Branched O-linked oligosaccharides ectopically expressed in transgenic mice reduce primary T-cell immune responses

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Core 2 β -1,6-*N*-acetylglucosaminyltransferase, C2GnT, is a key enzyme in O-linked oligosaccharide (O-glycan) biosynthesis and the resultant core 2 branch serves as a backbone for additional glycosylation to form oligosaccharide ligands such as sialyl Le^x. Since the expression of C2GnT is highly regulated during T-cell development and increases in pathological conditions such as the Wiskott–Aldrich syndrome, we have generated transgenic mice overexpressing C2GnT in the T-cell lineage. Surprisingly, T lymphocytes in the transgenic mice develop normally, but they exhibit a reduced immune response when assayed by delayed-type hypersensitivity, proliferation upon stimulation and cytokine production. Moreover, T lymphocytes from the transgenic mice adhere much less efficiently to ICAM-1 and fibronectin than do T lymphocytes from non-transgenic mice. These results indicate that overexpression of the core 2 branched O-glycans in T lymphocytes results in reduced immune responses due to impaired cell–cell interaction. Such an impaired immune response may be one of the causes for immunodeficiency in the Wiskott–Aldrich syndrome.

Keywords: immunodeficiency/*N*-acetylglucosaminyltransferase/O-glycan/T-cell response/Wiskott–Aldrich syndrome

Introduction

Glycoconjugates are major components of the cell surface of mammalian cells, and their carbohydrate structure changes dramatically during development. Specific sets of carbohydrates are characteristic of different stages of differentiation, and distinct carbohydrates are expressed in tissue- and cell-specific manners in both developing and mature organisms (Feizi, 1985). Aberrations in these cell-surface carbohydrates are often associated with malignant transformation and other pathological conditions including immunological deficiency (Hakomori, 1984; Fukuda, 1996; Rademacher *et al.*, 1988).

Among various cell surface carbohydrates, the functional significance of O-glycans is not well understood; however, several reports suggest that these molecules are involved in the modulation of adhesive processes. O-glycans expressed on eggs were shown to be a receptor for sperm in both mouse (Florman and Wassarman, 1988) and sea urchin (Kitazume-Kawaguchi *et al.*, 1997). The P- and L-selectins bind with higher affinity to sialyl Le^x

present in O-glycans than in N-glycans (Lasky *et al.*, 1992; Sako *et al.*, 1993). This higher affinity binding may be the result of ligand multimers binding to multimeric O-glycans.

In O-glycans, various ligands, including those functional in selectin–carbohydrate interactions, can be formed only in core 2 branched O-glycans, which are synthesized by core 2 β -1,6-*N*-acetylglucosaminyltransferase, C2GnT (Fukuda *et al.*, 1986; Bierhuizen and Fukuda, 1992). In T-cell development, the synthesis of the core 2 branch is restricted to cortical thymocytes, and core 2 branched oligosaccharides are no longer synthesized in medullary thymocytes (Baum *et al.*, 1995). This switching off of core 2 branchings is critical for proper apoptotic process in T-cell development, and continuous expression of the O-glycans apparently inhibits apoptosis occurring during thymocyte development (Perillo *et al.*, 1995). In peripheral T lymphocytes, quiescent T cells express exclusively simpler O-glycans while core 2 branched O-glycans appear only when T cells are activated by mitogens (Piller *et al.*, 1988). In these instances, it has been shown that the expression of core 2 branched O-glycans and C2GnT is well correlated (see Figure 1).

Expression of core 2 branched oligosaccharides is substantially increased in pathological conditions. In contrast to untransfected cells, rat fibroblasts and mammary carcinoma cells transfected with T24Hras express core 2 branched oligosaccharides as they become metastatic tumors (Yousefi *et al.*, 1991). In patients with immunodeficiency such as the Wiskott–Aldrich syndrome (WAS) (Higgins *et al.*, 1991; Piller *et al.*, 1991), AIDS (Saitoh *et al.*, 1991) and leukemia (Brockhausen *et al.*, 1991; Saitoh *et al.*, 1991), leukocytes in the peripheral blood express a substantial amount of core 2 branched oligosaccharides.

The majority of O-glycans in T lymphocytes is present in CD43 (leukosialin) (Andersson *et al.*, 1978; Carlsson and Fukuda, 1986). Strikingly, antibodies specific to the O-glycans attached to CD43 are produced in patients with AIDS (Ardman *et al.*, 1990). Since antibodies specific to leukosialin induce biochemical and functional changes in T lymphocytes *in vitro* (Mentzer *et al.*, 1987), human antibodies present in patients' blood may have similar effects on thymocytes, resulting in inappropriate activation of thymocytes during the process of maturation (Lefebvre *et al.*, 1994). However, it has not been determined whether auto-antibodies play a role in the pathogenesis of AIDS.

Several studies indicate that CD43 is directly involved in cell–cell interaction, and binding of CD43 to ICAM-1 has been demonstrated (Rosenstein *et al.*, 1991). Moreover, CD43 has been shown to provide a co-stimulation during T-cell activation (Sperling *et al.*, 1995) and to be a T-lymphocyte receptor for binding to high endothelial venules during lymphocyte recruitment to the lymph node

Fig. 1. Structure and biosynthesis of O-glycans attached to CD43. CD43 on resting T cells carries tetrasaccharides (115 kDa glycoform), and CD43 on activated T cells carries branched hexasaccharide, core 2 O-glycans (130 kDa glycoform) (Piller *et al.*, 1988). The expression of the core 2 O-glycan is directed by core2:β-1,6-N-acetylglucosaminyltransferase, C2GnT, which forms a crucial core 2 branch, denoted by boxes. The CD43-130 kDa glycoform carrying the core 2 O-glycans is dominantly expressed in resting T cells from Wiskott-Aldrich syndrome patients (Piller *et al.*, 1991).

(McEvoy *et al.*, 1997). In order to understand how expression of core 2 oligosaccharides in CD43 on T lymphocytes modulates cell-cell interaction, we have generated transgenic mice whose T cells overexpress C2GnT under the control of the T cell-specific *lck* promoter.

Here we show that lymphocytes overexpressing core 2 branched oligosaccharides exhibit reduced primary immune responses when assayed for delayed-type hypersensitivity, T-cell proliferation responses and cytokine production. Such reduced immune responses are associated with a significant decrease in adhesion of those T lymphocytes to ICAM-1 and fibronectin, demonstrating that the reduced immune responses are caused by impaired adhesion of those T lymphocytes expressing branched O-glycans.

