

Catalytic antibodies to HIV: Physiological role and potential clinical utility

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Abstract

Immunoglobulins (Igs) in uninfected humans recognize residues 421–433 located in the B cell superantigenic site (SAg) of the HIV envelope protein gp120 and catalyze its hydrolysis by a serine protease-like mechanism. The catalytic activity is encoded by germline Ig variable (V) region genes, and is expressed at robust levels by IgMs and IgAs but poorly by IgGs. Mucosal IgAs are highly catalytic and neutralize HIV, suggesting that they constitute a first line of defense against HIV. Lupus patients produce the Igs at enhanced levels. Homology of the 421–433 region with an endogenous retroviral sequence and a bacterial protein may provide clues about the antigen driving anti-SAg synthesis in lupus patients and uninfected subjects. The potency and breadth of HIV neutralization revives hopes of clinical application of catalytic anti-421–433 Igs as immunotherapeutic and topical microbicide reagents. Adaptive improvement of anti-SAg catalytic Igs in HIV infected subjects is not customary. Further study of the properties of the naturally occurring anti-SAg catalytic Igs should provide valuable guidance in designing a prophylactic vaccine that amplifies protective catalytic immunity to HIV.

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1. Introduction

Infection with human immunodeficiency virus-1 (HIV) causes acquired immunodeficiency syndrome (AIDS), characterized by depletion of CD4⁺ T cell, hyperglobulinemia and B cell hyperplasia. The major host cells are T cells and macrophages. Their infection occurs *via* binding of the coat protein gp120 trimer to CD4 receptors and chemokine co-receptors (mostly CCR5 and CXCR4) [1]. In addition, monomeric gp120 induces neuronal and CD4 T cell death, and the monomer shed from the virus may play a role in disease pathogenesis. Progression of infected humans to AIDS varies from a year to more than two decades [2]. Some repeatedly exposed humans remain free of infection [3]. The inability of the adaptive immune system to prevent and control infection derives from the structural variability of the HIV envelope. gp120 is composed of five comparatively constant (C) regions and five highly variable (V) regions. Most adaptive responses are directed against V domain immunodominant epitopes, which mutate rapidly. This allows emergence of escape viral mutants [4]. Adaptive cytotoxic T cell and neutralizing Ig responses only provide transient protection [5,6]. gp120 structural variability also underlies the failure to develop an effective HIV vaccine. gp120 V domain sequences expressed by diverse HIV strains found around the world are highly variable. Protein and DNA vaccination strategies aimed at inducing protective T cell and Ig responses have been unsuccessful [7,8].

We review here catalytic Igs to a conserved epitope in the B cell superantigenic site (SAg) of gp120. The Igs are found at variable levels in humans without infection and are likely to provide partial protection against HIV. The suitability of the SAg epitope as a target for HIV immunotherapy and prophylactic vaccination are discussed.

2. Superantigenic character of gp120

B cell SAgS are antigens recognized by Ig V domains without the requirement of adaptive sequence diversification. The paired V domains of the heavy (VH) and light (VL) chain subunits of physiological IgG, IgA and IgM class Igs bind [9] and catalyze the hydrolysis [10,11] of gp120 by recognizing its SAg site (Fig. 1A). Conserved framework regions (FRs) are involved in gp120 SAg recognition, assessed by V domain homology analysis and FR/complementarity determining region (CDR) swapping studies [12,13]. B cell receptor (BCR; surface Ig complexed to signal transducing proteins) engagement by SAgS is thought to downregulate B cells without dependence on T cells

[14]. The effect may be mediated by modulation of CD79b expression, a BCR associated signal transducing protein [15]. Most reported B cell SAg binding Igs contain VH3 family V domains found in the majority of expressed Igs of healthy adults. The binding is characterized by moderate affinity [16]. VH3 usage, however, is not the sole determinant of gp120 SAg recognition. A monoclonal catalytic VH2 family IgM from a human without infection recognizes the gp120 SAg site [10]. Rare L chain Ig subunits isolated from phage display libraries can bind and hydrolyze the gp120 SAg site independent of the H chain [17]. Igs with the most potent anti-viral activity are likely to be formed by pairing of VL and VH domains with the greatest gp120 binding capability. Ig catalytic sites are usually located in VL domains [18]. VH pairing with catalytic VL domains will also enhance anti-viral potency. A single catalyst molecule hydrolyzes thousands of antigen molecules and hydrolysis of polypeptides usually results in loss of their biological activity [19]. This is exemplified by observations of more rapid gp120 hydrolysis and superior HIV neutralization by salivary IgA from uninfected subjects compared to other Ig classes (Fig. 1B).

