The aberrant expression of core 2 O-glycans on T cell surface glycoproteins has been associated with various immunodeficient syndromes such as Wiskott-Aldrich syndrome and AIDS. To determine the effect of this aberrant expression of core 2 O-glycans on immune responses, we previously generated transgenic mice overexpressing core 2 β,1,6-N-acetylgalcosaminyltransferase (C2GnT) in T cells, and demonstrated that T cell primary immune responses mediated through interaction between T cells and antigen-presenting cells are impaired in the transgenic mice (Tsuboi, S., and Fukuda, M. (1997) EMBO J. 16, 6364–6373). In this study, we determined whether overexpression of core 2 oligosaccharides on T cells leads to impaired humoral immune responses by B cells using the same transgenic mice. When T cells were activated, both T and B cells from the transgenic and control mice expressed an equivalent amount of CD40L and CD40, which are, respectively, the receptor and counter-receptor for the interaction between T and B cells. However, activated T cells from the transgenic mice induced B cell proliferation less efficiently than those from control mice, regardless of whether B cells were isolated from control or the transgenic mice. This suggests that overexpression of core 2 O-glycans on T cell surface glycoproteins renders T cell-B cell interaction inefficient. Moreover, in the transgenic mice both immunoglobulin isotype switching and germinal center formation were also impaired. Taken together, these results indicate that aberrant expression of core 2 O-glycans on T cell surface glycoproteins results in impaired humoral immune responses due to an impaired interaction between T and B cells.

Carbohydrate structures of glycoproteins and glycolipids dramatically change during development and differentiation (1–3). Abnormalities in those cell surface carbohydrates are often associated with malignant transformation and other pathological conditions, including immunodeficiency (1–4). Among the various cell surface carbohydrates, the biological function of mucin-type O-glycans, which are attached to serine or threonine residues on cell surface glycoproteins, is not well understood. There are, however, several reports suggesting that O-glycans are involved in the modulation of cell adhesion processes. For example P- and L-selectins bind with higher affinity to sialyl Leα and sulfated sialyl Leα expressed in O-glycans than those expressed in N-glycans, possibly due to multivalency of O-glycans (5–7). In O-glycans of blood cells, various ligand carbohydrate structures can be formed only in a branched O-linked hexa-saccharide, NeuNαcα2→3Galβ1→3(NeuNαcα2→3Galβ1→4Glcnacβ1→6)GalNacα1→Ser/Thr, called a core 2 O-glycan. Core 2 β,1,6-N-acetylgalcosaminyltransferase (C2GnT) is essential to form core 2 O-glycans (8, 9). In T cell development, the expression of core 2 O-glycans is restricted to cortical thymocytes, and their expression is barely detectable in medullary thymocytes (10). This down-regulation of core 2 O-glycan synthesis is apparently critical for apoptotic processes in T cell development (11). In peripheral T cells, resting T cells express a simple tetrasaccharide, NeuNαcα2→3Galβ1→3(NeuNαcα2→6)GalNacα1→Ser/Thr. When T cells are activated by mitogens, the expression of C2GnT is induced, and as a result, O-glycans are converted to the core 2 O-glycans, NeuNαcα2→3Galβ1→3(NeuNαcα2→3Galβ1→4Glcnacβ1→6)GalNacα1→Ser/Thr (12).

Various pathological conditions, including certain immunodeficient syndromes, are associated with aberrant expression of core 2 O-glycans. Examples include Wiskott-Aldrich syndrome (13, 14), leukemia (15, 16), AIDS (16–18), and malignant transformation (19). In these diseases, core 2 O-glycans are highly expressed on resting T cells, while they are not expressed in normal conditions. To examine the influences of aberrant expression of core 2 O-glycans on the immune system, we have previously generated transgenic mice whose T cells overexpress core 2 O-glycans. We demonstrated that T cells from these transgenic mice exhibit reduced primary T cell immune responses, as assessed by delayed-type hypersensitivity, proliferation upon stimulation, and cytokine production (20). We demonstrated that reduced immune responses of T cells are due to impaired interaction between T cells and antigen-presenting cells (20). The next important question is whether overexpression of core 2 O-glycans affects humoral immune responses. In this study, we address this question by examining humoral immune responses in the transgenic mice compared with those of control mice. We demonstrate that overexpression of core 2 O-glycans impairs humoral immune responses to thymus-dependent antigen. We suggest that this reduced B cell immune response is due to inhibition of the interaction between T and B cells (T-B interaction).
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**EXPERIMENTAL PROCEDURES**

