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Core 2 *N*-acetylglucosaminyltransferase activity: a diagnostic marker for Wiskott–Aldrich syndrome

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Serine/threonine (*O*)-linked oligosaccharide on cell-surface CD43 has been reported to be abnormal in haemopoietic lineages of patients with the X-linked immunodeficiency, Wiskott–Aldrich syndrome (WAS). This defect largely appears to be the result of abnormal regulation of UDP-GlcNAc:Galβ1–3GalNAc-Rβ1–6-*N*-acetylglucosaminyltransferase (also known as core 2 GlcNAc-T), an enzyme in the Golgi apparatus that is subject to regulation during haemopoietic differentiation. To determine whether core 2 GlcNAc-T activity provides a reliable marker for WAS, we studied 12 unrelated WAS patients with respect to their expression of this enzyme activity. Compared with healthy subjects, the WAS patients showed levels of core 2 activity that were, on average, 2.5- and 3.9-fold higher in fresh lymphocytes and platelets respectively. These data suggest that altered core 2 GlcNAc-T activity is consistently found in lymphocytes and platelets of WAS patients and as such may provide a diagnostic marker for the disease. In view of the relatively limited amounts of blood sample generally available from infants and young children, we have also tested a more sensitive coupled assay that permits assessment of core 2 GlcNAc-T activity in very small samples of cells and which would therefore render this assay of wide clinical applicability.

Keywords: immunodeficiency, *N*-acetylglucosaminyltransferase, *O*-glycosylation, Wiskott–Aldrich syndrome

Introduction

The Wiskott–Aldrich syndrome (WAS) is an X-linked recessive disorder characterized by reduced platelet numbers, size and function, eczema, severe combined immunodeficiency and susceptibility to lymphoid malignancies [1–4]. Recurrent infections and a haemorrhagic diathesis begin at birth or shortly thereafter and are the major causes of early mortality in affected boys [5]. While a number of immune defects, such as altered levels of serum immunoglobulins, impaired T-cell mitogen responses and reduced expression of the B-cell surface glycoprotein CD23, have been detected in WAS patients, the presence of these and other immune cellular abnormalities varies considerably among affected boys [2, 3, 6]. Similarly, the disease is clinically heterogeneous, a phenomenon that has become increasingly appreciated in the context of recent genetic data suggesting that X-linked thrombocytopenia and WAS are allelic variants of the same disease [7, 8], and thus that a subgroup of boys presenting with congenital thrombocytopenia represent atypical or attenuated cases of WAS. This phenotypic heterogeneity may obscure the diagnosis of WAS, particularly in early life, a problem that is clinically important in view of the current availability of assays for genetic prediction,

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and prevention of WAS [8, 9], as well as advances in bone marrow transplantation technology. For this reason our group has been interested in developing biochemical assays which would further facilitate early detection of WAS patients.

While the nature of the primary gene defect in WAS is presently unknown, data from our group and one other have revealed an association between WAS and a defect in *O*-glycosylation [10–12], which is reflected in the size distribution and levels of lymphocyte CD43 [10, 11, 13, 14]. CD43 is a transmembrane glycoprotein that is expressed on all non-erythroid haemopoietic cells [15] and which has been implicated in antigen-independent lymphocyte activation [16]. The molecular weight of CD43 shows cell lineage-specific difference owing to the structural variation in the 90 *O*-linked carbohydrate chains found on the glycoprotein [15]. The size of the CD43 glycoprotein in phytohaemagglutinin (PHA)-stimulated lymphocytes from WAS patients and normals has been determined to be approximately 95 kDa and 115–95 kDa respectively, and these size differences have been shown to correlate with expression of the glycosyltransferase, core 2 GlcNAc-T [10, 11]. This enzyme utilizes UDP-GlcNAc to convert the core 1 (*i.e.* Galβ1–3GalNAcα-O) to the core 2 [*i.e.* Galβ1–3(GclNAcβ1–6)GalNAcα-O] structure, and its activity therefore defines an important branch point in the *O*-glycan biosynthesis pathway (Figure 1). Core 2 glycan structures can be further extended with polylactosamine and Lewis antigens (*e.g.* sialyl-Lewis^x), the latter of which have been shown to participate in leucocyte adhesion and homing when expressed on mucins such as GlyCAM-1, MAdCAM-1, CD34 and PSGL-1 (reviewed in Ref. 17). By contrast, in the absence of core 2 GlcNAc-T activity, *O*-glycans are limited to smaller sialylated core 1 oligosaccharide structures [11, 18, 19]. Therefore, it is possible that abnormal expression of core 2 and associated carbohydrate structures in WAS may contribute to lymphocyte and platelet dysfunction by altering adhesive interactions mediated by *O*-linked oligosaccharides and selectins.

