Thyroid Autoimmunity at 60: Where Next?

Paul Starr Award Lecture 2013

Tony Weetman University of Sheffield Medical School



PRESENTATION FROM THE 83rd ANNUAL MEETING OF THE AMERICAN THYROID ASSOCIATION, OCTOBER 16-20, 2013 (Tony Weetman)

Autoimmunity is the cause of Hashimoto's thyroiditis - 1956

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STUDIES ON ORGAN SPECIFICITY

V. CHANGES IN THE THYROID GLANDS OF RABBITS FOLLOWING ACTIVE IMMUNIZATION WITH RABBIT THYROID EXTRACTS¹

NOEL R. ROSE AND ERNEST WITEBSKY

Preliminary Communication

AUTO-ANTIBODIES IN HASHIMOTO'S DISEASE (LYMPHADENOID GOITRE) PATIENTS with Hashimoto's disease (lymphadenoid

> I. M. ROITT D.Phil. Oxfd DEBORAH DONIACH M.D. Lond. P. N. CAMPBELL Ph.D. Lond. R. VAUGHAN HUDSON F.R.C.S.



Connections and combinations in autoimmune thyroid diseases - 1963

Reg Hall (Paul Starr Award 1987) observed extended families with autoimmune thyroid disease and thyroid antibodies at higher than expected frequency in unaffected siblings

Deborah Doniach and Ivan Roitt observed clustering of thyroiditis in patients with pernicious anaemia





The end of thyroid autoimmunity history?



- Why does autoimmune thyroiditis run in families?
- Why are other diseases associated with it?
- What starts and what might stop the pathogenesis?

Autoimmunity – a shared genetic basis

Most autoimmune diseases are associated with HLA class I or II polymorphisms; HLA-DR3 haplotype associations are common to all autoimmune endocrinopathies

Pernicious anaemia is associated with HLA-A2, -A3, and -B7 so other genes are involved, identified by candidate approach

GWAS hint at other novel genes (Tomer et al; Taylor et al)



MHC Class-2 molecule loaded with a peptide (image by Dr. Roger Miesfeld)

Which genes are involved besides HLA?

CTLA-4 associated with AITD, T1DM, Addison's disease and vitiligo

PTPN22 associated with Graves' disease, T1DM, SLE, RA and vitiligo

IL-2Rα/CD25 associated with Graves' disease, T1DM, MS, JRA



(Zeitlin et al, 2008)

FCRL3 associated with AITD, RA, SLE; affects regulatory T cell function; *CD40*

Why do some patients get Hashimoto's thyroiditis and others Graves' disease?

Linkage disequilibrium structure encompassing *TSHR* allowed analysis of haplotype tagging SNPs

One haplotype was associated with Graves' disease but not Hashimoto's thyroiditis (P<1x10⁻⁶, OR 1.7)

Further analysis has refined the association with 2 SNPs in intron 1 giving the strongest association



(Dechairo et al, 2005; Brand et al, 2009)

Vitiligo: an exemplar disease association

- 0.4% overall prevalence but
 7% in AITD
- Also associated with pernicious anaemia, SLE, T1DM, RA, and Addison's disease
- Autoimmune mechanisms still unclear



Ariel Taub



http://www.dermpedia.org/dermpedia-textbook/vitiligo

Genetic associations in vitiligo

14 vitiligo susceptibility loci, half of them associated with other autoimmune diseases: *HLA* class I & II, *PTPN22, CD25, LPP, UBASH3A* and *C1QTNF6*.

Another locus, *TYR*, encodes tyrosinase, a vitiligo autoantigen, and may mediate target specificity of immune attack against melanocytes.



(Jin et al, 2010)



Figure 2. Genetic relationships of generalized vitiligo susceptibility genes and other autoimmune diseases. Circles indicate loci associated with susceptibility to a given autoimmune disease: yellow, shared risk alleles; orange, opposite risk alleles at same SNP; white, secondary association due to primary association with autoimmune disease epidemiologically associated with generalized vitiligo. SLE, systemic lupus erythematosus.

