Thyroid Autoimmunity as a Window to Autoimmunity:

An explanation for sex differences in the prevalence of thyroid autoimmunity

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Abstract

Autoimmune thyroid diseases (AITDs), predominately Graves' disease and Hashimoto's thyroiditis, comprise the most common autoimmune diseases in humans. Both have the production of anti-thyroid antibody as an important aspect and both are much more prevalent in females, being at least 10 times more common than in males. Using these two clues, a hypothesis for the initiation of thyroid autoimmunity is proposed that helps to make the case that the thyroid is one of the most sensitive sites for autoimmunity and helps account for the prevalence and the observed sex differences in AITDs and associated diseases, such as type 1 diabetes and Latent Autoimmune Diabetes in Adults (LADA).

The primary mechanisms proposed involve the underlying state of inflammation as a result of the adipokines, especially leptin, TNF- α , and IL-6, and the receptors able to recognize pathogen-associated molecular patterns (PAMP's) and damage-associated molecular patterns (DAMP's) through Toll-like receptors (TLR) and others receptors present on thyrocytes. The adipokines are produced by adipose tissue, but have hormone-like and immune modulating properties. As the levels of leptin are significantly higher in females, an explanation for the sex difference in thyroid autoimmunity emerges. The ability of the thyrocytes to participate in innate immunity through the TLR, provides an adjuvant-like signal and allows for the action of other agents, such as environmental factors, viruses, bacteria, and even stress to provide the initiation step to break tolerance to thyroid self-antigens.

Seeing the thyroid as one of the most sensitive sites for autoimmunity, means that for many autoimmune disorders, if autoimmunity is present, it is likely to also be present in the thyroid – and that that condition in the thyroid was probably earlier. The evidence is seen in multiple autoimmune syndrome.

Key words: leptin, thyroid autoimmunity, sex differences, Hashimoto's thyroiditis, Graves' disease

Introduction

The thyroid gland can host two common autoimmune diseases, Graves' disease and Hashimoto's thyroiditis (autoimmune thyroiditis). Graves' disease results in hyperthyroidism which has an estimated prevalence of 80/100,000/year in women and 8/100,000/year in men in Western countries (McGrogan *et al.*, 2008). The primary cause of hypothyroidism in the West (where dietary iodine is generally at sufficient levels) is Hashimoto's disease. Hypothyroidism has an estimated incidence of 2.2/100,000/year for men and 498.4/100,000/year for females in Western countries (McGrogan *et al.*, 2008). Taken together, autoimmune thyroid diseases (AITDs) are the most common autoimmune diseases in humans (Hayter and Cook, 2012).

Autoimmune thyroid disease results from the breaking of tolerance to self-antigens of the thyroid. There appears to be several ways in which this can happen, including infection, genetic predisposition (Medici *et al.*, 2014), environment, some drugs including IFN- α (Tomer *et al.*, 2007), and dietary iodine (Hansen *et al.*, 2006; Tomer & Huber, 2009). Although the mechanisms of the two diseases involve different arms of the immune response for their primary destructive result (antibody for Graves' and cell-mediated immunity for Hashimoto's), there are common pivotal anti-thyroid antibodies that both serve to characterize the nature of the diseases, and to allow monitoring of the disease progression as in other autoimmune diseases (Damoiseaux, 2013). As various anti-thyroid antibodies need to be generated in both diseases, the nature of their targets, and the propensity of aspects of the immune response to react through genetics and environmental influences (Burek & Taylor, 2009) are clearly involved, although the role of antibody in the autoimmune destruction of Hashimoto's thyroiditis is not understood. Additional avenues to the breaking of tolerance, probably involving cross reactivity of antibody due to molecular mimicry, have also been suggested. These include responses to *Candida albicans* (Vojdani *et al.*, 1996), *Bifidobacterium* and *Lactobacillus* species (Kiseleva *et al.*, 2011), *Helicobacter pylori* strains expressing CagA (Figura *et al.*, 1999), *Yersinia enterocolitica* (Guarneri *et al.*, 2011), *Clostridium botulinum* neurotoxin A (Gregoric, *et al.*, 2011), HCV (Tomer & Villanueva, 2004), and human parvovirus B19 (Mori *et al.*, 2007). Recently Gammazza *et al.*, 2014 have identified heat shock protein Hsp60 as another potential mimic which would add stress to the list of factors potentially involved. Although there is an association with several potential mimics and associated agents, it has not been possible to show a direct establishment of an autoimmune disease in the thyroid with any agent. Indeed, induced animal models (McLachlan & Rapoport, 2014) from agents are common (see for instance: Mori & Yoshida, 2010 and