**Transgenic Mice—**Transgenic mice (Lck-mC2GnT) were established in the previous study (20). Briefly, the transgene, mouse C2GnT, was cloned into pBluescript under the control of p56^cACK promoter (20, 21) and injected into FVB/N mouse zygote pronuclei. Stable lines of mice were generated by breeding founders with FVB/N mice (22). The resultant preparations were >95% pure T cells, as determined by flow cytometric analysis for αβ T cell receptor. B cells were purified from spleens by flow cytometric sorting. The resultant preparations were >95% positive for B220 by flow cytometric analysis. Purified T or B cells were homogenized in 0.1 ml of an extraction solution (0.4% Triton X-100, 150 mM NaCl), and the assay was performed essentially as described using Galβ1-3GlcNAcα1-p-nitrophenol as a substrate (9). Similarly, UDP-Gal:Galβ1-3GlcNAcβ1-4Galβ1-3-Gal:UDP-Galα1-Net, UDP-Galα1-Net, or UDP-Galα1-3-galactosyltransferase were used for measuring GALβ1-3GlcNAcα1-4Galβ1-3-Gal:UDP-Galα1-Net and UDP-Galα1-3-galactosyltransferase assays were used for measuring αβ T cell receptor. The membranes were incubated with the anti-CD43 monoclonal antibodies as described (20), and with anti-CD44 (IM7, 1:40 dilution; Ref. 25), anti-CD45RB (23G2, 10^5 M), anti-CD45ABC (23G2, 10^5 M), and anti-CD2 (RM2-5, 0.5 μg/ml). The antibodies except for anti-CD44 were purchased from PharMingen (San Diego, CA).

**Expression of CD45 in CHO Cell Lines—**CD45^ABC DNA containing the CHO region (26) was subcloned into pcDNA (pcDNA-CD45^ABC). CHO cells stably expressing leukosialin (CHO-leu) and CHO cells stably expressing leukosialin and C2GnT (CHO-leu-C2) (27) were transiently transfected with pcDNA-CD45^ABC using LipofectAMINE PLUS reagent (Life Technologies, Inc.). Two days after the transfection, whole cell lysates were prepared and subjected to Western blotting analysis. A monoclonal antibody reactive to all isoforms of the CD45 (90-P11, 10 μg/ml; Ref. 28) and a CD45-exon B specific antibody (23G2, 10 μg/ml), purchased from PharMingen, were used. CD45^ABC cDNA was kindly provided by Dr. Matthew Thomas (Washington University School of Medicine, St. Louis, MO).

**Flow Cytometry—**Monodispersed cells (1 x 10^6) from mouse tissues were stained with fluorescent-conjugated antibodies (1 μg/ml) as described previously. PE-conjugated anti-CD4 and anti-CD44 activation-associated isoforms, FITC-conjugated anti-β220 (RA3-6B2), FITC-conjugated anti-αβ T cell receptor (H57-597) or anti-CD40 (HM40–3), and PE-conjugated anti-CD40 ligand (MR1) were purchased from PharMingen. Anti-mouse CD23/16 (PharMingen) was included in all incubations to block antibody binding to Fc receptors. Analyses were done with a FACScan flow cytometer using the CellQuest program (Becton-Dickinson, San Jose, CA).

