting in 2001, six scientific cores were established to generate data, materials and new technologies. By the end of funding in 2011, the mouse phenotype core (MPC) submitted data to a website from the phenotype screen of 36 mutant mouse strains deficient in a gene for either a glycan-binding protein (GBP) or glycosyltransferase (GT). Each mutant strain was allotted three months for analysis and screened by standard phenotype assays used in the fields of immunology, histology, hematology, coagulation, serum chemistry, metabolism and behavior. Twenty of the deficient mouse strains had been studied in other laboratories, and additional tests were performed on these strains to confirm previous observations and discover new data. The CFG constructed 16 new homozygous mutant mouse strains and completed the initial phenotype screen of the majority of these new mutant strains. In total, >300 phenotype changes were observed, but considering the over 100 assays performed on each strain, most of the phenotypes were unchanged. Phenotype differences include abnormal testis morphology in GlcNAcT9- and Siglec-H-deficient mice and lethality in *Pomgnt1*-deficient mice. The numerous altered phenotypes discovered, along with the consideration of the significant findings of normality, will provide a platform for future characterization to understand the important roles of glycans and GBPs in the mechanisms of health and disease.

Keywords: glycan-binding protein / glycosyltransferase / homozygous mutant mice / knock-out mice / mouse phenotype

Introduction

Complex carbohydrate structures on proteins vary according to cellular development, tissue specificity, senescence and cellular activation (Taylor and Drickamer 2006). These carbohydrates play critical functional roles in host–pathogen interactions, progression of malignancies and embryologic development (Ohtsubo and Marth 2006; Varki et al. 2009). The study of carbohydrate structures and carbohydrate-binding proteins has been technically challenging because of the complexity of bound carbohydrate-ligand structures and the difficulties encountered in their analytical characterization. Reverse genetic studies, employing the construction of mutant mice lacking a specific gene of interest, have proved instrumental in the determination of the gene's biological function (s). Previous studies, using transgenic or "knock-out" mouse strains have elucidated the function of many genes in the glycomics field (Lowe and Marth 2003).

The CFG was a large-scale, 10-year research initiative funded in 2001 by the National Institute of General Medical Sciences (NIGMS). The CFG's goal was to provide resources and services to investigators studying the interactions between glycan-binding proteins (GBPs) and their ligands in cell-to-cell communication. The CFG established six scientific cores whose aims were to develop novel resources and reagents for use by participating investigators (PIs) within both the glycoscience and general scientific community. Since these six cores were not recharge facilities, they differed from core facilities in research centers or those supported by program project grants. Instead, the CFG's cores functioned as collaborating scientific groups supplying novel reagents and services. The CFG's mandate also included immediate access to all of the experimental data on a website (Raman et al. 2006). Currently several of the CFG's cores have remained in operation using alternate funding.

Two of the CFG's-scientific cores were dedicated either to the construction or analysis of mutant mouse strains. These

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strains were homozygous mutant mice lacking a single, specific gene for a GBP (ligand) or glycosyltransferase (glycan structures). The mouse-transgenic core (MTC) was responsible for the construction of novel mutant mice and, by the end of the grant funding, this core had generated 29 total and conditional mutant mouse lines. The construction schemes for these strains, including maps and rationale, can be found on the CFG's website. The mutant mice tested by the MPC, along with conditional strains and double knock-out strains, are currently available for distribution by the Mutant Mouse Regional Resource Center (MMRRC).

The CFG's second mouse core, the MPC, provided the initial phenotype screen of these genetically altered mice to serve as a stimulus for further research. The assay order for phenotype evaluation was designed similar to the human clinical situation. Blood tests to detect early organ abnormalities began the phenotype survey and histopathology completed the analysis. Over 100 tests common in the fields of hematology, coagulation, serum chemistry, immunology, metabolism and behavior were used to examine the static and functional (induced) differences between mutant and wild-type mice. The MPC completed the phenotype survey for each mutant mouse strain in approximately three months. Once completed, the experimental data and summary of significant differences were uploaded onto the CFG's website and made available to the scientific community within six weeks. All the experimental data and summaries of significant phenotypes are currently accessible to the general public on the website.

During the first years of the MPC's operation, participating investigators contributed 20 mutant strains for additional phenotype analysis. In many cases the MPC helped confirm and/or extend their original findings. Starting in 2006, the first of the CFG's newly constructed mutant stains became available for phenotype testing, and eventually sixteen of these new strains were surveyed for phenotype differences.

This report highlights only some of the phenotype differences observed in these mutant mouse strains during the 10 years of the MPC's operation. Over 300 phenotype changes, some unexpected and some corroborative, were uploaded and preserved in a public database. In addition, all the highly significant results that showed no phenotypic differences observed between mutant and wild-type strains have been uploaded. We hope the information provided here will stimulate additional efforts designed to elucidate the critical role(s) of cell-surface carbohydrate–protein interactions in cell-to-cell communication.

Results

This manuscript presents many of the abnormal phenotypes observed from the ten-year survey of 36 mutant strains of mice. These data are intended as a tool to lead interested investigators to peruse the website for additional data, and lead to further investigation of the abnormalities noted in the specific strains.

The order of mouse strains chosen for analysis was determined by the CFG's steering commitee with input from participating investigators (PIs). The mice tested were 7–16 weeks old, and four strains had additional tests performed after 12 months of age. In addition, one strain was tested again after a more congenic strain became available. The C57BL/6 strain was characterized at the beginning of the phenotype survey as a reference. All the mutant mice (in the 129 background) were compared with littermate controls during testing. In total, the MPC determined the phenotype profile of forty specific mouse strains.

The MPC's unique phenotype screen consisted of over 100 assays performed by four individual sub-cores with specific expertize (Table I). These sub-cores were termed: The immunology core, the hematology core (hematology/chemistry/coagulation), the histology core and the behavior core (metabolism/behavior). These cores performed experiments that included both static assays, such as the measurement of serum chemistry values, and assays used to test induced responses, such as lymphocyte proliferation experiments.

During the first years of funding, while new mutant strains were being constructed by the CFG, 20 preexisting mutant mouse strains were donated for testing by investigators in the glycobiology community. The goal was to ascertain whether additional phenotype changes could be identified, using the CFG's unique assays not readily available to individual investigators. In addition to corroborating several earlier findings, approximately ~200 phenotype differences were observed between homozygous mutant and wild-type littermates in the preexisting strains (Table II).

In 2001, the MTC began the construction of new homozygous and conditional mutant strains. The CFG's PIs selected which genes that would be targeted for mutation and phenotype characterization. The rationale behind the choice of which glycogene should be a target for deletion, as well as the strain construction details, can be found on the CFG's website. The majority of the new strains were constructed using C57BL/6 parental ES cells (Bruce4), except for the ST6GalNAc-II- and GlcNAcT9-deficient strains (129 background). The resulting C57BL/6 background eliminated the extended time necessary to backcross the new strains before the phenotype screen. After the MTC closed nine years later, it had constructed 29 total and conditional mutant mouse lines deficient in GBPs or glycosyltransferases.

In 2006, the first homozygous mutant strain constructed by the MTC became available for phenotype analysis. In total 16 of the new mutant strains were subjected to the phenotype survey, and over 100 phenotypic differences were observed between mutant and wild-type mice (Table III).

Because the MPC's phenotype assays had been planned as a general screen, there are many important caveats to consider when reviewing the data. For example, not all sub-cores analyzed animals of the same age or genders. Since the mandate from the CFG was to perform a rapid phenotype screen and upload data within six weeks after experimentation, many individual experiments that required a large number of mice were not repeated three times. Also in the preexisting mutant strains not constructed by the CFG, a few strains contained selectable markers, so the products of these markers may contribute to the observed phenotype. Another consideration was that no confirmation of the lack of protein product for many of the mutant strains phenotyped was performed.

Table I. List of assays used for mutant mice phenotype survey by the MPC

Hematology	Histology	Behavior	Immunology
WBCs	Brain	Weight	Delayed-type hypersensitivity (DTH)
Neutrophils	Heart & blood	Body temperature	T-dependant response
Lymphocytes	Vessels	Physical features	T-independent response
Monocytes	Lungs	3D activity levels	Inflammation
Eosinophils	Thyroid	Food intake	Phagocytosis
Basophils	Adrenal glands	Water intake	Opsonization
Red blood cells (RBCs)	Lymph nodes	Oxygen consumption	Serum immunoglobulin
Hemoglobin (HGB)	Spleen	Carbon dioxide production	T cell proliferation:
Hematocrit (HCT)	Thymus	Circadian rhythm	Anti-CD3
Mean corpuscular	Bone marrow	Heart rate	ConA
Volume (MCV)	Salivary glands	Respiration rate	B cell proliferation:
Mean corpuscular	Liver	Blood pressure	LPS
Hemoglobin (MCH)	Pancreas	Pulmonary function	Anti-IgM
MCH concentration	Esophagus	Fear conditioning	Tissues for flow Cytometry & apoptosis:
Red cell distribution	Stomach	Neurological screen	Spleen
Width (RDW)	Duodenum	Eyeblink	Thymus
Platelet count (PLT)	Ileum	Tail suspension	Lymph node
Mean platelet volume	Colon	Rotorod	Blood
(MPV)	Ovaries	Balance beam	Bone marrow
Prothrombin time (PT)	Testes	Wire-hang	Peyer's patch
Partial thromboplastin	Prostate	Grip strength	Antibodies used:
Time (APTT)	Uteri	Pole test	CD3, CD4, CD5, CD8, CD11b, CD19, CD20, CD22,
Antithrombin	Skin	Open field	CD24, CD25, CD40, CD41, CD43, CD44, CD45R,
Protein C	Bone & joints	Latency to move	CD62L, CD86, CD117, CD138, αIgM, αIgD, Sca-1,
Protein S	Skeletal muscle	Tail flick	Ter119, Gr-1, NK1.1, αI-A^b, αV_βTCR, αTCRγ/δ
Plasminogen	Kidneys	Hot plate test	*As needed*: ANA, Collagen-induced arthritis (CIA)
Alpha-2-antiplasmin	Bladder	Hearing	
Bleeding time	Breast	Prepulse inhibition of startle (PPI)	
Glucose	Adipose	Threshold to startle response (SR)	
Urea nitrogen	*As needed*:	Social dominance	
Creatinine	Luxol fast blue	Approaching object	
Bicarbonate	Alcian blue (two pH levels)	Passive avoidance	
Chloride & sodium	Periodic acid schiff (PAS)		
Potassium & calcium	Lectin histochemistry		
Bilirubin, direct & total	Immunohistochemistry		
Albumin & total protein			
Phosphorus			
AST (SGOT) & ALT (SGPT)			
Alkaline phosphatase			
As needed: Lipid panel			

Details of above protocols at: https://www.functionalglycomics.org/glycomics/publicdata/phenotyping.jsp.