Synthetic gp120 peptide studies have suggested that residues 421–433 are components of the SAg site [13]. This peptide region also contains amino acids that are essential for binding to host CD4 receptors determined by previous structural studies [20]. As CD4 recognition is obligatory for virus entry into host cells, the 421–433 epitope is relatively conserved in diverse HIV strains. IgMs, [10] IgAs [11] and isolated L chain subunits [17] from uninfected humans hydrolyzed the 432–433 peptide bond located within this epitope. Catalytic selectivity was indicated by lack of hydrolysis of irrelevant proteins. Proteolytic Igs utilize nucleophilic mechanisms akin to serine proteases [18]. We prepared a 421–433 peptide analog containing the strongly electrophilic phosphonate group (E-421–433) that binds covalently to the catalytic sites of serine proteases and proteolytic Igs [21]. E-421–433 specifically and irreversibly inhibited the activity of the proteolytic Igs. IgA neutralizing activity was inhibited by E-421–433 [11]. These observations indicate non-covalent recognition of the peptide epitope coordinated with nucleophilic attack on the polypeptide backbone as the mechanism of catalysis and HIV neutralization.

3. What drives catalytic Ig formation?

Ig catalysis [22] and SAg recognition [23] are germline V gene encoded functions. The expression of these functions is subject to deterioration or improvement due

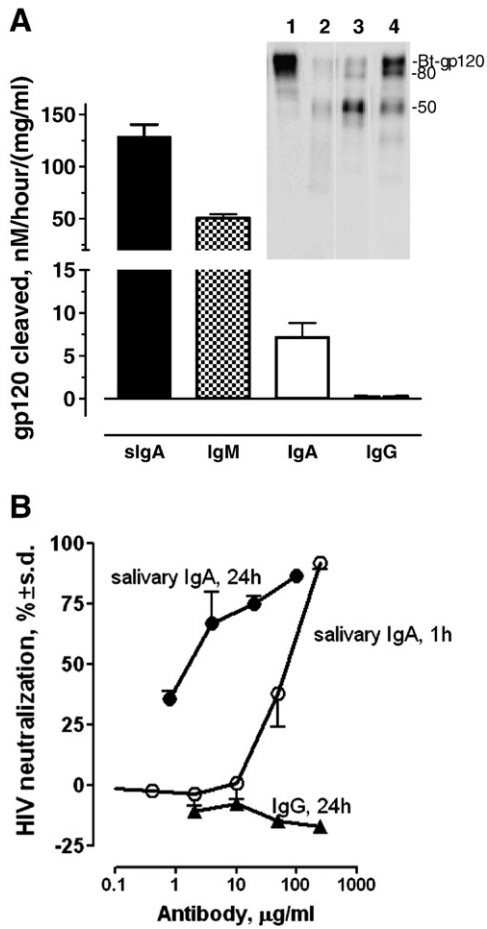


Fig. 1. A, Cleavage of biotinylated gp120 by salivary IgA, serum IgM, IgA and IgG from humans without HIV infection. Comparative gp120 cleaving activity of serum IgM (45 µg/ml), salivary IgA (32 µg/ml), IgA (144 µg/ml) and IgG (144 µg/ml) expressed per equivalent Ab mass. Each bar represent the mean of 4 human subjects. Reaction conditions: 17 h, 0.1 µM Bt-gp120. Inset, Streptavidin-peroxidase stained blots of reducing SDS-gels showing cleavage of Bt-gp120 (0.1 µM) incubated 23 h with diluents (lane 1), pooled polyclonal salivary IgA (lane 2, 32 µg/ml), serum IgM (Lane 3, 45 µg/ml), and serum IgA (lane 4, 160 µg/ml) from 4 humans. Reaction volume, 0.02 ml. B, HIV neutralization by Abs from HIV-seronegative humans. Neutralizing potency of salivary IgA and serum IgG Abs purified from pooled saliva or serum of 4 human subjects. HIV-1 strain, 97ZA009; host cells, phytohemagglutinin-stimulated PBMCs. Serum IgG was incubated with the virus for 24 h and salivary IgA was incubated 1 h (open circle) and 24 h (close circle). Values are expressed as percent reduction of p24 concentrations in test cultures compared to cultures that received diluent instead of the Abs (means ± s.d. of 4 replicates). Time-dependent neutralizing activity by salivary IgA suggests that catalysis is a possible mechanism of its neutralizing activity. Data from reference [11].

