CASE REPORT

Graves' disease and Hashimoto's thyroiditis in monozygotic twins: case study as well as transcriptomic and immunohistological analysis of thyroid tissues

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Abstract

Objective: To report on the rare simultaneous occurrence of Graves' disease (GD) and Hashimoto's thyroiditis (HT) in monozygotic twins.

Design: We compared the pattern of thyroid tissue-derived cDNAs to gain insight into previous and ongoing immune destruction and reconstruction processes using microarrays. The results were confirmed by immunohistology and real-time PCR.

Results: Destruction of thyroid tissue in HT reduced levels of thyrocyte-related cDNAs and cDNAs encoding extracellular matrix components, but increased levels of proteases involved in extracellular matrix degradation compared with GD. Lymphocytic infiltrates forming ectopic follicles replaced the thyroid tissue almost completely in HT. Thus, lymphocyte-related cDNA levels were higher in HT than in GD. The same was true for many chemokines and their receptors, which not only enable migration towards the thyroid but also maintain the lymphocytic infiltrate. HT also showed increased levels of cDNAs encoding molecules related to apoptosis than did GD. Surprisingly, the Th1- and Th2-specific cytokine profiles suggested for HT and GD respectively could not be confirmed. cDNAs encoding factors and receptors involved in angiogenesis were increased in GD compared with HT.

Conclusions: Comparison of gene expression reflects the cellular differences between the two types of autoimmune thyroid disease in twins with identical genetic and similar environmental background.

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Introduction

Autoimmune thyroid disease (AITD) manifests itself in various clinical forms such as classical Hashimoto's thyroiditis (HT) and Graves' disease (GD). Histologically, HT and GD are both characterized by lymphocytic infiltration. However, this infiltration varies in quantitative as well as qualitative respects, possibly reflecting the different underlying pathogenic mechanisms of the two diseases (1). In HT, lymphocytic infiltrates replace thyroid follicles, resulting in a loss of hormone-producing cells and thus hypothyroidism. The follicles remain intact in GD. Here, stimulating autoantibodies against the thyroid-stimulating hormone receptor TSHR (thyroid stimulating antibodies, TSABs) are the direct cause of hyperthyroidism.

Although GD and HT have very different phenotypes and the mechanisms leading to the dichotomy of GD and HT are unknown, they are generally believed to share a number of common etiological factors. GD and HT frequently occur in the same family; some

individuals progress from one form to another. Whole-genome linkage studies on multiplex families with AITD have shown that multiple genes are responsible for a predisposition towards GD and HT, and that some are common to both diseases and some are unique (2). Although rare, there have been reports on monozygotic twins in which one twin had GD and the other had HT (3–6).

Studies on twins help in understanding the close relationship between various forms of AITD and reveal the genetic influence on its development (7–9). The relatively low concordance in monozygotic twins indicates that AITD requires non-heritable etiological determinants as well. Environmental and hormonal factors are shown to be associated with increased risk of developing AITD in genetically predisposed individuals (10; reviewed in Prummel *et al.* (11)).

In the present study, we have examined thyroid tissues excised from a pair of monozygotic twins, one of whom had GD and the other HT, and compared tissue-derived cDNA patterns by microarray to gain

an insight into the past and present immune destruction and reconstruction processes in the thyroid. Since the thyroid tissues were removed on the same day, not only the genetic background but also age and processing of the thyroid were the same, which compensates for the disadvantage of analyzing only one typical case of HT compared with GD.

Materials and methods

Patients and assays

The monozygotic twin sisters were 18 years old on the day of surgery. GD and HT were diagnosed on the basis of clinical, biochemical and histological characteristics as well as by scintiscan. Thyroid dysfunction was confirmed by measurements of serum thyroid-stimulating hormone (TSH), free thyroxine (fT₄) and free triiodothyronine (fT₃). TSH binding inhibiting immunoglobulin (TBII) was determined using DYNtest TRAK human, thyroglobulin (Tg) antibodies using DYNtest anti-Tg, and thyroperoxidase (TPO) antibodies using DYNtest anti-TPO (all from B.R.A.H.M.S AG, Hennigsdorf/Berlin, Germany) in sera from the patients (12). TSA detection was carried out as described by Evans *et al.* (13). Bovine TSH (1 mU/ml, Sigma) was added for thyroid blocking antibody (TBA) detection (14).

