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Review

Tn-syndrome

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Abstract

The idiopathic Tn-syndrome, formerly called 'permanent mixed-field polyagglutinability', is a rare hematological disorder characterized by the expression of the Tn-antigen on all blood cell lineages. The immunodominant epitope of the Tn-antigen is terminal α-*N*-acetylgalactosamine, *O*-glycosidically linked to protein. Normally this residue is 3′-substituted by β-galactose thereby forming the core 1 structure known as the Thomsen–Friedenreich (TF) antigen (Galβ1 ⇒ 3GalNAcα1 ⇒ Thr/Ser). The cause of the exposure of the Tn-antigen appears to be due to the silencing of the gene expression of β1,3galactosyltransferase, since treatment of deficient Tn(+) lymphocyte T clones with 5′azacytidine or Na butyrate leads to reexpression of enzyme activity and the sialylated TF-antigen. The Tn-syndrome is acquired and permanent and affects both sexes at any age. Its origin is unknown. Pluripotent stem cells are affected since all lineages are involved but each one to a variable extent. Therefore, normal cells co-exist with Tn-transformed cells. Clinically, patients suffering from the Tn-syndrome appear healthy. Laboratory findings usually reveal moderate thrombocyto- and leukopenia and some signs of hemolytic anemia not warranting any treatment. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Galactosyltransferase; Polyagglutinability; Thrombocytopenia; Thomsen–Friedenreich antigen

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Abbreviations: TF, Thomsen–Friedenreich; 1,3GT, *N*-acetylgalactosaminide β1,3galactosyltransferase, also called 'T-transferase'; OSM, ovine submaxillary mucin; HPA, *Helix pomatia* agglutinin; FACS, fluorescence activated cell sorting; mAb, monoclonal antibody; UDPGal, uridine diphosphogalactose

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1. Introduction

The idiopathic Tn-syndrome occupies a unique position in the field of glycosylation disorders for two reasons: First, it is up till now the only known permanent, well defined disorder of *O*-glycosylation and second, the disorder is acquired and confined to a fraction only of hemopoietic cells of all lineages. The disorder is due to a complete absence of UDP-Gal:*N*-acetylgalactosaminideβ1,3galactosyltransferase activity (EC 2.4.1.122; 1,3GT) in the affected cells. Therefore, the Tn-antigen is exposed on blood cells. In addition to the idiopathic Tn-syndrome, Tn-antigens may also occur on cell surfaces in a variety of malignant disorders as a cancer-associated epiphenomenon. These aspects are not included in this review.

Several reviews on the Tn-syndrome have been published [1,2].

2. Pathways of *O*-glycosylation

Recent work has unravelled an astonishing diversity of *O*-glycosidic structures. The best known are the ubiquitous GalNAc core and its common substituents as shown in Fig. 1. Additional *O*-glycosidic glycans built on *O*-mannosyl- [3] or *O*-fucosyl-peptide [4] cores have recently been identified which are not relevant in the present context. Normally, peptide-linked *O*-αGalNAc is substituted by Gal in a β1→3 linkage catalyzed by 1,3GT to form the so-called core 1 structure (for review see [5]). Unsubstituted peptide-linked αGalNAc has antigenic properties and is designated as the Tn-antigen (Fig. 1). Unsubstituted Galβ1→3GalNAcα-R (core 1) is called the TF-antigen (formerly called T-antigen) according to Thomsen–Friedenreich.[1] Usually the core 1 structure is bisialylated as shown in Fig. 1. Removal of sialic acids unmasks the TF-antigen which, therefore, is called a cryptantigen. In some cases the Tn-antigen can be substituted by sialic acid in a α2→6 linkage to GalNAc thus forming the sialosyl-Tn-antigen. Other possible pathways are shown in Fig. 1. Note that core 2 formation does not occur in the absence of 1,3GT because of the absolute requirement of the GlcNAc-T branching β1→6 *N*-acetylglucosaminyltransferase for the core 1 substrate (for review see [5]).

3. Discovery and early work

The Tn-syndrome was originally described by Moreau and Dausset et al. [6,7] in 1957, based on the observation that certain red cells can be agglutinated by all human sera irrespective of their ABH blood group status. This phenomenon was usually ascribed to ‘aging’ of erythrocytes in sera obtained from patients with infections and was most probably due to a microbial neuraminidase activity which unmasked the TF-antigen. By contrast, these authors described a patient who exhibited the phenomenon of polyagglutinability for many years and who also had leukopenia, thrombocytopenia and a chronic hemolytic anemia. Careful serologic differentiation permitted to establish an antigen different from the TF-antigen: This antigen was then called Tn. This remarkable first description of a case with Tn-syndrome already contained the suggestion of an acquired immune hemolytic disease triggered by a microbial, probably viral infection which unmasks or modifies a latent antigen. The chemical nature of this antigen was unknown at that time.

4. Clinical findings

The prevalence of the Tn-syndrome is extremely

[1] The formerly used designation of T-antigen for the Thomsen–Friedenreich antigen should be abandoned because of possible confusion with retroviral T-antigens.

