Review and Hypothesis: Does Graves' Disease Develop in Non-Human Great Apes?

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Background: Graves' disease, caused by stimulatory thyrotropin receptor (TSHR) autoantibodies, has not been observed in animals. In contrast, Hashimoto's thyroiditis develops in chickens, rats, mice, dogs, and marmosets. Attempts to induce an immune response in mice to the luteinizing-hormone receptor suggested that autoantigen glycosylation was one parameter involved in breaking self-tolerance. Over evolution, TSHR glycosylation increased from three asparagine-linked-glycans (N-glycans) in fish to six N-glycans in humans and great apes. All other placental mammals lack one N-glycan in the shed TSHR A-subunit, the primary Graves' disease autoantigen. We hypothesized that (a) lesser TSHR A-subunit glycosylation reduces immunogenicity, accounting for the absence of Graves' disease in most placental mammals; (b) due to human-like A-subunit glycosylation, Graves' disease might arise in great apes. Here, we review and analyze the literature on this subject and report the results of a survey of veterinarians at primate centers and zoos in North America.

Summary: Previous experimental data from induced TSHR antibodies in mice support a role for A-subunit glycosylation in breaking self-tolerance. An extensive search of the great-ape literature revealed five reports of noncongenital thyroid dysfunction, four with hypothyroidism and one with hyperthyroidism. The latter was a gorilla who was treated with anti-thyroid drugs but is now deceased. Neither serum nor thyroid tissue from this gorilla were available for analysis. The survey of veterinarians revealed that none of the 979 chimpanzees in primate research centers had a diagnosis of noncongenital thyroid dysfunction and among ~1100 great apes (gorillas, orangutans, and chimpanzees) in U.S. zoos, only three were hypothyroid, and none were hyperthyroid.

Conclusions: Graves' disease appears to be either very rare or does not occur in great apes based on the literature and a survey of veterinarians. Although the available data do not advance our hypothesis, there is a paucity of information regarding thyroid function tests and thyroid autoantibodies in the great apes. In addition, these primates may be protected against TSHR autoimmunity by the absence of genetic polymorphisms and putative environmental triggers. Finally, larger numbers of great apes need to be followed, and tests of thyroid function and thyroid autoantibodies be performed, to confirm that spontaneous Graves' disease is restricted to humans.

Introduction

THYROID AUTOIMMUNITY, including Hashimoto's thyroiditis and Graves' disease, is common in humans. In the United States, 4.6% of the population has hypothyroidism (0.3% clinical and 4.3% sub-clinical), and 1.3% have hyperthyroidism (0.5% clinical and 0.7% subclinical) (1). Hypothyroidism is mostly due to Hashimoto's thyroiditis and is associated with autoantibodies to thyroid peroxidase (TPO) and thyroglobulin (Tg) [reviewed in Ref. (2)]. Most hyperthyroidism is due to Graves' disease, which is caused by stimulatory autoantibodies to the thyrotropin receptor (TSHR) [reviewed in Ref. (3)]. Thyroiditis resembling the

condition in humans develops spontaneously in chickens, dogs, rats, mice, and marmosets [e.g., Refs. (4–8)]. In contrast, Graves' disease has only been reported in humans. Hyperthyroidism that arises in aging cats is due to toxic nodular goiter and does not involve thyroid stimulating autoantibodies (TSAb) (9).

Autoimmunity involves breaking tolerance to self proteins, TPO and Tg in Hashimoto's thyroiditis and particularly to the TSHR in Graves' disease. Susceptible strains of mice can be induced to develop Graves'-like hyperthyroidism by immunization with DNA encoding the human TSHR in plasmid or adenovirus vectors [reviewed in Ref. (10)]. After intramolecular cleavage into A- and B-subunits, A-subunits are

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shed from the cell surface (11,12). Adenovirus immunization with the TSHR A-subunit is even more effective than the TSH holoreceptor for inducing thyroid stimulating antibodies and hyperthyroidism (13). This information, together with other evidence (14,15), supports the concept that the shed TSHR A-subunit is the primary autoantigen driving TSAb generation.

Insight into parameters that contribute to breaking tolerance toward thyroid antigens arose from our studies immunizing mice with adenovirus expressing the rat luteinizing hormone receptor (LHR), closely homologous to the TSHR. Possible reasons for the very poor antibody response include differences in mouse genetic background, high amino acid homology between the rat LHR and mouse LHR (self-protein), and glycosylation of the immunogen (16). Of note regarding the latter, glycosylation influences the antigenicity of infectious organisms such as Ebola virus (17) and simian immunodeficiency virus (18).