Results

Generation of transgenic mice overexpressing core 2 O-glycans on T cells

To overexpress core 2 O-glycans on T-cell surface glycoproteins, we used a well-established transgenic expression vector utilizing the proximal promoter of p56^{lck} (Chaffin *et al.*, 1990; Anderson *et al.*, 1993; Levin *et al.*, 1993; Alberola-Ila *et al.*, 1995; Swan *et al.*, 1995) to target the expression of mouse core 2 β-1,6-N-acetylglucosaminyltransferase (mC2GnT) to the T-cell lineage of transgenic mice (designated Lck-mC2GnT). Figure 2A provides a schematic diagram of the *lck*-mC2GnT expression construct, which includes a 3.2 kb fragment of the *lck* proximal promoter, a 2.0 kb mC2GnT genomic fragment and a 0.8 kb fragment derived from SV40, which provides a polyadenylation signal (Chaffin *et al.*, 1990; Klarsfeld *et al.*, 1991). A 6 kb fragment containing the expression cassette was injected into FVB/N mouse zygote pronuclei using standard techniques.

We established two transgenic mouse lines (Lck-

mC2GnT-1 and -2). Figure 2B shows Southern blots of genomic DNA from those transgenic lines. With a *Bam*HI digestion, the endogenous mC2GnT gene gives a 2.2 kb fragment and the transgene gives a 1.4 kb fragment. Comparison of the transgene signal with that of the endogenous gene indicates that ~10 copies of the transgene were present per mouse genome in both lines (Figure 2). In all of the experiments shown below, almost identical results were obtained using two independently generated transgenic lines. Therefore, we conclude that clonal variations played little or no role in determining the phenotype of either line. In both lines, thymocytes, splenocytes, lymph node cells and peripheral blood lymphocytes from the transgenic mice showed dramatically increased activities of mC2GnT (Table I). However, there was no detectable difference in C2GnT activity between the kidney of the transgenic and control mice, since the *lck* promoter is specific for the T-cell lineage (Table I).

The expression of core 2 O-glycans results in the high molecular weight glycoform of CD43, CD43-130 kDa, while cells expressing CD43 carrying simpler tetrasaccharides exhibit the low molecular weight glycoform, CD43-115 kDa. The two different forms of CD43 can be distinguished by two different monoclonal antibodies specific to each glycoform. As shown in Figure 3A, an increase in the number of cells expressing the CD43-130 kDa glycoform was observed in thymus, spleen, lymph nodes and peripheral blood lymphocytes from the transgenic mice and those expressing CD43-130 kDa glycoform were found to be αβTCR⁺ cells. No increase in the expression of the CD43-130 kDa glycoform was, however, observed in B220⁺ cells (data not shown).

Western blot analysis demonstrated that T lymphocytes derived from the transgenic mice express almost exclusively CD43-130 kDa, while CD43-115 kDa is the dominant form in the leukocytes derived from wild-type mice (Figure 2C). The immunoblot analysis of other O-glycoproteins such as CD44 and CD45RB revealed that

Fig. 2. (A) Diagram of *lck*-mC2GnT transgene. The transgene consists of a 3.2 kb fragment of the *lck* proximal promoter, a 2.0 kb mouse C2GnT gene fragment (containing the entire coding region) and a 0.8 kb fragment of a polyadenylation signal derived from SV40. (B) Southern blots of genomic DNA from Lck-mC2GnT transgenic mice and the control. Arrowhead indicates the 2.2 kb *Bam*HI fragment from the endogenous mC2GnT gene. Arrow indicates a 1.4 kb *Bam*HI fragment from the transgene. The probe used for hybridization is shown in (A). (C) Western blot analysis of thymocyte extracts from Lck-mC2GnT transgenic mice and the control. Monoclonal antibodies to CD43-115 kDa glycoform (S7), CD43-130 kDa glycoform (1B11), CD2 (RM2-5) and CD29 (9EG7) were used.

O-glycans on those proteins were also altered (data not shown). In contrast, no difference in the molecular weight of CD2 and CD29 was noted between wild-type and transgenic mice, in agreement with the fact that they do not contain *O*-glycans (Figure 2C).

In the Lck-mC2GnT transgenic mice, no gross developmental defects were observed and the mice developed and bred normally. We expected that core 2 *O*-glycan overexpression would influence T-cell development, since a decrease in C2GnT expression is associated with the transition from cortex to medulla in the thymus (Baum *et al.*, 1995). However, the number of thymocytes and the CD4 and CD8 subset population were comparable between normal and transgenic mice (Figure 3B). The expression levels of CD3, CD4, CD8, $\alpha\beta$ TCR, CD44 and CD45RB

Table I. Core 2 β -1,6-*N*-acetylglucosaminyltransferase (C2GnT) activity^a

Tissue	Control	Lck-mC2GnT
Thymus	0.263 $\pm$ 0.049	40.9 $\pm$ 10.9
Spleen	1.66 $\pm$ 0.12	14.5 $\pm$ 1.4
Lymph node	0.202 $\pm$ 0.041	3.66 $\pm$ 0.070
Peripheral blood lymphocytes	0.094 $\pm$ 0.027	0.460 $\pm$ 0.027
Kidney	8.26 $\pm$ 0.37	8.97 $\pm$ 0.075

^aC2GnT activities (nmol/h/mg) in crude extracts from tissues were measured as described in Materials and methods.

in thymi and spleens from the Lck-mC2GnT transgenic mice were also comparable with those of wild-type littermates (data not shown). We conclude that overexpression of core 2 *O*-glycans on T-cell surface glycoproteins did not affect thymocyte development.

***Lck*-mC2GnT transgenic mice showed reduced primary T-cell responses**

We then examined whether overexpression of core 2 *O*-glycans on T-cell surface glycoproteins in the transgenic mice affected T-cell function *in vivo*. In order to test whether primary T-cell responses were reduced in the transgenic mice, the mice were immunized with keyhole limpet hemocyanin (KLH). At 7 days after the immunization, mice were re-challenged with PBS and KLH in the right and left ears, respectively. After 24 h, the swelling of each ear was measured, and the delayed-type hypersensitivity (DTH) response was measured as ear swelling. The DTH responses in the transgenic mice were much reduced when compared with the control mice (Figure 4A and B).