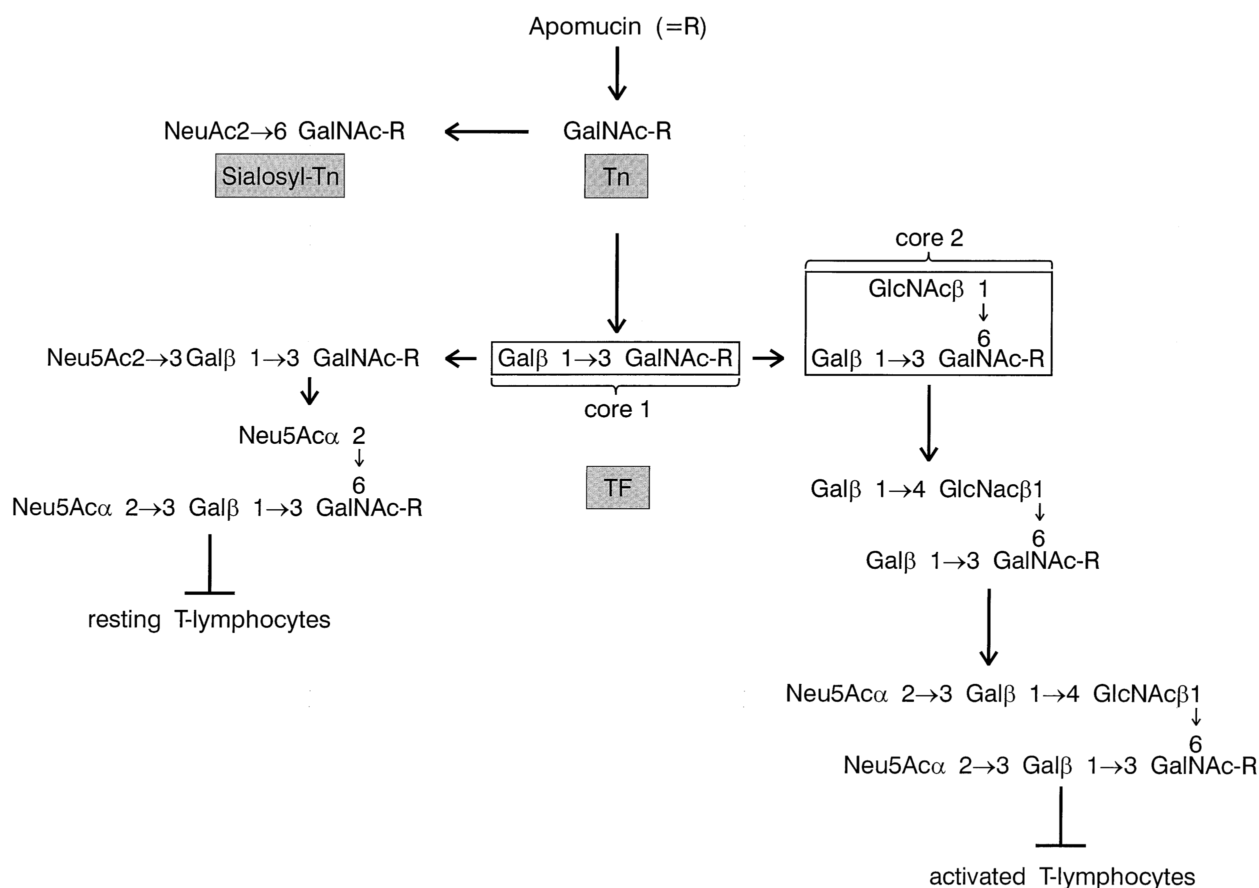

Fig. 1. Biosynthetic pathways of *O*-glycans and structures of TF-, Tn- and sialosyl-Tn-antigens. The main membrane component harbouring *O*-glycans on erythrocytes is glycophorin, on leukocytes leukosialin (or CD43). Further modifications of the core 1 structures are described in [5].

low; it is impossible to give a reliable figure but its occurrence among normal blood donors must be below 1 in 150 000 as we did not find a single case by screening this high a number [1]. Besides the classical case described by Moreau and Dausset [6,7], a few other clinical reports are available. They are summarized in Table 1. In summary, patients affected by the idiopathic Tn-syndrome generally do well except for minor signs of hemolysis or thrombocytopenia. They are usually found by alert technicians testing blood in normal blood donors or in patients for an unrelated clinical problem and observing the phenomenon of polyagglutinability. If referred to an expert in blood group serology, these cases may be further investigated. Often, a thorough work-up is not deemed important since therapeutic consequences are not warranted. Hence, many cases may escape undiagnosed. However, the patient reported by Haynes et al. [12] had a bleeding disorder as a consequence of thrombocytopenia. Interestingly, in this case survival of heterologous erythrocytes was found normal leading the authors to suggest a stem cell disorder since the exogenous red blood cells did not acquire the property of polyagglutinability during more than four months. In Tn(+) patients, the Coombs tests usually are negative despite the fact that anti-Tn Igs are ubiquitously present in serum.