**Analysis of CD40 Ligand (CD40L) Expression on T Cells—**CD4^+ T cells were purified from spleens with Mouse T Cell CD4 Subset Column Kit (R&D Systems, Minneapolis, MN) (22). The resultant preparations were >95% pure CD4^+ T cells, as determined by flow cytometric analysis for CD4. Purified CD4^+ T cells were activated for 6 h in 24-well plates coated with anti-CD3 (142C-11, 20 μg/ml) and mouse ICAM-1-IgG fusion protein (10 μg/ml) as described (20). In parallel, purified T cells were activated for 6 h with PMA and ionomycin as described (20). Activated T cells were subsequently harvested, washed, stained with PE-conjugated anti-CD40 ligand, and then analyzed by flow cytometer.

**B Cell Proliferation Assay—**Spleen cell suspension was prepared from a pool of spleens from eight mice (four males and four females). CD4^+ T cells were isolated from half of a spleen cell suspension as described above. B cells were isolated from the other half of the cell suspension by a combination of treatment with antibodies to Thy-1.1, CD4, and CD8 (PharMingen), followed by rabbit complement (ICN, Irvine, CA) and incubation on plastic dishes to deplete adherent cells (29). The resultant preparations were >95% pure B cells, as determined by flow cytometric analysis with B220. For B cell proliferation assays, purified B cells (2 x 10^5 cells/well, 200 μl) were cultured in RPMI 1640 medium containing 10% fetal calf serum, 2-mercaptoethanol (50 μM), penicillin (100 units/ml), and streptomycin (100 μg/ml) for 72 h in 96-well flat-bottomed tissue culture plates. In some experiments, the medium also contained 10 ng/ml IL-4 (PharMingen), 1 μg/ml lipopolysaccharide (Sigma), or 5 μg/ml anti-CD40 (HM40–3, PharMingen). In parallel, purified B cells (1 x 10^5 cells/well) were co-cultured with non-activated or T cells activated for 6 h (1 x 10^5 cells/well) in the same medium as described above. The T cells were fixed with 0.4% paraformaldehyde in PBS for 15 min at room temperature, then washed and used to stimulate B cells (30). Cultures were pulsed with 1 μCi/well [3H]Thymidine (NEN Life Science Products) for 5 h of a 72-h culture period and then harvested onto a glass fiber filter. [3H]Thymidine uptake was measured by liquid scintillation counting.

**Anti-KLH Antibodies Analysis by ELISA—**Mice were intraperitoneally immunized with 100 μg of KLH (Sigma) in complete Freund’s adjuvant. Mouse anti-KLH IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA levels in the serum were determined by an isotype-specific ELISA. Briefly, 96-well plates were coated with 10 μg/ml KLH for 24 h. Plates were then washed and blocked with 1% bovine serum albumin in PBS. Diluted serum samples were then added to wells, and plates were incubated for 1 h at 37°C. Anti-KLH IgM antibody titer was determined with biotin-conjugated goat anti-mouse IgM and streptavidin-conjugated horseradish peroxidase (PharMingen). The other antibody titers were determined using alkaline phosphatase-conjugated goat anti-mouse IgG, IgG1, IgG2a, IgG2b, IgG3, and IgA. AsA4s were developed by using 1-Step ABTS substrate (Pierce) for peroxidase and p-nitrophenyl phosphate substrate (PharMingen) for alkaline phosphatase (31, 32).

**Immunocytochemical Detection of Germinal Centers—**Mice were immunized with KLH as described above. Spleen flat sections (5 μm thick), 7 days after the immunization, were fixed with 4% paraformaldehyde in PBS. The sections were incubated with antibody or lectin in a humidified box (30). The sections were suspended in 10 mM HEPES buffer containing 0.15 mM NaCl, 1 mM CaCl_2, 1 mM MgCl_2, and 3% fetal calf serum, and stained with biotinylated peanut agglutinin (Vector Laboratories, Burlingame, CA) for 1 h at room temperature (33). Parallel sections were suspended in PBS supplemented with 3% fetal calf serum and stained with biotinylated goat anti-mouse IgG (BS–602, PharMingen). Biotinylated peanut agglutinin and anti-mouse IgM were stained with PE-conjugated streptavidin (PharMingen). The slides were evaluated and photographed under a Zeiss Axiosplan fluorescence microscope.

