

Molecular mimicry and autoimmune thyroid disease

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Abstract Hypothesized 40 years ago, molecular mimicry has been thereafter demonstrated as an extremely common mechanism by which microbes elude immune response and modulate biosynthetic/metabolic pathways of the host. In genetically predisposed persons and under particular conditions, molecular mimicry between microbial and human antigens can turn a defensive immune response into autoimmunity. Such triggering role and its pathogenetic importance have been investigated and demonstrated for many autoimmune diseases. However, this is not the case for autoimmune thyroid disease, which appears relatively neglected by this field of research. Here we review the available literature on the possible role of molecular mimicry as a trigger of autoimmune thyroid disease. Additionally, we present the results of *in silico* search for amino acid sequence homologies between some microbial proteins and thyroid autoantigens, and the potential pathogenetic relevance of such homologies. Relevance stems from the overlap with known autoepitopes and the occurrence of specific HLA-DR binding motifs. Bioinformatics data published by our group support and explain the triggering role of

Borrelia, *Yersinia*, *Clostridium botulinum*, *Rickettsia prowazekii* and *Helicobacter pylori*. Our new data suggest the potential pathogenic importance of *Toxoplasma gondii*, some *Bifidobacteria* and *Lactobacilli*, *Candida albicans*, *Treponema pallidum* and hepatitis C virus in autoimmune thyroid disease, indicating specific molecular targets for future research. Additionally, the consistency between *in silico* prediction of cross-reactivity and experimental results shows the reliability and usefulness of bioinformatics tools to precisely identify candidate molecules for *in vitro* and/or *in vivo* experiments, or at least narrow down their number.

Keywords Autoimmune thyroid disease · Molecular mimicry · Bioinformatics · Amino acid sequence homology

1 Introduction

Molecular mimicry was hypothesized about four decades ago and has been experimentally studied only in the last 20–25 years. Its intriguing implications, particularly the pathogenesis of autoimmune diseases, have led to a rapid growth of investigations in this field.

For sake of completeness and clarity, before discussing the role of molecular mimicry in thyroid autoimmunity, we believe that it is appropriate to briefly provide some historical data, definitions and technical information.

1.1 Mimicry in nature

The observation that different organisms can have similar morphological characters was firstly noticed during the eighteenth century by Carl Nilsson Linnaeus, who justified it as a consequence of physical and/or biochemical interaction with the environment. However, the term “mimicry” was used in

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biology only about one century later, in 1862, by the English entomologist Henry Walter Bates, in a study on Amazon butterflies [1]. He reported a surprising similarity between the color patterns of the wings of species belonging to different families, and suggested that this mechanism was used by the butterflies to deceive predators that relied on visual characteristics to identify edible preys. Subsequent studies by many researchers [2–5] confirmed the initial intuition of Bates, and indeed they demonstrated that mimicry is widely present in nature, and not only for defensive, but also for offensive purposes (“aggressive mimicry”). Other terms that, in addition to visual mimicry, appeared in the literature were acoustic, olfactory or behavioral mimicry [2, 5].

1.2 Molecular mimicry

Relatively recently, research has shown that similarities between molecules of different species (molecular mimicry) are common and important in a number of mechanisms of interaction, such as infection and immune response, in the context of pathogenetic processes that lead to several systemic or organ diseases, mainly the autoimmune ones. Molecular mimicry has multiple peculiarities. First, when considering an infectious process, microbes are predators of the organism infected but, in turn, they are preys of the host immune response. Thus, on one hand molecular mimicry follows the classical Batesian model, while on the other hand it can be qualified as aggressive mimicry. However, it is an atypical aggressive mimicry because the mimicked organism is also the prey. Another peculiarity is that the microbes’ aim is not to kill their host, but to take advantage of its resources for as long as possible. This is particularly true for viruses, as their survival depends entirely on the biosynthetic machinery of the infected cells. Indeed, many microbial molecules are similar to factors involved in the regulation of apoptosis, cell proliferation, inflammation and/or immune response, and use molecular mimicry to modulate in their favor these critical pathways of the host [6–8].

1.3 Molecular mimicry and autoimmunity

In the course of evolution, some countermeasures (not yet completely understood) have been developed by hosts to achieve a proper antimicrobial response in spite of the elusion mechanisms based on molecular mimicry. Because of genetic and external factors, as well as some intrinsic limits of the system, this can turn an immune response against “non-self” molecules into an autoimmune reaction against molecules similar to microbial ones.

