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Selenium status and over-expression of interleukin-15 in celiac disease and autoimmune thyroid diseases

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Summary. In celiac disease (CD), for its multifactorial nature, the target organs are not limited to the gut, but include thyroid, liver, skin and reproductive and nervous systems. Between the extraintestinal symptoms associated with CD, autoimmune thyroid diseases (AITDs) are more evident, underlining as CD-related autoimmune alterations can be modulated not only by gluten but also by various concurrent endogenous (genetic affinity, over-expression of cytokines) and exogenous (environment, nutritional deficiency) factors. In their pathogenesis a central role for over-expression of interleukin-15 (IL-15) is shown, by inhibiting apoptosis, leading to the perpetuation of inflammation and tissue destruction. Thyroid is particularly sensitive to selenium deficiency because selenoproteins are significant in biosynthesis and activity of thyroid hormones; besides, some selenoproteins as glutathione peroxidase are involved in inhibiting apoptosis. Thus, selenium malabsorption in CD can be thought as a key factor directly leading to thyroid and intestinal damage. Considering the complexity of this interaction and on the basis of available evidence, the aim of this review is to assess as preventive and therapeutic target the role of IL-15 and selenium in the pathogeneses of both CD and AITD.

Key words: celiac disease, autoimmune thyroid diseases, selenium, interleukin-15.

Riassunto *(Stato del selenio e sovra-espressione dell'interleuchina-15 nella malattia celiaca e nelle malattie autoimmuni tiroidee)*. Nella malattia celiaca (MC), per la sua natura multifattoriale, gli organi target non sono solo limitati all'intestino, ma includono la tiroide, il fegato, la pelle ed i sistemi riproduttivo e nervoso. Tra i sintomi extraintestinali associati alla MC, le malattie autoimmuni tiroidee (AITD) sono le più evidenti, sottolineando come le alterazioni autoimmuni associate alla MC possano essere modulate non solo dal glutine ma anche dalla coesistenza di vari fattori endogeni (ad esempio l'affinità genetica, la sovra-espressione di citochine) ed esogeni (ad esempio l'ambiente, le carenze nutrizionali). Nella loro patogenesi, viene mostrato un ruolo centrale della sovra-espressione della interleuchina-15 (IL-15) che, determinando l'inibizione dell'apoptosi, conduce alla perpetuazione dell'infiammazione e del danno tissutale. La tiroide è particolarmente sensibile alla carenza di selenio, poiché le selenoproteine (SeP) sono importanti nella biosintesi e attività degli ormoni tiroidei; inoltre, alcune SeP, come il glutatione perossidasi, sono coinvolte nella inibizione dell'apoptosi. Perciò, il malassorbimento del selenio nella MC può essere considerato un fattore chiave che conduce direttamente al danno tiroideo e intestinale. Considerando la complessità di questa interazione e sulla base delle evidenze disponibili, lo scopo di questo lavoro è di valutare, come target preventivo e terapeutico, il ruolo della IL-15 nella patogenesi della MC e AITD.

Parole chiave: malattia celiaca, malattie autoimmuni della tiroide, selenio, interleuchina-15.

INTRODUCTION

Celiac disease (CD) has increasingly become considered as a multi-organ disorder, and it has been linked to a number of diseases including autoimmune disorders. Besides for both diseases' multifactorial nature, the gene-environment interaction seems a fundamental process for their occurrence. Several studies have shown that CD is associated with an increased prevalence of autoimmune thyroid disease (AITD) and *vice versa* [1].

Sometimes the association does not reflect a clinical status of overt diseases, thus it must be carefully evaluated by further research. Therefore, pathological manifestations can be also modulated by various concurrent endogenous (*e.g.* genetic affinity, over-expression of cytokines, signaling mechanisms) and exogenous (*e.g.* environment, nutritional deficiency, gluten presence) factors.

In CD and AITD both HLA DQ2 and DQ8 and the gene coding for cytotoxic T-lymphocyte-associ-

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ated to antigen-4 (CTLA-4) are over-expressed. CD induces malabsorption, with consequent micronutrients deficiency as selenium, important element of selenoproteins. These enzymes as glutathione peroxidase act by mechanisms that include effects on gene expression, DNA damage and repair, signaling pathways, regulation of cell cycle and apoptosis.

Some intestinal glutathione peroxidases by modulating apoptosis defend the tissue from reactive oxygen species (ROS) by removing the cells affected by oxidative damage. Association factor in the pathogenesis of active CD and AITD is over-expression of interleukin-15 (IL-15) that, by inhibiting apoptosis, is a survival factor of intraepithelial lymphocytes (IELs), leading to progressive damage to enterocytes and thyrocytes. The association is confirmed by selenoproteins that are also involved in inhibiting apoptosis; thyroid is particularly sensitive to selenium deficiency because selenoproteins are significant in functions of RNA in biosynthesis and activity of thyroid hormones; thus, selenium malabsorption in CD can be thought as a key factor directly leading to thyroid and intestinal damage.

In light of the available evidence, the review aims to investigate the mechanisms whereby the lack of selenium due to CD-associated malabsorption may affect the health of the thyroid, by over-expression of inflammatory IL-15. Since, uncontrolled expression of IL-15 is critical in the pathogenesis and maintenance of refractory CD and AITD [2], it could be considered an attractive and sensitive target for development of early diagnosis and new therapies.

CELIAC DISEASE AND AUTOIMMUNE THYROID DISEASES

CD and AITD are autoimmune diseases that, despite the different origin of their antigens, the gluten in CD as exogenous environmental factor, and self-thyroid antigens in AITD as endogenous factors, are caused by abnormal immune response to these antigens; they are the outcome of a complex gene-environment interaction. In addition, it is important to stress that they are among the diseases frequently observed in association [3]; common autoimmune disorders tend to coexist in the same subjects and to cluster in families.