Other caveats to consider include the general genetic background (epitasis) or the chance that unlinked loci might alter the effects of the mutation. Moreover, the vivarium environment, including the pathogen load, enriched environment, diet, exercise, number of animals per cage, gender and age when tested, can also influence the observed phenotypes. Therefore, with these and other caveats in mind, caution should be exercised in attributing any phenotypic effect solely to the loss of the allele.

The data summarized below give brief descriptions of the significant phenotype differences observed in mice deficient in carbohydrate-binding proteins or certain carbohydrate structures. Owing to the large number of altered phenotypes discovered by the screen, only a limited number of the interesting phenotypes are presented below, categorized by gene family. Before any conclusions are made regarding function, investigators are strongly encouraged to consider all of the strain-specific data available on the CFG's website before additional experimentation.

Glycosyltransferases

The largest group of mutant strains tested by the MPC included mice lacking one of the many glycosyltransferase genes. The previously characterized glycosyltransferase-deficient strains subjected to further testing by the MPC include C2 GlcNAcT, FUT1, FUT2, FucT-IV, FucT-VII, Galgt-1, Galgt-2, Mgat3, Mgat5, ST3Gal-1, ST3Gal-II, ST6Gal-1 and ST8Sia-1. A brief description of findings from the CFG's newly constructed glycosyltransferase-deficient strains include GalNAcT3, GlcNAct9 (129 background), manic fringe, *Pomgnt1* (limited screen) and ST6GalNAc-II (129 background). The B3gnt5 mutant strain, although constructed by the CFG's MTC, was not screened because of lack of available experimental mice before the MPC closed.

GalNAcT3 (Galnt3). The *Galnt3* gene for UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase-T3 was first cloned in 1996 (Zara et al. 1996). The ppGaNTase-T3 transcript is abundant in the major salivary glands, gastrointestinal tract and

Table II. Phenotypes observed in 20 previously characterized mutant mouse strains

Mutant mice	Phenotype screen results[a]
C6ST-1 (*Chst3*)	[b]*Imm*: Reduced lymph node cell number, elevated T-cell proliferation, increased frequency of CD5 cells in bone marrow. [c]*MB*: Less aggressive in social dominance, hearing impaired PPI *Heme*: Decreases in neutrophils *Hist*: Delayed maturation ovarian follicles
Core2-GlcNAcT (*Gcnt1*)	*Imm*: Reduced frequency of B220 expression, inflammation reduced at 6 h&48 htimepoints *Heme*: Increased neutrophils, monocytes *Hist:* B220 cells in spleen not detected with immunostain.
Fut1 (*Fut1*)	*Imm*: Changes in Serum IgM, B-cell proliferation, T-cell proliferation, CD117 (c-kit), CD11b (Mac-1), lymph node cell numbers *MB*: Hypoactive with low initiation of movement. Changes in open field, tail flick, fear conditioning *Heme*: Increase in Protein S, α-2-antiplasmin
Fut2 (*Fut2*)	*Imm:* Changes in Ter119, IgG_1 *MB*: Conditioned fear context, conditioned fear cue (combined) *Heme*: Low RBC, antithrombin, plasminogen, urea nitrogen, calcium, total protein *Hist:* Spleens with extramedullary hematopoeisis
FucT-IV (*Fut4*)	*Imm*: Increased acute inflammation, serum IgM *MB*: Changes in O_2 consumption, CO_2 production, rotorod, cage top hang *Heme*: Small decreases in lymphocytes
FucT-VII (*Fut7*)	*Imm*: Reduced inflammatory $Gr1^+$ cells, reduced DTH, increased serum IgG_1& IgG_{2b}, neutrophilia, T-cells, lymph node cell number *MB*: Reduced startle response, vertical activity *HC*: Large increases in all WBC components *Hist*: Increased neutrophils in lymph nodes, kidneys and livers
Galectin-1 (*Lgals1*)	*Imm*: Changes in Sca-1, T-independent response, peritonitis, B-cell proliferation, T-cell proliferation *MB*: Low social dominance, initiation of movement, anxiety *Heme:* Decreased blood urea nitrogen
Galectin-3 (*Lgals3*)	*Imm*: Increased Inflammation *MB*: Conditioned fear, approaching object, social dominance *Heme*: Decreased Glucose
Galgt-1 (*B4galnt1*)	*Imm*: Decrease in T-dependent response, low static IgG2a *Heme*: Small increase in neutrophils, decrease in monocytes. *Hist*: Prominent germinal centers in lymph nodes
Galgt-2 (*B4galnt2*)	*Imm*: Increase in Gr-1, CD138, decrease in CD3, CD62L, $CD22^+$ B cells *MB*: Higher startle response, increased diastolic blood pressure *Heme*: Increase in neutrophils, alkaline phosphatase
Mgat-3	*Imm*: Small but significant increase in B-220 positive cells in blood and a decrease in the bone marrow. *MB*: Decrease in vertical activity
Mgat-5 (mixed 129 × C57BL/6) (*Mgat5*)	*MB*: Lower motor activity, lower latency to fall in rotorod test, higher prepulse inhibition, more sensitive to hotplate *Heme*: Small increase in monocytes & neutrophils, decreased Protein C *Hist*: Loss of LPHA lectin staining.
Mgat-5 (*Mgat5*) (C57BL/6)	*Imm*: T-cell proliferation, serum IgM, CD11b, Gr-1, CD41 *MB*: Impaired rotorod, abnormal wire hang, deficit in startle response, lower weight *Heme*: Increased neutrophils, lymphocytes, monocytes, eosinophils, AST, ALT, alkaline phosphatase. Decreased RBC, HGB, HCT, MCV, MPV, APTT, antithrombin, protein C, protein S, plasminogen, glucose, urea nitrogen, bicarbonate, calcium, albumin, total protein, *Hist*: Prominent germinal centers in lymph nodes and in spleen
mMGL (*Mgl1*)	*MB*: Social dominance, decreased startle response *Hist*: Smaller lymphoid follicles in spleen, large lymph nodes, liver granulomas
PSGL-1 (*Selpgl*)	*Imm*: Reduced inflammatory peritoneal $Gr1^+$ cells, increased serum IgG_1, IgG_{2b}, and IgG_3, decreased T-dependent response, neutrophilia, blood T-cells reduced
(40% agouti)	*MB*: Elevated social dominance, hearing impaired, less active. *Heme*: Increase numbers of neutrophils, eosinophils *Hist*: Small splenic lymphoid follicles,
Siglec-4a (*Mag*)	*Imm*: Increased B-cell proliferation, IgM, B220, CD19 *MB*: Reduced Social dominance, startle response, PPI *Heme*: Slight increase in AST
ST3Gal-I (*St3gal1*)	*Imm*: Loss of $CD8^+$ cells in spleen, decrease in CD3 & V_βTCR, decreased proliferation, apoptosis, increased CD40, CD44, CD22, IgM, IgD, B-cell proliferation, Ter119 *MB*: Lower startle response, blood pressure *Heme*: Decreased platelets, higher PT, APTT, bleeding time, decreased factor V, increased alkaline phosphatase *Hist*: Poorly defined lymphoid follicles in spleen, lymph nodes
ST3Gal-II (*St3gal2*)	*Imm*: Lower T-cell proliferation, higher frequencies of T-cell populations in spleen *Heme*: slight increase in bleeding time, slight decrease in antiplasmin concentration *Hist*: 10 of 15 ST6GalNAc-II homozygous mutant animals had prominent germinal centers compared with 6 of 16 wild-type animals.
ST3Gal-IV (*St3gal4*)	*Imm*: lower antibody response, delayed DTH, lower frequencies of V_βTCR T cells, lower mature B cells, $CD22^+$ cells, higher serum IgM levels *MB*: Reduced startle response *Heme*: Decreased platelets, Factor VIII, vWF, Increased platelet volume, bleeding time, alkaline phosphatase *Hist*: Increased megakarocytes in spleen and bone marrow. Follicular hyperplasia in lymph nodes, extramedullary hematopoiesis in liver.

Continued

Table II. (Continued)

Mutant mice	Phenotype screen results[a]
ST6Gal-1 (*St6gal1*)	*Imm*: Reduction in serum IgA and IgM, decreased T-cell proliferation, increase in total thymic cell numbers, decreased Gr-1, decreased TCR and CD8 markers in lymph node and spleen *MB*: Decreased social dominance *Heme*: Decreased neutrophils, eosinophils *Hist*: Smaller lymphoid follicles in spleen and lymph nodes
ST8Sia-1 (*St8sia1*)	*MB*: Muscle weakness with impaired wire hang, impaired hearing, and impaired social dominance *Heme*: Decreased antiplasmin *Hist*: Smaller follicles in spleen, prominent germinal centers in lymph nodes

[a]The following represent specific antibodies: CD3, CD5, CD8, CD11b, CD19, CD22, CD40, CD41, CD44, B220 (CD45R), CD62L, CD117, CD138, Ter119, Gr-1, V_{β}TCR, TCRγ/δ.
[b]Imm: Immunology Core; MB: Metabolism and Behavior Core; Heme: Hematology Core; Hist: Histology Core.
[c]Behavior analyses were not completed for all strains.

both the male and female reproductive systems. Additional studies have shown that ablation of the *Galnt3* gene leads to low concentration of circulating intact fibroblast growth factor-23 and hyperphosphatemia (Ichikawa et al. 2009). Phenotype results from the hematology core revealed that GalNAcT3 homozygous mutant mice exhibited decreased levels of monocytes and platelets. This might have an influence on the ability to elicit a chronic inflammatory reaction, and may also lead to defects in coagulation.

GlcNAcT9 (Mgat5b). The gene for mannoside acetylglucosaminyltransferase 5, isoenzyme B encodes a beta-1, 6-*N*-acetylglucosaminyltransferase that functions in the synthesis of complex cell surface glycans and is specifically expressed in the brain and testis (Inamori et al. 2003). Interestingly, the histology core discovered that four out of five GlcNAcT9-deficient mice showed degenerating cells and atrophic changes with cystic areas in the testis, while none of these changes was observed in wild-type males. In addition, the kidneys of five out of six male GlcNAcT9-deficient animals had many cortical tubules with clear cytoplasm, while only one out of six wild-type animal showed a similar result. The immunology analysis showed differences in B-cell bone marrow populations in GlcNAcT9-deficient mice (Table III). Additional characterization of older animals may reveal a role for GlcNAcT9 in immunologic responses, defects in reproduction due to the abnormal testicular maturation, an inability to concentrate urine or altered sodium/potassium ratios due to renal tubular abnormalities.

