Androgen-dependent expression of FcγRIIB2 by thyrocytes from patients with autoimmune Graves' disease: a possible molecular clue for sex dependence of autoimmune disease

VALÉRIE ESTIENNE,[*,†] CHRISTINE DUTHOIT,[†] MANUELA REICHERT,[*] ASJA PRAETOR,[*,‡] PIERRE CARAYON,[†] WALTER HUNZIKER,[*,‡,1] AND JEAN RUF[†,1]

[*]Institute of Biochemistry, University of Lausanne, CH-1066 Epalinges, Switzerland; [†]U555 INSERM, Faculté de Médecine Timone, F-13385 Marseille Cedex 5, France; and [‡]Institute of Molecular and Cell Biology, Singapore 117609, Republic of Singapore

ABSTRACT **Thyrocyte expression of HLA class I and class II antigens and related accessory molecules would convert these epithelial cells into functional antigen-presenting cells. Here we show that whereas normal thyrocytes express FcRn, Graves' disease thyrocytes also express FcγRIIB2. We further find that expression of FcγRIIB2, but not FcRn, is repressed by dihydrotestosterone. By mediating the uptake and transport of autoantibodies, we suggest that these IgG Fc receptors contribute in various ways to the onset and/or progression of autoimmune thyroid diseases. The androgen-mediated decrease of FcγRIIB2 expression in Graves' disease thyrocytes also provides a rationale for the predominant susceptibility of women to develop an autoimmune thyroid disease. Our findings open up a new prospect to autoimmunity, linking the role of the target organ to the sex dependence in autoimmune disease.—Estienne, V., Duthoit, C., Reichert, M., Praetor, A., Carayon, P., Hunziker, W., Ruf, J. Androgen-dependent expression of FcγRIIB2 by thyrocytes from patients with autoimmune Graves' disease: a possible molecular clue for sex dependence of autoimmune disease. *FASEB J.* 16, 1087–1092 (2002)**

Key Words: autoimmune diseases · IgG Fc receptors · sex dependence

Autoimmune diseases (AID) occur when the immune system turns against the body and targets some of its organs, tissues, and cells for destruction. As AID affect millions of individuals worldwide, intense study of these diseases and the underlying mechanisms has been carried out during the past two decades (1). However, the mechanisms involved in AID remain a matter of debate, notably the alternate role of the immune system and the targeted organ in the onset and progression of the disease. The current view of AID is that they all develop from common mechanisms involving genetic predisposition, environmental factors, and immune dysregulation (2). Therefore, a detailed understanding of any one of the different AID will provide insight into all the others, leading to establishment of unifying mechanisms. This view is heuristically alluring but finds some limitation in the sex dependence of AID known to vary widely from Sjogren's syndrome and autoimmune thyroid diseases (AITD) to diabetes mellitus (1).

AITD are regarded as prototypes of AID and cumulate irrefutable advantages for the study of the events leading to autoimmunity. These include the tissue accessibility from surgery and therefore the possibility to purify thyroid autoantigens such as thyroglobulin (Tg) and thyroperoxidase (TPO), the existence of several thyroid human disease types and experimental models, and the availability of a large body of clinical and experimental data in the literature (3). Thyrocytes were initially shown to express human leukocyte antigen (HLA) DR molecules (4) and to present antigen to T cells (5). They were further found to overexpress HLA class I molecules and associated molecules such as the transporter associated with antigen processing (6), the latent membrane proteins (LMP) -2 and LMP-7 (7) in AITD, and, more recently, to express the invariant chain that associates with HLA class II molecules in the endoplasmic reticulum and HLA-DM (8). These findings were suggestive of thyrocytes being directly involved in antigen presentation and in the onset and/or progression of AITD. We recently provided additional evidence to support this hypothesis by showing that Tg is fragmented into several immunoreactive peptides during hormone synthesis (9), and that the smaller of them binds and enters human cultured thyrocytes (10). This peptide contains a pathogenic T cell epitope whose production is enhanced by the internalization of Tg-bound monoclonal antibody complexes through a membrane Fc receptor (FcR) (11).

[1] Correspondence: U555 INSERM, Faculté de Médecine Timone, 27 Bd Jean Moulin, F-13385 Marseille Cedex 5, France (Jean.Ruf@medecine.univ-mrs.fr) and Institute of Molecular and Cell Biology, 30 Medical Drive, Singapore 117609, Republic of Singapore (Hunziker@imcb.nus.edu.sg).