to biased germline gene usage; V-D-J/V-J sequence diversification; VH-VL combinatorial diversification; and somatic hypermutation occurring at the FRs/CDRs. IgMs and IgAs preferentially express gp120 SAg hydrolyzing activity, with only low level activity evident

in IgGs [11]. The activity levels of polyclonal Igs from different humans of individual monoclonal Igs are highly variable. No obvious antigenic stimulus driving the synthesis of the catalytic Igs exists in humans who are not infected with HIV. Occam’s razor suggests consideration of a random distribution of the SAg-selective catalytic activity that is expressed fortuitously as the Igs mature adaptively in response to irrelevant antigenic stimuli. However, studies summarized below suggest that the gp120 SAg hydrolyzing (and neutralizing) activity is open to selection and improvement under certain circumstances.

Polyclonal IgGs from mouse lupus models and patients with lupus but no HIV infection [24] bind the 421–433 gp120 region at levels superior to controls without autoimmune disease. We isolated Ig light chain subunits (IgLs) and HIV neutralizing single chain Fv fragments that recognize the 421–433 epitope from lupus Ig libraries [17,25]. Mutations are frequent in the FRs and CDRs of these clones. Some of the Ig fragments catalyze the cleavage of gp120 [17]. We and others reported the tendency towards enhanced catalytic Ig synthesis in autoimmune disease [26,27]. Several groups have noted that HIV infection is infrequent in lupus patients [28,29]. Increased 421–433 binding and hydrolyzing Igs may be the reason. A generalized increase of polyreactive Igs to microbial proteins is an unlikely explanation, as the lupus Ig binding, hydrolytic and neutralizing activities are specific for the 421–433 region.

Concerning the antigenic stimulus driving synthesis of the 421–433 recognizing Igs without HIV infection, we found no human proteins in the databanks with sequence similarity to the gp120 421–433 region. However, 27 of 39 nucleotides that code for the consensus clade B residues 421–433 (CCGTATGTAACGAAAAGGATGAAAGACGGTGTACAAATA) are identical to a human endogenous retroviral sequence (HERV; TTAGATCTGATGAAAAGGATGAAAGAAATTTTCAAAA; identities in italics; nucleotides 303–341, rv 012650, family HERVL47) [17]. Expression of human endogenous retroviral sequences (HERVs), which constitute up to 8% of the human genome, is increased in lupus [30]. Interestingly, certain anti-gp120 Igs are reported to recognize a HERV expressed by placental tissue [31]. *S. gordonii* is a commensal bacterium that colonizes mucosal sites at varying densities. A probable transcription regulator-like (PRL) protein from this bacterium contains a peptide region homologous to gp120 residues 421–432 as shown below:

gp120	421	KQ-IINMQEYVVK	432
<i>S. gordonii</i> PRL protein	77	KQFIINMSQNVGK	89

Regrettably, amplification of protective catalytic Igs to the SAg is not routine in HIV infected subjects. However, we observed that prolonged infection in a subpopulation of HIV infected individuals who remained AIDS-free is associated with a modest increase of catalytic IgAs to gp120. No increase was observed at an earlier stage in infection (6 months). In contrast, robustly increased conventional Igs with binding activity directed to the non-SAg gp120 epitopes were evident at this time. Evidently, therefore, immunological pathways that generate antigen binding Igs do not favor the synthesis of anti-SAg catalytic Igs.