**RESULTS**

**Generation of Transgenic Mice Overexpressing Branched Hexaasccharidines on T Cells—**As shown previously, we have generated transgenic mice that express the mouse C2GnT gene under the control of the proximal promoter of p56^cACK. This promoter allowed increased activity of C2GnT in lymphoid tissues where T cells are present (20). However, the previous results were obtained on a mixture of T and B lymphocytes, and we could not exclude the possibility that B lymphocytes also contained increased C2GnT activity in the transgenic mice. To determine whether only T cells contain increased C2GnT activity, T and B cells were purified from spleens, and glycosyltransferases were assayed in cell lysates of purified T and B cells. As shown in Table I, T cells derived from the transgenic mice (Lck-mC2GnT) contained C2GnT activity approximately 50 times higher than did T cells derived from control mice. In contrast, the activities of β-1,3-galactosyltransferase, which forms core 1 in O-glycans, and β-1,4-galactosyltransferase, which forms N-acetyllactosamines, were almost equal in control and the transgenic mice (Table I). Moreover, no difference in C2GnT activities was found in B cells between the transgenic and control mice (Table I). These results indicate that C2GnT is specifically increased in T cells of the transgenic mice without an accompanying change in the activities of other glycosyltransferases.

In the above experiments, as well as those that follow, we found that results obtained from two independently generated transgenic lines (Lck-mC2GnT-1 and -2) were practically identical. Therefore, we conclude that clonal variations play little or no role in determining the phenotype of either line.
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**Table I**

Glycosyltransferase activities (nmol/h/mg) in the cell extracts of T and B cells isolated from spleens were measured as described under "Experimental Procedures" (mean ± S.E.). In each assay, two spleens from one male and one female were tested. The activities in the cell extracts from three independent assays are shown.

| Glycosyltransferase                        | Control       | Lck-mC2GnT     |
|-------------------------------------------|---------------|---------------|
| UDP-GlcNAc:Galβ1→3GalNAcβ-1,6-GlcNAc   | T cell 2.1 ± 0.3 | 116.9 ± 9.2  |
| transferase                               | B cell 1.2 ± 0.2 | 1.3 ± 0.09   |
| UDP-Gal:GalNAcβ-1,3-Gal transferase      | T cell 0.051 ± 0.002 | 0.042 ± 0.002 |
|                                           | B cell 0.019 ± 0.003 | 0.018 ± 0.005 |
| UDP-Gal:GalNAcβ-1,4-Gal transferase      | T cell 14.0 ± 0.6 | 14.9 ± 1.2    |
|                                           | B cell 6.6 ± 0.3  | 6.4 ± 0.7     |

**Fig. 1.** Mouse C2GnT overexpression resulted in a change in glycosylation of T cell surface glycoproteins. The expression of a CD44-hexasaccharide form carrying core 2 O-glycans in spleen and lymph node was analyzed by flow cytometry. Spleen and lymph node cells were stained with PE-anti-CD43-hexasaccharide form carrying core 2 O-glycans, increased in both spleens and lymph nodes derived from the transgenic mice (Fig. 1). In contrast, the number of B cells, which are positive for B220 staining, was similar in control (49–52%) and the transgenic (47–50%) mice (Fig. 1).

Fig. 2 shows the results of Western blotting of T cell extracts. On the T cells from control mice, most of CD43 was the 115-kDa tetrasaccharide form, while on T cells from Lck-mC2GnT transgenic mice, most CD43 was the 130-kDa hexasaccharide form and can be detected by the 1B11 antibody (34). Previously, we have shown that CD43 expressing the hexasaccharide was increased in the whole cell populations of the transgenic mice (20). As shown in Fig. 1, the population of T cells (αβ T cell receptor positive cells) displaying the CD43-hexasaccharide, which carries core 2 O-glycans, increased in both spleens and lymph nodes derived from the transgenic mice (Fig. 1). In contrast, the number of B cells, which are positive for B220 staining, was similar in control (49–52%) and the transgenic (47–50%) mice (Fig. 1).