Professional antigen-presenting cells such as macrophages and dendritic cells express various FcR involved in their biological responses (12). Antigen–antibody complexes are known to influence the immune response and facilitate generation of cryptic T cell epitopes through internalization via FcR (13, 14). Expression of IgG FcR in splenic macrophages is decreased by androgens (15) and increased with estrogens (16), suggesting a balanced role for sex steroids in the immune system. To further elucidate the putative role of thyrocytes in antigen processing and presentation, we investigated the expression of IgG FcR on normal and pathological human thyrocytes and their regulation by steroid hormones. We show that depending of the physiopathological status of the gland, thyrocytes express FcγRIIB2 in addition to FcRn and that during Graves' disease (GD), expression of FcγRIIB2 is regulated by androgen. These results provide a molecular clue for sex dependence of AITD and may have relevance to the reciprocal role of the immune system and the target organ in AID.

MATERIALS AND METHODS

Primary culture of human thyrocytes

Primary cell cultures were prepared as described by Rasmussen et al. (17). The tissue was minced into small fragments. The tissue fragments were washed in Coon's modification of Ham's F-12 medium (Sigma, St. Louis, MO) containing 2.6 g/L sodium carbonate (Merck & Co., Darmstadt, Germany), penicillin/streptomycin (100 μg/mL) (Life Technologies, Grand Island, NY), and kanamycin (100 μg/mL) (Life Technologies). To separate the epithelial cells from the connective tissue, the fragments were digested by collagenase I (1 mg/mL) (Life Technologies) and dispase II (2.4 U/mL) (Boehringer-Mannheim, Meylan, France) in a calcium- and magnesium-free phosphate-buffered saline (PBS), pH 7.4 at 37°C for 15 min. The enzymatic digestion was repeated for 60 min with fresh enzyme mixture. The digested tissue was filtered through sterile gauze. Medium was added and the cells were washed by successive centrifugations at 100 *g* for 5 min. Finally, the cells were pooled and counted with a Malassez cell. The cells were then seeded to a density of 2×10^6 cells per 250 mL Falcon tissue culture flask (75 cm^2; Becton Dickinson, Franklin Lakes, NJ) in a final volume of 20 mL of the initial medium supplemented with 5% fetal calf serum (Valbiotech, Paris, France) in the presence of bovine thyroid-stimulating hormone, 1 U/L (Sigma) to maintain the thyrocytes in a stimulating environment and five nutritionals: human insulin, 10 mg/L (Boehringer); somatostatin, 10 μg/L (Novabiochem, Läufelfingen, Switzerland); human transferrin, 6 mg/L (Boehringer); hydrocortisone, 10^{-8} M (Calbiochem, La Jolla, CA); and glycyl-histidyl-lysine acetate, 10 μg/L (Calbiochem). The culture medium also contained L-glutamine (2 mmol/L) (Life Technologies) and nonessential amino acids (ICN Pharmaceuticals, Costa Mesa, CA). The cells were cultured at 37°C in a humidified air atmosphere containing 5% CO_2.

After overnight growth, cells were incubated in the absence or presence of 17-β estradiol or dihydrotestosterone (DHT) (Fluka, Buchs, Switzerland). The concentration of 17-β estradiol and DHT ranged from 10^{-9} M to 10^{-6} M in the medium. To block the DHT effect, cells were cultured in the presence of DHT 10^{-6} M with equal or 10-fold concentration of flutamide (Sigma). DHT, 17-β estradiol, and flutamide were dissolved in ethanol to a final ethanol concentration of 0.01%. The same ethanol concentration was added to control medium.

Immunofluorescence

For direct staining, cells grown on coverslips were washed with PBS at pH 6 or 7.4 containing 0.9 mM $CaCl_2$ and 0.9 mM $MgCl_2$ (PBS+) and fixed for 2 min with cold acetone-methanol (1:1). Cells were incubated for 60 min with the first antibody (3 μg/mL TPO mAb 47 (18) or 10 μg/mL of Fab fragments of anti-FcγRII mAb; Medarex, Princeton, NJ), washed six times with PBS+, then incubated with a labeled secondary antibody. To monitor the internalization of IgG, cells were washed with PBS+ and incubated for 60 min at 37°C in the presence of 10 μg/mL of soluble or heat aggregated (30 min, 56°C) human IgG (Sigma) in medium supplemented with 0.5% bovine serum albumin, adjusted to either pH 6 or 7.4. Cells were then transferred on ice, washed with PBS+, fixed, and internalized IgG were detected with labeled secondary antibodies (Jackson, West Grove, PA). Stained cells were mounted in Mowiol (Calbiochem) and viewed in a Zeiss Axiophot fluorescence microscope using a 63× oil immersion lens. Pictures were acquired with a Color Cool View camera (Photonic Science) equipped with the Image Pro Plus software (v3.0; Media Cybernetic). Photoshop software (v5.02; Adobe System Inc.) was used for image editing.