Binding of conventional antigens to the BCR drives B cell division. BCR-catalyzed gp120 cleavage will result in release of the gp120 fragments, depriving the cells of the proliferative signal. In the conventional immune response, therefore, the catalytic activity can improve adaptively only to the extent that product release is slower than the proliferative transmembrane signal. IgAs/IgMs display superior proteolytic activity compared to IgGs. This is understandable if μ/α -BCRs generate more rapid transmembrane signals compared to γ -BCRs, a hypothesis that remains to be tested experimentally. A second scenario is that BCR catalysis itself induces cell proliferation. Peptide bond hydrolysis is highly exothermic and liberates large amount of energy. In theory, the catalytic energy could be utilized to induce favorable BCR signaling, e.g., by inducing a productive change in the BCR conformation or by increasing BCR diffusion in the lipid bilayer, thus increasing the probability of BCR cross-linking. Theoretically, BCR engagement by conventional antigens and SAgS could result in opposite cellular signaling, in that BCR-catalyzed SAg hydrolysis and product release may lessen the down-regulatory and apoptotic effects of prolonged BCR occupancy by the SAg. Accordingly, adaptive maturation of B cells producing proteolytic Igs may be less restricted than of cells expressing BCRs that bind the SAg site stably.

4. Implications for host-virus evolutionary relationship (Fig. 2)

HIV is thought to have originated *via* zoonotic transmission from monkeys to humans a few decades ago [32]. The sequence of HIV gp120 residues 421–433 is conserved in simian immunodeficiency virus (SIV) envelope sequences in Los Alamos database. No virus homologs of HIV in lower organisms have been identified thus far. The ability of Igs to recognize SAgS is encoded by germline V genes, and HIV is neutralized as a consequence of Ig recognition. Modern B cell SAgS have

probably helped shape the structure of germline Ig genes, in that the SAg recognition capability was presumably developed by Darwinian evolution over millions of years as a defense against microbial infection. These arguments support a hypothetical ancient homolog of HIV that drove the evolution of human germline-encoded anti-gp120 SAg Igs. Additional supporting evidence for the primordial HIV homolog is the existence of a HERV sequence homologous to the modern 421–433 region. HERVs are thought to be relics of viral sequences integrated into the host genome. They may fulfill a useful function in the evolution of novel host proteins with improved functions [33]. However, HERVs also hold the potential of serving as building blocks for novel pathogenic viruses if expressed as functional genomes with appropriately packaged capsid and infective envelope structures (Fig. 2).

5. Clinical prospects

We turn now to the utilitarian features of the catalytic anti-HIV Igs. gp120 SAg site binding by IgGs from uninfected humans (which are poorly catalytic) correlates inversely with the frequency of subsequent HIV infection [9]. The protective effects of infusion of IgG from uninfected monkeys against SIV [34] and of pooled IgG from uninfected humans (IVIg) against HIV has been investigated [35]. Proteolytic Igs inactivate antigens irreversibly and with potency superior to conventional Igs [36]. Catalytic salivary IgAs from uninfected humans neutralized primary, R5-dependent clade B and C strains. Saliva from healthy humans is available in abundant amounts, and pooled salivary IgA is a candidate for development as a passive immunotherapy formulation. HIV is transmitted mainly across mucosal surfaces. There is agreement that a safe and effective vaginally applied microbicide will help slow heterosexual HIV transmission. The salivary catalytic IgAs could be formulated as a vaginal microbicide. Similar considerations apply to the homogeneous single chain Fv constructs from the lupus library. These constructs display cross-clade HIV neutralizing activity, and their potency is superior to monoclonal Igs that have been advanced as candidate anti-HIV reagents [25,37]. This revive hopes for effective passive immunotherapy of HIV infection.

Developing a prophylactic vaccine is the highest priority in HIV research. Synthetic peptides are notorious for assuming conformations that differ from the corresponding epitope in native proteins. Structural elucidation of synthetic 421–433 analogs bound to the anti-SAg neutralizing Igs will be useful to reveal the neutralizing relevant conformation of the peptide.