Fig. 2 shows the results of Western blotting of T cell extracts. On the T cells from control mice, most of CD43 was the 115-kDa tetrasaccharide form, while on T cells from Lck-mC2GnT transgenic mice, most CD43 was the 130-kDa hexasaccharide form carrying core 2 O-glycans (Fig. 2A). Similar results were obtained for other O-glycosylated proteins such as CD44 and CD45RB. CD44 was detected as an 85-kDa protein on T cells from control mice, whereas T cells from the transgenic mice displayed CD44 as an 88-kDa band (Fig. 2A). CD45RB is an isoform of CD45. Western blotting using a monoclonal antibody, 23G2, identified CD45RB as a 200-kDa band on T cells from control mice, whereas on T cells from the transgenic mice, this 200-kDa band was faint (Fig. 2A). The antibody 23G2 reacts with a peptide portion that is heavily glycosylated with O-glycans (35, 36). It is likely that 23G2 cannot react efficiently with the same polypeptide when it is glycosylated with core 2 O-glycan containing oligosaccharides. In fact, the activity of 23G2 was weaker against CD45 expressed on CHO-leu-C2 cells, which express core 2 O-glycans (27), than it was against the parent CHO cells, which do not express core 2 O-glycans (lanes 5 and 6 in Fig. 2B). In contrast, almost equal reactivity was observed when another antibody, 30F-11, which reacts with all isoforms of CD45 (28), was used (lanes 3 and 4 in Fig. 2B). On the other hand, mouse C2GnT overexpression did not affect CD2, because CD2 does not contain O-glycans (Fig. 2A).

**Fig. 3** shows the results of Western blotting of C2GnT-overexpressing cell lines (37). These results strongly suggest that overexpression of mouse C2GnT in Lck-mC2GnT transgenic mice causes these T cell surface glycoproteins to carry substantially more core 2 O-glycans than do glycoproteins derived from control mice. These observations are consistent with the results obtained by Western blotting of C2GnT-overexpressing cell lines (37).

**Table II**

**No Difference in CD40 Ligand and CD40 Expression Levels between Lck-mC2GnT Transgenic and Control Mice**—We then asked whether overexpression of core 2 O-glycans affects T-B interaction. To address this question, we first examined whether overexpression of core 2 O-glycans affects expression levels of CD40 ligand (CD40L), since T-B interaction is mainly mediated by CD40L-CD40 interaction (26, 27, 38–40). When T cells were incubated without activators, no CD40L expression was observed in either transgenic or control mice (Fig. 3A, top panel). When T cells were activated for 6 h with anti-CD3 and mouse ICAM-1-IgG fusion protein, T cells from the transgenic mice expressed CD40L on the cell surface at a level equivalent to that of control mice (Fig. 3A, middle panel, 13–17% and 13–15%, respectively). T cells activated with PMA and ionomycin from either the transgenic or control mice expressed CD40L on the cell surface at a level equivalent to that of control mice (Fig. 3A, bottom panel, 13–17% and 13–15%, respectively). T cells activated with PMA and ionomycin from either the transgenic or control mice expressed similar amounts of CD40L as well (Fig. 3A, bottom panel, 64–69% and 63–69%, respectively). These results show that similar amounts of CD40L were induced in vitro activated CD4+ T cells derived from the transgenic mice and control mice (Fig. 3A). In addition, B cells purified from both the transgenic and control mice expressed comparable amounts of CD40 (Fig. 3B).
Activated T cells from Lck-mC2GnT transgenic mice inefficiently induce B cell proliferation compared with control—To further examine whether overexpression of core 2 O-glycans affects T-B interaction, we assayed T cell-dependent B cell proliferation in vitro. This assay is critical because CD40-CD40L interaction is too weak to be detected by adhesion (41). T cells were activated for 6 h with anti-mouse CD3 and a mouse ICAM-1-IgG fusion protein shown in Fig. 2A. T cells from the transgenic mice (closed symbols) were cocultured with activated T cells from control mice (shown as C) or the transgenic mice (shown as Lck). B cell proliferation was induced more by activated T cells from wild-type mice than by those from the transgenic mice (shown as Lck). The proliferative responses of B cells from the transgenic mice were enhanced by addition of IL-4. However, T cells derived from the transgenic mice yielded a reduced proliferative response even in the presence of IL-4 (Fig. 2A).