IgG-agarose precipitation and Western blot analysis

Cells were lysed in 5 mg/mL 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate in 50 mM phosphate buffer adjusted to either pH 6 or 7.4 (lysis buffer). Total proteins (1 mg) from thyrocytes and 0.2 mg of total proteins from Madin-Darby canine kidney (MDCK) cells transfected with the FcRn were incubated with 25 μL of IgG agarose (~10 mg IgG/mL agarose slurry) in lysis buffer pH 6 or 7.4 at 4°C for 12 h. The IgG agarose was washed three times with lysis buffer, pH 6 or 7.4, and bound proteins were eluted by boiling in sample buffer and analyzed by SDS-PAGE (10% acrylamide) in nonreducing conditions. For Western blot analysis, proteins were transferred to nitrocellulose and probed with a 1:200 dilution of polyclonal rabbit anti-FcRn peptide serum.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was prepared using a RNEasy Midi Kit (Quiagen, Hilden, Germany) according to the manufacturer's protocol. The integrity of the RNA was confirmed by electrophoresis in denaturing 1.5% agarose gels. RT-PCR was performed using 1 μg of total RNA, the primers listed in **Table 1** and the Titan One Tube RT-PCR System (Boehringer-Mannheim) according to the manufacturer's instructions. PCR products were analyzed by electrophoresis on 2% agarose gels and ligated into the pCRblunt vector (Invitrogen, Groningen, The Netherlands) for sequencing.

RESULTS

Characterization of thyroid cells

Normal thyroid tissue specimens were obtained from para-adenomatous tissues of 10 patients with macrofol-

TABLE 1. *Oligonucleotides of 5′ and 3′ primers*[a]

FcγR type	Sense	Sequence 5′–3′	Size of PCR product (bp)
FcγRI	+	GGA CAA CTC TGC TCC TTT GGG TT	896
	−	GGA AAC ATT ATT CCC ACT GCC AG	
FcγRII	+	GGC TCA TTC CCA CCC ACA CG	508
	−	GGA TTG GCT GAA ATC CGC TTT TT	
FcγRII B1, B2	+	GCT CCA GAC CCC TCA CCT G	B1: 532, B2: 475
	−	CTA AAT ACG GTT CTG GT	
FcγRIII	+	GGC TCC TCC CAA CTG CTC TG	674
	−	GGC ACA GAG AAA TAT AGT CCT GT	
FcRn	+	CTT GGG TCT GGG AAA AC	670
	−	GGA CTT GGC TGG AGA TT	
GAPDH	+	GGG CTC CTT CTG CTG AT	210
	−	GGG CTG GGT GGC AGT GAT	
VW factor	+	GGG TTA CAA GGA AGA AAA T	556
	−	TCA CTT GCT GCA CTT CC	

[a] +/− means that the oligonucleotide is sense or antisense relative to cDNA.

licular nontoxic adenomas based on histopathological examination at thyroidectomy. Autoimmune thyroid tissues were from seven patients undergoing thyroidectomy for GD previously treated by anti-thyroid drugs for 16–20 months. At least 2 wk before the operation, no patient was treated with drugs known to influence the thyroid gland. Each patient's tissue was used to derive a single primary culture of thyrocytes as described in Materials and Methods. After 8–10 days of culture, we obtained a confluent thyroid cell layer that homogeneously stained for TPO monoclonal antibody by immunofluorescence (**Fig. 1**).

Thyrocyte expression of IgG FcR

The expression of IgG FcR was investigated in 10 normal and 7 GD primary cultures of thyrocytes using RT-PCR. Total RNA was prepared from each primary culture and RT-PCR was performed with specific primers for FcRn, FcγR I, II, and III (Table 1). Positive controls were performed with the monocyte cell line U937 for FcγRI, II and III (19) and with transfected MDCK cells for FcRn (20) (data not shown). RT-PCR products were obtained from RNA of normal and GD thyrocytes with the FcRn primers (**Fig. 2*a***) and sequencing confirmed that the PCR product encoded FcRn. FcγRII-specific primers yielded a PCR product only for RNA from GD thyrocytes (Fig. 2*a*) and sequencing determined its identity as the FcγRIIB2 isoform. Primers for the others IgG FcR yielded no PCR product from either normal or GD thyrocytes. Primers for the Von Willebrand factor, a specific marker for endothelial cells, showed no RT-PCR product for both normal and GD thyrocytes (Fig. 2*a*), ruling out a

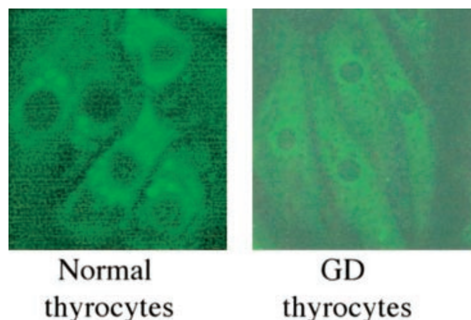

Figure 1. Normal and GD thyrocytes express TPO. Homogeneous fluorescent staining of TPO in each cell culture identifies the cells as differentiated thyrocytes.