Manic fringe (Drosophila homolog, O-fucosylpeptide 3-beta-N-*acetylglucosaminyltransferase)*. Previous work has shown that lunatic fringe (*Lfng*) and manic fringe (*Mfng*) cooperatively promoted marginal zone (MZ) B cell development and MZ B cell generation (Tan et al. 2009). It has also been shown that *Mfng* is not required for embryonic development (Moran et al. 2009) or murine pancreas development and function (Svensson et al. 2009).

Analysis by the MPC's immunology core indicated *Mfng*-deficient mice exhibited altered B cell and macrophage responses compared with wild-type mice. Since this observation was the result of a phenotype screen, the full delineation of MZ B-cell populations by other markers was not performed. However, in the acute inflammation assay, no significant differences between wild-type and *Mfng*-deficient mice were observed at time zero in the population of IgM^+, $CD5^+$ CD45R $(B220)^+$ cells. Additional phenotypes observed in this strain include an increased proliferative response of purified B cells following stimulation with anti-IgM antibody, and in the acute inflammation assay, fewer macrophages in the peritoneum 48 h following thioglycollate injection (Figure 1). There was also a decrease in the frequency of mature B cells in the bone marrow, an increase in the frequency of B cells ($B220^+$) cells in the blood, a higher frequency of $CD138^+$ plasma cells in the lymph node and higher amounts of the IgG_1 antibody isotype in serum. In the T-dependent antibody response to KLH, lower IgG_1 and IgG_3 isotype antibodies were noted (data not shown). These findings suggest a hyperproliferative early B-cell response and a defect in macrophage accumulation in the peritoneal cavity in response to antigen. The hematology core observed lower numbers of neutrophils, and increased numbers of lymphocytes, which correlates with the increased frequency of B cells in the blood observed by the immunology core. Also increased activity levels of the coagulation factors II, VII, IX and XI suggest abnormalities in the coagulation cascade.

Pomgnt1. The enzyme O-mannose beta-1, 2-*N*-acetylglucosaminyltransferase participates in O-mannosyl glycan synthesis and has been shown to function in diverse roles including reactive gliosis in the retina, neuroblastoma adhesion (Abbott et al. 2006), muscle-eye-brain disease (Manya et al. 2003, 2004) and the proliferation of myoblasts (Miyagoe-Suzuki et al. 2009). Two previously constructed homozygous mutant *Pomgnt1*-deficient strains have been constructed using parental 129 ES cells, and one of these published strains exhibited a 60% mortality rate prior to weaning (Miyagoe-Suzuki et al. 2009). The CFG's MTC constructed a mutant *Pomgnt1*-deficient strain using C57BL/6 Bruce4 parental ES cells. This Pomgnt1 mutant strain exhibited greater lethality than the other strains engineered in the 129 parental background. Of the 165 mice born to heterozygous breeders, only one animal was a homozygous Pomgnt1 mutant mouse, and this animal died before weaning. This increased mortality soon after birth should be subject to further investigation.

Table III. MPC phenotype survey of 16 novel strains constructed by the CFG

Mutant mice[a]	Phenotype screen results[b]
DCIR (*Clec4a2*)	*Imm*: Elevated IgG isotype antibodies (IgG_{2a}, IgG_{2b}, IgG_3) when stimulated with a T-dependent type-II antigen. Increased frequency of $CD22^+$ cells in the bone marrow, and higher frequency of CD86 (B7.2) positive cells in the lymph node. *Hist*: The lymph nodes were slightly larger. In an older cohort of mice, evidence of increased inflammation in salivary glands and degenerated tubules in the testes
DC-Sign (*Cd209a*)	*Imm*: Decrease in the frequency of mature B-cells ($B220^{hi}$, HSA^{lo}) in the bone marrow and higher frequency of plasma cells ($CD138^+$). In aged DC-Sign mutant mice (>6 mos) was observed a decrease in serum concentration of IgG2b and hyperproliferative T cells. *Heme*: Increased lymphocytes and monocytes, decreased blood urea nitrogen. *Hist*: Enlarged Germinal centers in the B-cell zones of the spleen. Older mutant mice exhibited a slight increased incidence of inflammation in the organs.
Galectin-2 (*Lgals2*)	*Imm*: Lower level of apoptosis was observed in the lymph nodes. *Heme*: Decrease in neutrophils, increase in lymphocytes. *Hist*: increased red pulp areas in spleen.
Galectin-9 (*Lgals9*)	*Imm*: Elevated antibody production and hyperproliferative B cells, greater cell numbers in lymph node, spleen, thymus and Peyer's patches. *Heme*: Increased lymphocytes and neutrophils. *Hist*: Prominent germinal centers in spleen and lymph nodes.
GalNAcT3 (*Galnt3*)	*Hem*: Decreased monocytes, decreased platelet count, increased bilirubin
GlcNAcT9 (*Mgat5b*)	*Imm*: Higher frequency of IgD+ single positive cells (and IgM+IgD+ cells) in the bone marrow; elevated static levels of the IgG2b antibody isotype; elevated antibody responses in the T-dependent antibody production assay; lower frequencies of Pre-B cells in bone marrow. *Heme*: Slight increase in glucose *Hist*: Degenerated testis tubules, vacuolations in the kidney tubule epithelial cells.
Langerin (*Cd207*)	*Imm*: Decrease in the static serum concentrations of IgA and IgG_1isotypes and a decrease in specific isotypes (IgG_3, $IgG2_a$, IgM and IgA) after injection with a T-dependent antigen. *Hist*: Small foci of inflammation near the islets.
LSECtin (*Clec4g*)	*Heme*: Increased circulating lymphocytes, monocytes, RBCs, decreased platelet count.
Mfng	*Imm*: Fewer peritoneal macrophages, elevated B-cell proliferative response, decrease in mature B cells in bone marrow, increase in B-cells in blood. *Heme*: Decreased neutrophils, increased lymphocytes, decreased PT and APTT with increased Factors II, VII, IX and XI
Mcl (*Clec4d*, *Clecsf8*)	*Imm*: Elevated frequencies of the following populations; $CD19^+$, $CD22^+$ cells; $B220^{hi}$, $CD19^+$ cells; IgD SP (IgM^{lo}) cells; $B220^{hi}$, $CD24^{lo}$ cells; Impaired proliferation in T-cell and B-cells. Mcl mutant mice at the lower concentrations of stimulants tested. *Hist*: Spleen shows extramedullary hematopoiesis.
Mincle (*Clec4e*, *Clecsf9*)	*Imm*: No phenotype differences observed. *Heme*: Significant decreases in all subtypes of WBCs. *Hist*: The heart was globular and the valves showed evidence of increased amounts of extracellular matrix. The heart valves in 12 of 14 mutant mice had large accumulations, with globular endings of valve leaflets.
mSignR1 (*Cd209b*)	*Imm*: Higher percentage of macrophages in the inflamed peritoneum. T-independent antigen elevation of IgM, IgG3, $IgG2_b$. Lower frequency of mature B cells in bone marrow. *Heme*: Decreased bilirubin levels. *Hist*: Thicker mesangium in kidney glomeruli.
mSignR3 (*Cd209d*)	*Imm*: Increased proliferation, greater frequencies of IgM^+ B cells, spleen *Hist*: Increased adipocytes in bone marrow: at 12 weeks & in aged mice.
Pomgnt1 (*Pomgnt1*)	Homozygous mutant not viable. 165 animals from het x het produced one mutant live animal.
Siglec-H	*Imm:* T-cell hyperproliferation with Con-A *Heme:* Increase in blood urea nitrogen *Hist:* Vacuolated kidney tubules, degenerated seminiferous tubules, testis.
ST6GalNAc-II (*St6galnac2*)	*Imm*: Lower body weights, decreased serum IgA, elevated B-cell proliferation, elevated numbers of pre-B lymphocytes, elevated Ter-119, lower frequency of Sca-hi cells *Heme*: Increased protein S and ALT, decreased creatinine *Hist:* Prominent germinal centers in splenic lymphoid follicles

[a]All CFG homozygous mutant strains were generated from Bruce4 ES cells (C57BL/6) except for ST6GalNAc-II and GlcNAcT9 (129).
[b]Specific antibodies used: CD19, CD22, CD24, B220 (CD45R), CD86, CD138, αIgM, αIgD, Ter119.

ST6GalNAc-II (St6galnac2). This enzyme transfers sialic acid onto underlying T and Tn structures and Sialyl-Tn structures are abnormally abundant on certain malignancies (Marcos and Pinho 2004; Takashima 2008). Analysis by the Immunology Core showed that ST6GalNAc-II-deficient male mice had significantly lower body weights. In response to LPS stimulation, purified splenic B cells demonstrated hyper-proliferation (Figure 2A). In the T-independent assay, ST6GalNAc-II homozygous deficient mice showed a significant decrease in serum IgA levels produced at day 14 ($P = 0.04$). In the T-dependent response to KLH, a higher level of the IgG_3 isotype was observed at day 14 ($P = 0.005$). Flow cytometry analysis revealed that ST6GalNAc-II-deficient mice had elevated Pre B-cells and lower frequencies of $CD5^+$ cells in the bone marrow ($CD5^+$, $B220^-$) and higher levels of $CD62L^+$ cells in the spleen. All flow cytometry data are available on the CFG's website. Further studies should determine whether there is a correlation between the lower body weights observed in the male mice with the altered T-dependent and T-independent responses.

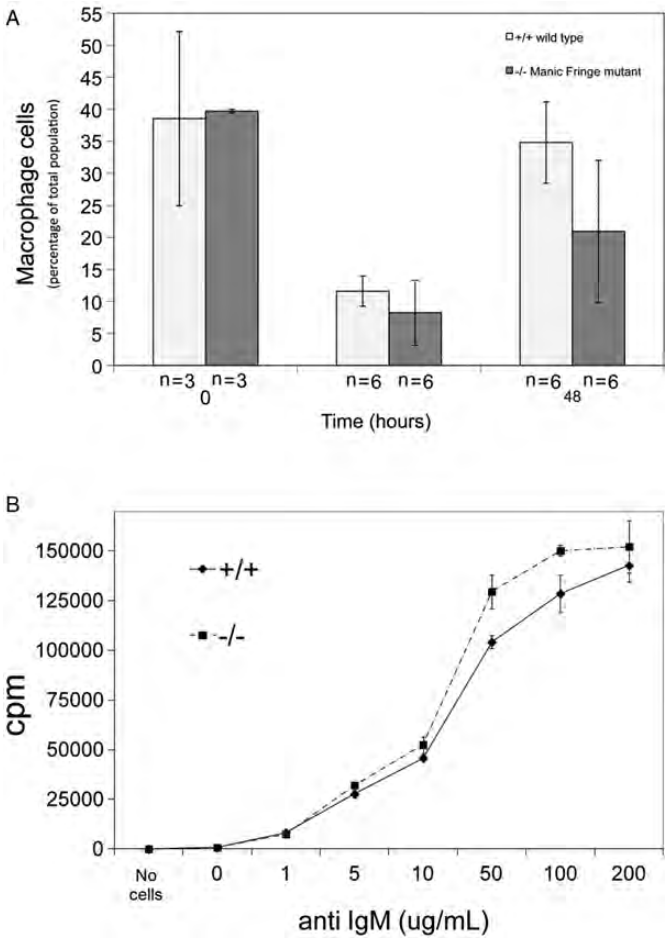

Fig. 1. Immunological phenotype of *Mfng*-deficient mice. (**A**) Macrophage populations in peritoneal exudates 48 h following thioglycollate injection. Macrophages are defined as Gr-1^{med}, CD11b^{hi}and F4/80^{hi}. Similar results were obtained in two experiments ($P = 0.032$, $P = 0.042$). (**B**) Anti-IgM stimulation of purified splenic B cells from *Mfng* mutant and wild-type mice.