The thyroid tissues were removed from the twins on the same day. Twin B's tissue sample was taken from the nodule surrounding tissue. The study was approved by the local Medical Ethics Committee.

Zygosity and HLA typing

Zygosity of the twins was evaluated by short tandem repeat (STR) typing using 16 unlinked autosomal microsatellite markers (PowerPlex16 System, Promega, Madison, WI, USA). PCR products were resolved and detected by capillary electrophoresis on an ABI Prism 3100 Avant Genetic Analyzer (Perkin Elmer, Foster City, CA, USA). Molecular typing of HLA-DRB1 and HLA-DQB1 was performed using the PCR sequence-specific primer (SSP) technique with commercial primer sets (Olerup SSP; GenoVision VertriebsgesmbH, Vienna, Austria) according to the requirements of the European Federation of Immunogenetics (EFI). HLA typing for Class I was performed by complement-dependent microlymphocytotoxicity testing.

Identification of lymphocytic infiltrates and germinal centers

Thyroid sections stained with hematoxylin and eosin (HE) were screened for focal lymphocytic infiltration (15). The ectopic follicle and germinal center areas ($n = 50$) were measured as described (1).

Microarray analysis

Total RNA was isolated from thyroid tissue using the RNeasy kit (Qiagen, Hilden, Germany). Ten micrograms were used to prepare ds cDNA (Superscript II, Life Technologies, Gaithersburg, MD, USA) primed with oligo-dT containing a T7 RNA polymerase promoter site (Genset SA, Paris, France). Phenol chloroform extraction was used to purify the cDNA before *in vitro* transcription using the ENZO BioArray RNA transcript labeling kit (Affymetrix, Santa Clara, CA, USA) to synthesize cRNA. The cRNA was fragmented and hybridized to Affymetrix GeneChip U95Av2. To detect differentially regulated genes, we selected genes that were characterized by the Affymetrix MAS5 software as 'increased' or 'decreased' and had a signal log ratio (SLR) ≥ 1.5 or ≤ -1.5 (that is, these genes are expressed at levels at least 2.828 times higher or lower in the GD than in the HT sample; arbitrarily chosen). If a certain gene is represented by more than one specific probe set on the chip, we displayed the probe set with the lowest SLR.

Real time RT-PCR

Superscript II RNaseH-reverse transcriptase (Invitrogen) was used to synthesize cDNA. Quantitative PCR was performed on a RotorGene (Corbett Research, Mortlake, Australia) real-time machine by using SYBR Green I. The PCR primers for CXCR4, CXCL13, CD20, CD3, TPO, Tg, MMP9, and TIMP3 have been published elsewhere (15–18). The primer pair for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was sense 5'-GCT TTC AAT AGC ACC TTG CC-3', antisense 5'-CTG CTC ACG TCA TCA TCA GT -3'.

Transcripts were quantified in three tissue samples from each patient as described (15). Data are expressed as ratio-specific cDNA:GAPDH cDNA, which was calculated from the same cDNA sample. Finally, log (2) ratios of the GD:HT data were calculated.

Immunohistology

Serial sections of thyroid tissue were cut at 6 μ m and fixed in ice-cold methanol for 10 min. The sections were incubated using the monoclonal antibodies Ki-67 (clone MIB1), CD45R0, CD8, CD22, laminin, collagen IV and vascular endothelial growth factor (VEGF) obtained from DakoCytomation GmbH (Hamburg, Germany), Acris Antibodies GmbH (Hiddenhausen, Germany), and Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Following incubation with the antibody at 4 °C overnight, bound antibodies were detected using a super-sensitive detection kit (Dako Cytomation).