Tomasi *et al.*, 2005) – but most, except in genetically susceptible individuals (ElRehewy *et al.*, 1981), require an adjuvant as part of the induction process. This can be an important hint if one looks for what could provide an adjuvant-like signal in addition to a mimic. In some sense, how the systems get to the point at which an autoimmune state eventually results may be more important to determine than which potential triggers could provide the initiation (the final step in a chain of steps). There may be many possible triggers, but unless the system can respond to them and initiate breaking of tolerance, there is no autoimmune disease.

In this paper, we focus on the foundation that makes possible AITDs. The hint that this is the fundamental place to begin is in the large sex difference in the disease. Why women are so much more likely to develop AITDs has been difficult to establish. The reason for the sex difference has been examined from many angles, including genetics related to the X chromosome, pregnancy, and hormones (Lleo *et al.*, 2008; Fortunato *et al.*, 2014), but none have been able to conclusively determine the cause except in genetically rare cases (McLachlan & Rapoport, 2014). Also, miRNA expression has been seen to show sex differences, providing yet another potential avenue for sex differences (reviewed in Dai & Ahmed, 2014). Associations between AITDs and type 2 diabetes (Coller & Huggins, 1927; Procaccini *et al.*, 2011) and suggestions that inflammation may be at the core of both and responsible for the sex differences in incidence (Thorand *et al.* 2007).

The sex differences in thyroid autoimmunity emerge at puberty. It has been noted (Whitacre, 2001) that at puberty, both leptin and estrogen rise in tandem. Both leptin (Procaccini *et al.*, 2012) and estrogen (Cutolo *et al.*, 1995) are potent modulators of the immune response. Leptin has also been suggested as being involved in some way in the sex differences in incidence of these diseases (Cojocaru *et al.*, 2013).

Here, we will try to establish an inflammatory cause for the development of the foundation for thyroid autoimmunity through leptin and other adipokines (Carbone *et al.*, 2012), and the ability of thyroid cells to participate in innate immunity (reviewed in Kawashima *et al.*, 2011). The thyroid also seems to be somewhat unique immunologically. The primary reason for this sensitivity seems due to the presence of lymphoid tissue akin to secondary lymphoid follicles with germinal centers in the thyroid autoimmunity (Armengol *et al.*, 2001). It is expected that this event happens early in the process after nonspecific responses result in increased lymphocyte infiltration into the thyroid (Armengol *et al.*, 2003). A second aspect that makes the thyroid unique among autoimmune target tissues is that it is the only one with a hormone (TSH) that stimulates its growth (and increased thyroid antigens). In addition, CD40 expression in thyrocytes – at least those that are stressed in some way – allows the thyrocytes to function

as antigen presenting cells. This aspect is implicated for Graves' Disease. In Hashimoto's the specificity may be also through the link with CD20 B cells which are both implicated in diabetes initiation and necessary for the production of spontaneous autoimmune thyroiditis in mice. B-cell depletion also seems to result in the production of increased Treg cells (and lower levels of autoimmunity in mice). So, the unique nature appears to be both structural and the special nature of thyroid antigens and markers.

Discovered in 1994 (Zhang *et al.*, 1994), leptin is one of several proteins produced in adipose tissue (known as adipokines) that are believed to play a role in regulating energy intake and energy expenditure. As such, an association with the thyroid would not be unexpected. Recent results, however, point to a role for leptin both as an immune modulator and as a player in the initiation of autoimmune thyroid disease (Duntas & Biondi, 2013). The nature of the inflammation seen with leptin's influence (Fantuzzi, 2005), the sex differences in leptin levels (Hickey *et al.*, 1996), innate immunity in the thyroid (using the Toll-like and other receptors), along with the sex differences in the nature of the immune response (Bouman, *et al.*, 2005), provide solid testable hypotheses for the observed sex differences in AITDs. It should be noted that recent results on an X-linked Toll-like receptor is claimed to determine sex differences in systemic lupus erythematosus (Umiker, *et al.*, 2014).