Previous studies of core 2 GlcNAc-T activity in relation to the activation state of normal T lymphocytes have revealed that this enzyme activity is relatively low in resting cells, but increases three- to fourfold following activation by interleukin 2 (IL-2) and anti-CD3 antibody [11, 20]. However, the results of a pilot study involving analysis of lymphocytes from WAS patients revealed an inversion of this phenomenon, such that

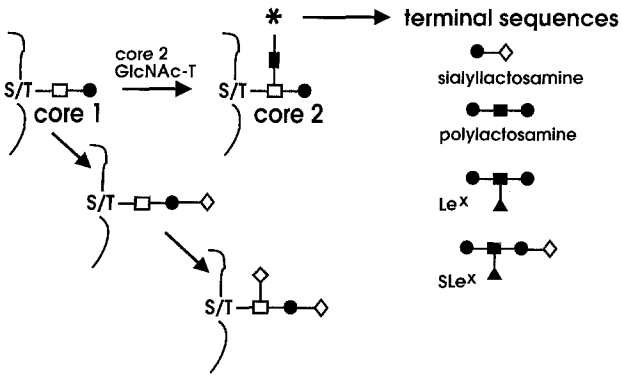

Figure 1. Schematic of the *O*-linked oligosaccharide pathways. Specific glycosyltransferases catalyse the sequential addition of monosaccharides to produce the core 1 and 2 structures as well as the terminal sequences. These glycans are added to a subset of serine and threonine (S/T) residues of newly synthesized proteins. The right side of the diagram shows a number of terminal extensions which can be added to the GlcNAc of the core 2 trisaccharide (*i.e.* the product of core 2 GlcNAc-T). In normal subjects, resting lymphocytes favour the core 1 pathway (the diagonal arrows), while in activated cells and platelets the core 2 pathway is favoured. The pattern is reversed in WAS patients. ■, GlcNAc; □, GalNAc; ●, Gal; ▲, Fuc; ◇, SA.

core 2 GlcNAc-T activity in resting cells from the patients was similar to that measured in activated T cells from normal subjects, and activity decreased by three- to fourfold following stimulation of WAS cells [11, 12]. We have also demonstrated that cultured lymphocytes from WAS patients show impaired proliferative responses to periodate [10, 21], a compound that reacts with sialic acid residues on the cell-surface glycoproteins. Our results indicate that periodate non-responsiveness provides a potential diagnostic test for the WAS phenotype. However, this analysis involves a time-consuming cell culture assay requiring numbers of lymphocytes which are sometimes difficult to obtain from very young and ill children. With this problem in mind, we have tested a coupled assay for evaluation of core 2 GlcNAc-T activity which can be performed quickly using very small samples [22, 23]. The results of the present study on a cohort of 12 unrelated WAS patients confirm the association between WAS and altered core 2 GlcNAc-T activity in platelets as well as resting and activated T lymphocytes, and indicate that the assessment of this enzyme activity using the sensitive coupled assay [22] should facilitate the diagnosis of WAS, even in very young children.

Materials and methods

Chemicals

Galβ1–3GalNAcα-pNp and GalNAcα-pNp were obtained from Toronto Research Chemicals. UDP-GlcNAc, UDP-Gal, bovine milk β1–4 Gal-T, buffer salts, Triton X-100, 2-mercaptoethanol, interleukin 2 (IL-2) and sodium periodate were from Sigma. High-performance liquid chromatography (HPLC)-grade KH_2PO_4 and acetonitrile were from Fisher, the Ultrahydrogel HPLC column (7.8 × 300 mm) and Sep-Pak C_{18} cartridges from Millipore–Waters. UDP-[6-^{3}H]*N*-acetylglucosamine (26.8 Ci/mmol) was purchased from Du Pont–New England Nuclear and UDP-[6^{3}H]galactose (18.9 Ci/mmol) was from Amersham. α-Modified Eagle minimum essential medium, RPMI-1640, Iscove's modified Dulbecco medium, fetal calf serum (FCS), gentamicin, transferrin, bovine serum albumin (BSA) and ethanolamine were from Gibco. Ficoll-Paque was from Pharmacia. Anti-CD3 antibody was purchased from Becton Dickinson and phytohaemagglutinin (PHA) from Difco.