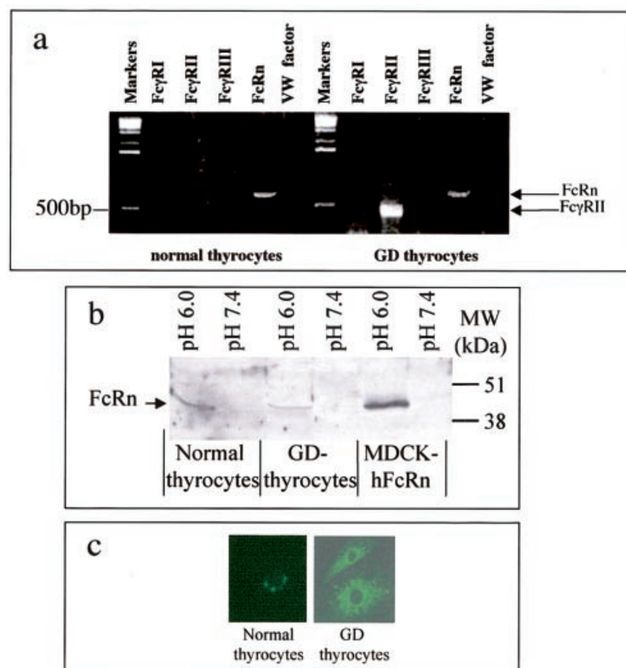

Figure 2. Thyrocytes express IgG FcR. *a)* RT-PCR products for FcRn were detected in normal and GD thyrocytes whereas those for FcγRIIB2 were present only in GD thyrocytes. *b)* FcRn protein was detected in normal thyrocytes and in smaller amounts in GD thyrocytes. FcRn from thyrocytes showed similar pH-dependent IgG binding properties as the FcRn from transfected MDCK cells. *c)* GD but not normal thyrocytes express FcγRIIB2.

contamination of the thyrocytes with endothelial cells. As expected, fibroblasts from parathyroid tissues did not express IgG FcR transcripts (not shown), further confirming the specificity of the detection of IgG FcR transcripts in thyrocytes.

Expression of FcRn by normal and GD thyrocytes was confirmed at the protein level by mixing cell lysates with IgG agarose at pH 6 or 7.4 and analyzing precipitates by SDS-PAGE and Western blot using an FcRn antiserum (21). A 42 kDa protein was detected when cell lysates were precipitated with IgG agarose at pH 6 but not at pH 7.4 (Fig. 2*b*), consistent with the known pH dependence of IgG binding by FcRn. The 42 kDa thyrocyte protein comigrated with FcRn from transfected MDCK cells and showed the same IgG binding properties (Fig. 2*b*). FcRn was present in normal and, in smaller amounts, in GD cells. Since there are no antibodies to FcγRIIB2 suitable for immunoblotting, we confirmed the expression of FcγRIIB2 by GD and its absence in normal thyrocytes at the protein level by indirect immunofluorescence on fixed cells using $F(ab)_2$ fragment of a specific manufactured monoclonal antibody (Fig. 2*c*).

Functional activity of the IgG FcR

To determine whether thyrocytes were able to internalize IgG, we used cells cultured for 4 days, reaching 80% confluence. The cells were incubated at a pH of 6 or 7.4 for 60 min at 37°C in the presence of soluble or aggregated human IgG. Normal and, to a lesser extent, GD thyrocytes internalized soluble but not aggregated IgG at pH 6 (**Fig. 3**). In contrast, at pH 7.4, aggregated IgG were internalized by GD but not normal thyrocytes (Fig. 3). In GD thyrocytes, aggregated IgG were delivered to distinct vacuolar compartments reminiscent of lysosomes (Fig. 3). The FcR-mediated uptake of IgG into thyrocytes is ascertained by the low concentration (10 μg/mL) of IgG used in the experiment and by the differences observed in the immunofluorescent patterns shown in Fig. 3.