A spectrum of autoimmunity doesn't exist

ORGAN-SPECIFIC

Hashimoto's thyroiditis, Graves' disease, pernicious anaemia, Addison's disease

Goodpasture's syndrome, pemphigus vulgaris, multiple sclerosis

Myasthenia gravis, ITP, idiopathic leucopenia

Primary biliary cirrhosis, ulcerative colitis, Sjögren's syndrome

SLE, dermatomyositis, scleroderma, RA

NON-ORGAN-SPECIFIC



Genetic associations in vitiligo

- A second GWAS (450 cases and 3,182 controls), an independent replication study (1,440 cases and 1,316 controls) and a meta-analysis (3,187 cases and 6,723 controls) identified 13 additional vitiligo-associated loci:
 27 susceptibility loci in total, comprising 20% of total (around 50 loci in T1DM, and 71 in Crohn's by 2010, comprising 25% of total!)
- As in AITD, most vitiligo susceptibility loci encode either immunoregulatory proteins or target cell melanocyte components (Jin et al, 2012)







Multiple genotypes of phenotypic autoimmune disease

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Conclusion and clinical significance (1)

- Part of the explanation for familial clustering of thyroid autoimmunity is genetic; by analogy over 100 genes may contribute susceptibility
- Disease associations are partially explained by shared genetic susceptibility
- This makes screening for thyroid autoimmunity worthwhile in SLE, RA, Sjögren's and systemic sclerosis, as well as more 'organ-specific' disorders
- The high frequency of thyroid autoimmunity makes screening for other rarer diseases much less useful

But non-genetic factors are important!

THYROID Volume 18, Number 4, 2008 © Mary Ann Liebert, Inc. DOI: 10.1089/thy.2007.0234

> Changed Presentation of Hashimoto's Thyroiditis in North-Eastern Sicily and Calabria (Southern Italy) Based on a 31-Year Experience



Salvatore Benvenga^{1,2} and Francesco Trimarchi¹

 In Austria, increase in HT between 1980 and 2010

(Ott et al, 2011)

 In USA, HT was rare up to 1940, then increased to 1970, static to 1990, then a further increase

(Caturegli et al, 2013)

Environmental factors

Antigen release or immunogenicity:

lodine, selenium Radiation damage

Altered immunological balance:

Stress (in Graves' disease) Drugs – lithium, α-IFN, reconstitution syndromes Allergens (in Graves' disease) Alcohol (protective) Smoking (Janus effect), especially in ophthalmopathy Toxins – 3MC, polychlorinated biphenyls, pesticides

Shared environmental predisposition?

Alemtuzumab depletes 95% of lymphocytes in multiple sclerosis but one third of patients get Graves' disease, ITP or other disorders (Coles et al, 1999)

In 3% of HIV patients, Graves' disease developed within 17 months after the end of HAART, during immune reconstitution ; also RA and SLE

(Chen et al, 2005)

Altered T cell immunoregulation causes AITD and other types of autoimmunity: a shared environmental factor



Existential factors

- Female sex (strongest risk factor: x8)
- Parity (a possible reconstitution phenomenon)
 - Age (up to 70yo)
- Stress (in Graves' disease)
- Hygienic environment: the microbiome
- Increased affluence

Swiss cheese model for autoimmune disease



Conclusion and clinical significance (2)

- Environmental and existential factors are increasingly important in thyroid autoimmunity
- Their interplay with complex genetic factors makes it unlikely we can predict disease in the near future
- Some environmental and existential factors are shared between autoimmune diseases, adding to the reasons for disease associations
- Certain environmental factors mandate screening for thyroid autoimmunity, including the use of novel immunomodulatory agents

Thyroid autoantibodies

- TG, TPO and TSH-R antibodies have pathogenic roles via ADCC and TSH-R blockade
- Role in management unclear: weakly predict future disease
- Other antibodies less well defined: 2nd colloid antigen, thyroid hormones, NIS:19.6% of GD patients and 13.5% of HT patients positive in recent study

(Brix et al, 2013, ETA)



(Vanderpump et al 1995)