Results

Case report

The monozygotic twin sisters were born in 1984 after an uncomplicated pregnancy. They lived in the same house until the age of fourteen. Thus, for the last four years the twins may be discordant for a number of environmental risk factors such as iodine intake, number of negative life events, and infections. Both patients were smokers (10 cigarettes/day). There may have been a family history of AITD. In 2001, the mother was diagnosed with HT (TSH 50.0 mU/l, anti-TPO > 1000 U/ml, TBII negative). Information on other family members was not available.

After birth, goiter was diagnosed in one of the twins (twin A; GD), which was treated with iodine. At the age of 15, hyperthyroidism was diagnosed in this twin. Endocrine orbitopathy was diagnosed three years later. Twin A was treated with methimazole (20 mg/day). Patient compliance was poor, however. Since hyperthyroidism was diagnosed in twin A, attention was given to her twin sister (twin B, HT) who was found to have mild transient hypothyroidism. A cold nodule was detected by scintiscan, which was the indication for thyroid surgery. Twin A (GD) was strongly positive for autoantibodies against the TSH receptor, which had TSAb quality, whereas no TBII was detectable in the HT patient (Table 1). Both patients' sera showed very high levels of antibodies against TPO, and were positive for antibodies to Tg.

Zygosity and HLA typing

The short tandem repeat (STR) typing result confirmed monozygosity in the twin sisters examined. The 16 STR markers investigated yielded completely identical allele profiles (data not shown). The twins bore HLA-A 3,11,

HLA-B 7, 35 (Bw6,-), and HLA-Cw 3,7. HLA-DRB1 *11, *15 and HLADQB1*03, *06 were determined by PCR-SSP.

Microarray analysis, immunohistology and real-time PCR

The two thyroid tissues showed differences in expression for a total of 2866 transcripts; 1088 showed higher expression in the GD patient, 1778 in the HT sample. Setting a cut-off at the signal log ratio, 522 cDNAs were increased in GD and 407 were increased in HT.

Thyrocytes

Thyrocytes remain intact in GD, while normal thyroid parenchyma disappears in HT (Fig. 1a). We found cDNAs from thyrocyte-related molecules such as the TSHR, TPO, Tg, and thyrocyte transcription factors 1 (TTF1) and 2 (FOXE1) or of molecules known to be present in thyrocytes such as type I iodothyronine 5' deiodinase (DIO1), at much lower levels - if present at all - in HT compared with GD tissue (Table 2). Real-time PCR results for Tg and TPO are shown as examples in Fig. 2.

Thyroid stroma

The levels of cDNAs for several collagen and laminin chains, including collagen IV (COL4), which is a component of the basal membrane, were lower in HT compared with GD tissue (Table 2). This result was confirmed by staining for collagen IV and laminin (Fig. 1g, h). On the other hand, the cDNA encoding matrix-metalloproteinase-9 (MMP9), which enables

Table 1 Patients' characteristics.

	Twin A (GD)	Twin B (HT)
First clinical diagnosis	GD with endocrine orbitopathy	Cold nodule
Histological diagnosis	GD	HT
Number of focal infiltrates ($n/226 \text{ mm}^2$)	60	*
Number of ectopic secondary follicles ($n/226 \text{ mm}^2$)	50	90
Area of a secondary follicle ($n = 50$; mean $\pm$ S.E.M.) (mm^2)	0.197 ± 0.002	0.601 ± 0.011
Area of a germinal center ($n = 50$; mean $\pm$ S.E.M.) (mm^2)	0.062 ± 0.001	0.263 ± 0.006
Relation between germinal center and mantle zone areas	1:3.2	1:4.4
Anti-thyroperoxidase (TPO) antibodies (U/ml); cut off: > 60 U/ml	64 400	52 000
Anti-thyroglobulin (Tg) antibodies (U/ml); cut off: > 60 U/ml	8720	168
TSH binding inhibiting immunoglobulin (TBII) (U/l); cut off: > 1.5 U/l	57.9	0
cAMP bioassay for		
Thyroid stimulating antibodies (TSAb), stimulation index (%), cut off: 1.5%	32	0.1
Thyroid blocking antibodies (TBAb), inhibition index (%), cut off: 40%	14	23
Thyroid hormones (three days before surgery)		
TSH (mU/l) (norm 0.4–4.0)	< 0.002	2.04
fT ₃ (pmol/l) (norm 2.3–6.3)	9.8	5.7
fT ₄ (pmol/ml) (norm 10.3–24.5)	16.4	19.9
Treatment	Methazole 5	None