Subjects

The cells and cell lines used in this study were obtained from 12 patients affected with WAS and 15 healthy donors including first-degree relatives of the patients as well as unrelated controls. Patients were diagnosed with WAS on the basis of presentation with the classical clinical triad: eczema, persistently low numbers of abnormally small platelets and recurrent infections. Among the 12 patients, however, one boy (WAS11) did not manifest recurrent infections and was referred with the diagnosis of probable WAS. Four other patients (WAS1, 3, 5 and 9), in whom the severity of infections was less than that observed in classic WAS patients, were referred with the diagnosis of attenuated WAS. All patients and controls were studied when not acutely ill or febrile. Where possible, carrier status for WAS was assayed in mothers of affected boys by determination of the lymphocyte X-chromosome inactivation state using restriction fragment length polymorphism (RFLP)–methylation analysis as previously described [24–26].

Activation of T lymphocytes

Peripheral blood from WAS patients and normal subjects was collected into heparinized tubes. Mononuclear cells and platelets were isolated by density-gradient centrifugation over Ficoll-Paque. Mononuclear cells were washed with RPMI-1640 medium, 20% FCS, 20 mM HEPES, 50 μg/ml gentamicin; platelets remaining in the supernatant after the first wash were pelleted at 20 000 ***g*** for 20 min. If not used immediately for glycosyltransferase assays, platelets were frozen at −20°C. The mononuclear fraction was depleted of macrophages by adherence to FCS-coated plastic dishes for 1 h and thereafter of red blood cells using the ammonium chloride method. T cells were cultured at 10^6 cells/ml in RPMI-1640 medium, 20% FCS, 20 mM HEPES, 0.55 μM 2-mercaptoethanol and 50 μg/ml gentamicin alone or in the presence of 2 μg/ml anti-CD3 antibody and 10 units/ml IL-2.

Proliferation assays

Mononuclear cells obtained from patients and controls (see above) were washed and treated with L-PHA or periodate as previously described [27]. Briefly, cells were resuspended at a concentration of 10^6 cells/ml in RPMI-1640 containing 5% heat-inactivated FCS, 2 mM L-glutamine and 5 mg/ml gentamicin, and cultured in 0.2-ml aliquots in flat-bottom microtitre platelets. Twenty hours before termination of the incubation, cultures were pulsed with [^{3}H]thymidine (2 μCi per well), harvested onto glass-fibre filters and incorporated radioactivity determined using a beta liquid scintillation counter. PHA was added directly to cultures at a final concentration of 2 μg/ml, while periodate stimulation was performed by incubating cells suspended at 10^7 cells/ml in 1 ml of phosphate-buffered saline with 2 mM periodate for 30 min at 0°C, followed by washing and resuspension in culture medium. The results of proliferation assays are expressed as the stimulation index (SI), which is the ratio of [^{3}H]thymidine incorporation in stimulated cells to [^{3}H]thymidine incorporation in unstimulated cells.

Glycosyltransferase assays

Core 2 GlcNAc-T (conventional method) and β1–3Gal-T enzyme assays were performed in resting and activated T cells, Epstein–Barr virus (EBV)-transformed B cells and platelets. In all cases, the starting material was washed three times in 0.9% saline and lysed in 0.25% Triton X-100, 0.15 M NaCl, at 4°C for 10 min. The lysates were adjusted to a protein concentration of 8–12 μg/μl with lysis buffer and used as a source of transferases.