In summary, the mechanisms by which molecular mimicry can interfere with physiological functions of the human organism and cause diseases are the emulation of regulatory molecules and the induction of autoimmunity. A typical example of

the first possibility is that of the human herpesvirus 8, which can induce Kaposi’s sarcoma because some of its proteins resemble human molecules which directly or indirectly prevent cell apoptosis (FLIP, i.e. FLICE Inhibiting Protein, and Bcl-2) or inhibition of growth in case of DNA damage (cyclin D), modulate local immune response (IRF, i.e. Interferon Regulating Factor) and switch it from the Th1 to the Th2 phenotype (interleukin-6, macrophage inhibiting protein I, II and III) [9].

The possible induction of autoimmunity is likely the most studied effect of molecular mimicry. The term “molecular mimicry” was used for the first time by Shapiro et al. back in 1976 [10]. They theorized molecular mimicry between HLA-B27 and microbial agents as one of the possible explanations for the association between this HLA haplotype and inflammatory arthropathies [10]. Three more articles on this topic were published in the 1970s [11–13] but, due to the technological limits of that time, it was substantially impossible to go beyond hypotheses. Initial experimental evidence started to appear in the subsequent decade, when the number of published articles increased to more than 90. In particular, Fujinami and Oldstone co-authored a fundamental paper which enunciated the modern theory of molecular mimicry as trigger of autoimmune diseases [14]. However, the real “coming of age” of this field of research occurred in the 1990s, thanks to the simultaneous availability, at relatively low cost, of certain technical advancements. These included improved laboratory techniques, which made available large amounts of pure biomolecules, electronic calculators with high data storage and processing power, and large-scale computer networks allowing quick and easy data exchange between researchers. These conditions led to many experimental confirmations of the original theory of Shapiro and colleagues [10], to a better understanding of the biological reasons for the existence of molecular mimicry and the mechanisms which make it possible, and to the creation of softwares which emulate and/or predict molecular interactions and their effects in living organisms.

The existence of molecular mimicry is an apparent contradiction in evolutionary terms, because it may cause dangerous and potentially fatal diseases. However, its reasons appear clearer when analyzing the mechanisms of immune recognition in the light of current knowledge. Schematically, antigen-presenting cells capture antigens and process them with their lytic enzymes. Next, some fragments are exposed on the cell membrane and interact with an MHC molecule. The MHC-antigenic fragment complex is then recognized by a TCR (T-Cell Receptor) molecule of a T lymphocyte. Complementarity of the three molecules (MHC, antigenic fragment, TCR) from a biochemical and physical point of view is necessary for immune activation, as this complementarity guarantees the specificity of the response. Even considering only protein antigens, and based on the size of the peptides that can be

presented by an MHC molecule, the number of possible fragments is comprised between 10^{12} and 10^{15} . The human T-cell repertoire, however, includes approximately 10^8 different clones. Consequently, the immune recognition system must have a certain degree of flexibility, so that each T-cell clone can recognize several antigenic peptides which have certain characteristics in common. In this system, immunity acquired against one pathogen is efficient against a vast range of other pathogens with similar antigenic characteristics, and it remains protective even if the original microbial strain mutates [15, 16]. Such advantages imply a certain loss of specificity. Indeed, two different molecules (even from different organisms, e.g. human and microbial) might not be distinguishable by the immune system if their antigenic peptides presented to the immune system are sufficiently similar. This indistinguishability allows improper immune reactions, and is the basis for the possible onset of autoimmunity. Thus, the actual level of flexibility has to be the best compromise between specificity and sensitivity. Indeed, an absolutely specific immune system would require much more encoding space in DNA (up to the entire human genome, according to some estimates) and would be easily circumvented by microbes with even minimal mutations, while a less specific one would be excessively sensitive, leading frequently to self-destructive reactions and unreliable antimicrobial defence [15–17].

The observed frequency of autoimmune diseases is, indeed, much lower than expected on the basis of the above figures, which suggests that each T-cell clone could react against 10,000 to 10,000,000 different molecular targets. This is due to several control mechanisms present in human organisms: deletion of autoreactive T-cell clones, peripheral induction of T-cell apoptosis, induction of anergy, action of regulatory T-cells [18]. Selective deletion of T-cells was initially considered the most important control mechanism, but more recent studies have shown that such deletion removes from the repertoire only the most dangerous clones, leaving many potential autoreactive elements available. Instead, it is the other three mechanisms that are quantitatively more important [7, 18–24].