The thyroid and primary antigens associated with thyroid autoimmunity

The thyroid has the primary task of producing two hormones, triiodothyronine (T_3) and thyroxine (T_4), that are responsible for the regulation of the metabolism of proteins, carbohydrates and fats. Also produced are calcitonin and small quantities of neuroendocrine peptides. The hormones T_3 and T_4 (especially the active T_3) affect every cell in the body through regulation of oxygen consumption and metabolism, but they directly and indirectly affect other systems including the maintenance of homeostasis in blood pressure, digestion, and reproduction. The control of the production of the thyroid hormones is through a feedback system involving two hormones in the brain and receptors in the thyroid. The primary driver of hormone production is TSH (thyroid stimulating hormone or thyrotropin) produced by the pituitary. TSH receptors, found primarily on thyroid epithelial cells, bind TSH, beginning the cascade leading to thyroid hormone production. The production of TSH is controlled by the hormone TRH produced in the hypothalamus. The control of thyroid hormone production is described as involving this HPT (hypothalamus – pituitary – thyroid) axis.

Several anti-thyroid antibody in AITDs have been identified. For the purpose of diagnosis, antibody is primarily aimed at thyroglobulin (Tg), the prohormone of T_3 and T_4 , thyroid peroxidase (TPO), the enzyme responsible for adding iodine to Tg, and the TSH receptor (TSHR). Antibody to any of these three primary antigens are diagnostic for AITDs. For Graves', antibody to TSHR causes an

agnostic signal to continue production of thyroid hormone with disturbing the normal feedback control. In Hashimoto's thyroiditis, the primary anti-thyroid antibody is against TPO. The rise of antibodies to TPO have been suggested to predict an eventual development of clinical disease (Hutfless *et al.*, 2011). The direct damage through the antibody-related complement system and antibody-dependent cellular cytotoxicity are not thought to be the primary cause of damage to the thyroid characteristic in the disease, however. Hashimoto's thyroiditis involves a complicated infiltration and interaction of immune and thyroid cells (reviewed in Iwona, 2011). Anti-Tg can be found in either condition, so is not diagnostic – except for the presence of an autoimmune disease (Acar *et al.*, 2013; McLachlan & Rapoport, 2013). A connection between TPO, TPO antibody, and breast cancer prognosis has been proposed (Muller *et al.*, 2014). A mathematical description of the clinical progression of Hashimoto's thyroiditis can be found in Pandiyan *et al.*, 2013.

Inflammation, leptin, and diabetes

The product of the *ob* gene, leptin, was discovered in 1994 (Zhang *et al.*, 1994). It was identified as a hormone involved in appetite control through the leptin receptor. Leptin, produced in white adipose tissue, affects appetite through a negative feedback loop involving the leptin receptor in the hypothalamus (reviewed in Arora & Arora, 2008). It is now known that leptin is one of a number of hormones, the adipokines, which have many functions – most having some impact on the thyroid (Cinar & Gurlek, 2013). One effect is both to alter thyroid function through disruption of the feedback loop of the HPT axis by altering TRH (Duntas and Biondi, 2013). A second effect is through inflammation and inflammatory molecules. The direct effect on the thyroid by inflammatory molecules (primarily TNF- α) can be seen in two studies, Zacccone *et al.*, 2003 and Chen *et al.*, 2007. In each, the role of the cytokine in generating autoimmune thyroid disease in the mouse is highlighted. In Zacccone *et al.*, the role of the adjuvant is also described – similar to the steps suggested in this paper.

In this paper, “leptin” will be used as referring to all of the adipokines in this context, which include leptin, adiponectin, TNF- α , and IL-6. The discovery that leptin is involved in low-level inflammation, provides both a marker of adipose tissue that can be measured, and a source of the inflammation is reviewed in Scotece *et al.*, 2014.