After α-IFN, 50% Ab+ developed AITD vs 5.4% Ab- (Koh et al, 1997)

Pendrin is a new thyroid autoantigen



Vitiligo – the search for autoantibodies

Antigen	% positive
Tyrosinase	11
TRP-1	5
TRP-2	5
Pmel17	5
SOX10	3
SOX10 in APS1	22
MCHR1	17



(Kemp et al, 1997, 1998, 2002; Hedstrand et al, 2001)

Cross-reacting antigens in alopecia explain the association with vitiligo

Antigen Trichohyalin Keratin 16 TH Tyrosinase TRP-1 TRP-2 Pmel17 Retinol-binding protein 4 Positive (%) 10/10 (100) 8/10 (80) 6/32 (19) 3/32 (9) 2/32 (6) 2/32 (6) 2/32 (6) 10/15 (67)



(Leung et al, 2010, Kemp et al, 2010; Ahn et al, 2011)

(Ramot et al, 2010)

Thyroid autoantibody patterns

- Some weak evidence of cross-reactivity e.g. TPO antibody subset binding to brain in encephalopathy (Blanchin et al, 2007)
- Disease clusters are now being defined by thyroid antibodies



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Thyrotropin Receptor Autoantibodies Are Independent Risk Factors for Graves' Ophthalmopathy and Help to Predict Severity and Outcome of the Disease

Anja K. Eckstein, Marco Plicht, Hildegard Lax, Markus Neuhäuser, Klaus Mann, Sebastian Lederbogen, Christian Heckmann, Joachim Esser, and Nils G. Morgenthaler

Thyroid antibodies and autoimmunity: effects independent of thyroid status?

TPO antibody positivity associated with depression in post menopausal women (Pop et al, 1997)

Meta analysis found ~2.5-fold increase in miscarriage in those who were TPOAb + (Prummel and Wiersinga, 2004)

Quality of life related to TPOAb not thyroid function and may increase fibromyalgia symptoms (Ott et al, 2011; Dardano et al, 2012; Watt et al, 2012)



Conclusion and clinical significance (3)

- Autoimmune disease engenders multiple autoantibodies: 'autoimmune escalation' (Rose)
- Cross reaction between antigens at an antibody and/or T cell level may explain some disease associations
- Thyroid antibodies are clinically useful surrogate markers for the presence of autoimmune thyroiditis but have weak clinical precision
- However they may mark immunological effects on health

What prevents autoimmune thyroid disease from progressing?

If we all have autoreactive T cells, why don't we all have autoimmune thyroid disease?

At least two reasons:

- regulation by T cells
- peripheral tolerance

Both have therapeutic potential

Immune dysfunction, polyendocrinopathy and X-linked enteropathy syndrome

IPEX is rare, onset in neonates and usually fatal without marrow transplant; typically have hypothyroidism, IDDM and thrombocytopenia

Caused by mutation in *FOXP3:* central to the development of CD4⁺, CD25⁺ T regulatory cells

Increasing evidence of a functional Treg defect in AITD (Glick et al, 2013)



HLA-DR expression & peripheral tolerance

- TFCs express HLA-DR in AITD: proposed as a disease initiator by Franco Bottazzo
- Always secondary to thyroiditis in animal models and only induced by γ-IFN, a T cell product
- DR+ TFCs can present antigen to memory T cells but paralyse naïve T cells

(Marelli-Berg et al, 1997)





HLA-DR expression & peripheral tolerance



Conclusion and clinical significance (4)

- T regulatory cells and peripheral tolerance are crucial to health, as thymic deletion is not 100% effective
- Genetic and environmentally induced defects in T regulatory cells give rise to autoimmune thyroid disease in animals and man
- The thyroid itself plays a major role in regulating lymphocytic accumulation and stimulation through complex pathways that will differ between individuals
- This role has therapeutic potential (*cf* carbimazole)