Celiac disease. CD in genetically predisposed individuals is a permanent intolerance to gluten, a protein present in some cereals such as wheat, barley and rye. CD is a chronic enteropathy marked by villous atrophy, Lieberkühn's crypt hyperplasia and lymphocytosis and is effectively an abnormal immune response to this protein that affects about 1% of the population in the Western world [3]. To date the only sure road to treatment is a lifelong gluten-free diet (GFD).

Fundamental inflammatory processes occur in the proximal part of the small intestine mucosa and the main symptoms are: diarrhea, abdominal swelling, abdominal pain, weight loss, retarded growth and malabsorption. The damage affects the surface of the mucosa, while the submucosa and muscularis mucosae of the small intestine are not involved. Because it leads to malabsorption, CD causes deficiencies of micronutrients such as iron, folic acid and vitamin K, which are essential for organogenesis, of fat-soluble vitamins that are important for spermatogenesis, of vitamin D and calcium, necessary for normal maintenance of bone structure, and of selenium, an important element for many systems such as nervous [4], cardiovascular [5] and endocrine [6]. Besides selenium, in endocrine system of the thyroid, other micronutrients (*e.g.* vitamins A, B1, B2, magnesium, manganese, potassium, copper, zinc, iron and mainly iodine) are required for thyroid hormone synthesis and function [7].

These deficiencies may in part explain the pathogenesis of complex extraintestinal symptoms associated with CD on account of their relationship with both a subclinical malabsorption of these nutrients and a common basis of immune and/or endocrine imbalances linked to the direct interference of gluten with these systems [8]. This important characteristic becomes evident when it is considered that CD is also associated with other disorders such as: osteoporosis [9], type 1 insulin-dependent diabetes mellitus [10], neuropathy [11], increased risk of intestinal lymphoma [12] and of autoimmune diseases of the liver [13], biliary system [14] and thyroid [15].

Studies on the incidence and prevalence of autoimmune diseases have demonstrated the protective effect of GFD in both adult and pediatric celiac individuals. Incidence is 5.4 per thousand patients/year who follow the GFD, compared with 11.3 per thousand patients/year who do not follow the GFD ($p = 0.002$). Besides, the patients most at risk are those with a familiar history of autoimmune diseases [16].

The assessment on the prevalence reports as these disorders increase in parallel with the process of ageing. For subjects diagnosed in the first two years of life and who therefore eliminate gluten from their diet at an early age the risk of developing autoimmune diseases is 5.1%, while for those diagnosed between 2 and 10 years and after 10 years the risk rises to 17% and 23.6% respectively [17]. These data emphasize how celiac patients with early diagnosis and treatment can reduce the risk of developing other autoimmune diseases, and show how the mechanisms underlying this association, such as environmental factors, immunological mechanisms and genetic predisposition, are all implicated in the pathogenesis of both.

With regard to genetic predisposition, human leukocyte antigen (HLA) class II haplotypes DQ2 and DQ8 are over-expressed in many autoimmune diseases and in CD; inheritance of these haplotypes together with the associated immunological phenotypes may further explain the simultaneous occurrence of these pathologies [18]. Another potential explanation may be found in the increased expression of the gene coding for cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4). CTLA-4 is a T cell surface molecule involved in control of T cell prolif-

eration and it is a candidate gene in susceptibility to the autoimmunity. In particular, the CTLA-4 CT60 A/G dimorphism has been associated with CD and is reported to be strongly linked to autoimmune thyroid disease (AITD). A significant effect of the CTLA-4 CT60G allele at the homozygous state on the risk of developing AITD in children with CD suggests that the reported association of the CTLA-4 CT60 A/G polymorphism with CD is limited to the subgroup of patients who are or will be complicated with AITD [19].

Autoimmune thyroid diseases. AITDs mainly include Graves' disease (GD), the most common cause of thyrotoxicosis, and Hashimoto's thyroiditis (HT), the most common cause of hypothyroidism. They affect up to 2-5% of the general population [20] and represent the classical model of organ-specific autoimmune disease. They are caused by abnormal immune response to self-thyroid antigens; in this process a key role is played by T lymphocytes when antigen recognition is mediated by receptors on the cell surface (T cell receptor, TC-R). This causes the breaking of tolerance by deficit of suppressor T cells and aberrant expression of DR region of HLA (HLA-DR), absent on normal thyroid cells. The contemporary expression of HLA-DR on thyroid follicular cells and auto-antigens causes the autoimmune reaction by direct or indirect cytotoxicity antibody-dependent complement-mediated.

TC-R are generated through a process of random rearrangement of gene segments, obtaining a large number of clones of T lymphocytes, each of which specifically recognizes a single antigen, but at the same time determines the appearance of clones of lymphocytes "auto-reactive" that are able to recognize and trigger an immune response against "self" mediated by T cells and local deposition of immune complexes. This leads to a cascade of destructive events of follicular cells. AITDs are characterized by the presence of high levels of circulating autoantibodies against thyroid cell antigens. Auto-antigen involved in GD is thyroid stimulating hormone receptor (TSH-R) while auto-antigens mainly involved in HT are thyroglobulin (Tg) and thyroid peroxidase (TPO).