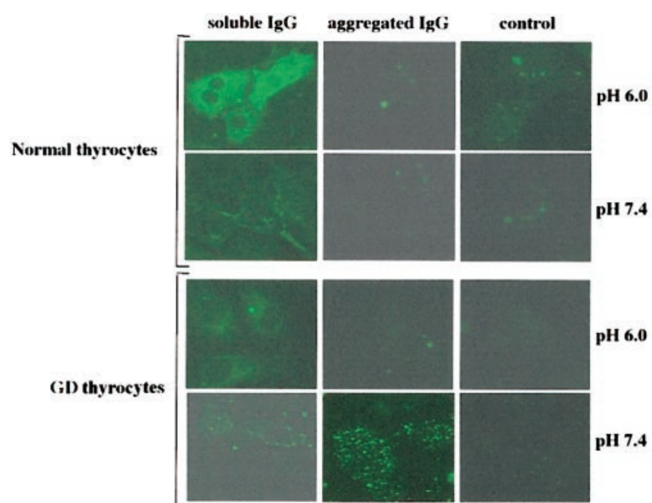

Figure 3. Thyrocytes internalize IgG. Normal and, to a lesser extent, GD thyrocytes internalized soluble IgG at pH 6 whereas GD thyrocytes internalized aggregated IgG at pH 7.4. Control experiments were made in the absence of IgG.

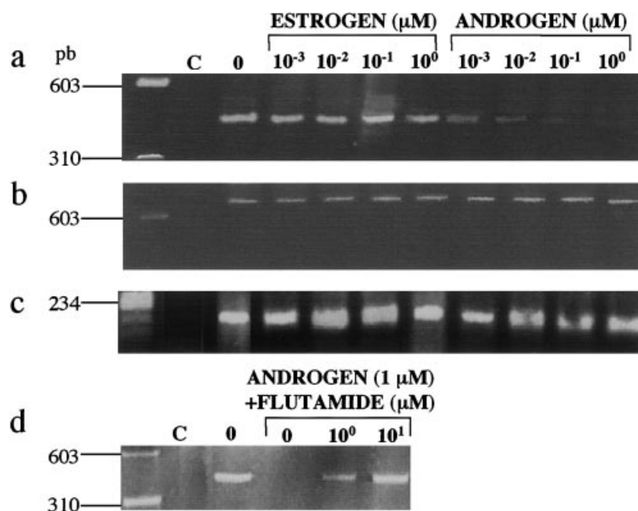

Figure 4. Sex hormones act on the FcγRIIB2 expression by GD thyrocytes. RT-PCR products for *a)* FcγRIIB2, *b)* FcRn, and *c)* GAPDH were tested from cultured GD thyrocytes in the absence (0) or presence of increasing amounts (10^{-3}-10^{0} μM) of estrogen or androgen hormones. C means control made in the absence of ARN from thyrocytes. We only observed the gradual decrease of FcγRIIB2 transcripts by increasing the doses of DHT. *d)* The effect of 1 μM dose of DHT was totally reversed by 10 μM flutamide, an anti-androgen.

Effect of sex hormones on IgG FcR expression

We assessed the effect of DHT and 17-β estradiol on IgG FcR expression by thyrocytes using RT-PCR. In GD thyrocytes, DHT led to a gradual decrease in FcγRIIB2 expression at doses ranging from 10^{-9} to 10^{-6} M whereas17-β estradiol did not (**Fig. 4*a***). Both sex hormones had no effect on FcRn expression in normal (not shown) and GD thyrocytes (Fig. 4*b*). Primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (22) were used to show that similar amounts of RNA were used in the RT-PCR (Fig. 4*c*). Flutamide, an anti-androgen that counteracts exogenous androgens in castrated animals (23), abrogated the DHT-mediated decrease of FcγRBII expression in thyrocytes in vitro (Fig. 4*d*), confirming that the repression was specifically mediated by the androgen.

DISCUSSION

Here we show for the first time that thyrocytes express two types of functional IgG FcR: FcRn and FcγRIIB2. FcRn, homologous to HLA class I, mediates the transport of IgG across epithelial barriers such as the intestine or the placental syncytiotrophoblast; this receptor binds IgG at a mildly acidic (pH 6) but not at neutral to basic pH (24). Three types of IgG FcR (FcγRI to III)

belong to the immunoglobulin superfamily (25) and mediate functions on immune cells as diverse as antibody-dependent cell cytotoxicity, endocytosis, and phagocytosis of IgG-coated antigens, release of inflammatory mediators, and regulation of lymphocyte proliferation and differentiation (12). Whereas a pH-dependent internalization of IgG is characteristic of FcRn, a preferential binding of aggregated vs. soluble IgG is typical for FcγRII. IgG complexes taken up by FcγRII are delivered to lysosomes (26) whereas ligands internalized via FcRn are not (27). The IgG internalization data we obtained for thyrocytes are thus consistent with an expression of FcRn in normal thyrocytes and FcγRII in GD cells.