The DTH response is a complex reaction that involves several steps. One possible mechanism that could account for the reduced DTH response in the transgenic mice was reduced T-cell activation, since molecules which bear core 2 *O*-glycans, such as CD43, CD44 and CD45, have been shown to participate in cell-cell interactions during T-cell activation (Rosenstein *et al.*, 1991; Chui *et al.*, 1994; Sommer *et al.*, 1995; Sperling *et al.*, 1995). In order to examine whether T-cell activation is impaired in the transgenic mice, draining lymph node cells from mice already primed with KLH were re-stimulated with KLH *in vitro*. KLH-specific T-cell proliferation was reduced in those T cells derived from the transgenic mice (Figure 5A). In parallel, we measured IL-2, IFN- γ and IL-4 production. As shown in Figure 5C-E, a substantial decrease in the production of IL-2, IFN- γ and IL-4 was observed in CD4⁺ T cells of transgenic mice compared with those from wild-type mice. In contrast, there was no significant difference in the response when T cells were stimulated with mitogens such as phorbol-12-myristyl-13-acetate (PMA) and ionomycin, which do not require cell-cell interaction for activation (Figure 5B). Taken together, these results indicate that CD4⁺ T cells in the draining lymph nodes from the Lck-mC2GnT transgenic mice were poorly activated. We suggest that this impaired activation leads to a reduced primary T-cell response.

Reduced proliferative responses of T lymphocytes from Lck-mC2GnT transgenic mice

The results obtained in the above *in vivo* and *in vitro* assays showed a reduction in the level of T-cell activation

Fig. 3. Phenotypical analysis of thymocytes, splenocytes, lymph node cells and peripheral blood lymphocytes in Lck-mC2GnT transgenic mice. (A) CD43-130 kDa glycoform expression. T cells from Lck-mC2GnT transgenic mice express CD43-130 kDa glycoform carrying core 2 O-glycans at high levels. Open histograms indicate the control. Closed ones indicate the Lck-mC2GnT transgenic mice. The numbers indicate the percentage of the total cells that express the CD43-130 kDa glycoform. (B) CD4 and CD8 expression. CD4 and CD8 expression on cells isolated from Lck-mC2GnT transgenic mice were comparable with those of their control wild-type littermates.

in response to an antigen in the transgenic mice. To further examine the differences in T-cell activation, we tested T cells for the ability to proliferate in response to T-cell activators. T-cell activation requires two distinct signals. The first signal is delivered through T-cell receptor-antigen interaction. The second signal, called a co-stimulatory signal, is delivered through other receptor-ligand

interactions, such as ICAM-1-leukocyte function-associated antigen-1 (LFA-1) and CD80-CD28 (Linsley and Ledbetter, 1993). We examined these interactions *in vitro* by incubating T cells from wild-type and transgenic mice on plates coated with anti-CD3 antibody and mouse ICAM-1-immunoglobulin G fusion protein (mICAM-1-IgG) to serve as a co-stimulatory molecule (Damle *et al.*,

Fig. 4. Lck-mC2GnT transgenic mice showed reduced delayed-type hypersensitivity (DTH). (A) Mice were sensitized with KLH. Seven days later, PBS and KLH were injected into the right and left ears, respectively. After 24 h, the thickness of ear swellings was measured. (B) ΔT^* = (ear thickness 24 h after re-stimulation with KLH) – (ear thickness 24 h after re-stimulation with PBS). The difference between sensitized control mice and Lck-mC2GnT transgenic mice is significant ($P < 0.0001$). In (B), four results were obtained from one transgenic mouse line and five results were obtained from another line, and five representative results out of these nine results were shown in (A). Each symbol represents a different mouse.

1992). T cells from the Lck-mC2GnT transgenic mice showed a reduced proliferative response compared with those from control mice, at various concentrations of mICAM-1 (Figure 6A). Those T cells from the transgenic mice or control mice did not proliferate on the plates coated with mICAM-1 alone (data not shown). In addition, we measured IL-2 production by T cells upon this stimulation. T cells from the transgenic mice produced a reduced amount of IL-2 compared with the control (Figure 6B). The reduced proliferation of T cells from the transgenic mice was restored by the addition of exogenous recombinant mouse IL-2 (Figure 6C, left). When T cells from wild-type and transgenic mice were activated with mito-

Fig. 5. Antigen-specific T-cell proliferative responses. (A) Mice were immunized with Keyhole Limpet Hemocyanin (KLH). Ten days later, the draining lymph node cells were prepared from the mice, and cells were then re-stimulated with KLH at 0, 0.1, 1.0 and 10 μg/ml. Recall proliferative responses of draining lymph node cells were measured by addition of 1 μCi of [³H]thymidine (³H-TdR; NEN, Boston, MA) per well for the final 6 h of a 96-h culture. (B) Lymph node cells were activated with PMA (10 ng/ml) and ionomycin (0.5 μg/ml). (C, D and E) IL-2 (C), IFN-γ (D) and IL-4 (E) production by the purified CD4⁺ T cells re-stimulated with 10 μg/ml of KLH. All experiments were done three times in triplicate and the results were expressed as the mean ± S.E. Open bars, control; closed bars, Lck-mC2GnT.

gens such as PMA and ionomycin, there was no significant difference between their proliferative responses (Figure 6C, right). The latter result indicates that T cells from the transgenic mice proliferate well if the stimulation signals bypass signal transduction through the T-cell receptor and co-stimulatory molecules. These results strongly suggest that stimulatory signals delivered through the interaction of T-cell surface receptors with cognate ligands are less efficient in the transgenic mice compared with the control mice, and that this inefficient signal transduction results in reduced T-cell activation in the Lck-mC2GnT transgenic mice. No difference was observed in the morphology of resting and blast T cells between control mice and the transgenic mice (Figure 6D).

Reduced adhesion of T cells from Lck-mC2 transgenic mice to ICAM-1 and fibronectin

To examine further the interaction of cell surface receptors on T cells with cognate ligands, we compared the adhesion of T cells from transgenic and wild-type mice with purified ligands, ICAM-1 and fibronectin, which are recognized by the T-cell surface receptors LFA-1 and VLA-5, respectively (Dustin and Springer, 1991; Ruoslahti, 1996). As shown in Figure 7, T cells from transgenic mice bound much less efficiently to ICAM-1 and fibronectin compared with

Fig. 6. Reduced proliferative response of T-lymphocytes from Lck-mC2GnT transgenic mice. (A) Mouse ICAM-1-IgG fusion proteins at various concentrations were co-immobilized with anti-CD3 (145-2C11, 0.1 $\mu\text{g/ml}$). Purified T cells were stimulated with anti-CD3 and mouse ICAM-1-IgG coated on plates. (B) IL-2 production by T cells stimulated with anti-CD3 (0.1 $\mu\text{g/ml}$) and mouse ICAM-1-hIgG (1 $\mu\text{g/ml}$) was measured using an ELISA kit from PharMingen. (C) Purified T cells were activated with PMA (10 ng/ml) and ionomycin (0.5 $\mu\text{g/ml}$). In parallel, 10 ng/ml of recombinant mouse IL-2 (PharMingen) was added to the culture of T cells from Lck-mC2GnT transgenic mice, then the proliferative response was assayed. Cell proliferation was measured by addition of [^3H]thymidine for the final 6 h of a 72 h incubation. All experiments were done three times in triplicate and the results were expressed as the mean $\pm$ S.E. (D) Purified T cells were stimulated with anti-CD3 and mouse ICAM-1-IgG (1 $\mu\text{g/ml}$) coated on plates. Cells were photographed after 72 h incubation. Bar = 20 μm .