The core 2 GlcNAc-T reaction contained 0.1 M TES buffer, pH 7.0, 0.125% Triton X-100, 0.1 M GlcNAc, 2 mM UDP-GlcNAc, 0.5 μCi of

UDP-[6-^{3}H]*N*-acetylglucosamine, 1 mM Galβ1–3-GalNAcα-pNp as acceptor and 20 μl of cell lysate [28, 29]. The β1–3 Gal-T reaction contained 20 mM $MnCl_2$, 0.1 M MES, pH 6.7, 0.5% Triton X-100, 1.6 mM UDP-Gal, 0.5 μCi of UDP-[6-^{3}H]galactose, 2 mM GalNAcα-pNp as the acceptor and 5 μl of cell lysate [30]. Reactions (total volume, 50 μl) were incubated for 2 h at 37°C and stopped by adding 0.5 ml of cold water. Tubes were processed immediately or stored at −20°C. Mixtures were diluted to 5 ml in water, applied to a Sep-Pak C_{18} column, and washed with 20 ml of water. Product was eluted with 5 ml of 100% methanol and counted in a beta scintillator counter. Endogenous glycosyltransferase activities were measured in the absence of acceptor and used to correct activity values determined in the presence of acceptor. The reactions were linear with time of incubation under the conditions used in each assay. Protein concentrations of cell lysates were determined with the bicinchoninic acid reagent (Pierce) using BSA as the standard.

Coupled assay for core 2 GlcNAc-T

The two-step assay for core 2 GlcNAc-T activity was performed essentially as described previously [23]. Briefly, samples were washed three times in saline solution and lysed in 0.25% Triton X-100, 0.15 M NaCl, on ice. Lysates containing 5–15 μg of protein were added to reaction mixtures containing 0.2 M TES, pH 7.0, 0.4 M GalNAc, 4 mM UDP-GlcNAc and 2 mM Galβ1–3GalNAcα-pNp, in a total volume of 50 μl. Incubations were carried out at 37°C for 2 h. The reactions were passed through C_{18} Sep-Pak cartridges, washed with water as described above, and product was eluted with 4 ml of methanol, dried, and the residue taken up in 80 μl of 10 mM HEPES, pH 8.0, 20 mM $MnCl_2$, 2 μCi of UDP-[^{3}H]Gal (18.9 Ci/mmol) and 90 mU of β1–4 Gal-T (in 20 μl of 25 mM HEPES, pH 8.0, and 2.5 mM $MnCl_2$) in a total volume of 100 μl. Samples were incubated for 2 h at 30°C, passed through C_{18} Sep-Pak cartridges, and the product was eluted with methanol. After evaporation of the methanol, the residue was resuspended in 150 μl of a 80:20 acetonitrile–water mixture and the product (*i.e.* Galβ1–3([^{3}H]Gal-β1–4GlcNAcβ1–6)GalNAcα-pNp) was separated on a 7.8 × 300 mm Ultrahydrogel HPLC column (Millipore–Waters) developed isocratically at 1 ml/min in the 80% acetonitrile solution. Fractions were taken at 1.5-min intervals and radioactivity counted in a beta-scintillation counter. Endogenous activities were measured in the absence of acceptor and were consistently not detected.

Results

The current study involved analysis of 12 unrelated boys who presented with clinical and immunological features of WAS. The affected boys ranged from 10 months to $11\frac{1}{2}$ years, and with the exception of one boy (WAS11) uniformly manifested variably severe eczema, recurrent infections and a bleeding diathesis consequent to thrombocytopenia. WAS patient 11 had mild recurring eczema, low numbers of small platelets and numerous male relatives with similar clinical features who had been variably diagnosed as having X-linked thrombocytopenia or probable WAS. Four other boys (WAS patients 1, 3, 5 and 9) were also considered as representing attenuated forms of WAS, based on the relatively mild nature of the episodic infections. While only a few boys included in this study had a positive family history for WAS, the results of RFLP–methylation analysis previously performed for the purposes of genetic counselling revealed non-random patterns of X-chromosome inactivation in lymphocytes of seven of nine mothers studied, a finding consistent with the diagnosis of WAS in these boys [24–26].