Importantly, recent work carried out in many laboratories has confirmed and extended work pioneered by Springer in identifying the Tn-antigen as a cancer-associated antigen, together with the TF- and the sialosyl-Tn-antigens [23]. It is beyond the scope of this review to critically assess and summarize this work. Suffice to note that unmasking of the Tn-antigen in cancer may induce a T-cell associated

Table 1
Tn-syndrome: clinical findings

Patient	Sex	Age (years)	Duration of Tn-trans-formation (years)	Ec[a] ($\times 10^6/\mu l$)	Ret[b] (%)	Lc[c] $\times 10^3/\mu l$	Thc[d] $\times 10^5/\mu l$	Hb[e] (% or g/100 ml)	Ht[f] (%)	Clinical symptoms	Obser-vations	Ref.
MT	m	65	9	2.7	–	2.87	1.12	60	30	Anemia requiring transfusions	Improvement upon treatment with prednisone	[6,7]
A	m	63	4 months	3	4.2	–	–	61	–	The patient suffered from bronchial carcinoma unrelated to the Tn condition	Acquired condition since all family members tested were negative	[8]
Vr	m	88	–	–	7.5	–	–	8.9	–	Signs of hemolytic anemia		[8]
B.A.	f	18	10 months	–	2	2	0.55, 0.70	13.8	–	Healthy	Persistence of Tn transformation 2.5 years later confirmed involvement of platelets demonstrated	[9–11]
N.N.1[g]	m	53	8	no[h]	no	2.4	0.18	–	–	No evidence for hemolysis, but bleeding disorder	Survival of heterologous erythrocytes in this patient was normal; splenectomy had no influence on thrombocytopenia	[12]
O.S.	m	28	> 4	1.3	–[i]	–	13.3	–	–	Healthy	50% of erythrocytes direct Coombs test negative	[13]
R.M.	m	56	> 3	no	no	no	no	no	–	Healthy	'Mosaic status'	[14]
R.K.	f	36	1	–	–	2.8	0.64	13.1	–	None	Bone marrow normal	[15]
R.R.	m		18	–	–	–	–	–	–	Healthy unrelated clinical problems due to accident	Mosaic status, 93% of erythrocytes Tn(−) transformed	[16,28]
D.J.H.	–	20	–	–	–	Lowered	Lowered	–	–	Urinary bleeding, stomatitis, chronic gingivitis	95% of erythrocytes Tn-transformed	[17]
C.C.	–	24	–	no	–	no	4–5	–	–	Asymptomatic moderate thrombocytopenia	100% of erythrocytes Tn-transformed	[17]

Table 1 (*continued*)
Tn-syndrome: clinical findings

Patient	Sex	Age (years)	Duration of Tn-trans-formation (years)	Ec[a] ($\times 10^6$/μl)	Ret[b] (%)	Lc[c] $\times 10^3$/μl	Thc[d] $\times 10^5$/μl	Hbe (% or g/100 ml)	Ht[f] (%)	Clinical symptoms	Obser-vations	Ref.
Az	–	–	–	–	–	–	0.70	–	–	–	Tn(+) transformed platelets identified	[11]
Du	–	–	–	–	–	–	0.53	–	–	–	Separation of normal from Tn-transformed platelets which lacked 1,3GalT activity	[11]
P.L.	m	27	ca. 1	no	Slightly elevated	2.9	0.45	no	no	–	Low haptoglobin bone marrow normal	[18]
M.Z.	f	61	Several years	4.5	–	3.5	1.56	13.7	39.8	–		[19]
R.P.T	f	35	0.5	–	–	–	–	–	–	–		[20]
Rob	f	22		no	no	no	no	no	no	–		[21]
N.N.2[g]	m	55		3.91	1.9	1.4	0.18			Bleeding disorder	Coombs test negative	[22]

[a]Ec, erythrocytes.
[b]Ret, reticulocytes.
[c]Lc, leucocytes.
[d]Thc, platelets.
[e]Hb, Hemoglobin, 100% defined as 14.5 g/100 ml.
[f]Ht, Hematocrit.
[g]N.N., not named.
[h]no, normal.
[i]Not determined or not reported.

Table 2
Reagents used to detect the Tn-antigen: monoclonal antibodies

Designation	Antigen	Biochemical characterization	Iso-type	Observations	Ref.
5F4 (mouse)	Asialo-OSM	αGalNAc and underlying structures, nc[a]	IgM	Binds Tn(+) T lymphocytes T	[30]
CU-1 (mouse)	Glycoprotein released by Lu65 human lung squamous carcinoma cells	Tn-antigen derived from immunogen, glycophorin A and asialo-OSM	IgG3	Not crossreactive with A antigen	[31]
6E6-H5-G9 (mouse)	Tn(+) erythrocytes	nc	IgM	Crossreacts with asialo-OSM	[32]
T9 2c (rat)	TF(+) erythrocytes; desialylation of normal erythrocytes uncovers small amounts of Tn-antigen	nc	IgM		[32]
12H5-C9-G11	Tn(+) erythrocytes	nc	IgM	Crossreacts with asialo-OSM	[32]
NCC-LU-81	Membrane fraction derived from human lung carcinoma Lu65 cells	αGalNAc-*O*-peptide	IgM	Crossreacts with A antigen	[32,33]
B72.3	Membrane fraction derived from human mammary carcinoma cells	nc	IgG1	Rel. low affinity to asialo-OSM recognizes also sialosyl-Tn	[29,34]
83D4 (mouse)	Human breast carcinoma cells	Mucin-like Tn-determinant containing membrane protein present in MCF-7 breast carcinoma cells			[35]
Etn 1.01	Tn(+) erythrocytes	nc	IgM		[36]
MLS128 (mouse)	Human colonic cancer cells LS180	Glycopeptides derived from LS 180 cells, OSM or asialo-OSM	IgG3	Not crossreactive with A antigen; GalNAc recognized even if 2→6 substituted by NeuAc	[37]
164H.1	Synthetic Tn-conjugates: αGalNAc-human serum albumin			All seven cases of colon carcinomas stained positive on frozen sections	[38]
Purified human anti-A antibodies	Do not react with Tn	Blood group A epitope	IgM		[15]