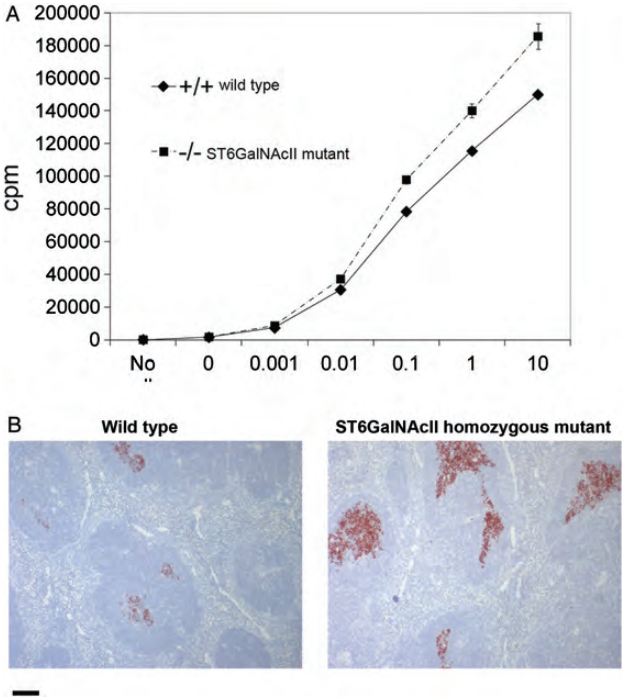

Fig. 2. B cell phenotypes observed in ST6GalNAc-II (*St6galnac2*)-deficient mice. (**A**) Purified B cell proliferation using the stimulant LPS. (**B**) Splenic germinal centers of B cell zones in lymphoid follicles of ST6GalNAc-II-deficient animals and wild-type animals as shown by PNA histochemistry. Increased PNA binding observed in spleen from mutant animals as shown on the right side of the panel (wild-type on the left).

The hematology core found ST6GalNAc-II-deficient mice exhibited a slight decrease in creatinine concentration and a slight increase in ALT. Previous studies have described an association between ST6GalNAc-II and kidney disease (Ding et al. 2009). These results, along with the observed lower amounts of IgA found by the immunology core, suggest further analysis for liver and kidney disease be performed on larger cohorts of mice and at different ages.

The histology core analysis of ST6GalNAc-II-deficient mice showed hyper-reactivity of the splenic follicles as confirmed by PNA lectin histochemistry (Figure 2B). It is not unexpected in wild-type mice to observe many areas of PNA binding within germinal centers of splenic follicles, but there was a remarkable increase in these areas in the spleens of the mutant mice, although the mice were not immunized or challenged. Infections are always possible, even in a clean facility, and this underscores the importance of phenotype analysis by multiple laboratories. Taken together, the CFG's findings suggest an abnormal B-cell response in this cohort of mice.

C6ST1 (Chst3, carbohydrate sulfotransferase 3). This group of sulfotransferases has been shown to play a role in neural stem cell maintenance (Akita et al. 2008). Chondroitin sulfates are found in cartilage, and the extent of sulfation varies with age. Chondroitin sulfates are also important in endothelial-lymphocyte interactions and may function in the maintenance of naïve T-cells in young mice (Uchimura et al. 2002). The immunology core analysis of C6ST1-deficient mice revealed reduced cell numbers in the lymph node, elevated T-cell proliferation and an increased frequency of CD5^{+} cells in bone marrow (both B220^{+} and B220^{-} populations). These findings correlate with the earlier observation that chondroitin sulfates are important in endothelial-lymphocyte interactions, suggesting abnormalities in the recirculation of T cells via high endothelial cells in lymph node. The behavior core observed that C6ST1-deficient mice were less aggressive in the social dominance assay. The histology core observed the expected loss of C6ST1 activity in the cartilage after immunohistochemistry analysis, and an unexpected finding of delayed maturation in the ovarian follicles, suggesting that the study of additional cohorts of animals may reveal defects in fertility.

Fucosyltransferase 2. Mice with targeted deletions of each of the two alpha-1-2 fucosyltransferase loci (*Fut1* and *Fut2*) have abnormalities in the gastrointestinal and genitourinary

tracts (Domino et al. 2001; Terahara et al. 2011). However, these mice are born at normal rates and exhibit no gross physical or behavioral abnormalities. The MPC's cores found additional phenotypes in Fut-2 homozygous mutant mice, including a decrease in red blood cell (RBC) numbers, extramedullary hematopoeisis in the spleen and a lower frequency of $Ter119^+$ cells in the blood. These results suggest a defect in hematopoiesis. Also the behavior core's phenotype survey revealed possible defects in fear conditioning.

FucT-VII (Fut7). Sialylated alpha-1-3-fucosylated glycans have been implicated as essential components of the counter-receptors for E, P and L-selectins (Maly et al. 1996). Two human alpha-1-3-fucosyltransferases, termed FUT4 and FUT7 (also termed FucT-IV and FucT-VII), are conserved among higher mammals and are expressed in cell types that elaborate selectin ligands. Thus, these enzymes have been implicated in the synthesis of the alpha-1-3-linked fucose associated with selectin counter-receptors (Homeister et al. 2001). The MPC survey of the FucT-VII homozygous mutant mice found large increases in all white cell populations and abnormal neutrophil infiltrates in the lymph nodes, spleen, kidney and liver. However, in the acute inflammation assay, a reduced number of immediate (6 h) inflammatory $Gr\text{-}1^+$ neutrophils were observed after peritoneal thioglycollate injection. Also in the contact hypersensitivity assay, where ear swelling is measured following the application of oxazolone, a delayed and reduced response was observed. Other phenotypes found in FucT-VII deficent mice include a higher frequency of $Gr1^+$ cells in the blood, bone marrow and spleen. There was also an increased frequency of $CD4^+$ T cells in the lymph nodes and spleen. The behavior core observed a reduction in the startle response that was not due to a deficit in hearing. Thus, the MPC screen detected increased white cell infiltrates in tissue, but delayed responses after inflammatory challenge, suggesting defects in the leukocyte trafficking.

Mgat5. The results from the analysis of this strain are mentioned to highlight the important influence of the number of back-crossed generations upon phenotype. The mannosyl alpha-1, 6-glycoprotein beta-1,6-*N*-acetylglucosaminyltransferase V is a Golgi enzyme that mediates branched N-glycosylation. Mgat5 activity has been associated with altering the severity of multiple sclerosis (Brynedal et al. 2010). Early studies using an Mgat5-deficient strain described changes in TCR signaling and the late onset of autoimmunity (Morgan et al. 2004). Mgat5 has also been shown to be important in tumor growth and cancer metastasis, cell proliferation and differentiation (Granovsky et al. 2000; Lau et al. 2007).

The MPC characterized two separate preexisting Mgat5 mutant strains (both in the 129 background) that differed in their level of backcrosses to C57BL/6 mice. The first Mgat5 mutant strain tested had the lowest number of backcrosses and the histology core confirmed the lack of L-PHA lectin binding on the Mgat5 organs, and the hematology screen showed several abnormalities. Others have observed that fewer backcrosses may influence the observed phenotypes, a possible result of interacting loci of the two mouse strains 129 and C57/Bl6 (Heidari et al. 2006). We examined another Mgat 5-deficient strain after a backcross of 10 generations to determine the possibility of additional phenotypes. The more congenic Mgat-5-deficient strain exhibited a greater number of altered phenotypes. These animals weighed significantly less than wild-type mice and exhibited impaired rotorod, abnormal wire-hang and pole test results, suggesting decreased muscle strength possibly because these mice were smaller than their littermates. The hematology core found a moderate increase in circulating white blood cells (WBCs) and a slight anemia. This strain also exhibited multiple reductions in the level of plasma proteins made in the liver, decreases in coagulation parameters and increases in the level of liver enzymes. These phenotype results suggest abnormal parenchymal liver function (Table II), which may manifest with more pathology as the mice age.

C-type lectins

Of the C-type lectin family members, the mMGL mutant strain was previously studied (Mizuochi et al. 1997; Onami et al. 2002), and a functional comparison of the mouse DC-SIGN, SIGNR1, SIGNR3 and Langerin C-tpe lectins was already described (Takahara et al. 2004). The new strains constructed by the CFG's MTC and screened by the MPC were: DCIR, DC-Sign, Langerin, LSECtin, mSignR1, mSignR3, Mcl and Mincle.

Mincle (Clec4e, Clecsf9). The first homozygous mutant strain constructed by the CFG was of the C-type lectin, Mincle. Mincle is expressed on macrophages and has been shown to play a novel role in response to infection with *Candida albicans* (Osorio and Sousa 2011). Mincle has also been studied as an ITAM-couple activating receptor that senses damaged cells (Yamasaki et al. 2008). Interestingly, histologic analysis revealed that five of twelve Mincle-deficient mice had globular hearts with bulbous abnormalities of the atrioventricular heart valves (Figure 3), which was not observed in the wild-type controls. Although this was a small percentage of animals expressing the abnormality, examination of older animals may reveal functional abnormalities, since heart valves with structural changes may contribute to dysfunction and heart disease as the mice age. Mincle mutant mice also showed significant reductions in several WBC populations (Table III). Continued examination of additional Mincle-deficient mice may reveal an abnormal ability to produce myeloid cells, which may affect the immune response to pathogens.

DCIR (Clec4a2). Previous experiments using DCIR-deficient mice (129 background) had indicated an autoimmune phenotype in older animals (Fujikado et al. 2008). DCIR mutant mice constructed by the CFG's MTC were in the parental C57BL/6 background and were tested for a similar autoimmune phenotype. However, many of the phenotype results found in our cohort of DCIR-deficient mice differed from those found in the previously characterized DCIR-deficient mice. For example, no elevated levels of ANA autoantibodies were observed in older mice (>1 year). Also joint abnormalities were not found, and no changes were observed in the $CD11c^+$ populations in the lymph node.