Our group published a comprehensive review of genetic and environmental factors that interact in the pathogenesis of thyroid autoimmunity [25]. In that paper, we pointed out that the molecular mimicry model can be considered a paradigmatic example of the multifactorial interaction that leads to autoreactivity. Indeed, structural similarity between self and non-self antigens is necessary, but not sufficient. For the onset of autoimmunity, it is also necessary that defensive reactions of the host be activated, that self-reactive T-cell clones be present in the repertoire, that the host possesses specific HLA alleles, and that mechanisms regulating the immune response be not fully functional [25].

2 Molecular mimicry and endocrine, nonthyroid autoimmune disease

The scientific interest on molecular mimicry, particularly in diseases with autoimmune pathogenesis, is shown by abundant literature data. As of March 26, 2016, a PubMed search (<http://www.ncbi.nlm.nih.gov/PubMed>) with the string “molecular mimicry” yields 7151 results (Table 1). The triggering role of molecular mimicry has been postulated, and often experimentally demonstrated, for many systemic or organ autoimmune diseases. A representative list of common autoimmune diseases and number of associated articles, as retrieved on PubMed, in which they are linked to molecular mimicry is shown in Table 1.

Surprisingly enough, molecular mimicry has been studied much less in endocrinology. The vast majority of papers concern diabetes, and, to a significantly lesser extent, thyroid, pancreas and infertility (Table 2). We aimed to review and summarize existing data on the possible role of molecular mimicry between microbial antigens and thyroid autoantigens as a trigger of autoimmune thyroid disease (AITD). Additionally, and prompted by clinical observations, we

Table 1 Number of articles indexed in PubMed concerning molecular mimicry and common non-endocrine autoimmune diseases (as of March 26, 2016)

Search string	No. of articles
“molecular mimicry”	7151
“molecular mimicry” AND “multiple sclerosis”	224
“molecular mimicry” AND “Guillain-Barré”	202
“molecular mimicry” AND “systemic lupus erythematosus”	143
“molecular mimicry” AND “rheumatoid arthritis”	127
“molecular mimicry” AND “autoimmune encephalomyelitis”	59
“molecular mimicry” AND “antiphospholipid syndrome”	50
“molecular mimicry” AND uveitis	44
“molecular mimicry” AND “Sjogren’s syndrome”	39
“molecular mimicry” AND scleroderma	30
“molecular mimicry” AND vasculitis	30
“molecular mimicry” AND “Crohn’s disease”	28
“molecular mimicry” AND (“celiac disease” OR “coeliac disease”)	26
“molecular mimicry” AND “inflammatory bowel disease”	20
“molecular mimicry” AND “ulcerative colitis”	16
“molecular mimicry” AND polymyositis	13
“molecular mimicry” AND “autoimmune myocarditis”	11
“molecular mimicry” AND dermatomyositis	10
“molecular mimicry” AND “Behçet’s disease”	9
“molecular mimicry” AND “arteritis”	5
“molecular mimicry” AND pemphig*	5
“molecular mimicry” AND “spondyloarthritis”	4

Table 2 Number of articles indexed in PubMed concerning molecular mimicry and endocrine autoimmune diseases (as of March 26, 2016)

Search string	No. of articles
“molecular mimicry” AND diabetes	277
“molecular mimicry” AND thyroid* AND autoimmun*	46
“molecular mimicry” AND pancreatitis	12
“molecular mimicry” AND infertility	8
“molecular mimicry” AND Addison’s disease	0
“molecular mimicry” AND adrenalitis	0
“molecular mimicry” AND hypoparathyroidism	0
“molecular mimicry” AND hypophysitis	0
“molecular mimicry” AND ovarian disease	0

present new *in silico* data which support the link with some specific infections.

3 Molecular mimicry and autoimmune thyroid disease

As of March 26, 2016, there are 46 articles indexed in PubMed which mention molecular mimicry in connection with thyroid autoimmunity (Table 2). Of these, 28 have been included in this review, because the remaining 18 are not relevant.