[a]nc, not characterized.

immune response, a finding currently exploited in immunotherapy trials [24].

5. Structure of the Tn-antigen

As early as in 1973, Sturgeon et al. [16] suggested on the basis of reduced electrophoretic mobility of Tn(+) erythrocytes and their reduced content of sialic acid that 'a biosynthetic defect common to all of these aberrations could be a block in the transfer of terminal D-galactose to *N*-acetylgalactosamine in a substantial proportion of heterosaccharide chains of red cell membrane glycoproteins'. Since the common *O*-linked bisialylated core 1 structure was already known to occur on glycophorin [25], it was a straightforward task to reveal the Tn-antigen as terminal αGalNAc by chemical analysis. This has been achieved by Dahr et al. [26] using β-elimination of the *O*-glycans followed by gas chromatography: Whereas glycans isolated from normal controls were identified as Galβ1→3GalNAc-ol, free *N*-acetylgalactosaminitol was found in material derived from two different Tn(+) patients (Ba, Am). Together with serological data, Dahr concluded that the Tn-antigen consists of terminal α-GalNAc. Subsequent work by Hesford et al. [27] confirmed the presence of terminal αGalNAc residues on glycoproteins derived from Tn(+) erythrocytes of the case RR: Tn(+) erythrocyte membranes could be used as acceptor sub-

strates for the newly identified erythrocyte membrane 1,3GT [28]: Normal donor erythrocyte membranes solubilized in Triton X-100 did not incorporate labeled galactose from UDP-[^{14}C]Gal, neither did Tn(+) erythrocyte membranes incorporate label. However, mixing normal with Tn(+) membrane lysates led to significant incorporation of label into glycophorin as shown by autoradiography of electrophoretically separated erythrocyte membranes. Tn(+) cell derived glycophorins have been investigated by Jokinen [13] and by Blumenfeld et al. [17]. Jokinen found substantial amounts of terminal *O*-linked GalNAc, but also, surprisingly, di- and tetrasaccharides. Since the Tn(+) pool of erythrocytes had to be separated by free-flow electrophoresis from the Tn(−) subset, some contamination may explain the occurrence of di- and tetrasaccharides in glycophorins of the Tn(+) subset. Alternatively, residual 1,3GT in Tn(+) cells might have given rise to these products. Blumenfeld et al. [17] analyzed the carbohydrate composition of isolated glycophorins without prior separation of Tn(+) from Tn(−) subsets and found less Gal and NeuAc in Tn(+) than in control glycophorins, while *N*-glycans seemed to be present. Interestingly, β-eliminated glycans contained a disaccharide composed of equal amounts of NeuAc and GalNAcol which, upon digestion with *Clostridium perfringens* neuraminidase, was cleaved whereas no cleavage was seen with Newcastle disease virus neuraminidase which preferentially cleaves $\alpha 2 \rightarrow 3$ linkages. Thus, the authors concluded that substantial amounts of the sialosyl-Tn structure were present and, in fact, confirmed a previous report by Kjeldsen et al. [29] of the occurrence of sialosyl-Tn on Tn-erythrocytes: these authors determined the presence of sialosyl-Tn using a panel of monoclonal antibodies to the Tn or the sialosyl-Tn epitope, respectively.

The development of mAbs and the use of more specific lectins are of considerable help in identifying the Tn-antigen (Tables 2 and 3).

6. All blood cells are involved

Early clinical work already led to the suggestion that Tn-transformation affects stem cells at an early stage of differentiation [12] since thrombocytopenia and leukopenia have frequently been observed (cf. Table 1). Involvement of platelets [11] and granulocytes [18] has been demonstrated by Cartron, Vainchenker and associates. A striking reduction of the sugar content of platelet membrane glycoproteins GP1b and glycocalicin was noted. In the Tn-patients, Cartron and Nurden [11] also found a dual population of platelets (normal and Tn(+), respectively) with a 'mosaic status' analogous to that already