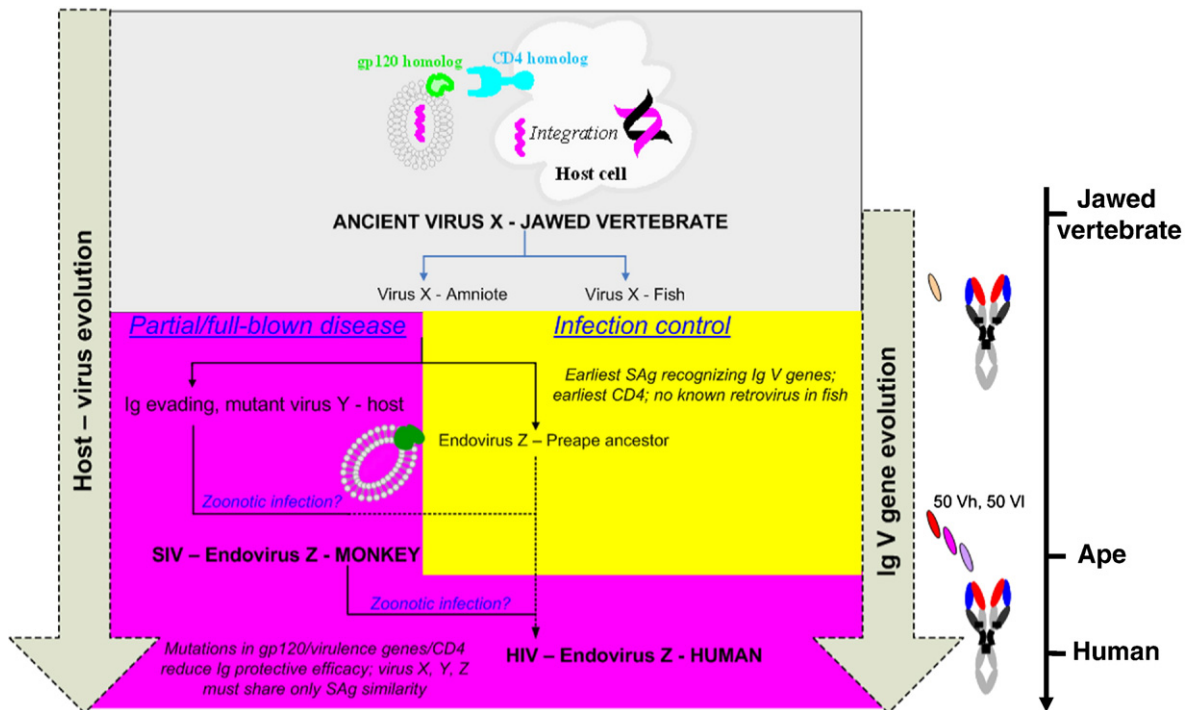


Fig. 2. A plausible phylogenetic explanation for the B cell superantigenic (SAg) character of modern HIV gp120. Preimmune murine and human antibody V domains bind and catalyze the hydrolysis of HIV gp120 [10,11], indicating its SAg character. The SAg site is essential for CD4 binding and HIV infection. Antibodies to this region from uninfected humans neutralize HIV infection, suggesting a pre-existing ability to protect against infection that arose over the course of V gene evolution. Jawed fish are the earliest vertebrates known to produce antibodies and CD4. Fish VH gene sequences are highly homologous to human VH3-family Igs known to recognize the SAg site, suggesting that the ability of preimmune antibodies to protect against HIV arose early in evolution. In turn, this argues for an ancient virus expressing a gp120 homolog. The homology between a HERV and the gp120 SAg site has been noted in our report describing antibody catalyzed gp120 hydrolysis [17]. HERVs are retroviral gene fragments integrated into the human genome over the course of evolution. The homologous HERV supports the existence of an ancient HIV related virus.

Constraining candidate peptide vaccines into the correct conformation will enhance the probability of inducing protective Igs. B cell differentiation pathways that favor adaptive improvement of Ig catalytic activity are virtually uncharted. Their elucidation will help design immunogens that induce protective catalytic immunity. Monoclonal Igs raised by immunization with an electrophilic analog of full-length gp120 (E-gp120) catalyze the hydrolysis of gp120 [38] and display cross-clade HIV neutralizing activity [39]. Some of these Igs recognize the 421–433 region. However, E-gp120 contains numerous antigenic epitopes, and the overall polyclonal Ig response is insufficiently focused at the 421–433 epitope. A KLH-conjugate of residues 421–436 induces Igs that recognize native gp120, [40] indicating that at least some peptide molecules assume the neutralization-relevant conformation of this epitope. The mucosal milieu supports the synthesis of Igs with superior catalytic and neutralizing activities compared to serum Igs [11]. Mucosal immunization,

therefore, may help improve the protective quality of the Ig response.