Leptin plasma levels correlated positively with body fat (Ostlund *et al.*, 1996), but the relationship with sex is important for this study. Hickey *et al.*, 1996 found that fasting serum leptin levels were over twice as high for females as males. This, along with possible effects of sex hormones, suggests that females should have more possibility of low-level chronic inflammation complications than men (Bouman *et al.*, 2005; Casimir *et al.*, 2010). It should be noted that the study by Casimir *et al.* was

completed on children before the effects of hormones would be expected. The potential suppressive effect of testosterone also suggests this result (Furman *et al.*, 2014). The human data on this connection is limited, however. One recent study by Harpsøe, et al., 2014 has identified an association between Body Mass Index (BMI) (an indirect measure of serum leptin levels), and several autoimmune diseases. Marzullo et al., 2010 have established that leptin levels are correlated with thyroid autoimmunity independent of bioanthromorphic, hormonal, or weight-related measures.

The interplay between thyroid disease and leptin in development of diabetes is complicated by several interacting systems (Hage et al., 2011). In terms of inflammation and thyroid, metabolic syndrome is clearly associated, for instance see Shantha et al., 2009 and Ruhla et al., 2010. The transition from metabolic syndrome to type 2 diabetes, is a well-known risk. A fraction (about 10%) of these type 2 or metabolic syndrome are eventually recognized as Latent Autoimmune Diabetes in Adults (Unger, 2007), an autoimmune disease associated (as is type 1 diabetes) with thyroid autoimmunity (Koch, 2011).

Innate immunity and the Pattern Recognition Receptors (PRR) in the thyroid

Besides the role of leptin in providing a signal which triggers inflammation and affecting the HPT axis, the native thyroid cells, thyrocytes, also play a role in the innate immune system. Thyrocytes have the ability to recognize and respond to pathogen-associated molecular patterns (PAMP) through PRR. The PRR's include Toll-like receptors, Nod-like receptors and many others (reviewed in Kawashima *et al.*, 2011). Thyrocytes also have the ability to respond to damage-associated molecular patterns (DAMP) coming from cellular damage (Kawashima *et al.*, 2011) – especially a response to double stranded DNA and/or RNA. The response of thyrocytes to these patterns plays two roles in this setting. One is to further increase the presence of inflammatory cytokines, TNF- α , IL-1, and IL-6, to promote a specific immune response. Toll-like receptors have been described as adjuvant receptors (Kaisho and Akira, 2002). The second is the presentation of self-antigens in association with MHCII expression on thyroid cells (Kawashima *et al.*, 2013). Inflammatory molecules have been recognized with a breaking of tolerance in the context of thyroid autoimmunity with a like-self (or self) antigen (Akeno *et al.*, 2011). The identified mimics now have the stage set to overcome self-tolerance. Note that if mimics are presented in the system in most animal models without an adjuvant signal, they are not able to generate autoimmunity.

Another aspect of this response may be in response to stress, where heat shock protein Hsp60, triggered as a response to the stresses of many conditions, is a mimic. In addition, the stress disruption of the Hypothalamic-Pituitary-Adrenal (HPA) axis is also seen to play a role in the altering of the immune response characteristics (Klecha *et al.*, 2008).

Multiple autoimmune syndrome and autoimmune polyglandular syndrome

One hint as to the relationship between the various autoimmune disorders useful here is through the idea of Multiple Autoimmune Syndrome (MAS) described by Humbert and Dupond, 1988. MAS is the coexistence of 3 or more autoimmune disorders in one individual. The useful grouping of autoimmune disorders of those that tend to be present together if they are present is useful in this discussion (Cojocaru et al., 2010).

- Type 1 MAS includes myasthenia gravis, thyoma, polymyositis, and giant cell myocarditis.
- Type 2 MAS includes Sjögren's syndrome, rheumatoid arthritis, primary cirrhosis, sclerodema, and autoimmune thyroid disease.
- Type 3 MAS includes autoimmune thyroid disease, myasthenia gravis and/or thyoma, Sjögren's syndrome, pernicious anemia, idiopathic thrombopenic purpura, Addison's disease, type 1 diabetes mellitus, vitiligo, SLE, and several others.