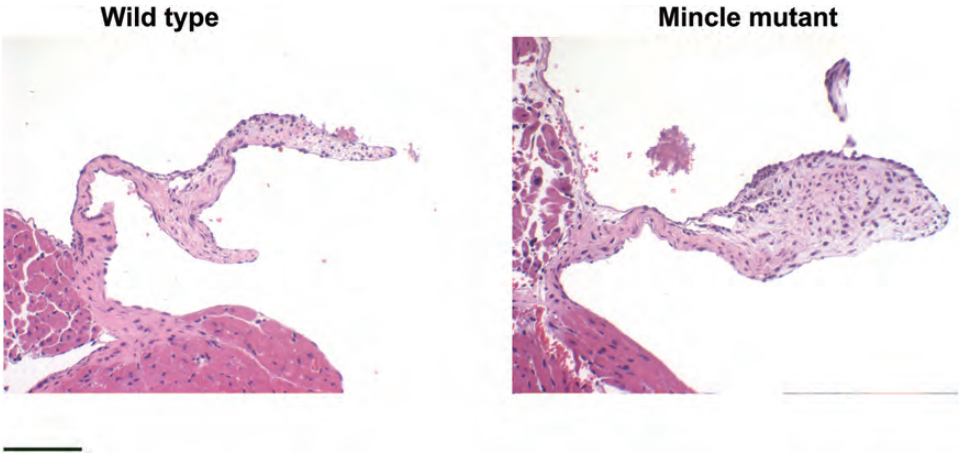

Fig. 3. Enlarged heart valves in Mincle (*Clec4e*)-deficient mice, shown on the right side of the panel, compared with wild-type mice. Scale bar = 100 µm.

These disparate results could possibly be due to the influence of interacting parental loci from either 129 or B6 background that are linked to an autoimmune phenotype, housing conditions or numerous other factors (Hickmann-Davis 2001; Barthold 2004; Heidari et al. 2006; Jelcick et al. 2011; Kiselycznyk and Holmes 2011). Other phenotypes exhibited by the CFG's DCIR mutant mice included an increase in the production of specific IgG isotype antibodies (IgG_{2a}, IgG_{2b}, IgG_3) when stimulated with a T-independent antigen, and elevated B-cell proliferation after stimulation by either anti-IgM or LPS (Table III). A screen of an older cohort of DCIR-deficient mice did not reveal additional pathology other than inflammatory foci in salivary glands, which is common in mice as they age. These results underscore the importance of the influence of strain variations on phenotype.

DC-Sign (Cd209a). The C-type lectin, DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin), mediates innate immune recognition of microbial carbohydrates and facilitates contact between dendritic cells and T cells that are important in initiating a primary immune response (Geijtenbeek et al. 2000). Distinct functions of DC-SIGN have been described in pathogen recognition and immune regulation (Koppel et al. 2005). Flow cytometry analysis revealed an increase in the frequency of mature B-cells in the bone marrow, and a higher frequency of plasma cells in the blood. In an aged cohort of animals, there was a decrease in the serum concentration of IgG_{2b} antibodies and hyper-proliferative T cells. The mutant mice also manifested lymphocytosis, monocytosis and evidence of inflammation. Histology studies revealed enlarged germinal centers in the B-cell zone of splenic lymphoid follicles, which correlates with the immunology core's findings. Clearly DC-SIGN is important in the immune response, and these mice merit additional studies.

Langerin (Cd207). The C-type lectin, Langerin, has been previously shown to function in antigen processing and presentation. Since Langerin is expressed in antigen presenting cells (Chang and Kweon 2010), it has been suggested that Langerin-deficient mice might be defective in contact hypersensitivity reactions (Romani et al. 2010). In the delayed-type hypersensitivity assay (DTH), a slight decrease in ear swelling was observed at 72 h, but no significant reduction was observed (data not shown). Also this cohort of Langerin-deficient mice had evidenced decreased antibody production. First, we observed a decrease in the static serum concentrations of IgA and IgG_1 antibody isotypes. Then in the T-dependent response to the antigen KLH, Langerin-deficient mice exhibited a decrease in the specific antibody isotypes IgG_3 and IgG_{2a} (Figure 4) and the IgM and IgA isotypes. In addition, flow cytometry revealed changes in the frequencies of B cell markers in cells isolated from the small intestinal Peyer's patch tissue (data not shown). The mechanism behind the decreased antibody production in this strain is unknown and requires additional investigation.

LSECtin. This recently identified member of the DC-Sign family has been shown to be important in the regulation of the T-cell immune response (Tang et al. 2009). Analysis by the hematology core showed increased circulating lymphocytes, monocytes, RBCs and decreased platelet counts (data not shown). No significant findings were noted in the immunology or histology screen.

Mcl (Clec4d). The second mutant strain constructed by the CFG was a homozygous mutant of the C-type lectin, Mcl (*Clec4d*, *Clecf8*), which is expressed on murine macrophages (Balch et al. 2002). Mcl-deficient mice exhibited impaired B-cell proliferation and T-cell proliferation when stimulants that crosslink the antigen receptor were used (data not shown). Also observed was a potential increase in the number of mature recirculating B-cells in the bone marrow (Table III). Histological examination showed extramedullary hematopoiesis in the spleen, which is a stress response commonly seen in mice and might be influenced by the abnormal B-cell and T-cell proliferation observed by the immunology core.

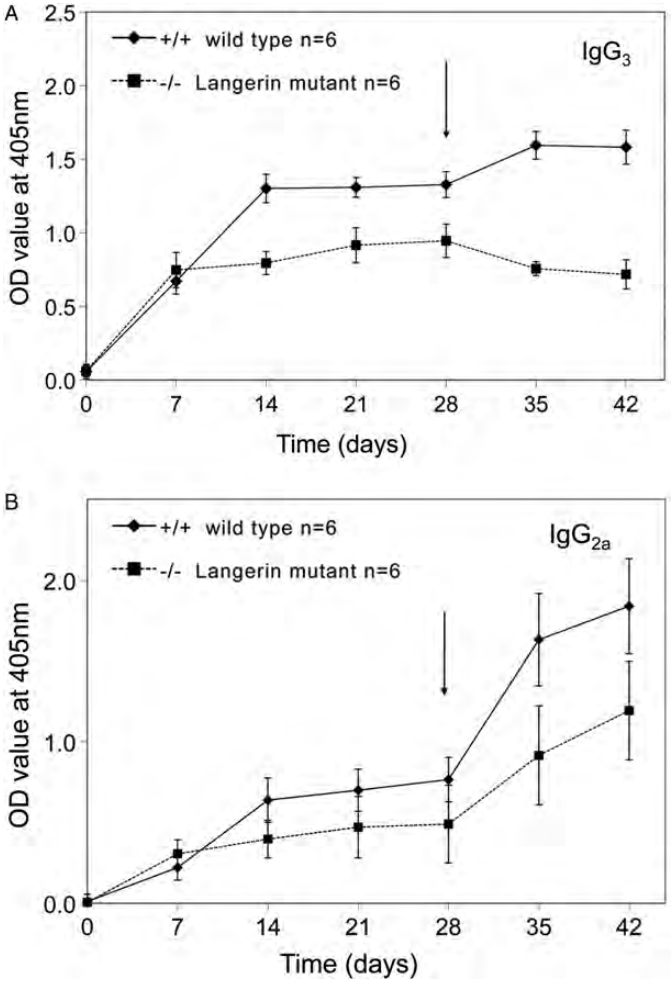

Fig. 4. Specific antibody production, following injection with the T-dependant antigen KLH, in Langerin (*Cd207*)-deficient mice. (**A**) Specific antibodies of the IgG_3 antibody isotype. Arrow indicates day of boost injection. (**B**) Specific antibodies of the IgG_{2a} isotype.

mSignR1 (Cd209b). Mouse mSignR1 is a murine homolog of DC-Sign and has a role in defense against pathogens (Geijtenbeek et al. 2002). Mouse SignR1 is expressed by specific macrophages, including MZ macrophages in the spleen and both subcapsular and medullary macrophages in the lymph nodes. Immunological testing revealed a greater percentage of macrophages in the peritoneum after thioglycollate stimulation, and an increase in several antibody isotypes (IgM, IgG_3, IgG_{2b}) in the T-independent assay (Table III). These findings indicate an increased ability of this cohort of mice to respond to inflammatory stimulation, suggesting an important role in defense against pathogens.

mSignR3 (Cd209d). The C-type lectin, mSignR3, is the mouse homolog of human DC-Sign. It is expressed by a subset of dendritic cells, macrophages and monocytes (Nagaoka et al. 2010). The immunology analysis showed an increase in the proliferation of purified splenic B-cells following anti-IgM stimulation, greater frequencies of IgG^+ IgM^+ B-cells in the spleen, and a slight increase in $B220^+$ $CD22^+$ cells, as well. The histology survey showed increased numbers of adipocytes in the bone marrow by two cohorts of mutant animals studied at 12 weeks, and again at one year of age. This result may indicate an abnormality in the production of hematopoietic cells.

Other GBPs

The Galectin superfamily consists of beta-galactoside-binding proteins that are important in immune regulation and homeostasis, as well as in tumor progression (Harregui et al. 2005; Liu and Rabinovich 2005; Stowell et al. 2008). Previously characterized Galectin-deficient strains reexamined for additional phenotypes include Galectin-1 and Galectin-3. The CFG constructed and analyzed two new homozygous mutant strains for Galectin-2 and Galectin-9. The behavior core observed several phenotypes in Galectin mutant mice, but this sub-core was eliminated after five years, so not all the CFG's newly constructed mutants were screened for behavior abnormalities.

Galectin-1 (Lgals1). The most interesting phenotype observed by the MPC's Behavior Core was an altered phenotype in the social dominance test by both Galectin-1 and Galectin-3 mutant mice. Galectin-1 mutant mice were not different from wild-type mice in gross physical assessment, sensory-motor reflexes, nociception, prepulse inhibition and threshold to acoustic startle, learning, memory, muscle strength and metabolic assays. However, Galectin-1 mutant mice performed statistically differently compared with littermate mice in the initiation of movement assay and the social dominance test. The initiation of movement assay is one of the motor assays and is a gross assessment to determine whether a mouse moves normally. Galectin-1 mutant mice took more time to move from the center to the edge of a 20-cm-diameter platform than wild-type littermates. Galectin-1 mutant mice did not show a statistically significant deviation from wild-type mice in the open field motor assay, suggesting that initiation of movement phenotype is not contaminated by an overall deficit in locomotion.

Galectin-3 (Lgals3). Galectin-3-deficient mice were also not different from wild-type littermates in multiple parameters of the behavior assays such as gross physical assessment, sensory-motor reflexes, nociception, prepulse inhibition, threshold to acoustic startle, motor activity, muscle strength and metabolic assays. Galectin-3 mutant mice performed statistically differently compared with wild-type mice in two assays, the single cue aspect of the conditioned fear task and the test of social dominance. The conditioned fear test is a form of classical conditioning and test of memory, whereby the mouse must remember a foot shock in the context of environmental cues. In this assay, the Galectin-3-deficient mice engaged in a significantly lower ability to appear immobile than did the control mice ($t_{(19)} = 2.27$, $P < 0.05$).