Similarly, B cells derived from control mice were cocultured with T cells from the transgenic and control mice (open symbols in Fig. 2A). T cells derived from the transgenic mice produced a reduced proliferative response of control B cells both in the absence and presence of IL-4 (Fig. 2A). These results indicate that T cells derived from the transgenic mice induced an impaired T-B interaction, regardless of whether the B cells were derived from wild-type or the transgenic mice.

In contrast, B cells from the control and transgenic mice showed comparable proliferative responses to lipopolysaccharide, to lipopolysaccharide plus IL-4, or to anti-CD40 treatment (Fig. 2B), where T-B cell interaction was not required. These results indicate that overexpression of core 2 O-glycans on T cell surface glycoproteins inhibits T-B interaction.

Isotype switching is impaired in Lck-mC2GnT transgenic mice—To investigate how overexpression of core 2 O-glycans in T cells influences antibody production, we tested primary and secondary antibody responses of the transgenic mice. IgM antibody is produced in primary immune responses, and production switches to other immunoglobulin subtypes, such as IgG, in secondary responses. As shown in Fig. 3, the production of anti-KLH IgG1, IgG2a, and IgG2b responses was significantly lower in Lck-mC2GnT transgenic mice (closed symbols) than in control mice (open symbols). In addition, the amount of IgM product was higher in transgenic mice than in the control mice.
due to impaired switching to other immunoglobulin isotypes (Fig. 5). The production of anti-KLH IgG3 and IgA in the transgenic mice was similar to that of control mice. These results, as a whole, indicate that isotype switching was impaired in Lck-mC2GnT transgenic mice.

**Impaired Germinal Center Formation in Lck-mC2GnT Transgenic Mice**—Germinal center formation is dependent on the interaction between CD40L and CD40 (30, 31, 42). We thus tested if germinal center formation in Lck-mC2GnT transgenic mice is reduced, since CD40L-CD40 interaction is impaired in transgenic mice as shown in Fig. 4A. Mice were immunized with KLH on day 0. Spleens from immunized mice were taken on day 7, the peak time of germinal center formation. Germinal centers from control mice detected by either anti-IgM antibody or peanut agglutinin were larger and denser than those from the transgenic mice (Fig. 6). Moreover, significantly fewer germinal centers were found in the transgenic mice than in controls (Table II). There are no obvious differences in numbers and ratios of T and B cells between the transgenic and control mice (Fig. 1). These observations are consistent with the phenotypes of CD40L-deficient or CD40-deficient mice. Mice carrying null mutations in either gene do not display abnormalities in the number and ratios of T and B cells, although germinal center formation is defective in both mice (30, 31, 42).