GD has an incidence of 1-2 cases per 1000 inhabitants per year and its prevalence is around 2.5-3%. It occurs most frequently in female (F/M: 5-10:1) in 30-40 years of life. In GD, the excessive production of antibodies anti TSH-R can mime action of thyroid-stimulating hormone (TSH or thyrotropin) by inducing an increase of thyroid cell growth and hormone secretion (thyrotoxicosis); this leads to the circulation increase of thyroid hormones responsible of disease characteristic symptoms.

HT has a high prevalence (0.6-0.8%) more frequently in females (F/M: 2-8/1) with probable genetic basis. In young subjects there is often an abnormal enlargement of the thyroid (goiter) and/or inflammatory destructive processes of gland (atrophy) that can lead to hypothyroidism.

An earlier study has estimated the risk of thyroid disease in individuals with CD from a general population cohort of 14 021 individuals, identified through the Swedish national registers, who were matched reference population of 68 068 individuals. The results have indicated that CD is associated with hypothyroidism ($p < 0.001$), thyroiditis ($p < 0.001$), and hyperthyroidism ($p < 0.001$); the highest risk estimates of these diseases is found in children. The relative risk of hypothyroidism in individuals with CD is higher in males than in females; the same gender difference could not be seen in hyperthyroidism and thyroiditis. The sex differences in the association of thyroid disease type with CD may be due to individual chance; alternatively, these differences may reflect sex-specific variation in the phenotypes of autoimmune disease [3].

The data from a study carried out in Northern Italy have also showed that 5.4% of patients with clinical, silent or latent CD had AITD, a significantly higher prevalence than found in the general population [15]. As for other autoimmune diseases, this prevalence is explained partly by the period of exposure to gluten and partly by the shared immunogenetic make-up. HLA-DQ2 and DQ8 haplotypes have a peak of association with Hashimoto's thyroiditis (HT), while the association with HLA-DQ2 is less evident in Graves' disease (GD). Patients with HT, moreover, are at greater risk of CD than patients with GD [21].

CD and AITD. Patients with CD and AITD are generally older than those with CD alone and in 77.5% of them thyroid disease is the first to appear [17]. This may be because the prevalence of HT increases with advancing age, and/or because these patients, who are untreated for a longer period of time, develop AITD.

GFD is thought to encourage a reduction in thyroid peroxidase antibodies (TPO-Ab) titers related to anti-tissue transglutaminase (anti-tTG) titers suggesting that anti-tTG could contribute to the development of thyroid disease in celiac disease [22]. Furthermore, the GFD reduces the need for levothyroxine for better absorption [23]. In clinical terms, the association CD/hypothyroidism is important, as both diseases can present with lethargy, macrocytic anemia, asthenia, intestinal disorders and malabsorption. Untreated hypothyroidism may hide weight loss and/or diarrhea, classical symptoms of CD, which may become evident only after administration of levothyroxine. For these reasons, thyroid function should be assessed at diagnosis in all celiac patients, assaying TSH and TPO-Ab [24]. Patients with AITD should be submitted to CD antibodies such as: anti-gliadin (anti-AGA), anti-endomysial (anti-EMA) and anti-tissue transglutaminase (anti-tTG) [21]. Patients with hypothyroidism and weight loss and/or diarrhea or who do not respond to thyroxine treatment should be given these tests. As already noted, when considering the association between CD and AITD, alongside im-

mune and genetic factors and the presence of gluten, other environmental factors such as selenium deficiency caused by CD-dependent malabsorption, may be important in this association.

The biomarkers, that are widely used for the assessment of selenium presence in human populations, include levels in blood, nails, urine and hair. The levels of selenium in plasma, serum, and whole blood tend to indicate recent selenium presence, whereas the levels of selenium in red blood cells are probably more sensitive for a long-term assessment of selenium status. It has been suggested that the reference values of selenium levels in the whole blood of healthy individuals from several countries are between 70-110 μg/l of blood [25] and are measured by mass spectrometry.

Selenium deficiency in combination with inadequate concentration of iodine contributes to the pathogenesis of disorders that include goiter, hypothyroidism and mental retardation. Iodine is a non-metallic trace element needful for animals and humans. About 800 million people are affected by iodine deficiency; iodine is an essential component of thyroid hormones T4 and T3 corresponding respectively to 65% and 59% of their molecular weights. Iodine supplementation, under form of iodized salt and iodized vegetable oil, produces dramatic improvements in many areas, even though iodine deficiency is still a problem for both developing countries and certain subpopulations like vegetarians that may not reach an adequate iodine intake even in countries considered iodine-sufficient. A reduction in dietary iodine content could also be related to increased adherence to dietary recommendations to reduce salt intake for preventing hypertension [7].

Although the importance of iodine in thyroid function is recognized, this review is focused on the importance of inadequate availability of selenium due to CD-associated malabsorption. Importance of selenium decrease is related to predisposition and/or manifestation of various human disorders such as impaired immune function, disturbances of the thyroid hormone axis, cancer, neurodegenerative age-related diseases and Keshan-Beck's disease [26].

SELENIUM AND SELENOPROTEINS

Some of the main biochemical features of selenium have emerged only in the last five years, although it has been known for nearly 40 years. Selenium was discovered by Berzelius in 1817. Although it is a toxic non-metallic essential trace element, it plays a fundamental part in regulating thyroid function for its structural and enzymatic roles in human biology.

Selenium is present in the organism in tiny quantities between 3 and 30 mg, according to diet in differing geographic areas [27]. As underlined, low selenium status may represent a risk factor for many diseases; an adequate selenium intake is needed to maximize the activity of glutathione peroxidase (GPx) and other selenoproteins (SePs). Endocrine tissues are the sites of greatest concentration; specifically, if it is deficient, the thyroid is a highly sensitive target because it plays an integral role in thyroid metabolism [28].