The human TSH holoreceptor contains six N-linked glycan motifs, five located on the shed A-subunit (19,20). Due to the polypeptide backbone of the shed TSHR A-subunit being less than half the size of the holoreceptor, the five N-linked glycans contribute nearly half the mass of the A-subunit (21). Moreover, the TSHR A-subunit, like Tg but unlike TPO, binds to the mannose receptor (22). Glycosylated antigen capture by mannose receptors on macrophages enhances antigen presentation to T cells for initiating or amplifying an immune response (23). Consistent with binding to the mannose receptor, in NOD.H2h4 mice that develop thyroiditis spontaneously, autoantibodies to Tg appear first, followed later by autoantibodies to TPO (24).

Review and Hypothesis

TSHR N-linked glycans in evolution

The number of N-linked glycans increases from three or four in fish to six in the TSH holoreceptor of humans (25). Western blotting demonstrated that all six sites in the human TSH holoreceptor are glycosylated (20). The TSHR A-subunits of great apes, namely gorillas, orangutans, and chimpanzees, resemble humans in having 5 N-linked glycan motifs (26) (Fig. 1). It is notable that all other placental mammals, including old world monkeys (25), lack the third N-linked glycan motif (amino acid residues 113–115) within the A-subunit. In two species of monkey, the second N-linked glycan (residues 99–101) is also absent.

We hypothesize that N-glycosylation is a major factor in the immunogenicity of the TSHL A-subunit, and, consequently, spontaneous Graves' disease could arise in chimpanzees, orangutans, or gorillas. This hypothesis was investigated, as described next, by reviewing previous experimental studies and the literature on thyroid function in primates and by consulting veterinarians at primate centers and zoos in North America.

Previous experimental evidence of a role for TSHR A-subunit glycosylation in immunogenicity

With hindsight, the contribution of N-linked glycosylation for TSHR antibody induction was provided by studies in which mice had been immunized with adenovirus expressing

the mouse A-subunit or the human A-subunit. As previously emphasized (27), the absence of one fewer N-linked glycan in the mouse A-subunit than in the human counterpart accounted for the lesser mass of the former glycoprotein. The observations from three published studies have been re-analyzed and summarized (Fig. 2).

First, immunizing BALB/c mice with high-dose mouse-TSHR A-subunit adenovirus (4 N-glycans) failed to break tolerance to the mouse-TSHR (27). In contrast, in transgenic BALB/c mice expressing low levels of human TSHR A-subunits (5 N-glycans) in the thyroid, high-dose immunization with adenovirus expressing the human A-subunit (a self antigen in these transgenic mice) overcame self-tolerance, and antibodies developed in 47% of mice [combined data from Refs. (28,29)] (Fig. 2a; $p=0.02$, Fisher's Exact test).

Second, TSHR knock-out mice (which lack TSHR self-antigen) were compared for antibody responses to immunization with adenovirus expressing the human- or the mouse- TSHR A-subunit (27). Mice immunized with human-TSHR A-subunit adenovirus developed a robust response to the receptor. In addition, more mice were positive for TSHR antibodies than mice immunized with adenovirus expressing the mouse-TSHR A-subunit (Fig. 2b; $p=0.038$, Fisher's Exact test).

In vivo evidence of the contribution of TSHR A-subunit glycosylation for inducing Graves' disease

Spontaneously arising endocrine diseases in humans have some counterparts in domestic cats and dogs, including hypercortisolism and hyperparathyroidism. However, despite the generation over many years of hundreds of different breeds, Graves' disease has not been observed in dogs or cats (30). As already mentioned, hyperthyroidism in aging cats is due to toxic nodular goiter (9). Together with the data just given for experimentally induced TSHR antibodies, these veterinary observations are consistent with our hypothesis that breaking tolerance to the TSHR is absent in mammals with less-well glycosylated TSHR A-subunits.