In addition to the analysis of glycosyltransferase activities, patients were also studied with respect to their lymphocyte proliferative responses to the T-cell mitogens PHA and periodate, reagents that normally induce extensive T-cell proliferation. WAS patient lymphocytes have been shown to proliferate in response to PHA, but show no response to periodate [10, 12]. The latter agent oxidizes sialic acid residues on cell-surface glycoproteins, including those of CD43, and induces a mitogen signal with stimulation indices of 5 or greater for normal subjects [10, 21]. Lymphocytes from all 12 WAS patients in the study were non-responsive to periodate treatment (Table 1).

While selective impairment in periodate-induced T-cell proliferation does appear to distinguish the spectrum of WAS patients, this assay is time-consuming and requires a substantial number of cells. For this reason, studies were undertaken to determine whether evaluation of core 2 GlcNAc-T activities provides a more sensitive and simpler strategy for diagnosis of this disorder. Accordingly, core 2 GlcNAc-T activity was assayed in the 12 WAS patients' T cells obtained either directly from peripheral blood or following 3 days of stimulation

Table 1. Clinical and laboratory findings in the WAS patients

WAS patient	Age (years)	Maternal X-inactivation status[a]	Platelets[b]		Lymphocyte proliferation[c]	
			Count ($\times 10^9$/l)	Volume	PHA	Periodate
1	0.8	NR	49	↓	48	0.9
2	4.2	NR	89	↓	58	1.3
3	11.6	R	25	↓	28	2.3
4	0.5	ND	15	↓	45	0.9
5	6.4	ND	10	ND	34	ND
6	0.5	NR	50	↓	75	0.8
7	8.3	NR	20	↓	27	1.9
8	0.5	NR	30	↓	116	1.0
9	16	R	19	↓	ND	ND
10	1.2	ND	19	↓	58	2.4
11	0.8	NR	38	↓	171	0.9
12	1.5	NR	14	ND	120	1.5

[a]X-chromosome inactivation state as determined by RFLP–methylation analysis of maternal lymphocytes is demarcated as non-random (NR) or random (R); ND, not done. As outlined in the text, a pattern of non-random X-chromosome inactivation is considered to demarcate the WAS carrier state, while random X-chromosome inactivation is observed in non-carriers.

[b]Platelet volume is indicated as being below the lower limit of normal (↓) for the particular laboratory; ND, not done.

[c]Numbers indicate stimulation indices (SIs) obtained $2\frac{1}{2}$ days following either PHA or periodate treatment of cultured lymphocytes pulsed with [^{3}H]thymidine. ND, not done.

in vitro with anti-CD3 antibodies + IL-2 (Table 2). As shown in Table 2, the average enzyme activity detected in unstimulated lymphocytes from the 12 affected boys (2.3 ± 0.43 nmol/mg/h) was 2.5-fold higher than the average of the activities measured in cells from unrelated normal subjects (*i.e.* 0.91 ± 0.42 nmol/mg/h). After stimulation, T cells from normal subjects showed a 2.6-fold increase in core 2 activity, while the cells from WAS patients showed a 3.9-fold decline in this activity (Table 2). Similar differences between WAS patients and control lymphocyte enzyme activities were observed when healthy relatives of WAS patients were used as the normal controls (Table 3). Core 2 GlcNAc-T activity was also found to be 2.5 times greater in platelets from WAS patients than in those from related and unrelated normal subjects (Tables 2 & 3). By contrast, no differences were detected between WAS patients and normal subjects with regard to activities of β1–3Gal-T,

Table 2. Core 2 GlcNAc-T and β3Gal-T activities in T lymphocytes from normal subjects and WAS patients[a]

Subjects	Core 2 GlcNAc-T (nmol/mg/h)			β3Gal-T (nmol/mg/h)		
	T cells		Platelets	T cells		Platelets
	Resting	Activated		Resting	Activated	
Normal	0.91 ± 0.42 (6)	2.38 ± 0.28 (6)	1.10 ± 0.28 (6)	26.8 ± 2.66 (4)	23.76 ± 1.77 (4)	10.78 ± 1.53 (4)
WAS	2.30 ± 0.43 (12) $P < 10^{-6}$	0.59 ± 0.23 (12) $P < 10^{-6}$	2.77 ± 0.46 (12) $P < 10^{-3}$	19.03 ± 2.99 (8) NS	22.15 ± 5.15 (8) NS	9.71 ± 1.43 (12) NS

[a]T cells were incubated with anti-CD3 antibody and IL-2 for 72 h as described in the Materials and methods section. The data shown represent the mean ± SD for the number of subjects as indicated in brackets below. Normal and WAS groups were compared using Student's *t*-test; NS, not significant.