Selenium enters in the food chain through vegetables (*e.g.* green vegetables, cereals), where its presence depends crucially on its availability in the soil and in sea water, which in turn influences its presence in animal food (*e.g.* poultry, fish).[27].

The association between the availability of selenium and individual health is a topic of considerable attention in European countries [29], particularly in regard to the development of thyroid disorders confirming that these pathologies are also linked to environmental determinants such as feeding, in the same way of CD that is linked to consumption of cereals.

This leads carefully to consider the uneven worldwide distribution of selenium. For example, extensive countries such as China, USA and Canada, have regions of excessively high and low but also normal selenium concentrations in the soil with consequently different intake of this element [30]. One example of a significant environmental deficiency of selenium comes from some areas of China, where it is associated with Keshan-Beck's disease, marked by growth retardation, muscular atrophy, spinal cord malformations and swollen joints. Because of the poor levels of selenium in the soil, the wheat in these areas is also deficient and, bearing in mind the importance of wheat in the local diet to check the progress of this disease, early dietary supplementations of selenium are advised [31].

For other countries such as middle Europe the major selenium sources are seafood, eggs and red meat, the latter due to the fact that production of life stock implies high selenium intake for growing healthy animals and therefore red meat from various sources is one of the major selenium sources in nutrition in middle Europe [32].

Selenium deficiency is caused not only by its scant environmental and/or nutritional availability, but also by diseases that lead to its malabsorption, such as CD. Selenium deficiency is particularly noteworthy because when GFD is adopted to control CD; this leads to the absence in food of cereals such as wheat and its derivatives, which are a source of selenium [27]. Apart from CD, other primary and secondary disorders lead to a poor absorption of micronutrients: primary disorders include chronic forms of the syndrome of malabsorption and Crohn's disease, while secondary disorders include cystic fibrosis, pancreatic insufficiency, biliary atresia, liver disorders and cholestasis. Other factors that can cause a reduced absorption of trace elements include interactions between nutrients, age, and certain drugs [33].

The daily allowance of selenium indicated for the Italian population by the LARN *(livelli di assunzione giornaliera raccomandati di nutrienti)* in agree-

ment with the USA's RDA (recommended dietary allowance), is about 55 μg for adults and 60 μg and 70 μg respectively for pregnancy and breast-feeding [34]. Overt human selenium in excess is rare; it depends on the chemical form of selenium [35]. It is right to observe that only for long-term chronic intakes of 400 μg/day may lead to intoxication, or selenosis, whose symptoms include degeneration of the cutaneous tissue, diarrhea and fever and, more rarely, vesicular dermatosis, neurological disorders (paresthesia, paresis) and liver damage [25].

Selenium occurs as inorganic (selenite and selenate) and organic (seleno-methionine, Se-met) forms. Both inorganic and organic forms can be metabolized to selenocysteine (Se-cys) and incorporated into selenoenzymes; the inorganic forms are available in dietary supplements. Selenium's beneficial effects are attributed to low molecular weight selenium compounds, as well as at its presence within SePs. Vegetables mainly contain Se-met, whereas meat and eggs mainly contain Se-cys. The recent identification of various distinct Se-cys-containing proteins, encoded by 25 human genes, provides information on the molecular and biochemical basis of beneficial and possible adverse effects of this trace element.

Incorporation of Se-cys into SePs employs a unique mechanism that involves decoding of the UGA codon; Se-met is incorporated unspecifically as methionine in proteins and only after degradation the selenium is available for SePs synthesis [36]. Se-cys incorporation sequence binding protein 2 (SBP2) represents a key trans-acting factor for the co-translational insertion of Se-cys into SePs [37]. The enzymatic function of the followings SePs have been identified and characterized:

Glutathione peroxidase (GPx). The fundamental role of GPx is to defend the organism from ROS through its glutathione-reducing action; the GPx reduce peroxides to water and alcohols. Five isoenzymes have been described: the cytosolic enzyme GPx1 is ubiquitously expressed in all types of mammalian cells; the GPx expressed in epithelial tissue of the gastrointestinal tract (GPx2) closely resembles GPx1 in the specificity of the substrate and its localization in the cytosol; the plasma GPx (GPx3) is a glycoprotein secreted in the thyroid follicular lumen where it may down-regulate thyroid hormone synthesis by decreasing hydrogen peroxide concentrations [38]. There is another fifth epididymis-specific nonseleno-GPx (GPx5) expressed in several tissue rodents and other mammals except humans. It appears to be up-regulated in mammalian epididymis in case of selenium deficiency and might act as a back-up system for the selenium-dependent GPx enzymes [28].

The ubiquitous phospholipid hydroperoxidase GPx (GPx4) can specifically reduce phospholipid hydroperoxidases and may be involved in moderating cell death through apoptosis and in the maturation of sperm; the olfactory epithelium-and embryonic tissue-specific GPx (GPx6) [39].

Thioredoxine reductase (TRx). It is a redox active enzyme in eukaryotic and prokaryotic organisms, where it reduces oxidized protein substrates through a NADPH-dependent pathway to maintain the cell's redox status. Two forms of TRx have so far been identified in mammals: TRx1 in the cytosol/nucleus and TRx2 in mitochondria, and other forms may also exist. Some of these SePs [40] are also involved in apoptosis, in cell proliferation and in modulating cell signaling systems and transcription factors.