The first paper which explicitly analyzed the issue of molecular mimicry and other theories proposed to explain the onset of thyroid autoimmunity dates back to 1992 [26]. The conclusion, based on a review of the scarce evidence existing at that time, was: “there is no valid evidence for viral involvement, and likewise the evidence for molecular mimicry as an initiating factor does not hold up to scrutiny”. Also, the author was “against an antigen-driven origin for AITD”, and even the idea of a genetic abnormality of thyrocytes appeared inappropriate; thyroid autoimmunity was considered as the result of “a disturbance of immunoregulatory mechanisms”, possibly related to alterations of the mechanism of antigen presentation. Similarly skeptical were the authors of a paper published two years later [27]. Even though they correctly acknowledged the existence of structural similarity between human thyrotropin receptor (TSHR) and *Yersinia enterocolitica* antigen O:3, they concluded that “in spite of this cross-reactivity, *Yersinia* seems not to be a major inducer of thyroid autoimmunity”.

The statements in the above studies [26, 27] were clearly due to the substantial lack of experimental data. However, in 1995, Tomer and Davies underscore that “data continue to accumulate in favour of infectious agents being important initiators of autoimmune disease”, adding that molecular mimicry could be considered an important possible mechanism to explain the onset of autoimmunity [28]. In 1999,

Martin and collaborators [29] presented new experimental data which showed that AITD are antigen-driven, thus disproving one of the affirmations previously made by Volpè [26]. In the same year, Rao et al. [30] reported the results of experiments in a murine model which suggested that, in some cases, nonimmunogenic viral peptides may play a potentiating role in the pathogenesis of AITD, i.e. induce strong response in specific T-cell clones already primed with the mimicked autoantigenic peptide.

Further insight into the mechanisms of induction of thyroid autoimmunity via molecular mimicry was provided by two groups [31–33]. Carayanniotis and Kong [31] presented a summary of the known thyroglobulin (Tg) epitopes, their microbial “molecular mimics” and the factors which promote the generation of pathogenic epitopes. Chen and coworkers [32, 33] investigated the biochemical characteristics necessary for the onset of the autoimmune reaction against TSHR in Graves’ disease and the immune dysregulation that occurs after immune reconstitution in patients with immunodeficiency due to human immunodeficiency virus-related infection, and that can cause AITD. An excellent picture of available data on the relationship between infections and autoimmune diseases, including data on thyroid, was given in a review article by Bach [34].

AITD has been linked in clinical and/or epidemiological literature to several microbial infections, which will be presented in detail hereafter. In many cases, experimental and/or *in silico* data exist which support and explain such associations in terms of molecular mimicry (Table 3). When no experimental and/or *in silico* data were found in literature, we used our usual bioinformatics approach to identify the protein antigens which are most probably responsible for disease triggering via molecular mimicry. In brief, we extracted from the Entrez Protein database (<http://www.ncbi.nlm.nih.gov/protein>) the amino acid sequence (precursor form) of human Tg, thyroperoxidase (TPO) and TSHR, and used the BLAST (Basic Local Alignment Search Tool) software (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to compare each of them with all the proteins of each microorganism associated to AITD. Next, we used the MotifFinder software [41] to search for the occurrence of HLA-DR binding motifs in the sequences shared between human thyroid autoantigens and microbial proteins. The results of this work are summarized in Table 4.

3.1 *Borrelia*

In 2002, we and colleagues reported a woman in whom hypothyroidism due to Hashimoto’s thyroiditis appeared three months after the appearance of skin lesions for which lichen sclerosus was diagnosed [35]. At observation by the dermatologists, this woman had serum antibodies against *Borrelia burgdorferi* of both the IgM and IgG class, proving recent infection by this spirochete which is the etiologic agent of Lyme disease [35].

Table 3 Summary of data published by our group on amino acid sequence homologies between microbial proteins and thyroid autoantigens [35–40]. Sequence numbering of thyroid autoantigens refers to precursors (TSH-R: GI 136448; Tg: GI 55770862; TPO: GI 4680721; NIS: GI 4507035). Bb = *Borrelia burgdorferi*, Bg = *Borrelia*

garinii, Bd = *Borrelia duttonii*, Bh = *Borrelia hermsii*, Ye = *Yersinia enterocolitica*, Yp = *Yersinia pestis*, Yp/p = *Yersinia pestis/pseudotuberculosis*. HLA-DR columns contain dashes when HLA-DR motifs were not investigated