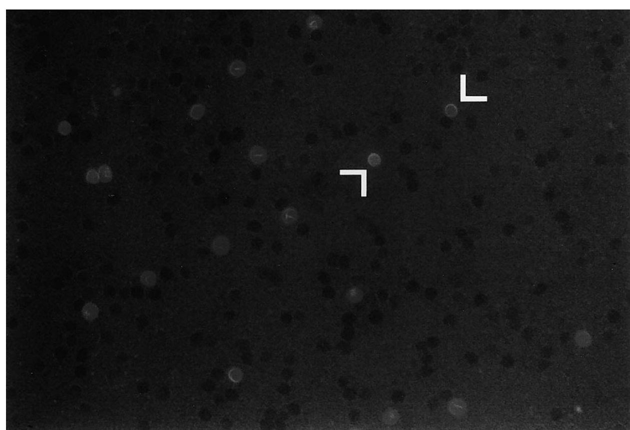

Fig. 2. Immunofluorescence of erythrocytes of patient R.R. Neuraminidase-treated erythrocytes were stained using a mAb to the TF-antigen as described [43]; examples are indicated with arrowheads. 7% of all red blood cells exhibit the TF-antigen, thus appear normal. 93% have been shown to be Tn(+), thus not reactive with the mAb [16,28].

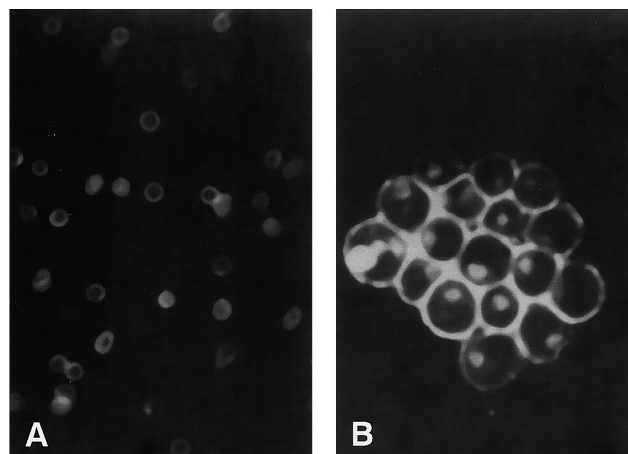

Fig. 3. Immunofluorescent detection of the Tn-antigen on erythrocytes (A) and burst forming units-erythroid colonies (B) using FITC-labeled HPA. As shown on panel B, all cells are positive indicating expression of Tn as a clonal feature. Reproduced from Vainchenker et al. [18] by copyright permission from Am. Soc. Clin. Invest.