FcRn is implicated in transcytosis of IgG across several epithelia as well as in the rescue of IgG from lysosomal degradation; it binds IgG on the cell surface when exposed to an acidic compartment (intestine) or in the acidic environment of endosomes after uptake of ligand internalized in the fluid phase (placenta, yolk sac) (24). TPO aAb (mostly IgG) have been detected in cytoplasmic vesicles and on the apical surface of thyroid cells, suggesting they are taken up by thyrocytes to reach their antigen (28, 29). Considering the follicular organization of the polarized thyrocytes with their apical side facing the enclosed colloidal space, it is unclear how in vivo IgG gain access to TPO, a membrane enzyme acting at the apical surface of thyrocytes. Although FcRn can transcytose in the basolateral to apical or, conversely, in the apical to basolateral directions (20) in vivo, the net transport of IgG has so far been observed only in the lumenal to the serosal direction (27). In liver, kidney, and mammary glands, FcRn is in contact with IgG on the serosal side but it does not transfer IgG to the lumen, suggesting that in these tissues the function of FcRn is restricted to the recycling of IgG (30). However, FcRn-mediated basolateral to apical transport of aAb in thyrocytes can not a priori be excluded since the intracellular transport of proteins in thyrocytes can differ from that in others epithelial cells (31). Alternatively, the normal function of FcRn in thyrocytes may be to recycle IgG internalized from the basal side. Recycling could be affected in AITD, leading to an alteration of the intracellular traffic of FcRn or retention of IgG in endosomes, which in turn could allow aAb to reach the apical compartment.

On monocytes and macrophages, FcγRIIB2 mediates the internalization and lysosomal degradation of IgG–antigen complexes (24). By facilitating uptake and lysosomal delivery, and thus proteolytic processing of antigen (32), FcγRIIB2 was shown to increase the efficiency of antigen bound to IgG to be presented by HLA class II (33). Intriguingly, the expression of HLA class II and accessory molecules required for antigen presentation is induced in thyrocytes in the course of GD (8). Although direct evidence for antigen presentation by GD thyrocytes has not been described (34), it is tempting to speculate that the expression of FcγRIIB2 on GD thyrocytes allows these cells to present autoantigen in HLA class II context.

Clinicians have long observed that AITD as well as other AID occur preferentially in women. Most of the evidence that human autoimmune response differs between males and females has been gathered from studies of animals. For example, androgens were found to prevent disease in experimental autoimmune encephalomyelitis (35) and thyroiditis (36). Sex hormones act on the immune systems, modulating antigen presentation, lymphocyte activation, and cytokine production (37, 38), but the molecular mechanism that supports their effect is still unknown. Metabolites of injected testosterone were found in thyrocytes of castrated-adrenalectomized male baboons, consistent with a direct action of androgen on the thyroid (39). Here we found that DHT down-regulated FcγRIIB2 expression on GD thyrocytes. This finding strengthens the idea of direct involvement of thyrocytes in autoimmune induction and provides the first clue to explain the sex dependence of AITD at the molecular level. More generally, our finding raises the prospect of a link between the role of the target organ in the autoimmune process and the sex dependence of AID. FJ