T cells from control mice. Adhesion to ICAM-1 could be inhibited by preincubation of T cells with anti-LFA-1 antibody, but not by isotype-matched control antibody. These results indicate that T lymphocytes overexpressing

core 2 *O*-glycans adhered much less to ICAM-1 and fibronectin. The adhesion to ICAM-1 could not be inhibited by preincubation of T cells with antibodies specific to CD43-115 kDa glycoform (S7) or CD43-130 kDa glyco-

Fig. 7. Reduced adhesion of T cells from Lck-mC2GnT transgenic mice to ICAM-1 and fibronectin. Purified T cells were labeled with [35 S]methionine, washed and resuspended at 5×10^5 cell per well in adhesion buffer (RPMI 1640 with 5% FCS) containing 50 ng/ml of PMA. Microtiter plates were prepared by coating overnight at 4°C with mouse ICAM-1-IgG, murine fibronectin or human IgG at 10 μ g/ml. T cells (5×10^4 per well) labeled with [35 S]methionine were incubated in the microtiter wells coated with the purified ligands at 37°C for 15 min, then unbound cells were removed by washing with adhesion buffer. In parallel, T cells were incubated with anti-mouse LFA-1 (M17/4), anti-mouse CD43-115 kDa glycoform (S7), anti-mouse CD43-130 kDa glycoform (1B11) or anti-mouse E-selectin (rat IgG_{2a}) as an isotype-matched control before adding to the ligand-coated plates. All assays were done three times in triplicate and expressed as the mean $\pm$ S.E.

form (1B11) (Figure 7), indicating that CD43 is not directly involved in the adhesion to ICAM-1.

Discussion

In the present study, we have generated transgenic mice expressing C2GnT under the control of the T-cell lineage specific *lck* promoter. T lymphocytes derived from transgenic mice express substantially increased amounts of C2GnT. In contrast, the level of C2GnT activity in the kidneys of the transgenic mice was essentially the same as that found in control mice. As a result, T-cell surface glycoproteins were decorated by bulky core 2 branched *O*-glycans and those T cells adhered much less to ICAM-1 and fibronectin, compared with T cells derived from wild-type mice. LFA-1 and VLA-5, counter-receptors for ICAM-1 and fibronectin, contain few if any *O*-glycans (Sastre *et al.*, 1986). Moreover, integrins which lack hybrid- and complex-type *N*-glycans were shown to be fully functional (Koyama and Hughes, 1992). Thus, it is apparent that T-cell adhesion mediated by integrins is not dependent on complexity of carbohydrate. The present results strongly suggest that overexpression of core 2 *O*-glycans on CD43 and other molecules interferes with adhesion of LFA-1 and VLA-5 with their counter-receptors. Interference with adhesion apparently results in impaired stimulation of T cells as measured by both *in vitro* and *in vivo* assays. On the other hand, neither defects in T-cell development nor changes in T-cell morphology were noted. These results, when combined, indicate that expression of the branched core 2 *O*-glycans on

T cells reduces the T-cell immune response by interfering with the interaction of T cells and antigen-presenting cells.

Increased expression of core 2 *O*-glycans may occur on any T-cell glycoproteins containing *O*-glycans in the transgenic mice. However, CD43 may be more crucial for modulation of T-cell adhesion to ligands than other glycoproteins on the T-cell surface, because CD43 molecules are present in large numbers ($\sim 3 \times 10^6$ per cell) on the cell surface (Carlsson and Fukuda, 1986) and extend far from the cell surface (Cyster *et al.*, 1991). In addition, CD43 is thought to be involved in the initial interaction of T cells with other cells (for example, antigen-presenting cells) (Rosenstein *et al.*, 1991), and was reported to be a T-cell co-stimulatory receptor (Sperling *et al.*, 1995). Leukocytes from CD43-deficient mice showed stronger adhesion to ICAM-1 (Manjunath *et al.*, 1995), while leukocytes from CD18-deficient patients showed no binding to ICAM-1 (Marlin and Springer, 1987). In addition, anti-LFA-1 antibody blocked binding of T cells to ICAM-1 almost completely, while anti-CD43 antibodies did not block adhesion to ICAM-1 (Figure 7). These results suggest that CD43 may be involved only in the initial binding of T cells to antigen-presenting cells, but eventually this binding is replaced by other counter-receptors such as LFA-1 to attain firm adhesion. As CD43 contains a large number (~ 80) of *O*-glycans per molecule (Carlsson and Fukuda 1986; Fukuda, 1991), it is likely that bulkier *O*-glycans such as core 2 *O*-glycans interfere with the initial interaction between CD43 and ICAM-1.

In later events during T-cell stimulation, overexpression of core 2 *O*-glycans on CD43 as well as the other glycoproteins containing *O*-glycans probably impedes interactions between T cells and antigen-presenting cells (Figure 7). This apparently results in poor activation of T cells (Figure 6), as exemplified by lower production of cytokines (Figures 5 and 6) and reduced T-cell proliferation and DTH response (Figures 4 and 5).

Our results are consistent with those reported on mice which are deficient in various adhesion molecules through gene knock-out (Sharpe, 1995). ICAM-1-deficient mice have been reported to exhibit reduced DTH response because of impaired interaction of T cells with antigen-presenting cells (Sligh *et al.*, 1993; Xu *et al.*, 1994), and mutant mice which have 2–16% of wild-type levels of CD18 expression have been reported to exhibit an impaired inflammatory response and delayed rejection of cardiac allografts because of impaired interaction between LFA-1 (CD11a/CD18) and ICAM-1 (Wilson *et al.*, 1993). Similarly, proliferation of T cells responding to antigen-presenting cells was markedly reduced in CD28-deficient mice (Green *et al.*, 1994). Immunosuppression can be also achieved by administering a soluble form of CTLA4, a homolog of CD28 (Linsley *et al.*, 1992; Lin *et al.*, 1993). These results indicate that interference with the T cell–antigen-presenting cell interaction through those co-stimulatory molecules leads to reduced T-cell primary responses. These results suggest that soluble CD43 containing core 2 *O*-glycans may be clinically applicable for suppressing unnecessary inflammatory responses.