The next 60 years: Precision Medicine





Autoantibodies to predict disease

- Risk in subclinical hypothyroidism is refined by antibody level
- IgG4-related sclerosing disease
- Proteomic technology in future will allow measurement of a number of autoantibodies (multiplexing) based on addressable microbeads or nanobarcoded particles
- The phenomenon of 'autoimmune escalation' may be used to identify which antibody clusters mark temporary and permanent damage



The next 60 years

- 'The complexity of the etiology of autoimmune thyroid disease is gravely underestimated' (Brix & Hegedüs, 2011)
- 'Further elucidation of the molecular mechanisms mediating immune dysregulation should improve our understanding of disease pathogenesis...' - no longer will this appear at the end of papers!
- Definitive therapy for Graves' disease will be available which will be targeted at the thyroid cell's role in the autoimmune process or at T regulatory cells

Collaborators

Sheffield

Phil Watson Helen Kemp Russell Metcalfe Raju Gottumukkala Liz Waterman Nikos Gavalas Ramzi Ajjan David Gawkrodger

Mentors

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Others

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Thyroid cells are immunologically promiscuous

HLA class I and II – antigen presentation, including peripheral tolerance

Adhesion molecules (ICAM-1, LFA-1) and chemokines – infiltration, cytotoxicity

Cytokines (IL-1, -6, -8, -12, -13, -18) - diverse effects

Soluble mediators (prostaglandins, NO) – cytotoxicity, TFC function

Fas, FasL and other death-related molecules - cytotoxicity

CD40 – B cell stimulation

Complement regulatory proteins – defence against complement attack



Vitiligo – search for novel autoantigens

- Enrich melanocyte cDNA phage display library on vitiligo patient sera
- 51% of 61 patients reacted with at least one of 8 new autoantigens (TIF2, HSP90, osteopontin, α- and γ- enolase, ubiquitin BP, rab38)
- No reactivity found in patients with segmental vitiligo

(Waterman et al, 2009)



Hashimoto's thyroiditis is heterogeneous

- One subtype is characterized by an increased number of IgG4+ plasma cells, high serum IgG4 levels and thyroid fibrosis
- Associated with rapid hypothyroidism and high levels of thyroid autoantibodies
- Plus a systemic form of IgG4related sclerosing disease which, when it affects the thyroid, is Riedel's thyroiditis





(Li et al, 2012)

Back to Hashimoto: Iymphocytic infiltration is the hallmark





Leppänen A, University of Helsinki

Chemokines

Chemokine gene expression in autoimmune thyroid disease

Chemokine gene expression analysed by RT-PCR in thyroid tissue samples from patients with:

Hashimoto's thyroiditis, lanes 1-4.

Graves' disease, lanes 5-10.

Multi-nodular goitre, lanes 11-14.



Cytokine expression by thyroid cells



IL-6 mRNA expression by TFC

Hashimoto's thyroiditis

Hakaru Hashimoto, a surgeon, described similar histological appearances in 4 patients which he termed *struma lymphomatosa* in 1912: he recognised the similarity to Graves' disease and Sjögren's syndrome but was largely ignored due to the advent of WW1, the publication in German and the rarity of the syndrome compared to endemic goitre



Zur Kenntniss der lymphomatösen Veränderung der Schilddrüse (Struma lymphomatosa)

х.

Von

Dr. H. Hashimoto, Assistent der I. chirurg. Universitätsklinik in Kiushiu, Japan (Director: Prof. Dr. H. Miyake) (Hierzu Tafel VI.)

Autoimmune hypothyroidism

'Myxoedema is dependent on a destructive affectation of the thyroid gland....the thyroid is converted into delicate fibrous tissue, which is here and there infiltrated with clumps of cells....seen as small islets of round cells, which evidently replace the gland vesicles....there are seen minute nodules about 1 to 2 mm in diameter which are found to be composed of leucocytes'

WM Ord (1877, and again in 1888 as a Report on Myxoedema for the Clinical Society of London)



Autoimmune hypothyroidism

In 1900 George Murray was writing confidently:

'The fibrosis may be primary, occurring as a result of chronic inflammation leading to a secondary atrophy of the epithelial cells' and described both the familial predisposition and the occurrence 'particularly in women who have borne children'