* Lymphocytic infiltrates almost completely replaced the thyroid tissue.

Figure 1 Pattern of cellular organization in the thyroid from monozygotic twins affected by Graves' disease (twin A, GD) and Hashimoto's thyroiditis (twin B, HT). In GD, we found focal lymphocytic infiltrates (arrow) lacking germinal center reactions and fewer as well as smaller ectopic follicles with typical germinal centers compared with the HT tissue (a). In twin B, the thyroid tissue is nearly completely replaced by lymphocytic infiltration forming many large ectopic follicles (a). Thus, more cells were labeled for CD45R0 (b), T-cell (CD8; c) and B-cell (CD22; d)-related antigens in the HT as in the GD tissue. Proliferation demonstrated here by Ki-67 staining was particularly evident in the germinal centers of secondary lymph follicles (e). VEGF expression, characteristic of higher angiogenic activity, was mainly found in the GD tissue (f). Several components of the extracellular matrix (laminin, collagen type IV; g, h) nearly disappeared in HT tissue but remained intact in GD. Scale bars: 200 μ m except in (e) 100 μ m.

degradation of extracellular matrix especially collagen IV, was overexpressed in HT, whereas higher levels of TIMP3, a tissue inhibitor of MMPs, were expressed in GD. PCR results for MMP9 and TIMP3 are shown in Fig. 2. Thus, not only were thyrocytes destroyed in HT tissue, the basal membrane also disappeared (Fig. 1g, h).

Intrathyroidal lymphocytes

Lymphocytic infiltrates almost completely replaced thyroid tissue in HT (Table 1, Fig. 1a). Although lymphocytes formed ectopic follicles with germinal centers in both HT and GD tissue, they were higher in number and spread in HT than in GD tissue (Table 1). Thus, many transcripts specific for T-cells and B-cells or other molecules preferentially found in these cells, such as several cell surface receptors,

transcription factors or cell signaling and activation molecules, were present at higher levels in HT than in GD tissue (data not shown). For example, only transcripts for the different CD3 and the T-cell receptor (TCR) chains are included in Table 2. As examples for increased lymphocytic accumulation in HT, the staining results for CD45R0, CD8 as a T-cell subpopulation, and CD22 as a B-cell marker are shown in Fig. 1b–d. PCR results for CD3 and CD20 are shown in Fig. 2.

Chemokines and chemokine receptors

The cDNA levels of many chemokines and their corresponding receptor(s) were much higher in HT compared with those in the GD thyroid (Table 3). For example, we found higher cDNA expression for

Table 2 Thyrocyte, lymphocyte and extracellular matrix-related transcripts differentially expressed between HT and GD tissue (SLR = signal log ratio: ≥ 1.5 or ≤ -1.5 ; GD:HT).