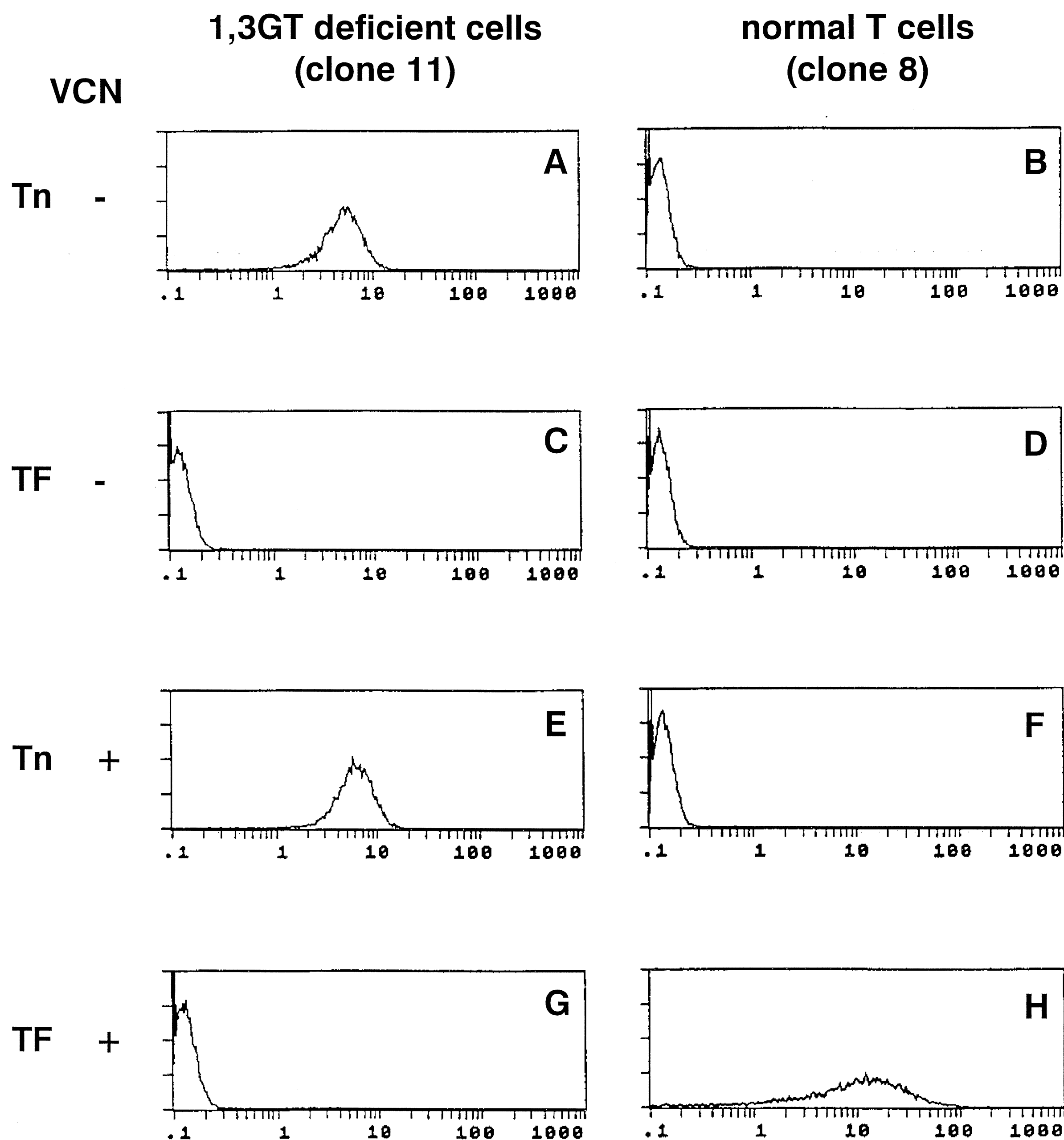

Fig. 4. Flow cytometric analysis of ex vivo T cell clones from patient R.R. VCN designates treatment with *Vibrio cholerae* neuraminidase prior to analysis. Analysis has been carried out using mAb TKH6 to the Tn-antigen (panels, A,B,E,F) or mAb 22.19 to the TF-antigen (panels C,D,G,H). Reproduced from Thurnher et al. [43] by copyright permission from Eur. J. Immunol.

found for erythrocytes (see Fig. 2). The biochemical phenotype of Tn-platelet membrane glycoproteins was also compared with those derived from a patient with Bernard–Soulier syndrome and a patient with thrombasthenia, respectively; in both these conditions 1,3GT activity was normal [11]. The glycoprotein GP1b of a patient with Tn-syndrome (Patient B.A., Table 1) was more thoroughly investigated by 2-dimensional gel electrophoresis and lectin binding studies and shown to be the major membrane component exposing the Tn-antigen [44].

In a series of elegant studies the clonal expression of the Tn-antigen was then demonstrated in erythroid (as expected) and granulocyte colonies [18,44]:

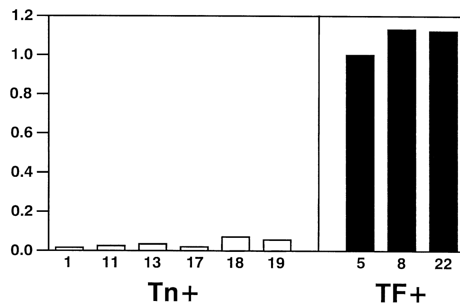

Fig. 5. β1,3 Galactosyltransferase activity in 6 different Tn(+) and 3 TF(+) T cell clones. 1,3GT activity is given as nmol/min per mg of protein and was measured using asialo-OSM as substrate [28]. Reproduced from Thurnher et al. [43] by copyright permission from Eur. J. Immunol.

peripheral blood and bone marrow cells were grown ex vivo from patients P.L. and B.A. (Table 1) under conditions to expand clonally as burst-forming units-erythroid and colony-forming units-granulocyte-macrophage. These cells were then stained using HPA-FITC and shown to uniformly express the Tn-antigen or to lack it thereby demonstrating clonality of Tn-expression (cf. Fig. 3). These data also confirmed the presence of two types of stem cells explaining the dual expression pattern of normal and Tn-transformed cells. As a spin-off of this study the authors also showed clonality of expression of the Tn-antigen in the megakaryocyte colony assay. Subsequent studies carried out by flow cytometry confirmed these data [44]. Since all these lineages are involved, a somatic mutation at the pluripotent stem cell level must be assumed. In this respect this condition recalls paroxysmal nocturnal hemoglobinuria (for review see [45,46]). More recently, erythroid cells from a donor with Tn-syndrome (patient N.N., see Table 1, [22]) were expanded in vitro and co-cultured with cells from a normal donor. In this in vitro system of mixed cultures the normal cells proved to have a growth advantage over those expressing the Tn-antigen. By contrast, in a culture of patient cells only, which initially had only 1.4% of Tn(+) cells, the Tn(+) subset expanded to 62.9% on day 9. This re-

Table 3
Reagents used to detect the Tn-antigen: lectins

Type	Epitope recognized	Crossreactive	Inhibitable by (concentrations indicate 50% inhibition)	Observation	Ref.
Dolichos biflorus	αGalNAc > βGalNAc	A_1	200 nmol/200 μl α-methylGalNAc		[40]
Moluccella laevis	αGalNAc	A, N	GalNAc	Binds to Tn lymphocytes	[30]
		May weakly bind to Gal	Asialo-OSM, asialo-agalacto-glycophorin		[41]
		As above, sialosyl-Tn		Agglutination of Tn erythrocytes	[39]
Vicia villosa isolectin B_4	αGalNAc			Co-crystallization of B_4 lectin with GalNAc	[42]
Salvia sclarea	αGalNAc	Tn-specific, not crossreactive with A			[39,40]
Helix pomatia	αGalNAc > αGlcNAc > βGalNAc > βGlcNAc > αGal	A	2.2 nmol/200 μl α-methylGalNAc; 1.9 nmol/200 μl methyl *N*-bromoacetyl αGal		[40]
Lima bean	αGalNAc > βGalNAc				[40]
Soy bean agglutinin	α,βGalNAc > α,βGal		2.5 nmol/200 μl GalNAc; 2.5 nmol/200 μl methyl βGalNAc; 2 nmol/200 μl methyl *N*-bromoacetyl αGalNAc		[40]