**Fig. 4.** Activated T cells from Lck-mC2GnT transgenic mice inefficiently induce B cell proliferation. A, CD4+ T cells were purified from spleens, derived from control mouse (C) or the transgenic mouse (Lck), activated with anti-CD3 and mouse ICAM-1-IgG, and fixed with paraformaldehyde. B cells, purified from control mice (open symbols) or the transgenic mice (closed symbols), were cocultured with activated CD4+ T cells for 72 h in 96-well tissue culture plates with or without exogenous IL-4 (10 ng/ml). B, purified B cells were cultured for 72 h with or without addition of lipopolysaccharide (LPS, 1 μg/ml), lipopolysaccharide plus IL-4 (10 ng/ml), or anti-CD40. Each symbol indicates a different set of experiments using T and B cells derived from a pool of three spleens. Bars indicate the average numbers. The experiments were independently performed three times using two different transgenic lines (Lck-mC2GnT-1 and -2), and representative results in one experiment are shown.
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Fig. 5. Isotype switching is impaired in Lck-mC2GnT transgenic mice. Mice were immunized with KLH in complete Freund’s adjuvant (100 μg, intraperitoneally), boosted 14 days later, and bled at the indicated time after the first injection. Mouse anti-KLH IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA levels were determined by isotype-specific ELISA. Sera were diluted 1:10,000 to assay IgM and IgG1 and diluted 1:1000 to assay IgG2a, IgG2b, IgG3, and IgA. Open symbols, control mice; closed symbols, Lck-mC2GnT transgenic mice. Each symbol represents three different sets of experiments using three different controls and transgenic mice. The experiments were independently repeated three times using offspring derived from Lck-mC2GnT-1 (four females and three males) and Lck-mC2GnT-2 (two females), respectively. A representative result from one experiment is shown here. The differences observed in the amount of IgM at 7 days and IgG1, IgG2a, and IgG2b at 20 days obtained in three separate experiments were statistically significant (p < 0.05).

DISCUSSION

This study demonstrated that B cells derived from the transgenic mice overexpressing core 2 oligosaccharides in T cells exhibit reduced humoral immune responses to thymus-dependent antigen, which depend on T-B interaction. B cells derived from the transgenic mice exhibited delayed immunoglobulin isotype switching (Fig. 5) and impaired germinal center formation (Fig. 6). Moreover, T cells derived from the transgenic mice inefficiently stimulated B cell proliferation, regardless of whether B cells were derived from wild-type or the transgenic mice (Fig. 4A). Overexpression of core 2 O-glycans on various surface glycoproteins of T cells apparently renders T-B interaction inefficient, resulting in reduced humoral immune responses in B cells.

B cell proliferation is induced by T-B interaction, which is mainly mediated by CD40L and CD40 (29–31, 38–40). Binding of CD40L, a member of the tumor necrosis factor gene family, to CD40, a protein related to tumor necrosis factor receptor, is thought to trigger signal transduction in B cells (43). A soluble CD40-IgG fusion protein was shown to reduce T cell-dependent B cell proliferation by inhibiting the interaction between T and B cells (39). No significant difference was detected in the expression levels of CD40L and CD40 induced in vitro on CD4+ T cells derived from the transgenic and control mice (Fig. 3). This observation was supported by the fact that anti-CD40 antibody treatment induced almost identical proliferation responses in B cells from the transgenic and control mice (Fig. 4B). CD40L expression is very rapid and transient. CD40L expression reaches maximal levels 6–8 h after activation and then returns to resting levels in 24 h (40). In contrast, B cell proliferation stimulated by T cells from the transgenic mice, assayed after a 72-h incubation, was decreased compared with that stimulated by T cells from control mice (Fig. 4A). CD40L contains few if any O-glycans (44). Core 2 O-glycans overexpressed on various glycoproteins on the T cell surface interfere with the interaction between T and B cells most likely in later stages, resulting in reduced T cell-dependent B cell proliferation (Fig. 4). These results, taken together, suggest that T cell activation in early stages is not impaired, but T-B interaction in later stages, which may be mediated by other than CD40-CD40L interaction, is impaired in the transgenic mice.

Previously, CD40L-deficient mice were shown to lack completely the ability to undergo immunoglobulin isotype switching in response to KLH (31). Similarly, B cells from CD40-deficient mice were unable to undergo isotype switching in response to thymus-dependent antigens (30, 42), and no germinal centers were found in CD40L- or CD40-deficient mice (30, 31, 42). In CD40L- and CD40-deficient mice, the impairment in immune responses such as isotype switching or germinal center formation is complete. In contrast, delayed immunoglobulin isotype switching and reduced formation of germinal centers were observed in the transgenic mice overexpressing core 2 O-glycans (Figs. 5 and 6). The results strongly suggest that the overexpression of core 2 O-glycans interferes with the interaction between T and B cells and CD40L-CD40 interaction is reduced but not abolished in the transgenic mice.