Iodothyronine deiodinase (ID). It is remarkable that the production as well as the metabolism of thyroid hormone are dependent on two trace elements: iodine and selenium. The thyroid gland requires iodine for the synthesis of thyroid hormones: thyroxine (3,5, 3',5'- tetraiodothyronine or T4) and triiodothyronine (3,5,3'- triiodothyronine or T3). T3, the physiologically active thyroid hormone, is produced either directly by the thyroid and by means of conversion catalyzed by selenium-containing ID from the circulating T4 in peripheral tissues.

Three iodothyronine deiodinases (ID1, ID2, ID3) have been identified, all of them are membrane-integrating proteins (29-33 kDa) sharing 50% sequence identity; each has a Se-cys residue at its active centre. The deiodinases differ in the specificity of their substrates and in their distribution in tissues regulating thyroid hormone homeostasis. ID1 and ID2, in particular, catalyze local and systemic deiodination of T4 into the biologically active form T3; while ID3 catalyzes the conversion of T4 into the biologically inactive form, reverse-3,5,3'-triiodothyronine (rT3) and of T3 into the inactive form, 3,3'-diiodothyronine (T2). ID1 and ID2 can also convert rT3 into T2 [41]. The iodothyronine deiodinases initiate or terminate thyroid hormone action and therefore are critical for the biological effects mediated by thyroid hormone. The activating of ID2 and the inactivating of ID3 can locally increase or decrease thyroid hormone signaling in a tissue- and temporal-specific fashion, independent of changes in thyroid hormone serum concentrations. This mechanism is particularly relevant because deiodinase expression can be modulated by a wide variety of endogenous signaling molecules [42].

Other SePs have recently been identified and while they have been less fully characterized, the possibility that they have important roles cannot be excluded such as:

- selenophosphatase synthetase (SPS2) is needed for the biosynthesis of selenophosphate, the precursor of Se-cys for the synthesis of SePs;
- selenoprotein W (SePW), an antioxidant protein necessary for muscular and cardiac functions [38] and abundantly expressed in the colon [43].

All this shows how the action of selenium is expressed through its incorporation in these proteins for its many enzymatic activities; thus, it is important that the organism should always have adequate availability of this element.

INTERLEUKIN-15 AND IMMUNE SYSTEM

Cytokines are molecules that influence activation, growth, and differentiation of several target cells. They are pro-inflammatory mediators, regulate the systemic inflammatory response, play a crucial role in AITD because of their involvement in autoimmune system, modulate development and growth of normal and neoplastic thyroid cells.

In patients with thyroid cancer cytokines, as well as chemokines, show to generate antitumor response and can be useful as serum biomarker. Cytokine assay should be used in the clinical evaluation of patients with indeterminate fine-needle aspiration cytology. Several cytokines, such as interleukin-6 (IL-6), leukemia inhibiting factor (LIF), and thyroid transcription factor-1 (TTF-1) are expressed in thyroid cancer cell lines and can be used for evaluating the inhibitory effects of numerous drugs in redifferentiation therapies [44].

In patients with AITD, between cytokines, uncontrolled expression of IL-15 is a critical factor in the pathogenesis and maintenance of disease *(Table 1)*; over-expression of IL-15 is crucial in refractory CD. IL-15 is a 114 amino acid (14 kDa) glycoprotein produced by various types of cell but not by T cells.

On account of its various roles in different immune and non-immune cells, which also explain the use of different signaling mechanisms, IL-15 is a key regulatory cytokine that modulates homeostasis of the innate and adaptive immunity. This characteristic is largely correlated with its unusually powerful anti-apoptotic property, which controls the homeostasis and growth of different cell population targets; this is confirmed by the wide distribution of IL-15 receptor (IL-15R) mRNA in these sites [45].

Signaling mechanisms. IL-15R is a trimeric structure composed of IL-2/15β/γ subunits, in common with IL-2, and a specific subunit IL-15Rα. Signaling by IL-15 can occur not only through binding to its trimeric receptor, but also through the complex formed between IL-15 and IL-15Rα. IL-2Rα is expressed mainly on activated T cells and binds IL-2 with low affinity, but cannot transduce a signal, for which reason it is presumed that its role is to recruit IL-2 to the cell surface and present it to IL-2Rβ/γ in cis. IL-15Rα, in contrast, by acting both in cis and in trans, has a relatively high affinity for IL-15, and indeed IL-15Rα mRNA is more widely distributed; it can be expressed on the cell membrane and presented to other types of cells expressing IL-2β/γ in trans [46]. IL-15-mediated signaling in T lymphocytes results in the activation of Janus kinase (JAK); this is a family of intracellular non-receptor tyrosine kinases, ranging from 120-140 kDa in size, that plays critical signaling roles for a number of members of the cytokine receptor by activating signal transducer and activator of transcription (STAT) proteins. Therefore, JAK-STAT signaling by cytokines (such as: IL-2, IL-4, IL-7, IL-9, IL-15) is critically important in the generation of immune and inflammatory responses and in T-cell expansion and differentiation. Lack of signaling by these cytokines, as occurs, for example, in genetic deficiency of either IL-2Rγ or JAK1/JAK3, results in severe combined immunodeficiency in humans and mice. Knock-out mice lacking STAT5a/b, STAT5b and JAK3 are NK cell deficient, showing the importance of the JAKs in signaling by γc chain-utilizing cytokines. The following are the IL-15R-mediated signal transduction events in different cells:

- in lymphocytes (T cells, NK cells, granulocytes) that express the whole trimeric IL-15R complex, IL-15 activates the JAK1/JAK3, STAT3/STAT5 pathways, Syk kinase, PLCγ, Lck kinase and Shc, leading to activation of the P13K/Akt and Ras/Rat/MAPK cascades. These pathways subsequently lead to expression of the bcl-2, c-myc and c-fosljun genes and activation of JF-kB;
- in mastocytes that express different isoforms of IL-15R, IL-15 involves the Jak2, Tyk2 and Syk kinase, SAT3, STAT5 and STAT6, which lead to expression of Bcl-x_L and c-myc and secretion of IL-4;
- in fibroblasts that express the subunits IL-15Rα and γ/c, stimulation of IL-15 leads to transactivation of Axl receptor tyrosine kinase pathway, which includes the phosphorylation of P13K/Akt and up-regulation of Bcl-x_L and Bcl-2 and the recruitment of TRAF2 to IL-Rα and activation of NF-kB [45].