Table 3. Core 2 GlcNAc-T and β3Gal-T activities in T lymphocytes from WAS patients and healthy relatives[a]

Subject	Core 2 GlcNAc-T (nmol/mg/h)			β3Gal-T (nmol/mg/h)		
	T cells		Platelets	T cells		Platelets
	Resting	Activated		Resting	Activated	
(a) Mother	0.65	1.85	1.60	25.77	29.23	9.14
Healthy son	0.73	1.83	1.05	20.80	15.65	12.68
WAS son (WAS3)	1.93	0.38	3.15	17.45	22.89	9.24
(b) Mother	1.01	2.24	1.02	17.43	21.94	7.65
WAS son (WAS7)	2.00	0.78	2.29	14.67	15.44	9.90
(c) Mother	1.41	2.33	1.56	16.54	25.61	9.00
WAS son (WAS8)	3.28	0.65	2.78	23.21	29.45	12.19
(d) Mother	0.68	2.00	ND	ND	ND	ND
WAS son (WAS9)	2.10	0.25	ND	ND	ND	ND
(e) Father	0.73	1.83	1.75	23.04	ND	ND
WAS son (WAS10)	1.85	0.48	2.48	22.35	22.39	ND

[a]T cells were incubated with anti-CD3 and IL-2 for 72 h as described in the Materials and methods section. The letters in parentheses indicate family groups. Data shown represent the mean of duplicate determinations which were within 10% of average. ND, not done.

another glycosyltransferase in the *O*-linked pathway, as evaluated in either resting of stimulated lymphocytes.

Since the quantity of peripheral blood lymphocytes from infants, and in particular those with bleeding disorders such as WAS, is often very limited, the practical applicability of any diagnostic assay becomes very dependent on its sensitivity. In this context, we have developed a strategy for evaluating core 2 GlcNAc-T activity which involves the use of a coupled enzyme assay that is approximately 100 times more sensitive than the currently available standard assay based on using UDP-[^{3}H]GlcNAc as a sugar donor [22]. In this new assay, core 2 GlcNAc-T reactions are done with unlabelled UDP-GlcNAc as donor and Galβ1–3GlcNAcα-pNp as acceptor. The product, Galβ1–3(GalNAcβ1–6)GalNAcα-pNp, is then further reacted with purified bovine β1–4Gal-T and UDP-[^{3}H]Gal to produce Galβ1–3([^{3}H]Galβ1–4GlcNAcβ1–6)GalNAcα-pNp, which can be separated by C_{18} Sep-Pak cartridge and/or Ultrahydrogel HPLC column. The two reactions were performed in series as follows:

$$\text{Gal}\beta1\text{–}3\text{GalNAc}\alpha\text{-pNp} \xrightarrow[\text{UDP-GlcNAc}]{\text{Cell lysate}} \begin{matrix}\text{Gal}\beta1\text{–}3\text{GalNAc}\alpha\text{-pNp}\\ \text{GlcNAc}\beta1\text{–}6'\end{matrix} \quad (1)$$

$$\begin{matrix}\text{Gal}\beta1\text{–}3\text{GalNAc}\alpha\text{-pNp}\\ \text{GlcNAc}\beta1\text{–}6'\end{matrix} \xrightarrow[\text{UDP-[}^3\text{H]Gal}]{\text{Bovine }\beta1\text{–}4\text{Gal-T}} \begin{matrix}\text{Gal}\beta1\text{–}3\text{GalNAc}\alpha\text{-pNp}\\ [^3\text{H}]\text{Gal}\beta1\text{–}4\text{GlcNAc}\beta1\text{–}6'\end{matrix} \quad (2)$$

As previously reported, standard curves for the second reaction (Gal-T) show that product is directly proportional to acceptor concentration (*i.e.* product of the first reaction) in the range 0–500 pmol and that core 2 GlcNAc-T activity measured in cell lysates is directly proportional to added lysate in the range 1–25 μg of protein [22]. The data in Table 4 show that the coupled and conventional assays give comparable results even when the former assay is carried out using approximately 1/25–1/100 the lysate protein required for the latter assay.