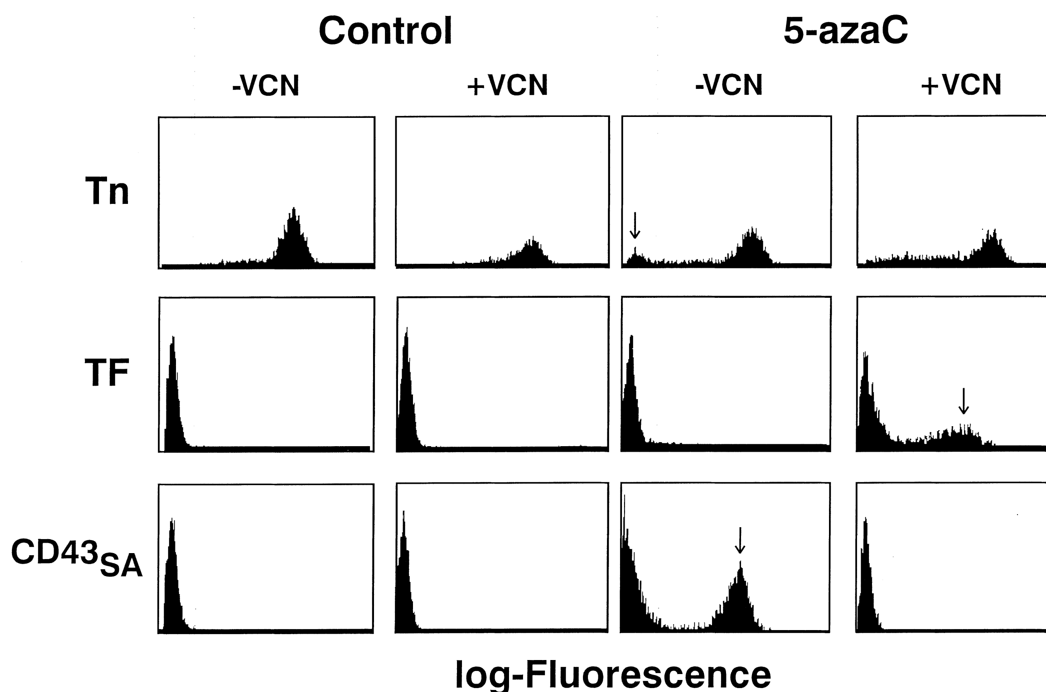

Fig. 6. Reexpression of TF-antigen and $CD43_{NeuAc}$. Reactivation in Tn(+) cells by treatment with 5′azacytidine. Tn(+) lymphocytes T derived from patient R.R. were treated with 5′azacytidine and analyzed by flow cytometry for the expression of the Tn-antigen (upper panels), TF-antigen (middle panels) and $CD43_{NeuAc}$ using the mAbs TKH6, HH8 and DF-T1, respectively, with and without prior treatment with neuraminidase. The arrows indicate 5′azacytidine-induced changes. Upper row: appearance of Tn(−) subset; middle row: appearance of a TF(+) subset (binds antibody after neuraminidase only); lower row: appearance of CD43 (using a mAb specific for sialylated epitopes). Reproduced from Thurnher et al. [47] by copyright permission from Am. Soc. Clin. Invest.

sult recalls somewhat Thurnher's data [47] showing overgrowth of Tn(+) lymphocytes in a culture previously treated with 5′azacytidine and reexpressing the TF-antigen.

The same group also provided evidence for the involvement of B and T cells in the Tn-syndrome [48] at a time when CD markers were not yet available. Therefore, quantitation was done by microscopic counting of HPA-labeled cells double stained with antibodies to Ig chains. While the percentage of Tn-transformed B-cells was around 50%, that of T cells was quite low, around 1 to 2%, a finding later confirmed by flow cytometry in case R.R. [43]. The possibility to immortalize B cells by Epstein–Barr virus infection was applied to the investigation of Tn-transformation by Gahmberg et al. [49]: EBV-transformed cells derived from a patient with blood-type 0 were stained with the HPA lectin and cloned by FACS. The resulting clones were uniformly lacking or expressing the Tn-antigen for over 1 year. The membrane proteins were analyzed and a 105 kDa glycoprotein was shown to react strongly with galactose oxidase prior to neuraminidase treatment of Tn(+) cells and to bind HPA, suggesting the presence of terminal αGalNAc. For reasons not understood, further cultivation of these B lymphoblastoid cells seemed difficult [50], a finding confirmed by Thurnher et al. (unpublished). Availability of mAbs to the TF and the Tn-antigen rendered isolation and cloning of Tn(+) T lymphocytes possible: Thurnher et al. generated a number of T cell clones from patient R.R. (Fig. 4) and showed absence of 1,3GalT activity in lysates of Tn(+) cell clones [43] (Fig. 5). Moreover, he showed that CD3(+), CD4(+) and CD8(+) subsets occurred among both Tn(+) and Tn(−) populations. Using *Limax flavus* lectin flow cytometry, the amount of sialic acid on Tn(+) T cells was estimated to be reduced by 50%. Thus, these cells can be considered as constitutively hyposialylated and were used as models to study binding of various lectins including *Amaranthus caudatus, Maackia amurensis, Sambucus*