Evidence for abnormal thyroid function in great apes

Is there any evidence for abnormal thyroid function in great apes? An exhaustive literature search (including formal publications, zoological society proceedings, and newsletters) for thyroid dysfunction in primates revealed five case reports of spontaneous disease: noncongenital hypothyroidism in a gorilla (31,32), a chimpanzee (33), and two orangutans (34,35) and noncongenital hyperthyroidism in one gorilla (36) (Table 1). All reports were for adults housed in zoos. Among lower primates, two geriatric rhesus monkeys developed hyperthyroidism due to multinodular goiter (37), and two newborns had compensated congenital hypothyroidism (38). The causes of hypothyroidism or hyperthyroidism were not always identified, and thyroid autoantibodies were rarely measured. However, autoantibodies to thyroglobulin or thyroid microsomes (TPO) were undetectable in the hypothyroid chimpanzee studied, and TSHR antibodies were undetectable in the geriatric hyperthyroid rhesus monkeys.

To provide insight into the numbers of great apes with thyroid dysfunction (information not available from published

FIG. 1. Alignment of amino acid sequences in the TSHR A-subunit of humans, great apes, old world monkeys, and other placental mammals. The dashed box indicates the signal peptide (amino acid residues 1–21); N-glycans are highlighted in blue. Species are identified by coloring and letters: Red, H, *Homo sapiens* (AAA36783) (50); Black, great apes: G, gorilla, *Gorilla gorilla* (ENSGGOTP0000008256) (26), T, chimpanzee, *Pan troglodytes* (ENSPTRP000000011214) (26), P, Orangutan, *Pongo pygmaeus* (ENSPYPY000000006869) (26); Blue, Old World monkeys: C, *Chlorocebus aethiops* (AAO11782) and M, *Macaca mulatta* (NP-001182324) (25); Green, other placental mammals: S, pig (*Sus scrofa* (AAL92560) (51), D, dog (*Canis lupus familiaris* (AAA30902) (52), F, cat (*Felis catus*, AF218264) (9); m, mouse (*Mus musculus*, AAB60455 764 (53). TSHR, thyrotropin receptor.

reports), inquiries were extended to veterinarians at primate centers and zoos in North America. Five US primate centers (University of Texas, MD Anderson; University of Lafayette, NIRC; SNPRC, Texas; Yerkes NPC; and Alamagordo) care for 979 chimpanzees. Four US zoos house ~1100 great apes, in-

cluding gorillas, orangutans, and chimpanzees (Smithsonian, Washington DC; North Carolina Zoological Park; St. Louis; San Diego and Atlanta Zoos). Veterinarians are treating presumed hypothyroidism in a gorilla (Dr. Bonnie Raphael, Bronx Zoo) and two orangutans (Dr. Haley Murphy, Atlanta Zoo),

FIG. 2. Glycosylation of the TSHR A-subunit in relation to the induction of TSHR antibodies. Data from published studies (27–29) have been combined or re-plotted. Mice were immunized twice with adenovirus expressing the A-subunit of the mouse or human TSHR. Autoantigens: the mouse TSHR in wild-type mice, the human A-subunit in transgenic mice, TSHR knockout (KO) mice lack any TSHR autoantigen; all mice are on the BALB/c background. Data shown are the number of mice that were positive (+ve, black bars) or negative (–ve; open bars) for TSHR antibodies (TSHR Ab). Significance of differences was tested by Fisher's exact test. (a) Wild-type mice immunized with high-dose

mouse-TSHR A-subunit adenovirus (4 N-glycans) (27) versus human A-subunit transgenic mice immunized with high-dose human TSHR A-subunit (5 N-glycans) adenovirus (28,29). Antibodies to the mouse TSHR were measured by flow cytometry by using mouse TSHR-expressing Chinese hamster ovary (CHO) cells; antibodies to the human TSHR were determined by TSH binding inhibition. The figure shows the combined numbers of TSHR antibody positive mice from two studies (28,29). (b) TSHR KO mice immunized with adenovirus expressing the mouse TSHR A-subunit or the human-TSHR A-subunit versus the mouse-TSHR A-subunit. Antibodies were measured by flow cytometry by using CHO cells expressing the mouse TSHR or the human TSHR, respectively, depending on the immunogen. Data plotted from Nakahara *et al.* (27), © 2010 The Endocrine Society.

TABLE 1. NONCONGENITAL HYPOTHYROIDISM OR HYPERTHYROIDISM IN GREAT APES AND RHESUS MONKEYS AND CONGENITAL HYPOTHYROIDISM IN RHESUS MONKEYS

	n	Hypo/hyper	Sex	Age (years)	Diagnosis	Treatment	References
<i>Noncongenital thyroid dysfunction</i>							
<i>Great apes</i>							
Gorillas	1	Hyper	M	34		Faviston + T4, MMI + T4	(36)
	1	Hypo	F	26	Primary hypo	T4	(31,32)
Chimpanzees	1	Hypo	F	16	Primary hypo		(33)
Orangutans	2	Hypo	F	30	Ab neg ^a	T4	(34,35)
<i>Lower Primates</i>							
Rhesus monkeys	2	Hyper	1F, 1M	31 ^b	Multinodular goiter	Lobectomy; tapazole (F)	(37)
<i>Congenital thyroid dysfunction</i>							
Rhesus monkeys		Hypo (comp)		Newborn	Congenital goiter	None	(38)

^aNegative for antibodies to thyroglobulin of thyroid microsomes.