Interestingly, both mutant strains lacking Galectin-1 or Galectin-3 were dominant in the social dominance assay (Figure 5). Galectin-1 mutant mice won 20/26 bouts (80%) and control wild-type mice won 6/26 bouts (23%), a lower percentage than would be expected by chance ($\chi^2_{(1)} = 15.08$,

$P < 0.001$). The results from the social dominance test of Galectin-3 mutant mice are similar to the data from the analysis of the Galectin-1 mutant mice. Galectin-3 mutant mice won 24/29 bouts (83%) and wild-type mice won 5/29 bouts (17%), lower than would be expected by chance. Supporting this observation, 60% of Galectin-3 mutant mice and 20% of wild-type littermates also exhibited an abnormal, aggressive attack response to the presence of a cotton-tip applicator in the neurological exam.

Galectin-2 (Lgals2). The carbohydrate-dependent binding of Galectin-2 induces apoptosis in activated T cells (Sturm et al. 2004; Stowell et al. 2008). Investigation of the molecular mechanisms and intracellular pathways involved in the pro-apoptotic activity has revealed binding to cell surface β-integrins (Loser et al. 2009). Studies of cytokine secretion by activated T cells incubated with Galectin-2 revealed a significant shift toward the T helper, Th-2 profile (Loser et al. 2009). So there are substantial differences between Galectin-2 and Galectin-1 in terms of cell-surface ligands and intracellular signaling pathways leading to cell death.

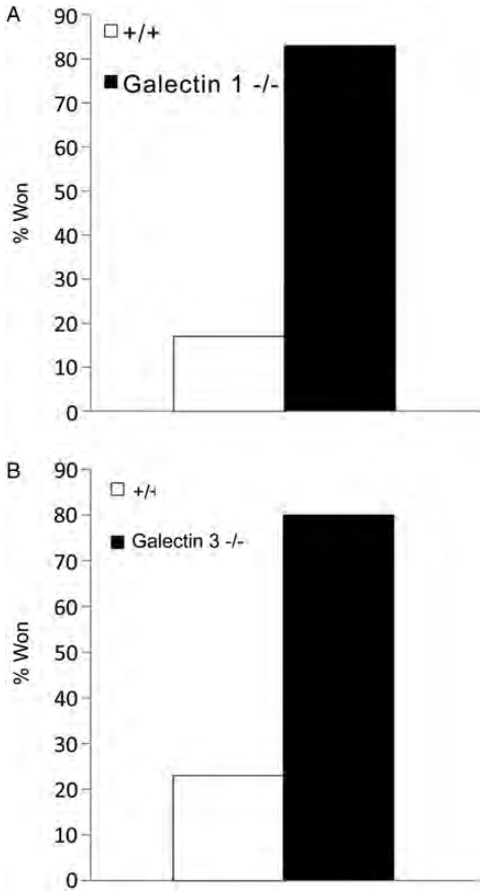

Fig. 5. Social dominance behavioral assay of Galectin-deficient mice. (**A**) Social dominance in Galectin-1 mutant mice (chi-square = 15.08, $P < 0.001$) and (**B**) Social dominance of Galectin-3-deficient mice (chi-square = 24.9, $P < 0.001$). These data indicate the total percent won, not an average, and so there are no error bars.

The CFG's immunology core observed that Galectin-2-deficient mice exhibited elevated frequencies of $CD8^+$ T-cells in the lymph node and elevated frequencies of $CD3^+$ $CD62L^+$ cells in the blood. Other T-cell related phenotypes include a slight increase in $CD4^+$ cells in the spleen and a higher frequency of TCR-Vβ staining in the lymph node. In addition, a lower frequency of apoptotic cells was observed in Galectin-2 mutant lymph nodes in comparison with wild-type mice. The observation that the Galectin-2 mutant mice had a higher frequency of $CD8^+$ cells supports the hypothesis that Galectin-2 may regulate activated $CD8^+$ cells, suggesting that in the absence of Galectin-2, the $CD8^+$ T cell population may expand. We observed significant, elevated DTH response in Galectin-2 mutant mice at 24 h, supporting the previous observation that systemic administration of Galectin-2 could suppress DTH reactions by inducing apoptosis in $CD8^+$ T cells (Loser et al. 2009).

Galectin-2 mutant mice also demonstrated elevated T-dependent (TD) and T-independent (TI) antibody responses (Figure 6). In the TI assay, both IgG_{2b} and IgG_3 antibody isotypes were elevated in relation to wild-type mice. The same isotypes were also higher in comparison with wild-type mice in the TD assay. Examination of blood samples from Galectin-2 mutant mice showed higher frequencies of $CD45R^+$ $CD22^+$ B-cells and slightly elevated populations of $CD128^+$ cells. Also cells isolated from the small intestinal Peyer's patch from Galectin-2-deficient animals exhibited elevated frequencies of $CD11b^+$ cells in comparison with wild-type mice. Since CD11b (Mac-1) is expressed on certain B-1 cell and myeloid cell populations, Galectin-2 mutant mice should be examined further for altered B-1 cell phenotypes. Since there was an increase in both the IgG_3 and IgG_{2b} isotypes in the T-independent assay and the major cells responsible for TI-2 response to carbohydrate antigens are B-1 cells, B-1 cell populations in the Galectin-2-deficient mice should be characterized further. Hematological assessment of Galectin-2 mutant mice showed a 33% decrease in neutrophil count and a 24% increase in lymphocyte count (data not shown). There was also a 10% decrease in alpha-2-antiplasmin levels. These findings may point to unusual coagulation abnormalities if additional cohorts of mice are examined (Takei et al. 2002; Matsuno et al. 2003; Okada et al. 2004). Histological analysis showed increased amounts of colon mucin in ~50% of the Galectin-2-deficient mice. Additional animals must be analyzed at different stages of development to determine whether these findings contribute to abnormal mucin production in models of inflammatory bowel disease.

Galectin-9 (Lgals9). Galectin-9 has been shown to be important in attracting eosinophils in allergic disease (Hirashima et al. 2004), and was shown to be important in glycosylation of the Glut2 transporter on pancreatic beta cells regulating glucose transport via galectin-9 interaction (Ohtsubo et al. 2005). The Galectin-9 mutant mice screened by the MPC had elevated levels of neutrophils and lymphocytes in the blood, prominent germinal centers in splenic lymph nodes as a result of activation and elevated antibody production with hyperproliferative B cells. Also

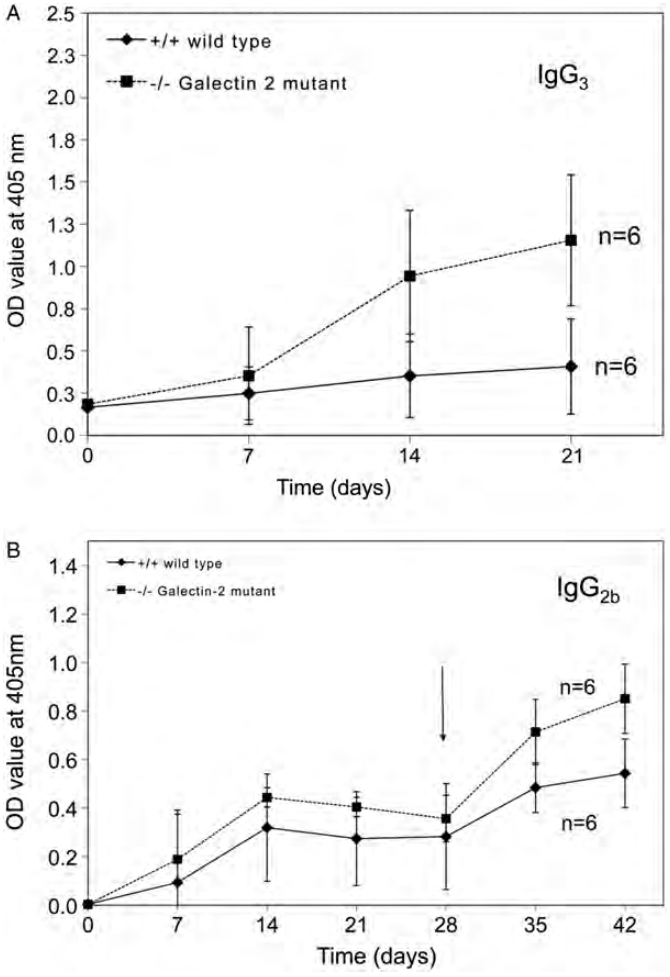

Fig. 6. Specific antibody production in Galectin-2 (*Lgals2*)-deficient mice. (**A**) Specific IgG_3 isotype production in the T-independent assay (ficoll) (**B**) Specific IgG_{2b} production in the T-dependent assay (KLH). Arrow indicates day of boost injection.

greater cell numbers were observed in comparison with wild-type mice in the lymph node, spleen, thymus and Peyer's patches. These findings need follow-up analysis on additional cohorts of mice. The obesity phenotype that was observed in other studies (Ohtsubo et al. 2005) was not remarkable in our screen, and may have been because only one random cohort of animals were examined due to time constraints.

Sialic acid-binding lectins

Siglec-4a (Mag, Siglec-G). Siglecs (sialic acid-binding immunoglobulin-like lectins) bind sialic acids that are expressed on many different types of immune cells. Siglec-4a, also known as Myelin associated glycoprotein (MAG), has been shown to stabilize myelin-axon interactions (Sun et al. 2004). In the Siglec-4a-deficient mice surveyed by the MPC, the immunology core observed an increased proliferative response of purified splenic B-cells to stimulation using anti-IgM or LPS. Siglec-4 has since been identified as expressed by the B1 subset of B cells and is known to inhibit Ca^{2+}-dependent signaling. The behavior core demonstrated that Siglec-4a-deficient mice lost more 'battles' in the social dominance task than would be expected by chance. In addition, Siglec-4 mutant mice were impaired in the startle response as measured by both the startle stimulus threshold task and prepulse inhibition task. Siglec-4a-deficient mice were impaired in prepulse inhibition assay, but the interpretation of this impairment is confounded by their impaired startle response. This startle impairment could be due to a hearing deficit rather than a deficit in the startle reflex *per se*.