IL-15 is thus at the apex of a cascade of inflammatory factors. It increases its production in response to a variety of stimuli, including TNF-α, which in turn induces the expression of other inflammatory cytokines and chemokines involved in the pathogenesis of autoimmune diseases. It is interesting to underline that serum IL-15 levels are directly correlated with the seriousness of tissue damage [47] such as in CD and AITD.

In celiac disease. In CD pathogenesis the exposure to gluten of the small intestine mucosa leads to over-production of cytokines by T cells, which trigger an inflammatory process that includes the release of IL-2, IL-6, IFN-γ and TNF-α and the activation of B lymphocytes with the production of specific antibodies and the triggering of autoim-

Table 1 | *Effect of IL-15 on the immune system*

Immune cells involved	Outcome of the effect of IL-15
T cells	The proliferation of activated T cells, helps to maintain the memory of CD8+ T cells
Natural killer (NK) cells	Activates
B cells	Stimulates the proliferation
Intraepithelial lymphocytes (IELs)	Controls
Dendritic cells	Promotes the development
Neutrophils	Activates
Macrophages	Induces the production of pro-inflammatory cytokines

Table 2 | *Active celiac disease: factors of cell damage expressed in enterocyte apoptosis levels correlated with over-expression of IL-15*

Factors of cell damage	Effects of over-expression of IL-15
IL-15Rα	Over-regulation on IELs, which express CD94+ cells
TNF-α	Increases
IFN-γ	Increases
Perforin/Granzyme molecules	Increases of IELs
IELs apoptosis	Inhibition
Villous atrophy	Increase of cytotoxicity

mune mechanisms. The role of IL-15 in the damage to the mucosa and the persistent inflammatory state is held to be of particular significance.

In the normal intestine, where enterocytes are exposed to a wide variety of insults, the role of IL-15 produced by macrophages, dendritic cells and intestinal epithelial cells is to increase the cytotoxicity of intraepithelial lymphocytes (IELs) and to modulate apoptotic signals that, by eliminating damaged cells, maintain a healthy epithelium.

In pathologies such as active CD, there is an over-expression of IL-15 by enterocytes and lamina propria mononuclear cells (LPMCs) which is associated with factors of cell damage *(Table 2)* [48].

In studies of C57BL/6 transgenic mice lacking IL-15 and IL-15Rα the occurrence of lymphocytopenia and the specific lack of NK T cells, CD8+ T cells and IELγ/δ suggests that signals transmitted via IL-15Rα are critical for the development, activation and/or survival of these cells [49]. The role of IL-15 in generating epithelial damage in active CD is thus crucial; its ability to promote the survival of IELs in CD may predispose to the emergence of clonal T cell proliferation. The blocking of IL-15 or of its receptor, thereby suppressing the activation and uncontrolled survival of IELs, could offer a new therapeutic tool to prevent not only tissue damage, but also the complications of CD [48].

In vivo studies have assessed the significance of the presence of some SePs in the apoptotic process in the intestine; specifically, SePs modulate apoptosis to maintain a healthy target tissue by eliminating cells affected by oxidative damage. The importance of the lack of the GPx antioxidant system in the intestinal mucosa is also demonstrated by the fact that its absence may set in motion a continuous cycle of ROS and inflammation that can lead to the accumulation of genetic mutations [50]. The phenotype of GPx1/GPx2 knock-out mice includes diseases of the colon and increased susceptibility to colitis [43], suggesting that these enzymes are important in determining both the level of antioxidant protection and the inflammatory response in the colon. GPx2 is also active in the tissues of the small intestine, notwithstanding differences in the selenium-dependent expression of these intestinal tracts. The activity of GPx2 was studied in the gastrointestinal tract of two groups of Wistar rats fed respectively with a selenium-deficient (5-10 μg kg^{-1}) and a selenium-adequate (300 μg kg^{-1}) diet. Although the activity of GPx2 and selenium levels were reduced in the tissues of both groups, in the selenium-deficient group both GPx2 activity and selenium levels were higher in the small intestine than in the colon [51]. These observations indicate that SePs such as GPx are crucial in producing the protective effect of selenium in the gastrointestinal tract. The colon, in particular, is more effectively protected from oxidative stress when selenium levels are adequate.

Data from studies on human colon adenocarcinoma cells (Caco 2) and colon tissue of weaned male Hooded Lister/Rowett rats in the presence or absence of adequate selenium concentrations revealed differences in the effects of selenium deficiency on SePs expression. The deficiency produced a statistically significant decrease ($p < 0.05$) in levels of GPx1 (60-83%) and of SePW (73%) mRNA, a small but significant reduction in GPx4 mRNA (17-25%), but no significant change of the levels of GPx2 mRNA. Thus, notwithstanding the maintenance of GPx2 expression, the effect of selenium deficiency was apparent on the gene expression of SePW and GPx1 and less so on GPx4, reducing antioxidant protection in the colon, which would consequently increase susceptibility to the development of inflammatory responses in the gastrointestinal system [43].