As described above, leukocytes from patients with immunodeficient syndromes such as WAS (Remold-O'Donnel *et al.*, 1984; Higgins *et al.*, 1991; Piller *et al.*, 1991) and AIDS (Saitoh *et al.*, 1991) show an increased

activity of C2GnT and increased expression of the core 2 O-glycans. C2GnT activity of the peripheral blood lymphocytes from WAS patients (0.4 nmol/h/mg) is 8-fold higher than that of normal individuals (0.05 nmol/h/mg) (Piller *et al.*, 1991). This C2GnT activity and its difference between WAS patients and normal individuals are similar to those found in peripheral blood lymphocytes of the Lck-mC2GnT transgenic mice and the control (Table I). Moreover, T cells of WAS patients showed reduced proliferation in response to immobilized anti-CD3 antibody and produced much less IL-2 (Molina *et al.*, 1993), which is similar to the T-cell phenotypes seen in the transgenic mice (Figure 6). Based on our present study, it is tempting to speculate that aberrant expression of core 2 O-glycans on T cells in these patients reduces primary T-cell responses, resulting in immunodeficiency. The gene defective in WAS has been identified (Derry *et al.*, 1994), and the protein made by the gene interacts with both the cytoskeleton and intracellular signaling systems (Symons *et al.*, 1996). Future studies will be of significance to determine if the mutated WAS gene in those patients causes constitutive activation of the gene encoding C2GnT, resulting in overexpression of core 2 O-glycans.

It has been shown that activated T cells also express core 2 O-glycans (Piller *et al.*, 1988). Similarly, the changes in O-glycans attached to mouse CD43 during T-cell activation was detected using *Vicia villosa* lectin (Kimura *et al.*, 1979). It is likely that the expression of core 2 O-glycans is a modulatory mechanism which helps to avoid excess T-cell activation and constitutive proliferation of antigen-specific T cells, thereby regulating the immune response under normal conditions. In relation to this hypothesis, it is noteworthy that in the recently reported CD43 null mice, cytotoxic T-lymphocyte activity was enhanced compared with that in wild-type mice (Manjunath *et al.*, 1995). Our study, however, presents evidence that the glycosylation status of CD43 directly influences the T-cell immune response. Considering that CD43 is the major carrier for O-glycans, the knock-out results can be interpreted that the loss of the core 2 branched O-glycans on CD43 may enhance the immune response. Future studies will be of significance to determine whether that is the case.

Materials and methods

Transgenic mice generation

Mouse C2GnT genomic DNA clones were isolated by standard hybridization techniques using human C2GnT cDNA (Bierhuizen and Fukuda, 1992) as a probe. The Lck-mC2GnT transgene was produced by ligation of a 2.0 kb *PstI*-*XbaI* mouse C2GnT gene fragment (containing the entire coding region) downstream of the p56^{lck} proximal promoter. Purification and injection at 2 ng/ml of the *NotI* fragment containing the Lck-mC2GnT transgene into FVB/N mouse zygote pronuclei was performed. Transgenic founders were detected by hybridization of genomic DNA with the mC2GnT probe, and stable lines of mice were generated by breeding founders with FVB/N mice.

Western blot analysis

Whole-cell lysates of thymocytes from Lck-mC2GnT transgenic mice and the control were prepared by solubilization in 50 mM Tris-HCl buffer, pH 7.5, containing 1% Triton X-100, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride and 1 µg/ml each aprotinin, leupeptin and pepstatin. The lysates were resolved by SDS-PAGE on a 7.5% gel, and transferred to nitrocellulose filters. The filters were incubated with the monoclonal antibodies, anti-CD43-115 kDa (S7, 2 µg/ml), anti-CD43-

130 kDa (1B11, 2 µg/ml) (Barran *et al.*, 1997), anti-CD2 (RM2-5, 0.5 µg/ml) or anti-CD29 (9EG7, 10 µg/ml). These antibodies were purchased from PharMingen (San Diego, CA). The filters were incubated using an anti-rat secondary antibody conjugated to horseradish peroxidase and the ECL detection system (Amersham Life Science Inc., Cleveland, OH).

Core 2 β-1,6-N-acetylglucosaminyltransferase assay

Mouse tissues were homogenized in 0.1–1 ml of extraction solution (0.4% Triton X-100, 150 mM NaCl) with a glass tissue grinder. Extracts were clarified by centrifugation of 16 000 g for 5 min. Clarified supernatants were directly used for assays. The assay was performed essentially as described (Bierhuizen and Fukuda, 1992). The reaction mixture containing 50 mM MES, pH 7.0, 0.5 µCi of UDP-[³H]GlcNAc (NEN) in 1 mM UDP-GlcNAc, 0.1 M GlcNAc, 10 mM Na₂EDTA, 1 mM *p*-nitrophenyl-Galβ1-3GalNAc (Toronto Research Chemicals) as acceptor and 25 µl of crude extracts from the tissues (250 µg protein) was incubated for 1 h at 37°C. The radiolabeled C2GnT reaction product was purified by C₁₈ Sep-Pak (Waters) column chromatography and counted.

Flow cytometry

Monodispersed cells (1×10⁶) from mouse tissues were incubated in a final volume of 200 µl with fluorescent-conjugated antibodies (1 µg/ml) at 4°C for 30 min. Phycoerythrin (PE)-conjugated anti-CD43 activation-associated isoform (1B11, 130 kDa glycoform-specific antibody) was purchased from PharMingen. Anti-CD4-PE (GK1.5) and anti-CD8-fluorescein-isothiocyanate (FITC) (53-6.7) were obtained from Becton-Dickinson Collaborative Biomedical Products (Bedford, MA). All incubations and washes were performed on ice. Analyses were done with a FACScan flow cytometer using the CellQuest program (Becton-Dickinson, San Jose, CA).

In vivo primary T-cell responses

For delayed-type hypersensitivity (DTH) responses, mice were immunized with 100 µg of Keyhole Limpet Hemocyanin (KLH) (Sigma, St Louis, MO) in a 1:1 emulsion with complete Freund's adjuvant (CFA) at the base of the tail. Seven days after immunization, the thickness of both ears was measured with a dial thickness gauge (Mitsutoyo Corp., Tokyo, Japan). 5 µl of PBS and KLH (5 mg/ml) were then injected into the right and left ears, respectively. Ear thickness was measured again 24 h later (Xu *et al.*, 1996). For antigen-specific T-cell responses, Lck-mC2GnT transgenic mice and wild-type mice were injected at the base of the tail with 100 µg of KLH in CFA. After 10 days, the draining lymph nodes were removed and the lymph node cells were cultured in 96-well plates at 5×10⁵ cells per well in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 µg/ml) and 2-mercaptoethanol (50 µM). The cells were re-stimulated with KLH at 0, 0.1, 1.0 and 10 µg/ml, or activated with phorbol-12-myristyl-13-acetate (PMA) (Sigma) at 10 ng/ml and ionomycin (Calbiochem, San Diego, CA) at 0.5 µg/ml. Cell proliferation was measured by addition of 1 µCi of [³H]thymidine (³H-TdR; NEN, Boston, MA) per well for the final 6 h of a 96-h culture (Green *et al.*, 1994).