nigra and *Triticum vulgare* lectins, respectively [51]: binding characteristics were compatible with defective *O*- but intact *N*-glycosylation. Moreover, these cells were also investigated for their binding of human *siglecs* (for definition see [52]) such as sialoadhesin (Sn), myelin-associated glycoprotein (MAG) and CD22. MAG and Sn binding, respectively were shown to be reduced to 19% and 33%, respectively, of control cells.

7. Molecular basis of the Tn-syndrome

Little is yet known about the causes leading to Tn transformation. A block in the transfer of Gal has been postulated by Sturgeon et al. based on serological observations [16]. Indeed, using asialo-OSM or *p*-nitrophenyl GalNAc as acceptor substrates, Berger and Kozdrowski, [28] and Cartron et al. [53], respectively, confirmed this prediction using detergent-solubilized membranes of affected erythrocytes. Importantly, both groups were able to detect the enzyme activity in serum [53,54]. Thus, absence of 1,3GalT seems to be confined to blood cells. Further analysis of this enzyme deficiency is hampered by lack of antibodies and genetic information. The enzyme has been remarkably resistant to all attempts at purification to homogeneity as well as cloning but has been characterized quite thoroughly on the basis of activity measurements (for review see [2]).

The main question to be answered in any case of enzyme deficiency is its origin: is it the consequence of a structural mutation or of silencing of gene expression?

This question can only be answered with some confidence once the sequence of the corresponding cDNA and the gene will have been elucidated. A tentative answer has been given by Thurnher et al. [47], who treated Tn(+) lymphocytes T with 5′azacytidine or butyrate, respectively: treated cells reexpressed the TF-antigen along with sialylated CD43 both as a result of reactivated and measurable 1,3GalT in lysates of these cells (Fig. 6). Re-expressing cells were kept in culture for several weeks and shown to revert to the original phenotype for reasons not understood. Recently qualitative similar results, i.e., some re-expression of the TF-antigen following treatment of lymphocytes T by the agents mentioned above were obtained in another patient (Patient M.Z.) [19]. Strikingly, the proportion of Tn(+) erythrocytes in the peripheral blood is, in most cases, over 50% and, in some cases, nearly 100%. By contrast, Tn(+) lymphocytes in the circulating blood do not exceed a few percent. The reason for this discrepancy is unknown. Two possibilities may be discussed: either Tn(+) transformation is associated with some proliferative advantage in the case of erythroid lineages and/or disadvantage in the case of lymphoid cells. Alternatively, expression of the Tn-antigen may be associated with the behaviour of blood cells in circulation, leading to sequestration of Tn(+) lymphoid cells in lymphatic organs.

8. Outlook

Recent developments in molecular biology of glycosyltransferases have revealed an unexpected diversity of these enzymes since probably all of them exist as families comprising five to over ten related molecular species. Sensu stricto these are isoenzymes since they are encoded by different, often syntenic genes. Examples include the family of α1,3fucosyltransferases (for review, see [55]), α2,3sialyltransferases (for review, see [56]), β1,4galactosyltransferases [57] and the recently cloned β1,3galactosyltransferases [58]. While some of the recombinant enzymes within this family of β1,3GalTs incorporate to a low extent βGal into terminal αGalNAc, convincing demonstration of a high catalytic activity has not been achieved. Therefore, 1,3GT may be unrelated to any of the known structures of this family and perhaps even belong to another family of core 1 structure forming galactosyltransferases. Circumstantial evidence for this possibility is the ubiquity of the core 1 structure and the fact that in the Tn-syndrome only blood cells are affected. In the case of the existence of a family of 1,3GTs, all members except one are completely inactive in blood cells since in Tn-transformed cells, no residual activity could be measured. In any case, after treatment of Tn-transformed cells with 5′azacytidine or Na butyrate, re-expression of activity may not necessarily reflect reappearance of the silenced isoenzyme. In fact, the reactivated 1,3GT could be due to one or more members of the putative gene family. However, the findings of

Jokinen [13] suggest the possibility of residual activity in affected cells, compatible with a leaky mutation. In this case the underlying cause of the enzyme deficiency can as well be a mutation in the protein-coding part leading to loss of function. Obviously, these questions to be answered need unequivocal cloning and expression of the 1,3GT operating in blood cells.

If the assumption of an acquired silencing of 1,3GT proves correct, elucidation of the mechanisms on how an expression of a defined glycosyltransferase is down-regulated may be of considerable interest to understand developmental regulation of glycosyltransferase genes.

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