Siglec-H (Siglech). Siglec-H was described as a novel endocytic receptor expressed on murine plasmacytoid dendritic cell precursors (Zhang et al. 2006). The Siglec-H-deficient mice screened by the MPC showed T-cell hyperproliferation after Concavalin-A stimulation. Serum chemistry analysis showed an increase in the blood urea nitrogen levels, indicating abnormal kidney function, which correlates with the abnormal morphology of the kidney tubules observed in all the five mutant mice, by the histology core. The testis of the Siglec-H homozygous mutant animals showed degenerating cells and atrophic changes with cystic areas (Figure 7). Since only a small cohort was screened here, examination of additional older animals may reveal a role of Siglec-H in kidney disease and/or fertility problems.

Website links

The construction scheme and diagram for each of the CFG's mutant strains can be found under "targeted genes" link at: http://www.functionalglycomics.org/static/consortium/resources/resourcecoref.shtml.

All the experimental details, protocols and summaries of phenotype results can be found under the individual strain name listed at: http://www.functionalglycomics.org/glycomics/publicdata/phenotyping.jsp.

Mutant mice constructed by the CFG's MTC can be obtained from the Consortium for Functional Glycomics collection at the Mutant Mouse Regional Resource Center (MMRRC) at the University of California, Davis. Individual mutant mice can be found using the strain catalog search (major strain collection) at: http://mmrrc.ucdavis.edu/.

Discussion

One of the great challenges since the human genome has been sequenced is to define the biological function of all human genes. Since 99% of mouse genes have human homologs, experimentation upon mouse models obtained by either reverse or forward genetics has been useful to reveal gene function. In the reverse genetics methodology, mouse genes are manipulated by employing transgenic methodologies, gene deletions or gene traps (Brown et al. 2006).

In the gene deletion model, mice lacking specific genes are engineered, and then investigators search for altered phenotypes in order to assign gene function(s). Historically, an individual laboratory would create a mutant strain, and then test the strain for phenotypes restricted to that laboratory's area of

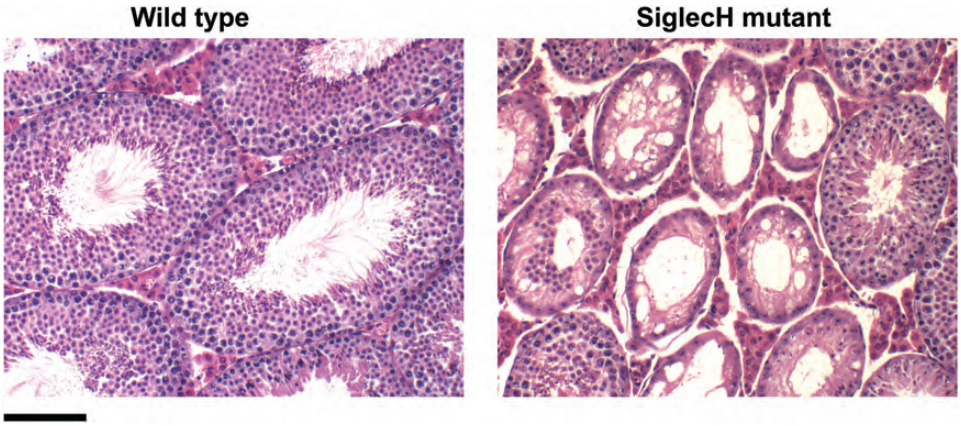

Fig. 7. Altered seminiferous tubules in the testes of SiglecH (*Siglech*)-deficient mice. Seminiferous tubules from Siglec H mutant mice (right) shows altered structure with poor maturation, compared with wild-type mice (left). Scale bar = 100 μm.

expertise. As a result, these mutant strains were often subjected to a limited phenotype screen, and negative data were rarely published.

Later multi-investigator groups were formed to phenotype mice lacking specific genes thought to play a role in defined human diseases, such as cancer or cardiovascular disease. In this case, general assays were used to screen the animals, but often, additional phenotype analysis was restricted to assays unique to the specific diseases in the group's area of study.

Several years after the MPC started operation in 2001, large worldwide endeavors were formed to delete or alter every gene in the mouse genome. For example, the KOMP project (Knock-Out Mouse Project) in the United States has currently constructed 307 mutant strains out of a planned 5000 strains (www.komp.org). These large-scale deletion projects spurred the creation of standard phenotype protocols beginning with general assays and then moving into higher tiers of specialized assays based upon the phenotype results from earlier screens (http://www.mousephenotype.org). This form of phenotype analysis follows the specific paradigms established earlier by veterinary pathologists working on the phenotype characterization of gene-altered mice (Brayton et al. 2001; Barthold 2004; Cardiff 2007; Crawley, 2007).

Previous studies of mice deficient in genes of relevance to the glycoscience community had often revealed pleiotropic effects in other organ systems and stages of development, such as cell surface communication, pathogen recognition, embryological development and carcinoma progression (Ohtsubo and Marth 2006; van Kooyk and Rabinovich 2008; Speradino et al. 2009; Varki et al. 2009). As a result, the MPC formed four sub-cores to utilize the expertize available within specific cores and to create a broad phenotype screening strategy consisting of a large number of common assays. The behavior core characterized general features (e.g. weight), metabolism and behavior. The immunology core was responsible for mouse husbandry and all immunological assays. The hematology core was responsible for the analysis of serum chemistry, coagulation and hematological parameters. Finally, the histology core analyzed tissue samples, including any necessary special stains or lectin assays (Maronpot et al. 1999; Ward et al. 2000; Martin et al. 2002).

As a result, the MPC was able to screen mutant mice to evaluate the numerous physiologic changes that influence the whole organism when specific genes are "knocked-out". The MPC's comprehensive phenotype program was designed to test both the static and functional aspects of multiple biological systems, and over 100 assays were used. During the operation time-span of ten years, the MPC completed a screen of 36 homozygous mutants of interest to the glycobiology community, and each strain was completed within three months. The four sub-cores were staffed with one dedicated technician, and one additional technician was responsible for the mouse husbandry. Later during the second five years of operation, the MPC worked directly with individual investigators to perform additional analyses beyond the standard phenotype survey. The MPC was also unique because the CFG mandated that all of the phenotype results were to be available to participating investigators on the website within six weeks of experimental completion, and then accessible six weeks later to the general public. Data posted on the CFG's website included summaries, experimental data and protocols. Moreover, links were created for every mouse tested with information describing the genotype, gender and age when tested.

The MPC's results revealed a considerable number of phenotype differences between mutant and wild-type mice. Almost all the strains exhibited some evidence of phenotype abnormality, which will need follow-up analysis to reveal the underlying pathology. Interesting phenotypes in ST6GalNAc-II-deficient mice observed here include lower body weights in male mice, elevated B cell proliferation and elevated frequencies of pre-B lymphocytes and immature RBC markers, which indicate hyper reactive hematologic parameters. The DC-Sign mutant mice exhibited a higher frequency of plasma cells in a younger cohort of mice. These mice also exhibited lymphocytosis, monocytosis and enlarged germinal centers in the spleen, while an older group of mice possessed hyper-proliferative T cells, which indicates a tendency to hyperproliferation and maturation of lymphocytes as a response to stress. The *Mfng*-deficient

mice showed an elevated B cell proliferative response, with decreased B cells in bone marrow, and higher numbers of B cells in circulation. The Galectin-9-deficient animals demonstrated elevated antibody production, hyper-proliferative B cells and higher total cell numbers in spleen, lymph node, thymus and Peyer's patch lymphoid cells. This may correlate with development of autoimmune diseases such as diabetes as has been reported by others (Ohtsubo et al. 2005). Unexpected findings for follow-up studies also include kidney and testis abnormalities in the GlcNAcT9 and Siglec-H mutant mice. Caveats in interpretation of mouse phenotype results should always include the influence of strain-specific modifier genes on observed differences (Kile et al. 2003; Heidari et al. 2006; Jelcick et al. 2011).

One drawback to the CFG's phenotype paradigm was that in the beginning several participating investigators suggested the CFG perform additional analyses and pursue conventional publication. It took several years before other investigators took advantage of the initial phenotypes and proceeded to expand upon the analyses, which then resulted in conventional publications. Examples include a study reporting impaired resistance to tuberculosis in mSignR3 mutant animals (Tanne et al. 2009) and increased susceptibility to systemic candidiasis in Mincle-deficient animals (Wells et al. 2008).

The MPC closed in the fall of 2011; however, two subcores are currently in operation at the University of California, San Diego (http://mousepheno.ucsd.edu). Hopefully in the future, the database centers collecting data from the numerous large-scale phenotype projects currently underway will be able to mine the CFG's database. This should stimulate additional experimentation and analyses to determine the function of each known mouse gene. Then along with an extended characterization of inherited defects in glycosylation and glycan structure, the understanding of glycan function(s) will significantly expand. This effort will generate new insights into the mechanisms by which glycans modulate development, differentiation, physiology and disease, and suggest new targets for drug discovery.

Materials and methods

Protocols for all experiments can be downloaded from the CFG's website at www.functionalglycomics.org. The various phenotype assays used to examine mutant mouse strains are listed in Table I. The assays fall into two general categories. The first category contains static assays that measure the current condition of the animal, and include hematology, serum chemistry, flow cytometry and histological analysis. Assays in the second category test the abilities of mutant mice, in comparison with their wild-type littermates, in a variety of functional response assays. Examples include the stimulation of acute inflammation (peritonitis), antibody responses to T-dependent antigens and behavior assays that evaluate learning and memory.

To complete a phenotype survey of a large number of mutant strains per year, effective use of mice is essential. Animals were bred for experimentation by the immunology core and then distributed to the other three cores. A set of mutant and wild-type mice of both sexes, between seven and 10 weeks of age, were sent first to the hematology core. After the hematology and coagulation experiments were completed, this group of mice was passed on to histology for sectioning. The immunology core used the second set of mice of similar ages for all experiments, except for DTH experiments where only females were used. The behavior core received the final cohort of mice consisting of three-month-old male mice. The behavior test battery assays were ordered in an attempt to place the more aversive tasks at the end of the battery or before the weekend when mice were not tested.

The assays that are performed in the MPC were chosen to serve as a non-hypothesis-driven initial examination designed to detect large group differences. These assays were usually not repeated more than three times. Assays that required a large number of mice, such as the inflammation assay, were performed only once. If an altered phenotype was observed, these assays were repeated. Therefore, it is important to consider that the results from our significant but limited phenotype screening could have missed important roles of glycans and GBPs in biological processes that were not studied, and those results that were observed represent preliminary data as a basis for further investigation.

Mice

Mutant mouse strains constructed in other laboratories were sent to the CFG's MTC for distribution to PIs, while mice that were used for experiments by the MPC core were bred by the immunology core. The extent of backcross to C57BL/6 varied between these mutant mouse strains; however, most preexisting strains analyzed had a backcross of greater than twenty. A limited number of strains were analyzed by total genome analysis (genescan).