Discussion

In an earlier study of *O*-glycosylation in WAS patients, core 2 GlcNAc-T activity was observed to be abnormally high in resting and activated lymphocytes for five boys manifesting the characteristic clinical features of WAS [11]. We have now studied 12 additional WAS patients and found the same aberrant pattern of core 2 GlcNAc-T activity in resting and activated T lymphocytes, as well as

Table 4. Comparison of core 2 GlcNAc-T using the conventional and the coupled assay methods

Sample	Core 2 GlcNAc-T (nmol/mg/h)[a]	
	Conventional assay	Coupled assay
T cells (WAS1)	2.67	2.59
T cells (N1)	0.74	0.71
Platelets (WAS1)	2.79	2.90
Platelets (N3)	1.19	1.13

[a]Glycosyltransferase activities were measured on resting lymphocytes and platelets as described in the Materials and methods section. Data shown represent the mean of triplicate determinations.

platelets from these patients. Averages of enzyme activities observed in patients versus healthy subjects showed highly significant differences between the two groups with at least three standard deviations distinguishing the mean enzyme activities detected in each group (Table 2). As the group of patients studied here included those expressing both classical and attenuated forms of WAS, it appears that the evaluation of core 2GlcNAc-T activity in either resting or activated T cells and/or platelets provides a reliable and sensitive marker for diagnosis of the spectrum of WAS patients.

Core 2 GlcNAc-T activity has also been found to be reduced in WAS relative to normal EBV-transformed B cells, this being the case in five of six WAS patients reported in a previous study [11]. However, in the current study, a wide range of core 2 GlcNAc-T activities was observed in both WAS patient and control EBV-transformed B-cell lines, and activities of the enzyme did not appear to correlate with disease (data not shown). These discrepant results may relate to the use of transformed cell lines, as B cells from normal subjects have been found to display a heterogeneous response to EBV infection [31]. Furthermore, B cells of WAS patients have been shown to have variably reduced levels of receptors for EBV and consequent highly variable growth patterns after transformation. It is possible in this context that serial passage of EBV-immortalized WAS cells selects for emerging subpopulations in which somatic mutations confer a growth advantage. Together, these observations suggest that the properties of EBV-B cell lines derived from WAS patients may change over time, a phenomenon that renders such lines unreliable as a source of cells for defining biochemical characteristics of WAS B cells.

Most of the data on core 2 GlcNAc-T activity obtained in this study were derived using the conventional enzyme assay which requires 20 ml or more of blood. By contrast, the coupled assay permits accurate measurement of lymphocyte and platelet core 2 activities using only 1 ml or less of blood (Table 4). The additional sensitivity of the coupled assay is partly the result of the use of relatively high concentrations of purified β1–4Gal-T enzyme in the second step and the favourable substrate K_m values for β1–4Gal-T, which are much lower than that for the core 2 GlcNAc-T enzyme [22]. The coupled assay can be adapted by changing the hydrophobic aglycon linker at the reducing end, which allows for other methods of trapping and detecting the product. For example, a fluorescent compound such as 4-methylumbelliferonyl at the reducing end of Galβ1–3GalNAcα- (supplied by Toronto Research Chemicals) can be used, and the reaction product detected by separating the mixture on HPLC or the new fluorophore assisted carbohydrate electrophoresis (FACE) polyacrylamide gel electrophoresis method for carbohydrates [32] (GLYKO). This approach may further enhance the sensitivity of the assay provided the fluorescent label products and substrate can be separated completely. In any case, it appears that cellular core 2 GlcNAc-T activity can readily be evaluated using very small numbers of cells and thus, in terms of clinical practicality, this assay has a clear advantage over the previously described periodate proliferation assay in relation to detection of WAS patients.

In conclusion, five patients investigated in a previous pilot study [11] and 12 patients examined in the current study show a highly reproducible pattern of abnormal core 2 GlcNAc-T activity in lymphocytes and platelets. Taken together, these data suggest that core 2 GlcNAc-T assessment may be of value in the diagnosis of this immunodeficiency disease.

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