Gene symbol	Gene description	SLR
Thyrocytes		
DIO1	Deiodinase, iodothyronine, type 1	6.7
TPO	Thyroid peroxidase	3.9
FOXE1	Forkhead box E1 (thyroid transcription factor 2)	2.9
TSHR	Thyroid stimulating hormone receptor	2.1
PAX8	Paired box gene 8	1.9
TG	Thyroglobulin	1.7
TTF1	Thyroid transcription factor 1	1.5
Lymphocytes (only some T-cell related examples shown)		
CD3E	CD3E antigen, epsilon polypeptide (TiT3 complex)	-2.3
TRA	T-cell receptor alpha locus	-2.0
CD3D	CD3D antigen, delta polypeptide (TiT3 complex)	-1.8
TRB	T-cell receptor beta locus	-1.7
Extracellular matrix		
TIMP3	Tissue inhibitor of metalloproteinase 3	3.1
COL4A2	Collagen, type IV, alpha 2	2.6
COL18A1	Collagen, type XVIII, alpha 1	2.4
COL4A1	Collagen, type IV, alpha 1	1.9
LAMA4	Laminin, alpha 4	1.9
HSPG2	Heparan sulfate proteoglycan 2 (perlecan)	1.7
CHI3L1	Chitinase 3-like 1 (cartilage glycoprotein-39)	-2.8
MMP9	Matrix metalloproteinase 9 (92 kDa type IV collagenase)	-2.3

CCR7 and its ligand, CCL19. Of the unique chemokine/chemokine receptor pairs CXCL13/CXCR5 and CXCL12/CXCR4, the transcript of one binding partner showed a signal log ratio ≥ 1.5 (CXCL13, CXCR4; Table 3). Real-time PCR results for CXCL13 and CXCR4 are shown as examples in Fig. 2.

Classical cytokines and cytokine receptors

There were no differences in interferon (IFN)- γ cDNA levels between the thyroid samples. Interestingly,

Figure 2 Semiquantitation of eight mRNAs by real-time PCR in twin A (GD) and twin B (HT) tissues. Data are expressed as ratio-specific cDNA:GAPDH cDNA, which was calculated from the same cDNA sample. Finally, log (2) ratios of the GD:HT data were calculated ($n = 3$, means $\pm$ S.E.M.).

interleukin (IL)-4, IL-5, IL-10, IL-12p35, and IL-13 cDNA were absent in both thyroids. We only found differences between GD and HT tissue in some cytokine receptor cDNAs (Table 3). In most cases, these transcripts were present in the HT but not in the GD sample.

Adhesion molecules

The cDNAs of adhesion molecules involved in migration, localization and retention of lymphocytes into tissues such as CD62L (SELL), CD54 (ICAM1), and integrin $\alpha 4$ (ITGA4) were present at higher levels in HT than in GD (Table 3). However, the cDNAs of adhesion molecules known to be involved in angiogenesis such as integrin $\beta 3$ (ITGB3), integrin $\beta 5$ (ITGB5), and ephrin A3 (EPHA3) were higher in GD than in HT.

Apoptosis

The cDNAs of many apoptosis-related genes such as the key regulator caspase 3 (CASP3) were more heavily expressed in HT compared with GD (Table 4). In contrast, the cDNAs of repressors of cell death such as Bcl-2-associated athanogene 1 (BAG1) were found at higher levels in the GD thyroid.

Angiogenesis

Higher levels of VEGF-A and VEGF-C cDNAs were present in GD thyroid tissue; this was confirmed by staining for VEGF (Fig. 1f). Similarly, we found a higher cDNA level

Table 3 Transcripts for chemokines and classical cytokines and their receptors as well as adhesion molecules differentially expressed between GD and HT tissue (SLR = signal log ratio: ≥ 1.5 or ≤ -1.5 ; GD:HT).