Cytokine assays

CD4⁺ T cells were purified by passage through a nylon wool column (WAKO, Osaka, Japan) and complement-mediated cytotoxic treatment with anti-B220 and anti-CD8a (53-6.7) antibodies (PharMingen). Splenic cells from unimmunized wild-type mice were treated with 0.5 mg/ml of mitomycin C (Sigma, St Louis, MO) at 37°C for 30 min. Assays for cytokine production by CD4⁺ T cells from KLH-immunized mice were conducted by culturing 2×10⁵ purified CD4⁺ T cells with 5×10⁵ mitomycin C-treated splenic cells and 10 µg/ml of KLH. After 24 h culture, 100 µl of culture supernatant was removed from each well for IL-2 assay. For IFN-γ and IL-4 measurement, supernatants were removed after 4 days of culture. ELISA was used to determine the levels of IL-2, IFN-γ and IL-4 in culture supernatants using a kit from PharMingen (Kamogawa *et al.*, 1993; Xu *et al.*, 1996).

T-cell proliferation assay

T cells from spleens were purified as described above. Microtiter plates were coated overnight at 4°C with anti-CD3 (145-2C11, PharMingen) at 1.0 µg/ml and mouse ICAM-1-human IgG fusion protein at 0, 0.001, 0.01 0.1 and 1 µg/ml. Purified T cells in 100 µl of complete RPMI 1640 medium were cultured in the above microtiter plates (1.2×10⁵ cells per well) for 72 h. Cell proliferation was measured by addition of [³H]thymid-

ine for the final 6 h of a 72 h incubation (Damle *et al.*, 1992). Cells were photographed ($\times 400$ magnification) after 72 h incubation.

In parallel, purified T cells were activated with PMA and ionomycin, and cultured for 72 h. For the preparation of mouse ICAM-1-IgG fusion protein, a cDNA encoding soluble mouse ICAM-1 fragment (Sui *et al.*, 1989) and the human IgG1 hinge plus constant region fragment (Tsuboi *et al.*, 1996) were ligated into pcDNA1 to yield pcDNA1-mouse ICAM-1-IgG. Three days after transfection of COS-1 cells with pcDNA1-mouse ICAM-1-IgG, mouse ICAM-1-IgG fusion protein was purified from the conditioned medium using anti-human IgG-agarose (Sigma).

Adhesion assay

T cells purified from spleens were labeled with [35 S]methionine (NEN), washed in RPMI 1640 medium containing 5% fetal calf serum (adhesion buffer) and resuspended at 5×10^5 cells/ml in adhesion buffer containing PMA at 50 ng/ml. Microtiter plates were coated with mouse ICAM-1-IgG fusion protein, murine fibronectin (Gibco-BRL, Grand Island, NY) or human IgG at 10 μ g/ml. The adhesion assay was carried out as described (Chan and Springer 1992; Manjunath *et al.*, 1995). T cells (5×10^4 per well) labeled with [35 S]methionine were incubated in microtiter wells coated with purified ligands at 37°C for 15 min, then unbound cells were removed by washing with adhesion buffer. For antibody inhibition experiments, cells were incubated with anti-mouse LFA-1 (M17/4), anti-mouse CD43-115 kDa glycoform (S7), anti-mouse CD43-130 kDa glycoform (1B11) or anti-mouse E-selectin (10E9.6) (PharMingen) as an isotype-matched control (rat IgG_{2a}) at room temperature for 10 min before adding to protein-coated plates.

All results described in Table I and Figures 4–7 were expressed as the mean $\pm$ standard error of triplicate experiments, unless otherwise indicated. All experiments were repeated three times and the results of a representative experiment are shown. Wild-type littermates were used in all the experiments as control mice.