Cojocaru et al., 2010 note that in the medical literature, a dermatological disease, usually vitiligo, is first to be diagnosed, but in most cases, autoimmune thyroid disease was also present (Ai et al., 2003). Although diagnosis of the syndrome indicates some genetic or environmental pre-disposition for autoimmunity, it does suggest that in Type 2 and Type 3, that if an autoimmune condition is present, it is likely that autoimmune thyroid disease is also present – and was likely present at the time of diagnosis.

It is likely that Type 2 MAS and Type 3 MAS are those autoimmune diseases where inflammation similar to that in AITD plays a role. It should be noted that in several autoimmune disorders, *e.g.* autoimmune hemolytic anemia and autoimmune thrombocytopenia, do not seem to require inflammation, although they can be associated with other autoimmune conditions that do. Also, certainly, inflammation does not always cause autoimmune disease.

A similar view of the relationship between autoimmune disorders is through autoimmune polyglandular syndrome where AITD is considered "Type 2" (Betterle et al., 2004). Type 2 APS is defined by the occurrence of Addison's disease with thyroid autoimmune disease and/or Type 1 diabetes mellitus. There is some evidence (Krysiak et al., 2014) that treatment with anti-inflammatory agents may be a useful treatment in Type 2 APS.

Discussion

The leptin levels rise in young girls as they approach puberty while in boys, leptin declines along with testosterone increase (Garcia-Mayor *et al.*, 1997). This increase begins a life-long dance between leptin and low-grade inflammation. Leptin's inflammatory effects are first felt in fat and liver (Li *et al.* 2006). In the thyroid context, leptin disrupts the HPT axis controlling thyroid function. Leptin also interferes with other systems as well. But unlike other systems, the interaction between thyroid disruption/weight gain and adipose tissue production/leptin production is unique to the thyroid. Many organs feel the inflammatory effects of leptin, but the disruption of control along with the inflammation is likely a unique condition. The dance continues in postmenopausal women as described by Teixeira *et al.*, 2009 where serum leptin is elevated in postmenopausal women with subclinical or overt hyperthyroidism. IL-6 is also seen as elevated in this population (Siemińska *et al.*, 2010). As events and pathogens that trigger the PAMP and DAMP response of the thyrocytes over time, the situation in the thyroid can be ripe for an autoimmune trigger. In men, with the level of underlying leptin-caused inflammation lower (due to leptin and suppressed by testosterone), the thyroid is not in a state that is as conducive to autoimmunity. An additional factor, not discussed here, is the relationship between pregnancy and the triggering of thyroid disruption and autoimmunity – primarily Graves' disease (Carney *et al.*, 2014). A further step to the breaking of tolerance allowing the establishment of an autoimmune disease is further facilitated by the effect of leptin on regulatory T (Treg cells) (Matarese *et al.*, 2010), where the Treg numbers are seen to be inversely correlated with serum leptin.

The specificity of the thyroid to the immune response seems due to the presence of lymphoid tissue akin to secondary lymphoid follicles with germinal centers in the thyroid autoimmunity (Armengol *et al.*, 2001). It is expected that this event happens early in the process after nonspecific responses result in increased lymphocyte infiltration into the thyroid (Armengol *et al.*, 2003). A second aspect that makes the thyroid unique among autoimmune target tissues is that it is the only one with a hormone (TSH) that stimulates its growth (and increased thyroid antigens). In addition, CD40 expression in thyrocytes – at least those that are stressed in some way – allows the thyrocytes to function as antigen presenting cells. This aspect is implicated for Graves' Disease. In Hashimoto's the specificity may be also through the link with CD20 B cells which are both implicated in diabetes initiation and necessary for the production of spontaneous autoimmune thyroiditis in mice. B-cell depletion also seems to result in the production of increased Treg cells (and lower levels of autoimmunity in mice). So, the nature appears to be both structural and the special nature of thyroid antigens and markers.