Gene symbol	Gene description	SLR
Chemokines and chemokine receptors		
CCR6	Chemokine (C-C motif) receptor 6	-2.1
CXCL14	Chemokine (C-X-C motif) ligand 14	-2.1
CCR7	Chemokine (C-C motif) receptor 7	-1.9
CXCL13	Chemokine (C-X-C motif) ligand 13	-1.9
CCL18	Chemokine (C-C motif) ligand 18	-2
CCL19	Chemokine (C-C motif) ligand 19	-2
CCR2	Chemokine (C-C motif) receptor 2	-1.6
CXCR4	Chemokine (C-X-C motif) receptor 4	-1.6
Cytokines and cytokine receptors		
D2S448	Interleukin-1 receptor antagonist, melanoma associated gene	1.7
IL1RL1LG	Interleukin 1 receptor-like 1 ligand	1.6
IL2RG	Interleukin 2 receptor, gamma	-2.4
IL7R	Interleukin 7 receptor	-2.2
IL6R	Interleukin 6 receptor	-2.1
IL21R	Interleukin 21 receptor	-1.9
IL27RA	Interleukin 27 receptor, alpha	-1.9
IL16	Interleukin 16 (lymphocyte chemoattractant factor)	-1.8
IL2RB	Interleukin 2 receptor, beta	-1.6
Adhesion molecules		
NCAM1	Neural cell adhesion molecule 1	2
ITGB3	Integrin, beta 3 (platelet glycoprotein IIIa, CD61)	2
MCAM	Melanoma cell adhesion molecule	2
EPHA3	EphrinA3	1.7
SELL	Selectin L (CD62L, lymphocyte adhesion molecule 1)	-3.7
ICAM1	Intercellular adhesion molecule 1 (CD54)	-1.8
ITGA4	Integrin, alpha 4 (CD49D, alpha 4 subunit of VLA-4 receptor)	-1.7
ITGA1	Integrin, alpha 1	4.2
ITGB5	Integrin, beta 5	2.4
ENPEP	Glutamyl aminopeptidase (aminopeptidase A)	2.4

of one of the VEGF receptors, FLT-1, in the GD thyroid. Transcripts of placental growth factor, another angiogenic stimulator, were also increased (Table 4).

Discussion

AITD is a multistep process initiated by the interaction of genetic and environmental factors. Thyroid samples obtained at surgery in most cases represent the chronic phase of AITD; lymphocytes were accumulated, ectopic lymphoid follicles had formed and, in the case of HT, thyrocytes had disappeared. However, maintenance of the lymphocytic infiltrate in AITD tissues is not static. The presence of activated T-cells in the thyroid (19, 20) and increasing variability of T-cell clones and auto-antibody affinity during the course of AITD (1, 21, 22), even after treatment, reflects the long-standing active immunological processes in the thyroid. Thus, comparison between HT and GD thyroid transcript patterns within identical genetic background

Table 4 Angiogenesis and apoptosis related transcripts differentially expressed between GD and HT tissue (SLR = signal log ratio: ≥ 1.5 or ≤ -1.5 ; GD:HT).

Gene symbol	Gene description	SLR
Angiogenesis		
PGF	Placental growth factor, vascular endothelial growth factor-related protein	2.7
VEGF	Vascular endothelial growth factor	2.6
FLT1	Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	1.9
VWF	Von Willebrand factor	1.9
PDGFRB	Platelet-derived growth factor receptor, beta polypeptide	1.8
VEGFC	Vascular endothelial growth factor C	1.8
Apoptosis		
BCL2L2	BCL2-like 2	2.1
BAG1	BCL2-associated athanogene	1.7
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10; TNF-related apoptosis-inducing ligand (TRAIL)	-2.5
TOSO	Regulator of Fas-induced apoptosis	-2.3
CASP3	Caspase 3, apoptosis-related cysteine protease	-2.1
TRAF1	TNF receptor-associated factor 1	-1.9
CASP1	Caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	-1.7
BIK	BCL2-interacting killer (apoptosis-inducing)	-1.6