Take-home messages

- Adaptive immune responses do not generally control HIV infection because of the mutability of immunodominant gp120 epitopes. In contrast, pre-existing catalytic Igs in uninfected humans directed to the gp120 B cell SAg site neutralize HIV and probably provide partial protection against the virus.
- The catalytic Igs recognize residues 421–433, which are essential for host CD4 receptor binding. Mucosal Igs express highest catalytic activity. Serum IgG is poorly catalytic, suggesting distinctive B cell differentiation pathways favoring catalytic Ig synthesis.
- Amplification of catalytic anti-421–433 Ig synthesis in HIV infected subjects is generally proscribed. However, catalytic Igs are produced at enhanced levels by uninfected lupus patients and after prolonged infection

in subjects who do not develop AIDS. Sequence homology of the 421–433 epitope with a HERV sequence and microbial proteins may help shed light on host-virus evolutionary relationships.

- Guidance for design of candidate peptide vaccines can be obtained by: (a) Determining the neutralization-relevant 421–433 conformation complexed with an anti-SAg Ig; and (b) identifying the antigen driving catalytic Ig synthesis in uninfected humans.
- Catalytic Igs to the 421–433 epitope neutralize HIV more potently and broadly than conventional Igs. They are candidate passive immunotherapeutic reagents and vaginal microbicides.

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T lymphocytes in patients with primary vasculitis: expansion of CD8+ T cells with the propensity to activate polymorphonuclear neutrophils.

To gain insight into the immune pathogenesis of primary ANCA-associated vasculitides, the prevalence of circulating T lymphocytes expressing CD11b as a marker for activation, was analyzed in patients with WG or microscopic polyangiitis. **Iking-Konert C. et al. (*Rheumatology* 2008; 47: 609–16).** Receptor expression and IFN γ synthesis were measured in T cells of patients with active disease by cytofluorometry and compared with expression in patients in remission and in healthy donors. During active disease, a small but conspicuous population of CD8+CD28+CD11b+ was found which produced IFN γ . In healthy donors and patients in remission or undergoing immunosuppressive therapy, CD11b was exclusively associated with CD8+CD28- cells, the latter being more frequent in patients with long-standing or severe diseases. In vitro experiments confirmed that CD11b is up-regulated when T cells are activated. After multiple rounds of re-stimulation, the CD11b expression persists whereas CD28 expression is host, compatible with the notion that CD8+CD28+CD11b+ represents a transient phenotype in the course T-cell activation. The IFN γ -producing T cells activated polymorphonuclear neutrophils (PMN) to express MHC class II, thus generating the same PMN phenotype as in patients with active ANCA-associated vasculitis. A similar PMN phenotype could be generated by cultivation with supernatants of activated T cells or by IFN γ alone, but not by antibodies to proteinase 3. Thus, in active primary vasculitis, a small population of CD8+ T cells, identified by the expression of CD11b, expands, producing IFN γ . These T cells could activate PMN, thus generating a long-living and potentially destructive PMN phenotype.

Anti-Scl-70 antibodies in autoimmune hypothyroidism.

The relationship between autoimmune thyroiditis and systemic sclerosis is controversial. Data exist on the presence of thyroid autoantibodies in patients with systemic sclerosis but, so far, anti-Scl-70 antibodies, which are highly specific to systemic sclerosis, have not been investigated in autoimmune hypothyroidism. This study, **Ugurlu S. et al. (*J Inter Med Res* 2008; 36:152–6)** compared the presence of anti-Scl-70 in females with autoimmune hypothyroidism (n=24) and in healthy age-matched female controls (n=26). Free thyroxine levels were similar in both groups. Thyroid stimulating hormone (TSH), antithyroid peroxidase (anti-TPO), antithyroglobulin (anti-Tg) and index values for anti-Scl-70 levels were significantly higher in patients with autoimmune hypothyroidism compared with controls, although anti-Scl-70 test was negative in both groups. Anti-TPO, anti-Tg and TSH significantly correlated with anti-Scl-70. In conclusion, autoimmune hypothyroidism seems to be associated with a higher index level of anti-Scl-70, yet a negative anti-Scl-70 antibody test. This suggests that autoimmune hypothyroidism might have common aetiological factors with systemic sclerosis.