^bGeriatric Rhesus monkeys.

n, number of animals; Hypo, hypothyroidism; Hyper, hyperthyroidism; MMI, methimazole; T4, thyroxine; M, male; F, female; comp, compensated.

Sources: Refs. (31–38).

but no thyroid function data were made available to us. No other veterinarians reported current or past instances of overt hyperthyroidism or hypothyroidism in the great apes for which they were responsible.

Published data for thyroid hormone levels and treatment in primates with thyroid dysfunction

Thyroid hormone values have been reported for some primates with thyroid dysfunction together with information

for euthyroid animals. Data from such reports and the small number of other primate studies (39,40) suggest that the human serum reference ranges for thyroxine (T4, total and free), triiodothyronine (total), and TSH are often suitable for the great apes (summarized in Table 2).

Euthyroidism was restored in three hypothyroid great apes by using synthetic thyroxine (Table 2). Hyperthyroidism in the geriatric female rhesus monkey was treated by thyroid lobectomy followed by antithyroid drugs (37) (Table 3). Antithyroid drugs (faviston or thiamazole, later methimazole)

TABLE 2. THYROID HORMONE AND THYROTROPIN LEVELS IN GREAT APES

Noncongenital Hypothyroidism					Euthyroid great apes					References
T4 μg/dL	F-T4 ng/dL	T-T3 ng/dL	TSH mIU/L	n	T4 μg/dL	F-T4 ng/dL	T-T3 ng/dL	TSH mIU/L		
Gorillas										
1F	<1.1 ^a 8.7 ^b	<0.04 1.8	85.4 4	3	8.2, 5.1, 5.9	1.4, 1.5, 1.9		6.7, 10.8, 8.9	(31)	
Kit (hu)				5	(5.1–12.6)				(32)	
Range					5.1–8.1			6.7–10.8	(40)	
Kit (hu)					(4.5–12.5)					
Chimpanzees										
1F	0.2	0.05	3.8	1	7.5	2.75		3.0	(33)	
Kit (hu)					(5–12)	(1.1–4.1)		(1.9–5.4)		
Adult M				5	4.8±0.8		109±27		(39)	
Range					(3.7–5.9)		(87–155)			
Adult F				17	5.8 ^c		148 ^c		(49)	
Adult M				9	9.0 ^c		70 ^c			
Orangutans										
1F	3.3 ^a 10.8 ^b		139 274						(34)	
Kit					(4.5–12.0)		(230–420)	(0.35–5.50)		
				4	2.0–4.7			0.4–8.6	(40)	
Kit					(5.0–12.6)			(0.4–5.0)		

Parentheses indicate the range of values.

^aBefore treatment.

^bAfter T4 treatment.

^cMean estimated from Ref. (49).

TSH, thyrotropin; T3, triiodothyronine; Kit (hu), range for humans in assay kit.

Sources: Refs. (31–34,39,40).

TABLE 3. THYROID HORMONE AND THYROTROPIN LEVELS IN RHESUS MONKEYS

	Congenital hypothyroidism			Euthyroid animals			References
	T4 $\mu\text{g/dL}$	T-T3 ng/dL	TSH $\mu\text{IU/L}$	n	T4 $\mu\text{g/dL}$	T-T3 ng/dL	TSH mIU/L
M	1.2		17.1		9.3 (mo)		1.6
M	2.6		7.8		5.6 (fa)		<1
					9.4 (inf)		2.6
					10.2 (inf)		6.9
Infants M				2–3		210–237	2.3–3
Infants F				1–2		150–241	6.9
<i>Noncongenital hyperthyroidism</i>							
M	28.2	379					(37)
F	22.2 ^a	433 ^a					
F	7.0 ^b	194 ^b					
Adult M				7	3.8–6.6	54–115	(39)
Adult F				24	1.3–7.6	63–295	

^aBefore treatment.^bAfter lobectomy and anti-thyroid drugs.

mo, mother; fa, father, inf, infant.