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REFERENCES

1. Whitacre, C. C. (2001) Sex differences in autoimmune disease. *Nat. Immunol.* **9,** 777–780
2. Ermann, J., and Fathman, C. G. (2001) Autoimmune diseases: genes, bugs and failed regulation. *Nat. Immunol.* **9,** 759–761
3. Weetman, A.P., and McGregor, A. M. (1994) Autoimmune thyroid disease: further developments in our understanding. *Endocr. Rev.* **6,** 788–830
4. Pujo-Borrell, R., Hanafusa, T., Chiovato, L., and Bottazzo, G. F. (1983) Lectin-induced expression of DR antigen on human cultured follicular thyroid cells. *Nature (London)* **304,** 71–73
5. Londei, M., Lamb, J. R., Bottazzo, G.F., and Feldmann, M. (1984) Epithelial cells expressing aberrant MHC class II determinants can present antigen to cloned human T cells. *Nature (London)* **312,** 639–641
6. Sospedra, M., Tolosa, E., Armengo, I. P., Ashhab, Y., Urlinger, S., Lucas-Martin, A., Foz-Sala, M., Jaraquemada, D., and Pujol-Borrell, R. (1997) Hyperexpression of transporter in antigen processing-1 (TAP-1) in thyroid glands affected by autoimmunity: a contributing factor to the breach of tolerance to thyroid antigens? *Clin. Exp. Immunol.* **109,** 98–106
7. Vives-Pi, M., Vargas, F., James, R. F., Trowsdale, J., Costa, M., Sospedra, M., Somoza, N., Obiols, G., Tampe, R., and Pujol-Borrell, R. (1997) Proteasome subunits, low-molecular-mass polypeptides 2 and 7 are hyperexpressed by target cells in autoimmune thyroid disease but not in insulin-dependent diabetes mellitus: implications for autoimmunity. *Tissue Antigens* **50,** 153–163
8. Catalfamo, M., Serradell, L., Roura-Mir, C., Kolkowski, E., Sospedra, M., Vives-Pi, M., Vargas-Nieto, F., Pujol-Borrell, R., and Jaraquemada, D. (1999) HLA-DM and invariant chain are expressed by thyroid follicular cells, enabling the expression of compact DR molecules. *Int. Immunol.* **11,** 269–277
9. Duthoit, C., Estienne, V., Delom, F., Durand-Gorde, J. M., Mallet, B., Carayon, P., and Ruf, J. (2000) Production of