In genetic-environmental diseases, such as CD, in which inflammatory damage is present in the small intestine, the consequent deficiency of selenium may modulate the expression of a series of SePs genes, including GPx, leading to recurrent inflammation and increased mucosal damage. In CD patients the presence of gliadines in the intestinal mucosa leads to a cascade of events triggered by hyperproduction of IL-15, that leads to apoptotic cell death of enterocytes. In this context, deficiency of selenium may also lead to inhibition of apoptosis of IELs and, consequently, to their over-production; this increase in lymphocytes determines increased apoptosis of enterocytes. Thus, because of the inadequate formation of SePs, lack of selenium can be considered an important direct factor in intestinal damage not only in individuals with CD, but also in healthy subjects with an inadequate intake of selenium due to other environmental and/or nutritional causes.

In autoimmune thyroid disease. The role of cytokines is important for the thyroid in the development of AITD. Cytokines, produced by thyroid follicular cells (TFCs) and infiltrating inflammatory cells, are essential for T and B cell growth and differentiation and may directly affect TFCs, leading to expression of molecules of HLA class II region (the same of CD region). In addition, cytokines can alter the growth and function of TFCs [52]. In AITD such as GD and HT, the systemic cytokine mRNA expression

of the Th1-type (TFN-γ, IL-18, IL-15, IL-2) and Th2-type (IL-4, IL-6, IL-10, IL-13) is changed resulting in a heterogeneous cytokine pattern in these diseases [53]. The serum cytokine profiles in these diseases are characteristic: the Th1 pattern of immune response characteristic of cellular immunity is dominant in HT, while in GD the predominance of Th2 cytokines indicates a humoral pattern of immune reaction [54], unlike CD in which cell damage is triggered by cell-mediated Th1/Th2-type reactions [55].

In vitro studies using cultured primary TFCs from patients with non-toxic multinodular goiter (NTMG), GD and HT show increased IL-12, IL-13 and IL-15 mRNA expression. Of these cytokines, the role of IL-15 in the pathogenesis of AITD is significant, especially in HT patients in whom thyroid tissue is more expressed. Besides, in GD and HT, the IL-1 and IFN-γ increases are particularly evident after TSH stimulation [56]. The over-production of IL-15 leads both to an inhibition of apoptosis, on account of its role in lymphocyte survival, and to an increase in the proliferation and differentiation of various types of non-immune cells. As with other autoimmune diseases [47], this may lead to an extension of damage of the thyroid cells, in direct correlation with serum levels of IL-15, as in CD, where the levels of IL-15 correlate with the degree of mucosal damage [48].

Bearing in mind that SePs are involved in inhibition of apoptosis, the presence of environmental factors such as selenium deficiency can be considered an additional element directly involved in thyroid damage in AITD. It should nonetheless be remembered that these diseases may also have other environmental causes such as: the consumption of immunotherapeutic agents, viral infections, lack of other trace elements (*e.g.* iodine, iron).

Patients with a particular genetic background, as HLA-DR3 and DR5 and polymorphism of CTLA-4 promoter and in presence of environmental factors, are more susceptible to the development of AITD compared with the normal population [57]. An interaction between genes and environment is thus necessary to trigger the onset of AITD, because the combination of genetic susceptibility and environmental factors is an important cause in prompting the autoimmune response to thyroid antigens.

Epidemiological studies, including family and twin data, support the strong genetic influence on the etiology of AITD. Several loci that are linked with AITD have been mapped and in some of these loci putative, AITD susceptibility genes have been identified. Some of these loci predispose to a single phenotype, while others are common to both GD and HT, indicating the existence of a shared genetic susceptibility to both diseases. The putative GD and HT susceptibility genes include both immune-modifying genes, such as HLA and CTLA-4, and thyroid-specific genes such as TSH-R and Tg and it is likely that the final phenotype of the disease is the result of an interaction between these loci, in combination with environmental factors [58].

It is useful to emphasise that the lack of selenium plays an important role among the environmental factors and that when it is caused by CD-linked malabsorption its clinical significance is even greater, given that CD and AITD are often present in association. In this context selenium deficiency can be considered an additional common cause of damage. The importance of selenium in the thyroid is demonstrated by the fact that one third of the total is localized in this gland in the form of Se-cys and that even when it is deficient the thyroid constitutes an important reserve [59].

Selenium is essential in thyroid because it regulates T3 production and antioxidant activity. Thyrocytes are constantly exposed to potentially toxic concentrations of H2O2 and lipid hydroperoxidase produced in high concentrations during thyroid hormone synthesis. When selenium intake is adequate the GPx and TRx intracellular systems protect thyrocytes from ROS. ROS normally exert their cytotoxic effects on thyroid cells at levels that are too low to induce necrosis via caspase-3-dependent apoptosis. When selenium is deficient the increase in ROS leads to an heightened apoptotic response and subsequently to greater damage. Selenium deficiency, by altering SePs production, leads to reduced antioxidant protection and triggers the autoimmune mechanisms in genetically predisposed subjects, thus, representing a major direct cause of thyroid damage. Therefore, due to the importance of selenium deficiency, subjects with AITD present with new or nonspecific symptoms should be screened for other autoimmune diagnoses [60].

CONCLUSIONS

AITDs are the result of complex factors relating to gene-environment interaction; the endogenous factors account for about 70-80% of liability to develop AITDs; however, at least 20-30% is attributed to environmental exogenous factors, which include certainly smoking (at least for Graves' disease and orbitopathy), probably stress, iodine and selenium intake, several drugs, irradiation, pollutants, viral and bacterial infections, allergy, pregnancy and post-partum [61].