Over time, the thyroid provides an adjuvant-like setting if specific mimic or self- antigens are present, resulting in autoimmunity in the thyroid. It is at this time that the molecular mimics described in the Introduction play a role. The fact that the specific agent or agents present at the overcoming of tolerance indicate why it has been difficult to identify a specific one in this case. The nature of this response will be determined by the agents which triggered the response and indicated by the anti-thyroid antibodies produced. This process is summarized in Figure 1.

Figure 1. Diagram of the hypothesized process by which thyroid autoimmunity is created.

Conclusion

The triggers of AITDs discussed contribute to the disease in many ways with different weights and relative importance. They cause the hormone deregulation and ultimately result in clinical malfunction of thyroid. The regulation and physiological responsiveness of the thyroid is also influenced by many factors, the susceptibility of thyroid to autoimmunity due to the unique regulation system and response axis to maintain delicate homeostasis and the structural integrity. The humoral and cellular immunity are dynamic and autoimmunity can also be influenced by a multitude of factors. In certain circumstances, the predominant status can be fragile, influenced by one factor which overwhelms the others -- affecting the overall autoimmune balance. Throughout the inflammatory progress and/or persistent stimulation, secondary autoimmune disorders in distal relatively resistant organs, such as pancreas, rheumatoid arthritis, etc. may be seen as starting with a thyroid malfunction/disorder.

With many mimics and factors, it would seem that the initiation of AITDs is very complicated – but at its heart, it may very well be a natural result of inflammation caused by leptin, and the presence of a trigger through PAMP or DAMP, along with a mimic or cell damage. The increased incidence in females can be easily seen in this context through increased leptin. There can be other aspects, suppression through testosterone in men, and the specifics of a stress response further shape a complete picture. In individuals with several autoimmune diseases, it is likely that thyroid autoimmunity also exists (and likely was existing at the time of the first diagnosis).

With the stage set by 1) inflammation, 2) the ability of the thyrocytes to respond to patterns, 3) a large collection of mimics and other agents, and 4) disruption of thyroid and Treg function (and resulting feedback to adipose tissue), the stage is set for AITDs. And, a process that results in an increased incidence of AITDs in females.

Testable hypotheses:

Several aspects of this theory are directly testable. Some of the most critical are listed here.

- 1) Central to the hypothesis is that inflammation due to leptin is a contributing agent to the development of AITD. Since there is an increase in obesity in the US, there should be a shift in the development of AITD to younger populations. There is a partial test of this idea in Popławska-Kita et al., 2014 where the Body Mass Index (BMI) was examined in Hashimoto's thyroiditis patients, both in euthyroid (normal T4 levels) and hypothyroid conditions. It was found that Hashimoto's patients had higher BMI and fat mass than

controls. This is not quite a test as disruption of the thyroid hormone can result in weight gain. But note that the result hold for euthyroid patients as well.

- 2) The rate of Hashimoto's in men should increase along with increasing obesity. This may involve a description of the level of obesity needed to generate an increase in the disease.
- 3) One should find the presence of high concentration of adipokine inflammatory molecules as predictive for the development of AITD. This has been explored by Mikoś et al., 2014 who found that in children, levels of IL-1 β , TNF- α , and IL-6 are predictive of AITD and the level of IL-1 β can be specifically used to distinguish Hashimoto's from Graves' disease. This was done in normal and diagnosed (but not treated) children, so it is not a predictive test.
- 4) Patients who develop Hashimoto's over time subclinical hypothyroidism should show inflammatory differences, or differences in BMI from those who do not.

Both obesity and Hashimoto's increase post menopause, but is Hashimoto's caused by obesity (i.e., leptin increasing)? There should be an easy proof that is likely an epidemiology study not yet completed- With the dramatically increasing obesity rates in the US there should be a "shift" in Hashimoto's from occurring almost exclusively post-menopause to occurring earlier in life (excluding Graves' disease which occurs by a different mechanism/ Ab-mediated receptor activation). If leptin is critically important then there should also be a "shift" to more men developing Hashimoto's since obesity is also increasing for males.

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