Microorganism	Foreign protein	Endogenous (thyroid) protein	Homology (% identity, % similarity)	HLA-DR molecules containing binding motifs for the pair of homologous proteins										
				1	2 (15)	3 (17,18)	4	5 (11,12)	6 (13,14)	7	8	9		
<i>Borrelia</i> spp.	DNA recombinase/ATP dependent helicase [Bb] aa 42-117	TSH-R aa 620-697	30%, 40%	✓	✓	✓	✓				✓	✓	✓	
	Hypothetical protein BBJ08 [Bb] aa 12-88	TSH-R aa 299-383	27%, 48%		✓	✓	✓					✓	✓	✓
	Hypothetical protein BBG02 [Bb] aa 164-288	TSH-R aa 141-260	27%, 49%	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Flagellar motor rotation protein A (motA) [Bb] aa 16-39	TSH-R aa 127-150	50%, 75%		✓	✓	✓					✓		
	Outer surface protein A (OspA) [Bg] aa 134-221	TSH-R aa 112-205	32%, 47%	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	vlp25 gene homologue [Bd] aa 82-168	Tg aa 2674-2758	30%, 49%	✓	✓	✓	✓					✓	✓	
	Ribosomal protein S12 [Bb] aa 12-34	Tg aa 1836-1904	37%, 48%											
	Hypothetical protein BB0400 [Bb] aa 10-29	TPO aa 625-644	48%, 57%			✓	✓					✓		
	S-adenosylmethionine-tRNA ribosyltransferase-isomerase [Bb] - aa 80-114	TPO aa 596-632	38%, 59%		✓		✓			✓	✓	✓	✓	✓
	Hypothetical protein [Bh] aa 185-243	TPO aa 174-232	28%, 49%		✓	✓	✓					✓	✓	✓
	Hypothetical protein BG0605 [Bg] aa 182-202	NIS aa 523-543	52%, 66%									✓		
	Hypothetical protein BB0592 [Bb] aa 178-202	NIS aa 519-543	48%, 64%									✓		
	Protein-export membrane protein [Bg] aa 383-431	NIS aa 74-120	32%, 53%	✓	✓	✓	✓					✓		✓
	Integral membrane protein BB0316 [Bb] aa 179-244	NIS aa 62-126	37%, 44%	✓	✓	✓	✓					✓	✓	✓
	Panthenate permease [Bg] aa 6-272	NIS aa 47-312	20%, 43%	✓	✓	✓	✓			✓	✓	✓	✓	✓
	Panthenate permease [Bb] aa 6-272	NIS aa 47-312	29%, 45%	✓	✓	✓	✓			✓	✓	✓	✓	✓
	<i>Yersinia</i> spp.	SpyA [Ye] aa 292-359	TSH-R aa 684-749	29%, 45%		✓	✓	✓				✓	✓	
Exopolysaccharonase [Ye] aa 368-405		TSH-R aa 319-363	31%, 48%	✓	✓	✓					✓		✓	
Surface protein B [Ye] aa 12-155		TSH-R aa 186-330	23%, 40%	✓	✓	✓	✓				✓	✓	✓	
Outer membrane protein M [Ye] aa 81-322		TSH-R aa 22-272	26%, 43%	✓	✓	✓	✓				✓	✓	✓	
Yts1H protein [Ye] aa 95-145		Tg aa 2475-2523	38%, 50%			✓	✓					✓	✓	
Hypothetical protein [Yp] aa 109-226		Tg aa 2131-2250	27%, 37%	✓	✓	✓	✓					✓	✓	✓
Putative tail fiber component T of bacteriophage P2 [Yp] aa 691-782		TPO aa 625-708	24%, 41%	✓		✓	✓					✓		✓
Putative AMP-binding enzyme [Yp] aa 93-199		TPO aa 302-412	25%, 39%	✓	✓	✓	✓					✓	✓	✓
MFS family efflux protein [Yp/p] aa 35-103		NIS aa 146-210	27%, 53%	✓	✓	✓	✓					✓	✓	✓
Arsenical efflux pump/H ⁺ antiporter, arsB [Yp/p] aa 292-334		NIS aa 138-181	31%, 54%	✓		✓	✓					✓		✓
ABC type branched-chain aa transport system [Yp/p] aa 117-330		NIS aa 114-306	23%, 35%	✓	✓	✓	✓	✓	✓			✓	✓	✓
Putative ABC sugar transporter (permease) [Yp/p] aa 66-145		NIS aa 112-189	18%, 47%	✓	✓	✓	✓					✓	✓	✓
Putative cysteine-type peptidase [Yp/p] aa 76-198		NIS aa 98-216	29%, 43%	✓	✓	✓	✓					✓	✓	✓
RosA [Ye] aa 235-351		NIS aa 98-215	24%, 41%	✓	✓	✓	✓					✓	✓	✓
Transport ATP-binding protein [Yp/p] aa 150-208	NIS aa 80-136	30%, 47%	✓	✓	✓	✓	✓	✓			✓	✓	✓	
Na/panthotate symporter [Yp/p] aa 13-385	NIS aa 24-402	23%, 42%	✓	✓	✓	✓			✓		✓	✓	✓	
Multidrug resistance protein B [Yp/p] aa 169-312	NIS aa 23-173	20%, 39%	✓	✓	✓	✓					✓	✓	✓	
Putative sodium:phenylacetate symporter, SSS family protein PhaJ [Yp/p] aa 38-219	NIS aa 22-201	26%, 45%	✓	✓	✓	✓	✓	✓			✓	✓	✓	
Proline permease [Yp/p] aa 11-416	NIS aa 20-428	21%, 40%	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	