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References

- Alberola-Ila, J., Forbush, K.A., Seger, R., Krebs, E.G. and Perlmutter, R.M. (1995) Selective requirement for MAP kinase activation in thymocyte differentiation. *Nature*, **373**, 620–623.
- Anderson, S.J., Levin, S.D. and Perlmutter, R.M. (1993) Protein tyrosine kinase p56lck controls allelic exclusion of T-cell receptor beta-chain genes. *Nature*, **365**, 552–554.
- Andersson, L.C., Gahmberg, C.G., Kimura, A.K. and Wigzel, H. (1978) Activated human T-lymphocytes display new surface glycoproteins. *Proc. Natl Acad. Sci. USA*, **75**, 3455–3458.
- Ardman, B., Sikorski, M.A., Settles, M. and Staunton, D.E. (1990) Human immunodeficiency virus type 1-infected individuals make autoantibodies that bind to CD43 on normal thymic lymphocytes. *J. Exp. Med.*, **172**, 1151–1158.
- Barran, P., Fellinger, W., Warren, C.E., Dennis, J.W. and Ziltener, H.J. (1997) Modification of CD43 and the other lymphocyte O-glycoproteins by core 2 N-acetylglucosaminyltransferase. *Glycobiology*, **7**, 121–136.
- Baum, L.G., Pang, M., Perillo, N.L., Wu, T., Delegeane, A., Uittenbogaart, C.H., Fukuda, M. and Seilhamer, J.J. (1995) Human thymic epithelial cells express an endogenous lectin, galectin-1, which binds to core 2 O-glycans on thymocytes and T lymphoblastoid cells. *J. Exp. Med.*, **181**, 877–887.
- Bierhuizen, M.F. and Fukuda, M. (1992) Expression cloning of a cDNA encoding UDP-GlcNAc: Gal β 1-3-GalNAc-R (GlcNAc to GalNAc) β 1-6GlcNAc transferase by gene transfer into CHO cells expressing polyoma large tumor antigen. *Proc. Natl Acad. Sci. USA*, **89**, 9326–9330.
- Brockhausen, I., Kuhns, W., Schachter, H., Matta, K.L., Sutherland, D.R. and Baker, M.A. (1991) Biosynthesis of O-glycans in leukocytes from normal donors and from patients with leukemia: increase in O-glycan core 2 UDP-GlcNAc: Gal β 3GalNAc α -R(GlcNAc to GalNAc) β (1-6)-N-acetylglucosaminyltransferase in leukemic cells. *Cancer Res.*, **51**, 1257–1263.
- Carlsson, S.R. and Fukuda, M. (1986) Isolation and characterization of leukosialin, a major sialoglycoprotein on human leukocytes. *J. Biol. Chem.*, **261**, 12779–12786.
- Chaffin, K.E., Beals, C.R., Wilkie, T.M., Forbush, K.A., Simon, M.I. and Perlmutter, R.M. (1990) Dissection of thymocyte signalling pathways by *in vivo* expression of pertussis toxin ADP-ribosyltransferase. *EMBO J.*, **9**, 3821–3829.
- Chan, P.-Y. and Springer, T.A. (1992) Effect of lengthening lymphocyte function-associated antigen 3 on adhesion to CD2. *Mol. Biol. Cell*, **3**, 157–166.
- Chui, D., Ong, C.J., Johnson, P., Teh, H.-S. and Marth, J.D. (1994) Specific CD45 isoforms differentially regulate T cell receptor signaling. *EMBO J.*, **13**, 798–807.
- Cyster, J.G., Shotton, D.M. and Williams, A.F. (1991) The dimensions of the T lymphocyte glycoprotein leukosialin and identification of linear protein epitopes that can be modified by glycosylation. *EMBO J.*, **10**, 893–902.
- Damle, N.K., Klussman, K., Linsley, P.S. and Aruffo, A. (1992) Differential costimulatory effects of adhesion molecules B7, ICAM-1, LFA-3 and VCAM-1 on resting and antigen-primed CD4⁺ T lymphocytes. *J. Immunol.*, **148**, 1985–1992.
- Derry, J.M.J., Ochs, H.D. and Francke, U. (1994) Isolation of a novel gene mutated in Wiskott–Aldrich syndrome. *Cell*, **78**, 635–644. [Erratum published in *Cell*, **79** (1994).]
- Dustin, M.L. and Springer, T.A. (1991) Role of lymphocyte adhesion receptors in transient interaction and cell locomotion. *Annu. Rev. Immunol.*, **9**, 27–66.
- Feizi, T. (1985) Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are onco-developmental antigens. *Nature*, **314**, 53–57.
- Florman, H.M. and Wassarman, P.M. (1985). O-linked oligosaccharides of mouse egg ZP3 account for its sperm receptor activity. *Cell*, **41**, 313–324.
- Fukuda, M. (1991) Leukosialin, a major O-glycan-containing sialoglycoprotein defining leukocyte differentiation and malignancy. *Glycobiology*, **1**, 347–356.
- Fukuda, M. (1996) Possible roles of tumor-associated carbohydrate antigens. *Cancer Res.*, **56**, 2237–2244.
- Fukuda, M., Carlsson, S.R., Klock, J.C. and Dell, A. (1986) Structures of O-linked oligosaccharides isolated from normal granulocytes, chronic myelogenous leukemia cells and acute myelogenous leukemia cells. *J. Biol. Chem.*, **261**, 12796–12806.
- Green, J.M., Noel, P.J., Sperling, A.I., Walunas, T.L., Gray, G.S., Bluestone, J.A. and Thompson, C.B. (1994) Absence of B7-dependent responses in CD28 deficient mice. *Immunity*, **1**, 501–508.
- Hakomori, S. (1984) Tumor-associated carbohydrate antigens. *Annu. Rev. Immunol.*, **2**, 103–126.
- Higgins, E.A., Siminovich, K.A., Zhuang, D., Brockhausen, I. and Dennis, J.W. (1991) Aberrant O-linked oligosaccharide biosynthesis in lymphocytes and platelets from patients with the Wiskott–Aldrich syndrome. *J. Biol. Chem.*, **266**, 6280–6290.
- Kamogawa, Y., Minasi, L.-A.E., Carding, S.R., Bottomly, K. and Flavell, R.A. (1993) The relationship of IL-4 and IFN γ -producing T cells studied by lineage ablation of IL-4-producing cells. *Cell*, **75**, 985–995.
- Kimura, A., Wigzel, H., Holmquist, G., Ersson, B. and Carlsson, P. (1979) Selective affinity fractionation of murine cytotoxic T lymphocytes (CTL). Unique lectin specific binding of the CTL associated surface glycoprotein, T-145. *J. Exp. Med.*, **149**, 473–484.
- Kitazume-Kawaguchi, S., Inoue, S., Inoue, Y. and Lennarz, W.J. (1997) Identification of sulfated oligosaccharic acid units in the O-linked glycan of the sea urchin egg receptor for sperm. *Proc. Natl Acad. Sci. USA*, **94**, 3650–3655.
- Klarsfeld, A., Bessereau, J.-L., Salmon, A.-M., Triller, A., Babinet, C. and Changeux, J.-P. (1991) An acetylcholine receptor α -subunit promoter conferring preferential synaptic expression in muscle of transgenic mice. *EMBO J.*, **10**, 625–632.
- Koyama, T. and Hughes, C. (1992) Functional integrins from normal and glycosylation-deficient baby hamster kidney cells. *J. Biol. Chem.*, **267**, 25939–25944.
- Lasky, J.A. *et al.* (1992) An endothelial ligand for L-selectin is a novel mucin-like molecule. *Cell*, **69**, 927–938.