Some environmental factors are typical in autoimmune diseases. Patients with type 1 diabetes mellitus, atopic dermatitis, multiple sclerosis, CD and AITD have a different pattern of seasonality of birth than the general population. Girls with the diagnosis of CD and patients of both sexes with a family history of CD have a peak of birth in September. This can be indicative of a perinatal virus infection typical of this month which can be a plausible candidate for the primary trigger [62].

Between the environmental factors, the nutritional deficiencies assume importance for CD and AITDs. In untreated CD the malabsorption of trace ele-

ments is a common occurrence, given that this disease principally affects the proximal small intestine, presumably on account of the greater concentration of gluten in this region [63], where essential micronutrients are absorbed [9]. This is why it is necessary to initiate GFD as soon as possible, in order not to affect the absorption of these elements, given that the pathogenesis of the complex extraintestinal manifestations of CD is traceable not only to the immune and/or endocrine imbalance caused by the direct interference of gluten with these systems [8], but also by the subclinical malabsorption of nutrients.

Bearing in mind AITD, iodine deficiency is a problem for certain subpopulations of many countries, for increased adherence to dietary recommendations to reduce salt intake in hypertensives and for improper diet [7].

An adequate intake of selenium is today rightly recognized as indispensable to health; the organism should always receive an adequate amount with food and should be able to use it. The importance of selenium, as a component of SePs, is evident in the thyroid, where the action, metabolism and synthesis of hormones need it to preserve homeostasis of pathways hormone-dependent.

SePs involved in cellular antioxidative defence systems and redox control, such as the GPx and the TRx family, are involved in protection of the thyroid gland from excess hydrogen peroxide and ROS produced by the follicles for biosynthesis of thyroid hormones. In addition, three key enzymes (ID1, ID2, ID3) are involved in activation and inactivation of thyroid hormones.

While the nutritional selenium supply for the protection of the thyroid gland and synthesis of some SePs of the GPx and the TRx family might be limiting their proper expression under pathological and physiological conditions, nutritional Se supply is normally sufficient for adequate expression of functional ID enzymes with exception of certain diseases, such as CD, impairing gastrointestinal absorption of Se compounds [26]. In regard to the evident association between AITD and CD, the selenium deficiency in CD is an important factor in the pathogenesis of AITD, together with genetic, environmental and immune factors [15]. Studies of the link between selenium deficiency, autoimmune system and inflammatory processes have shown that selenium supplementation (200 μg/day) modulates the production of anti-TPO in HT patients [64].

The benefits of supplements in terms of serum anti-TPO and cytokine levels are nevertheless more evident in patients with very active disease than in those with moderate disease [65]. GD patients benefit from selenium supplementation at doses > 120 μg/day [52], while the usefulness of supplementation has not yet been clarified for other autoimmune endocrine disorders [57]. Hence, the importance of a careful assessment when administering selenium supplements as the treatment for specific AITD, especially when these are associated with CD, which in any case causes a deficit of this micronutrient if GFD is not adopted or is incorrectly followed.

In the development of autoimmune disorders other endogenous factors, such as the cytokines, play an important role because influence activation, growth, and differentiation of several target cells.

In AITD, uncontrolled expression of IL-15 has a critical role in the pathogenesis and maintenance of refractory disease. A compelling rationale is that selective targeting of IL-15 represents a potentially valuable approach in CD treatment, where currently the only available treatment for this autoimmune disease is a life-long GFD or for a subset of CD patients who develop resistance to GFD and progress to type II refractory CD with no effective treatment.

In thyroid cancer cell lines, IL-6, LIF, and TTF-1 show a generate antitumor response that can be used both as serum biomarker and for evaluating the inhibitory effects of several drugs in redifferentiation therapies [44].

Because this cytokine is at the apex of a cascade of inflammatory events, it increases its production in response to a variety of stimuli whose serum levels are directly correlated with the seriousness of tissue damage [47]. In the pathogenesis of AITD and CD, in particular, the over-production of IL-15, by inhibiting apoptosis, is a survival factor of IELs, leading to progressive damage to thyrocytes [54] and enterocytes [48]. Increased levels of IL-15 can thus be considered a common factor in both diseases. This association is further confirmed by the consideration that SePs are also involved in inhibiting apoptosis; in this context selenium deficiency, a risk factor of CD, can be directly involved in intestinal and thyroid damage.

In conclusion, CD is associated with thyroid disease and *vice versa*; this association is seen regardless of temporal sequence; this indicates a shared etiology and that affected individuals are more susceptible to autoimmune diseases. The increased risk of association between CD and thyroid disease is an expression of a more general increase in autoimmunity that characterizes many individuals that may be due to shared genetic or immunological traits. Thus, the positive association should not be seen as an isolated phenomenon but as an integral component of genetic-environmental events of which the selenium deficiency is a key related factor. For this reason it is necessary not only to initiate appropriate pharmacological treatment and consider the administration of selenium supplementation on a case-by-case basis, but also to diagnose CD at an early stage in order to adopt GFD in time to prevent further increasing the risk of developing AITD. Such an approach can not only help to offset the malabsorption of micronu-

trients, but also reduce the risk of interference by gluten in the immune and/or endocrine systems, which is an important factor in the development of CD-related autoimmune diseases such as AITD.

The review aims to provide elements that could be useful to study preventive and therapeutic target as the iper-production of IL-15 and the selenium deficiency in the pathogenesis of CD and AITDs. The development of therapeutic agents able to block the receptor and/or signaling elements and the control of selenium availability mainly CD-associated could provide an effective treatment for these disorders.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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