immunoreactive thyroglobulin C-terminal fragments during thyroid hormone synthesis. *Endocrinology* **141,** 2518–2525
10. Duthoit, C., Estienne, V., Giraud, A., Durand-Gorde, J. M., Rasmussen, A. K., Feldt-Rasmussen, U., Carayon, P., and Ruf, U. (2001) H2O2-induced production of a 40-kDa immunoreactive thyroglobulin fragment in human thyroid cells: the onset of thyroid autoimmunity ? *Biochem. J.* **360,** 557–562
11. Dai, Y., Carayanniotis, K. A., Eliades, P., Lymberi, P., Shepherd, P., Kong, Y., and Carayanniotis, G. (1999) Enhancing or suppressive effects of antibodies on processing of a pathogenic T cell epitope in thyroglobulin. *J. Immunol.* **162,** 6987–6992
12. Daeron, M. (1997) Fc receptor biology. *Annu. Rev. Immunol.* **15,** 203–234
13. Lanzavecchia, A. (1995) How can cryptic epitopes trigger autoimmunity? *J. Exp. Med.* **181,** 1945–1948
14. Amigorena, S., and Bonnerot, C. (1998) Role of B-cell and Fc receptors in the selection of T-cell epitopes. *Curr. Opin. Immunol.* **10,** 88–92
15. Gomez, F., Ruiz, P., Lopez, R., Rivera, C., Romero, S., and Bernal, J. A. (2000) Effects of androgen treatment on expression of macrophage Fcgamma receptors. *Clin. Diag. Lab. Immunol.* **7,** 682–686
16. Gomez, F., Ruiz, P., Bernal, J. A., Escobar, M., Garcia-Egido, A., and Lopez-Saez, J. J. (2001) Enhancement of splenic-macrophage Fcgamma receptor expression by treatment with estrogens. *Clin. Diag. Lab. Immunol.* **8,** 806–810
17. Rasmussen, A. K., Kayser, L., Perrild, H., Brandt, M., Bech, K., and Feldt-Rasmussen, U. (1996) Human thyroid epithelial cells cultured in monolayers. I. Decreased thyroglobulin and cAMP response to TSH in 12-week-old secondary and tertiary cultures. *Mol. Cell. Endocrinol.* **116,** 165–172
18. De Micco, C., Ruf, J., Chrestian, M. A., Gros, N., Henry, J. F., and Carayon, P. (1991) Immunohistochemical study of thyroid peroxidase in normal, hyperplastic, and neoplastic human thyroid tissues. *Cancer* **67,** 3036–3041
19. Fleit, H. B., Wright, S.D., and Unkeless, J. C. (1982) Human neutrophil Fc gamma receptor distribution and structure. *Proc. Natl. Acad. Sci. USA* **79,** 3275–3279
20. Praetor, A., Ellinger, I., and Hunziker, W. (1999) Intracellular traffic of the MHC class I-like IgG Fc receptor, FcRn, expressed in epithelial MDCK cells. *J. Cell Sci.* **112,** 2291–2299
21. Kacskovics, I., Wu, Z., Simister, N. E., Frenyo, L.V., and Hammarstrom, L. (2000) Cloning and characterization of the bovine MHC class I-like Fc receptor. *J. Immunol.* **164,** 1889–1897
22. Dufour, V., Arnauld, C., Lantz, O., Peguillet, I., Morvilliers, K., Emmanuel, A., and Jestin, A. (1999) Quantification of porcine cytokine gene expression using RT-PCR, a homologous internal control and chemiluminescence for microplate. *J. Immunol. Methods* **229,** 49–60
23. Schroder, F. H. (1993) Cyproterone acetate-mechanism of action and clinical effectiveness in prostate cancer treatment. *Cancer* **72,** 3810–3815
24. Hunziker, W., and Kraehenbuhl, J. P. (1998) Epithelial transcytosis of immunoglobulins. *J. Mammary Gland Biol. Neoplasia* **3,** 287–302
25. Raghavan, M., and Bjorkman, P. J. (1996) Fc receptors and their interactions with immunoglobulins. *Annu. Rev. Cell. Dev. Biol.* **12,** 181–220
26. Hunziker, W., and Mellman, I. (1989) Expression of macrophage-lymphocyte Fc receptors in Madin-Darby canine kidney cells: polarity and transcytosis differ for isoforms with or without coated pit localization domains. *J. Cell Biol.* **109,** 3291–3302
27. Ghetie, V., and Ward, E. S. (2000) Multiple roles for the major histocompatibility complex class I-related receptor FcRn. *Annu. Rev. Immunol.* **18,** 739–766
28. Zimmer, K. P., Schmid, K. W., Bocker, W., Scheumann, G. F., Dralle, H., Bramswig, J., and Harms, E. (1997) Transcytosis of IgG from the basolateral to the apical membrane of human thyrocytes in autoimmune thyroid disease. *Curr. Top. Pathol.* **91,** 117–128
29. Zimmer, K. P., Scheumann, G. F., Bramswig, J., Bocker, W., Harms, E., and Schmid, K. W. (1997) Ultrastructural localization of IgG and TPO in autoimmune thyrocytes referring to the transcytosis of IgG and the antigen presentation of TPO. *Histochem. Cell Biol.* **107,** 115–120
30. Cianga, P., Medesan, C., Richardson, J. A., Ghetie, V., Ward, E. S. (1999) Identification and function of neonatal Fc receptor in mammary gland of lactating mice. *Eur. J. Immunol.* **29,** 2515–2523
31. Arvan, P., Kim, P. S. Kuliawat, R., Prabakaran, D., Muresan, Z., Yoo, S. E., and Abu Hossain, S. (1997) Intracellular protein transport to the thyrocyte plasma membrane: potential implications for thyroid physiology. *Thyroid* **7,** 89–105
32. Lanzavecchia, A. (1990) Receptor-mediated antigen uptake and its effect on antigen presentation to class II-restricted T lymphocytes. *Annu. Rev. Immunol.* **8,** 773–793
33. Amigorena, S., Bonnerot, C., Drake, J. R., Choquet, D., Hunziker, W., Guillet, J. G., Webster, P., Sautes, C., Mellman, I., and Fridman, W. H. (1992) Cytoplasmic domain heterogeneity and functions of IgG Fc receptors in B lymphocytes. *Science* **26,** 1808–1812
34. Sospedra, M., Obiols, G., Babi, L. F., Tolosa, E., Vargas, F., Roura-Mir, C., Lucas-Martin, A., Ercilla, G., and Pujol-Borrell, R. (1995) Hyperinducibility of HLA class II expression of thyroid follicular cells from Graves' disease. A primary defect? *J. Immunol.* **154,** 4213–4220
35. Dalal, M., Kim, S., and Voskuhl, R. R. (1997) Testosterone therapy ameliorates experimental autoimmune encephalomyelitis and induces a T helper 2 bias in the autoantigen-specific T lymphocyte response. *J. Immunol.* **159,** 3–6
36. Ansar Ahmed, S., Young, P.R., and Penhale, W. J. (1986) Beneficial effect of testosterone in the treatment of chronic autoimmune thyroiditis in rats. *J. Immunol.* **136,** 143–147
37. Eidinger, D., and Garrett, T. J. (1972) Studies of the regulatory effects of the sex hormones on antibody formation and stem cell differentiation. *J. Exp. Med.* **136,** 1098–1116
38. Weinstein, Y., Ran, S., and Segal, S. (1984) Sex-associated differences in the regulation of immune responses controlled by the MHC of the mouse. *J. Immunol.* **132,** 656–661
39. Sheridan, P. J., Aufdemorte, T. B., Triplett, R. G., Holt, G., and Martin, P. M. (1988) A differential nuclear uptake and retention of 3H-androgens in the thyroids of baboons. *J. Endocrinol. Invest.* **11,** 571–575

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