Sources: Refs. (37–39).

and T4 were used to treat the hyperthyroid gorilla (36). In this gorilla, the doses of anti-thyroid drug and T4 were altered in accordance with clinical symptoms; when treatment stopped, the hyperthyroid symptoms (abnormal appetite and hair loss) recurred, and therapy was continued. This approach was symptomatically effective for many years (~1984 to at least 1995, personal communication, Dr. Eva Ziemsen, Dresden Zoo). Neither serum nor thyroid tissue from gorilla "Benno" had been stored in the Dresden zoo, where he developed hyperthyroidism or after his transfer in 1995 to Zoo Leon, Mexico, where he died in 2004.

A definitive diagnosis of Graves' disease or toxic nodular goiter could not be established in this gorilla. However, it is important to note that toxic multinodular goiters responsible for hyperthyroidism were observed in geriatric rhesus monkeys (37), are common in aging cats (9), and increase in humans with advancing age. In contrast, gorilla Benno was younger when hyperthyroid symptoms were first manifest, about 34 years of age, and he survived (on treatment) until 43 years old. The normal life span for gorillas in captivity is 40 years, and a few survive until they are 50 years old (Dr. Ryan Devoe, North Carolina Zoological Park; personal communication). Consequently, we cannot rule out the possibility that gorilla Benno had Graves' disease.

Genetic susceptibility for Graves' disease in humans

Predisposing factors in human thyroid autoimmunity involve genes related to immune function and thyroid-specific genes [reviewed in Ref. (41)]. Polymorphic immune-related proteins that confer genetic susceptibility include the major histocompatibility complex (MHC), which is responsible for presenting peptides processed from intact proteins to T cells; genes involved in immune regulation (CTLA-4, CD25 or FoxP3), co-stimulation (CD40); inhibition of T cell activation (PTPN22) (42), B cell signaling (FCRL3), the interleukin 23-Receptor, and interferon-induced Helicase (IFIH1). Thyroid-specific genes associated with thyroid autoimmunity include

Tg and the TSHR. Moreover, it is possible that TSHR splice variants play a role in A-subunit shedding and thereby contribute to the development of Graves' disease (43). Polymorphisms have been described for MHC in chimpanzees (44–46), and there are some similar data for gorillas and orangutans (46). However, it is not known whether polymorphisms in any other susceptibility genes listed above occur in great apes.

Environmental triggers of thyroid autoimmunity

Postulated environmental triggers or exacerbators of thyroid autoimmunity include infections, iodine, medications, smoking, and, in Graves' disease, stress [reviewed in Ref. (41)]. Of these parameters, infections could contribute to the development of thyroid autoimmunity in great apes, and stress could play a role for such animals in captivity. According to the "hygiene hypothesis," the reduction in infectious diseases in western societies may be causally related to the enhanced frequency of autoimmunity (as well as allergy) in humans [e.g., Ref. (47)]. Conversely, great apes living in less hygienic conditions, either in the wild or in captivity, might be protected against developing autoimmunity. In this context, lymphocyte activity is lower in chimpanzees (in captivity) than in humans (48). Moreover, spontaneous autoimmunity in general seems to be rare in chimpanzees; neither rheumatoid arthritis nor type 1 diabetes have been reported (44).

Glycosylation in humans and great apes

All great apes have higher levels of transthyretin and a change in haptoglobin isoforms compared with humans (49). However, plasma protein expression and glycosylation for the proteins studied are conserved in humans and the great apes.

Summary and Conclusions

We hypothesized that the lesser glycosylation of the TSHR A-subunit (4 vs. 5 N-glycans) leads to decreased immunogenicity and could account for the absence of spontaneous Graves'

disease in placental mammals other than great apes. Although supported by previous experimental evidence involving induced TSHR antibodies in mice, a comprehensive literature review supplemented by a survey of facilities housing great apes was unable to confirm the hypothesis. It is possible that these animals may be protected against TSHR autoimmunity by the absence of genetic polymorphisms and putative environmental triggers. However, due to the relatively small number of great apes in captivity, a greater number of these animals needs to be followed, and levels of thyroid hormones and thyroid autoantibodies need to be measured to definitively conclude that spontaneous Graves' disease is restricted to humans.

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Data plotted in Fig. 2 was derived from Nakahara *et al.* (27), with permission from The Endocrine Society © 2010, all rights reserved.

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Disclosure Statement

The authors declare that no competing financial interests exist.

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