and management may give an insight into different immune and remodeling processes. However, comparison between HT and GD transcript expression primarily reflects the varied cellular composition of the lesioned tissue. Thyrocytes were replaced by enlarged lymphocytic infiltrates in HT. Thus, thyrocyte-related transcripts were lower and lymphocyte-related cDNAs higher in HT than in GD. Thyrocytes remain intact in GD, which unsurprisingly showed higher levels of basal membrane component cDNAs than did HT. Increased apoptosis has been proposed as the mechanism for thyroid cell destruction in HT. Besides thyrocytes, lymphocytes located in the ectopic follicles undergo apoptosis during somatic mutation in their immunoglobulin variable-region genes to provide antibodies with higher affinity. Interestingly, we found higher cDNA levels for the major components of the TRAIL/Apo2L, caspase 8 and caspase 3 apoptosis signaling pathway in HT compared with GD. Thus, an alternative pathway killing cells in HT besides that initiated by the activation of the Fas death receptor by binding Fas-ligand (FasL), (reviewed in Salmaso *et al.* (23)), might be involved. Transcripts of chemokines/chemokine receptors involved in lymphocyte migration into the thyroid and in the maintenance of infiltrates were more heavily expressed in HT than in GD thyroid. We found higher cDNA levels for CCR7 and one of its ligands, CCL19. This chemokine, together with CCL21, enables migration of T-cells into secondary lymphoid tissues or, as in the case of thyroid tissue, into ectopic follicles. Moreover, we have confirmed recent data demonstrating higher expression of

CXCL12 or its receptor CXCR4 as well as CXCL13 or its receptor CXCR5 in HT compared with GD thyroids (15, 18, 24) by microarray and real-time PCR. In addition to chemokines, classical cytokines may reflect ongoing immune processes in AITD. IL-2, INF- γ and tumor necrosis factor (TNF)- α -secreting Th1 cells stimulated by IL-12 were proposed to be involved in cell-mediated inflammatory reactions as seen in HT. A Th2-type cytokine secretion pattern characterized by IL-4, IL-5, IL-10 and IL-13 should prevail in GD. Here, the absence of many cytokine cDNAs in one or even both thyroid samples was surprising at first sight. However, the role of the Th1/Th2 balance in AITD is still controversial, especially after the publication of data from clinical studies and newly established mouse models (reviewed in Rotondi *et al.* (25), Dogan *et al.* (26), and Nagayama *et al.* (27)). Moreover, older studies have investigated cytokine cDNAs in thyroids using RT-PCR, which enables detection of traces of cytokine mRNAs (28–31). If low cytokine mRNA levels reflect protein expression of cytokines, their role during pathogenesis of AITD remains unclear.

Hypervascularity and increased blood flow in the thyroid is a diagnostic feature in GD (32, 33). Microvascular density per follicle is significantly higher in GD tissues than in normal thyroid (34). The mechanism of blood vessel formation – angiogenesis – is modulated by both promoters and inhibitors. In our study, upregulation of the principal promoter of endothelial cell growth and migration, VEGF, and one of its receptors, Flt-1, was seen in the GD tissue. Our data agree with those of Nagura *et al.* (34).

Until now, there have only been a few studies on monozygotic twins with AITD; one of these twins had either hyperthyroidism or hypothyroidism (3–6, 35, 36). All twins were female. The diagnosis of AITD in older studies was based on measurements of thyroid hormones, and, in some cases, on fine-needle aspirates. The diagnosis of GD in two of the studies was confirmed by the presence of LATS (long-acting thyroid stimulator). In two pairs of twins, the GD patient was TSAb-positive, while TBABs were detected in the patient with hypothyroidism (5, 6). In the case presented here, TBII was only detected in the GD patient, who showed TSAb but not TBAB activity. The HLA phenotype in our twins is not associated with any increased relative risk for Caucasians to develop AITD, although the diagnosis of HT in the mother suggests a family history of AITD. Increased frequency of HLA-DR3 (DRB1*03) and HLA-DQA1*0501 haplotypes has been demonstrated in Caucasian GD patients. The relative risk for people with DR3 is approximately 2–4 (37).

Altogether, comparison of equally managed thyroid tissue from monozygotic twins reflects the ongoing destruction and reconstruction processes within the thyroid at the cellular level. These processes are clearly different between the two clinical forms of AITD, GD and HT.

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