Table 3 (continued)

Microorganism	Foreign protein	Endogenous (thyroid) protein	Homology (% identity, % similarity)	HLA-DR molecules containing binding motifs for the pair of homologous proteins										
				1	2 (15)	3 (17,18)	4	5 (11,12)	6 (13,14)	7	8	9		
<i>Clostridium botulinum</i>	Neurotoxin A aa 60-76, 518-529, 97-103, 773-799, 621-692, 462-482, 1070-1078, 1073-1090, 186-208, 1205-1244, 521-533, 636-641	TSH-R aa 372-388, 328-339, 146-152, 645-671, 586-656, 354-377, 211-219, 170-187, 138-164, 152-188, 149-161, 225-230	18-66%, 41-100%	✓	✓	✓	✓				✓	✓	✓	
	Neurotoxin A aa 415-446, 952-988, 353-368, 906-914, 272-289, 209-218, 777-796, 211-224, 141-169, 272-298, 28-51, 48-65, 350-370, 1199-1241, 1072-1099, 2-15, 56-73, 257-268, 47-61, 1134-1145	Tg aa 1960-1993, 315-351, 496-511, 1856-1864, 1398-1415, 1121-1130, 98-117, 2414-2427, 2318-2346, 195-222, 1035-1058, 1809-1832, 1608-1628, 1332-1374, 817-844, 2237-2250, 1464-1481, 623-634, 385-399, 1676-1687	25-60%, 35-77%	✓	✓	✓	✓					✓	✓	✓
	Neurotoxin A aa 207-312, 985-1013, 884-896, 1061-1065, 48-62, 936-971, 175-192, 153-158, 897-910	TPO aa 391-492, 428-456, 45-57, 924-928, 777-792, 125-160, 701-718, 457-462, 104-117	20-100%, 36-100%	✓	✓	✓	✓	✓				✓	✓	✓
<i>Rickettsia prowazekii</i>	ATPase aa 75-133	TSH-R aa 351-405	31%, 49%			✓								
<i>Helicobacter pylori</i>	Trigger factor HP0795 aa. 20-108	TSH-R aa 74-157	27%, 42%		✓	✓	✓					✓	✓	✓
	Putative type I restriction enzyme aa. 387-466	TSH-R aa 281-364	25%, 43%		✓	✓	✓					✓	✓	
	Nonheme-iron ferritin aa. 60-133	TSH-R aa 286-364	27%, 48%		✓	✓	✓					✓	✓	✓
	Protein-export membrane protein secD aa. 79-132	TSH-R aa 85-141	26%, 50%		✓	✓	✓					✓	✓	
	Putative type III restriction endonuclease aa. 546-616	TSH-R aa 85-153	30%, 42%		✓	✓	✓					✓	✓	✓
	Putative vacuolating cytotoxin (VacA) paralog aa. 256-313	TSH-R aa 165-221	32%, 46%			✓	✓					✓	✓	
	Urease accessory protein (ureF) aa. 74-166	TSH-R aa 262-355	28%, 43%		✓	✓	✓					✓	✓	✓
	Hypothetical protein HP1142 aa. 133-189	TSH-R aa 287-345	29%, 55%		✓	✓						✓	✓	
	DupA aa. 126-262	TSH-R aa 244-365	21%, 45%		✓	✓	✓					✓	✓	✓
	DNA transfer protein aa. 126-262	TSH-R aa 244-365	21%, 46%		✓	✓	✓					✓	✓	✓
	Pyridoxal-phosphate biosynthetic protein aa 23-50	TPO aa 559-586	39%, 64%	-	-	-	-	-	-	-	-	-	-	-
	Putative transporter aa 68-165	NIS aa 381-461	28%, 43%	-	-	-	-	-	-	-	-	-	-	-
	Hypothetical protein HP0758 aa 320-403	NIS aa 419-514	29%, 49%	-	-	-	-	-	-	-	-	-	-	-
Sodium/proline symporter aa 13-436	NIS aa 23-452	23%, 41%	-	-	-	-	-	-	-	-	-	-	-	