IL-4 and IFN-γ produced in activated T cells also play critical roles in B cell differentiation and immunoglobulin isotype switching (41). In Lck-mC2GnT transgenic mice, both in vitro and in vivo T cell activation was reduced, resulting in reduction of IL-4 and IFN-γ production (20). However, the difference in T cell-dependent B cell proliferation between the transgenic and control mice was observed even after addition of IL-4 (Fig. 4A). We conclude that overexpression of core 2 O-glycans on T cell surface glycoproteins results in impaired T-B cell interaction and reduced production of cytokines, leading to impaired humoral immune responses in Lck-mC2GnT transgenic mice.

The results obtained in this present study are consistent with those of other reports demonstrating the effect of glycosylation on cell-cell interaction. In particular, cell adhesion of A375 melanoma cells was disrupted after cells were induced to express ASGP-1 mucin glycoprotein, a protein similar to leukosialin (CD43) (45). In contrast, it was reported that T cells derived from the N-acetylgalactosaminyltransferase V null mice produced hyperimmune responses to T cell mitogens (46). Since the side chain synthesized by N-acetylgalactosaminyltransferase V is further modified to produce poly-N-acetyllactosamines, the results strongly suggest that bulky N-glycans are essential to weaken unnecessary hyperimmune responses. These, and the findings presented here, indicate that the complexity and amount of O-glycans and N-glycans play critical roles in cell adhesion.

Leukocytes from patients with immunodeficient syndromes such as Wiskott-Aldrich syndrome (WAS) (13, 14, 47) and AIDS (16–18) show increased activity of C2GnT and levels of core 2 O-glycans. C2GnT activity of peripheral blood lymphocytes from WAS patients (0.4 nmol/h/mg of protein) is 8-fold higher than that of normal individuals (0.05 nmol/h/mg of protein).
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![Diagram](image129x610 to 475x729)

**Fig. 6. Germinat center formation is impaired in Lck-mC2GnT transgenic mice.** Spleen flat sections were prepared from control mice (A–D) and the transgenic mice (E–H) 7 days after injection of KLH. The sections were stained with biotinylated peanut agglutinin (PNA), followed by PE-streptavidin (A, C, E, and G). Parallel sections were stained with biotinylated anti-mouse IgM (anti-IgM), followed by PE-streptavidin. The slides were evaluated by a Zeiss Axioplan fluorescence microscope and photographed. Bar = 50 μm.

**TABLE II**
Number of germinal centers in spleens from control and Lck-mC2GnT transgenic mice.

|                     | Control | Lck-mC2GnT |
|---------------------|---------|------------|
| Number of germinal centers (per section) | 24.8 ± 3.4* | 13.5 ± 3.5* |

(13). This difference is similar to that seen in peripheral blood lymphocytes of the Lck-mC2GnT transgenic mice (0.46 nmol/h/mg of protein) compared with control mice (0.09 nmol/h/mg of protein) (20). Moreover, it has been shown that immunoglobulin isotype switching and IL-4 and INF-γ production are impaired in WAS patients (48, 49). These results suggest that overexpression of core 2 O-glycans on cell surface glycoproteins may be a cause of impaired B cell immune responses in WAS patients. The gene defective in WAS has been identified (50), and that gene product interacts with both the cytoskeleton and intracellular signaling molecules (51). Our preliminary studies indicate that introduction of a normal WAS gene into lymphocytes from WAS patients results in dramatically reduced levels of core 2 oligosaccharides.2 In future studies, it will be important to determine whether the mutated WAS gene promotes constitutive activation of the C2GnT gene, resulting in aberrant expression of core 2 O-glycans in T cells of those patients.

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