The CFG's MTC constructed sixteen novel homozygous mutant strains. For each strain, construction diagrams and polymerase chain reaction (PCR) protocols can be found on the website under CFG resources, mouse lines. For construction, C57BL/6J mouse BAC clones were used for the following strains: *Mfng*, (*Mnfg*, RP23-129N22), DCIR (*Clec4a2*, RP24-250H2), Langerin (*CD207*, RP24-75C14), *Pomgnt1* (RP23-60E24), Galectin2 (*Lgals2*, RP23-305P10) and Mcl (*Clecsf8*, RP23-284A5). These BAC clones were obtained from BACPACResources Center at Children's Hospital Oakland Research Institute, Oakland, CA. Homologous fragments were amplified by PCR using the above BAC DNA as template and then inserted sequentially into the replacement KO vector *pKO* for Galectin-2 and Langerin, or the conditional vector *pKO-3loxp* for *Mfng*, DCIR, *Pomgnt1* and Mcl. For the GalNAcT9, ST6GalNAc-II mutant strains, mouse 129/SvJ BAC clones spanning the genes were identified from a BAC library by southern blotting. Homologous fragments were amplified by PCR using the BAC DNA as template, and then inserted sequentially into the conditional KO vector *pKO-3loxp*. The resulting targeted vectors were used to transfect either Bruce 4 embryonic stem (ES) cells, a C57BL/6 ES cell line, or the EV cell line, based on the 129 genomic background. The homologous recombinant ES cell clones for the targeted gene were identified by Southern blotting analyses. The selected ES clones from Langerin and Galectin-2 replacement vectors were directly injected into Albino B6 blastocysts

to generate chimeras. The ES clones from the rest of the conditional vectors were further transfected with the *pMC-Cre* vector encoding the Cre recombinase and cultured with 2 μM ganciclovir (Sigma-Aldrich). The ganciclovir-resistant ES cell subclones with floxed alleles, were injected into Albino B6 blastocysts, while the EV cell line-derived subclones (GalNAcT9 and ST6GalNAc-II) were injected into C57BL/6 blastocysts to generate chimeric mice. Male chimeras were then crossed with Albino B6 females, or C57BL/6 females, respectively. The resulting replacement KO mice (Langerin and Galectin-2) and the remaining floxed allele KO mice were crossed with zp3-cre mice to remove the neo gene or generate delta allele knock-out mice, respectively, which were further intercrossed to produce homozygous delta knock-out mice. For Siglec H-deficient mice, three homologous fragments were amplified by PCR from C57BL/6 genomic DNA. Then they were inserted sequentially into the Ozgene plasmid 467-H3-T. The resulting targeted vectors were used to transfect Bruce 4 embryonic stem (ES) cells (C57BL/6). The homologous recombinant ES cell clones for the targeted Siglec H gene was identified by Southern blotting analyses. The selected ES clones were injected into blastocysts to generate chimeric mice. Male chimeras were crossed with C57BL6 females. All Siglec H mutant mice were generated by Ozgene Pty. Ltd. (www.ozgene.com). The resulting homologous recombinant allele mice were crossed with FLPeR transgenic mice (Jackson laboratory stock number 003946) and zip3-cre mice to generate Siglec H floxed allele and delta allele knock-out mice, respectively.

Immunology assays

For immunological characterization, static, developmental and functional aspects of the immune response were tested. Assays examining baseline values included the determination of serum immunoglobulin levels and organ apoptosis (spleen, thymus, lymphnode, blood, bone marrow).

Flow cytometry. Briefly, single-cell suspensions were obtained from spleen, thymus, bone marrow, blood and lymph node (axial and brachial). One million cells were placed in 100 μLs of FACs buffer (phosphate-buffered saline (PBS), 0.1% sodium azide, 1% bovine serum albumin (BSA)) and incubated with the appropriate antibodies for one hour at 4°C. Data were acquired using a FACSCalibur and analyzed using Cell Quest Pro software (Becton Dickinson). Unstained and isotype-stained control cells were used to determine positive cells. For flow cytometry analysis, a positive phenotype was determined if a minimum of three out of three littermate pairs showed similar altered expression. Data are expressed as a percentage of cells out of 10,000 events collected.

Delayed-type hypersensitivity (DTH). Sensitization was induced by topical administration of 2% oxazolone, and 5–6 days later ears were challenged again. Twenty-four hours later, ear swelling was measured for three days.

T-dependent and T-independent antibody production. Six mice were used per genotype, were sex matched and were between 6–12 weeks old. For T-dependent, mice injected with DNP-KLH in complete Freund's adjuvant, and boosted on day 28 using DNP-KLH emulsified in Freund's incomplete adjuvant. For T-independent (type-II) reactions, animals injected with DNP-Ficoll in 10% aluminum potassium sulfate and tail blood collection at indicated days.

T cell and B cell proliferation. Purified splenic B cells or T cells were obtained using the appropriate MACs columns. Proliferation was determined by ^{3}H-Thymidine encorporation following cell-specific stimulation, T proliferation (anti-CD3, ConA) and B cell proliferation (LPS, anti-IgM).

Inflammation. Six mice were used per genotype and examined at 6 and 72-h timepoints following peritoneal injection of 10% protease peptone.

Hematology, chemistry and coagulation assays

The hematology assays examined the phenotypes of blood cell populations of at least 32 mice, male and female, with wild-type controls included for each gender. Briefly, ethylenediaminetetraacetic acid (EDTA)-anticoagulated whole blood samples were analyzed in duplicates for complete blood cell count with leukocyte differential and platelet count on a Hemavet 850FS Multi Species Hematology System (Drew Scientific). When indicated, blood cells and platelet morphology were evaluated microscopically on Giemsa stained smears. For serum chemistry, the samples were analyzed with a Beckman CX-7 or a Roche automated analyzer. Chemistry panels include AST (Asparatate aminotransferase), ALT (Alanine aminotransferase), Bilirubin, Albumin, Total Protein, (to assess liver function) BUN, Creatinine, (to assess kidney function), Glucose, Na^{+}, K^{+}, Cl^{-}, HCO_3^{-}, Calcium, Phosphate and ALP (Alkaline phosphatase for bone abnormalities). Clotting assays were performed on a ST4 coagulation analyzer (Diagnostica Stago). Antigen assays were performed using conventional ELISA methods. The initial hemostasis evaluation include measurements of: Bleeding Time, prothrombin yime (PT), activated partial-thromboplastin time (APTT), Protein C, Protein S, Antithrombin, Plasminogen and α-2-Antiplasmin. When indicated, clotting factor activity levels and von Willebrand antigen levels were determined.

Histology assays

Complete protocols for all experiments can be downloaded from the CFG website. The histology core performed necropsies on 24 animals after the hematology core had done terminal bleeds. This included six wild-type male and six wild-type female mice, for comparison with six mutant male and six mutant female mouse organs. About 30 organs were examined for all mice using standard hematoxylin and eosin stains. Initial analysis involved a survey of hematoxylin and eosin stained sections with digital photomicrographs. Special stains were then performed if the first analysis showed some abnormality that needed further investigation (Kiernan 2001).

Histochemical stains. Periodic acid Schiff (PAS) were used for analysis of glycogen levels in the liver, mucin content in colon sections, and basement membrane abnormalities in

3-micron sections of kidney. Alcian Blue stains were used to visualize mucin content at two different pH levels (pH 2.5 for acidic mucins and pH 1.0 for sulfated mucopolysaccharides). Luxol Fast Blue stains (LFB) were used for analysis of myelin content, degeneration or loss.

Lectin histochemistry. Specific lectins were used to confirm loss of enzyme in the mutant animals, such as using L-PHA in organs to confirm the loss of *Mgat5* activity. Peanut agglutinin lectin (PNA) was used on spleen sections to determine hyper-reactivity within B-cell areas of splenic lymphoid follicles.

Immunohistochemistry. Specific antibodies were employed on paraffin sections using standardized methods. Examples of antibodies used were: Anti-GFAP (Glial Fibrillary Acidic Protein) to determine changes in numbers of astrocytes in brain samples from the ST3Gal-II homozygous mutant animals, anti-B220 antibodies on splenic samples from Core2-GlcNAcT mutant mice (expect loss of staining) and anti-C6ST1 antibody in the C6ST1 homozygous mutant animals (expect loss of expression).

Metabolism and behavior assays

The test battery is designed to provide a gross overall assessment of mutant mice on a variety of parameters. Ten male mutant mice and 10 littermate wild-type mice were tested in the metabolism and behavior test battery over a period of weeks. Assays were ordered in an attempt to place the more aversive tasks at the end of the battery or before the weekend when mice were not tested.

Social dominance tube test. Two mice of different genotypes are placed at opposite ends of a plastic tube 30 cm in length with a 3.5 cm inside diameter. The mouse that remains in the tube after a one-minute interval is considered "dominant" and "won". The mouse that backs out of the tube is the nondominant animal that lost. Each mouse was confronted with up to three different combatants within a five-minute interval. Results are presented as percent won. Significance is determined by the chi-square test.

Open field. Exploratory locomotor activity in a 30-min test period was measured in an open field (45 × 45 cm) Digiscan apparatus (Accuascan Electronics, Columbus, OH). Horizontal activity (locomotor activity), vertical activity (rearing), total distance (cm) and center distance were recorded. The center distance divided by the total distance is an indicator of anxiety-related behavior. Room lighting was 200 Lux. The center distance is defined as the area of the maze at least 1 cm away from the wall. The area of the entire arena is 2024 cm^2 and the center area is only 1849 cm^2. Thus, the center area is equal to about 91% of the total area and the outside perimeter is about 9% of the total area. Significance is reported as a P value from the Student's t-test.

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Conflict of interest

None declared.

Abbreviations

ALP, alkaline phosphatase; ANA, anti-nuclear antibodies; APTT, activated partial-thromboplastin time; BSA, bovine serum albumin; CBC, complete blood cell differential counts; CFG, consortium for functional glycomics; CIA, collagen-induced arthritis; DTH, delayed-type hypersensitivity; ES, embryonic stem; GBP, glycan-binding protein; GFAP, glial fibrillary acidic protein; GT, glycosyltransferase; HCT, Hematocrit; HGB, hemoglobin; KLH, keyhole limpet hemocyanin; LFB, luxol fast blue stains; *Lfng*, lunatic fringe; MAG, myelin associated glycoprotein; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; *Mfng*, manic fringe; MMRC, Mutant Mouse Regional Resource Center; MPC, mouse phenotype core; MPC, mouse phenotype core; MPV, mean platelet volume; MTC, mouse transgenic core; MZ, marginal zone; NIGMS, National Institute of General Medical Sciences; PAS, periodic acid Schiff; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PIs, participating investigators; PLT, platelet count; PNA, peanut agglutinin lectin; PPI, prepulse inhibition of startle; PT, prothrombin time; PT, prothrombin time; RBCs, red blood cells; RDW, red cell distribution width; SR, threshold to startle response; TD, T-dependent; TI, T-independent.

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