- Lefebvre, J.C., Giordanengo, V., Limouse, M., Doglio, A., Cucchiari, M., Monpoux, F., Mariani, R. and Peyron, J.F. (1994) Altered glycosylation of leukosialin, CD43, in HIV-1-infected cells of the CEM line. *J. Exp. Med.*, **180**, 1609–1617.
- Levin, S.D., Anderson, S.J., Forbush, K.A. and Perlmutter, R.M. (1993) A dominant-negative transgene defines a role for p56lck in thymopoiesis. *EMBO J.*, **12**, 1671–1680.
- Lin, H., Bolling, S.F., Linsley, P.S., Wei, R.Q., Gordon, D., Thompson, C.A. and Turka, L.A. (1993) Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA-4Ig. *J. Exp. Med.*, **178**, 1801–1806.
- Linsley, P.S. and Ledbetter, J.A. (1993) The role of the CD28 receptor during T-cell responses to antigen. *Annu. Rev. Immunol.*, **11**, 191–212.
- Linsley, P.S., Wallace, P.M., Johnson, J., Gibson, M.G., Greene, J.L., Ledbetter, J.A., Singh, C. and Tepper, M.A. (1992) Immunosuppression *in vivo* by a soluble form of the CTLA-4 T cell activation molecule. *Science*, **257**, 792–795.
- Manjunath, N., Correa, M., Ardman, M. and Ardman, B. (1995) Negative regulation of T-cell adhesion and activation by CD43. *Nature*, **377**, 535–538.
- Marlin, S.D. and Springer, T.A. (1987) Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for leukocyte function-associated antigen-1 (LFA-1). *Cell*, **51**, 813–819.
- McEvoy, L.M., Sun, H., Frelinger, J.G. and Butcher, E.C. (1997) Anti-CD43 inhibition of T cell homing. *J. Exp. Med.*, **185**, 1493–1498.
- Mentzer, S.J., Remold, O.D.E., Crimmins, M.A., Bierer, B.E., Rosen, F.S. and Burakoff, S.J. (1987) Sialophorin, a surface sialoglycoprotein defective in the Wiskott–Aldrich syndrome, is involved in human T lymphocyte proliferation. *J. Exp. Med.*, **165**, 1383–1392.
- Molina, I.J., Sancho, J., Terhorst, C., Rosen, F.S. and Remold-O'Donnell, E. (1993) T cells of patients with Wiskott–Aldrich Syndrome have a restricted in proliferative responses. *J. Immunol.*, **151**, 4383–4390.
- Perillo, N.L., Pace, K.E., Seilhamer, J.J. and Baum, L.G. (1995) Apoptosis of T cells mediated by galectin-1. *Nature*, **378**, 736–739.
- Piller, F., Piller, V., Fox, R.I. and Fukuda, M. (1988) Human T-lymphocyte activation is associated with changes in O-glycan biosynthesis. *J. Biol. Chem.*, **263**, 15146–15150.
- Piller, F., Le Deist, F., Weinberg, K., Parkman, R. and Fukuda, M. (1991) Altered O-glycan synthesis in lymphocytes from patients with Wiskott–Aldrich syndrome. *J. Exp. Med.*, **173**, 1501–1510.
- Rademacher, T.W., Parekh, R.B. and Dwek, R.A. (1988) Glycobiology. *Annu. Rev. Biol.*, **57**, 785–838.
- Remold-O'Donnell, E., Kenney, D.M., Parkman, R., Cirna, L., Savage, B. and Rosen, F.S. (1984) Characterization of the human lymphocyte surface sialoglycoprotein that is defective in Wiskott–Aldrich Syndrome. *J. Exp. Med.*, **159**, 1705–1723.
- Rosenstein, Y., Park, J.K., Hahn, J.K., Rosen, F.S., Bierer, B.E. and Burakoff, S.J. (1991) CD43, a molecule defective in Wiskott–Aldrich Syndrome, binds ICAM-1. *Nature*, **354**, 233–235.
- Ruoslahti, E. (1996) RGD and other recognition sequences for integrins. *Annu. Rev. Cell Dev. Biol.*, **12**, 697–715.
- Saitoh, O., Piller, F., Fox, R.I. and Fukuda, M. (1991) T-lymphocytic leukemia express complex, branched O-linked oligosaccharides on a major sialoglycoprotein, leukosialin. *Blood*, **77**, 1491–1499.
- Sako, D. *et al.* (1993) Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell*, **75**, 1179–1186.
- Sastre, L., Kishimoto, T.K., Gee, C., Roberts, T. and Springer, T.A. (1986) The mouse leukocyte adhesion proteins Mac-1 and LFA-1: studies on mRNA translation and protein glycosylation with emphasis on Mac-1. *J. Immunol.*, **137**, 1060–1065.
- Sharpe, A.H. (1995) Analysis of lymphocyte costimulation *in vivo* using transgenic and 'knockout' mice. *Curr. Opin. Immunol.*, **7**, 389–395.
- Sligh, J.E., Jr, Ballantyne, L.M., Rich, S.S., Hawkins, H.K., Waynesmith, C., Bradley, A. and Beaudet, A.L. (1993) Inflammatory and immune responses are impaired in mice deficient in intercellular adhesion molecule 1. *Proc. Natl Acad. Sci. USA*, **90**, 8529–8533.
- Sommer, F., Huber, M., Röllin, M. and Lohoff, M. (1995) CD44 plays a co-stimulatory role in murine T cell adhesion: ligation of CD44 selectively co-stimulates IL-2 production, but not proliferation in TCR-stimulated murine T_H1 cells. *Int. Immunol.*, **7**, 1779–1786.
- Sperling, A.I., Green, J.M., Mosley, R.L., Smith, P.L., DiPaolo, R.J., Klein, J.R., Bluestone, J.A. and Thompson, C.B. (1995) CD43 is a murine T cell costimulatory receptor that functions independently of CD28. *J. Exp. Med.*, **182**, 139–146.
- Sui, G., Hedrick, S.M. and Brian, A. (1989) Isolation of the murine intercellular adhesion molecule 1 (ICAM-1) gene. *J. Immunol.*, **143**, 3813–3820.
- Swan, K.A., Alberola-Ila, J., Gross, J.A., Appleby, M.W., Forbush, K.A., Thomas, J.F. and Perlmutter, R.M. (1995) Involvement of p21ras distinguishes positive and negative selection in thymocytes. *EMBO J.*, **14**, 276–285.
- Symons, M., Derry, J.M., Karlak, B., Jiang, S., Lemahieu, V., McCormick, F., Franke, U. and Abo, A. (1996) Wiskott–Aldrich Syndrome protein, a novel effector for the GTPase CDCHs, is implicated in actin polymerization. *Cell*, **84**, 723–734.
- Tsuboi, S., Isogai, Y., Hada, N., King, J.K., Hindsgaul, O. and Fukuda, M. (1996) 6'-Sulfo sialyl Le^x but not 6-sulfo sialyl Le^x expressed on the cell surface supports L-selectin-mediated adhesion. *J. Biol. Chem.*, **271**, 27213–27216.
- Wilson, R.W., Ballantyne, C.M., Smith, C.W., Montgomery, C., Bradley, A., O'Brien, W.E. and Beaudet, A.L. (1993) Gene targeting yields a CD18-mutant mouse for study of inflammation. *J. Immunol.*, **151**, 1571–1578.
- Xu, H., Gonzalo, J.A., St Pierre, Y., Williams, I.R., Kupper, T.S., Cotran, R.S., Springer, T.A. and Gutierrez-Ramos, J. (1994) Leukocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice. *J. Exp. Med.*, **180**, 95–109.
- Xu, J., Grewal, I.S., Geba, G.P. and Flavell, R.A. (1996) Impaired primary T cell responses in L-selectin deficient mice. *J. Exp. Med.*, **183**, 589–598.
- Yousefi, S., Higgins, E., Daoling, Z., Pollex-Krütger, A., Hindsgaul, O. and Dennis, J.W. (1991) Increased UDP-GlcNAc: Gal β 1-3GalNAc-R (GlcNAc to GalNAc) β 1-6-N-acetylglucosaminyltransferase activity in metastatic murine tumor cell lines. *J. Biol. Chem.*, **266**, 1772–1782.

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