Segments of thyroid autoantigens are typed in boldface on gray background when completely overlapping a known autoepitope, in normal font on gray background when partially overlapping a known autoepitope, in normal font on white background when not overlapping a known autoepitope

To convert the amino acid numbering of the precursor form of thyroid autoantigens, used in this table, into the mature protein numbering, subtract 20, 19 and 19 residues from the numbering appearing in the table for TSHR, Tg and TPO, respectively

One patient with associated Lyme disease and hypothyroidism had been reported previously [42], but in that patient thyroid disease was already present before the bacterial infection. Our suspicion that molecular mimicry between *Borrelia burgdorferi* proteins and thyroid autoantigen was at issue [35], received support subsequently [36]. In detail, we found amino acid sequence homology between five proteins of *Borrelia burgdorferi* and five segments of TSHR coinciding with (or including) known human T-cell epitopes. Our hypothesis was challenged by Völzke et al. [43], who did not find a statistically significant association between anti-*Borrelia* IgG and AITD in a study on 4256 persons from a German region (Pomerania) with endemic borreliosis. AITD was defined as the combined presence of a hypoechoic thyroid pattern in thyroid ultrasound and positive TPO-Ab levels.

Subsequently [37], we updated our work [36]. We demonstrated significant amino acid sequence homologies not only with TSHR, but also with Tg, TPO and sodium iodide symporter (NIS) for 16 *Borrelia* proteins [37]. These 16 proteins represent a mere 0.14 % of the 11,198 *Borrelia burgdorferi* proteins that were deposited in the data bank at that time. Homologies between the four thyroid autoantigens and other microbes were absent or much fewer, and such data conferred specificity to the homology with *Borrelia burgdorferi* proteins. For instance, only one protein of *Clostridium histoliticum* shared local sequence homology with only one thyroid autoantigen (TPO), and only two proteins of *Pneumocystis carinii* did so with only two segments of other thyroid autoantigens (Tg).

Table 4 Results of search for amino acid sequence homology between thyroid autoantigens and proteins of microbes associated in literature to autoimmune thyroid disease. Sequence numbering of thyroid autoantigens refers to precursors (TSH-R: GI 136448; Tg: GI 55770862; TPO: GI

4680721; NIS: GI 4507035). HLA-DR columns contain dashes when HLA-DR motifs were not investigated, because homology involved segments of thyroid autoantigens not overlapping a known autoepitope

Microorganism	Foreign protein	Endogenous (thyroid) protein	Homology (% identity, % similarity)	HLA-DR molecules containing binding motifs for the pair of homologous proteins									
				1	2 (15)	3 (17,18)	4	5 (11,12)	6 (13,14)	7	8	9	
<i>Toxoplasma gondii</i>	Calcium binding egf domain-containing protein aa 2610-2654	TPO aa 794-839	48%, 63%	-	-	-	-	-	-	-	-	-	-
	Calcium binding egf domain-containing protein aa 2706-2750	TPO aa 794-839	47%, 61%	-	-	-	-	-	-	-	-	-	-
	Calcium binding egf domain-containing protein aa 27-146	TPO aa 754-896	29%, 39%	√	√	√	√					√	√
	Calcium binding egf domain-containing protein aa 119-184	TPO aa 756-835	32%, 45%			√	√					√	√
	Acetylcholinesterase, putative aa 4-163	Tg aa 2279-2441	35%, 54%	√	√	√	√					√	√
	GCC2 and GCC3 domain-containing protein aa 1008-1045	Tg aa 1440-1497	44%, 56%	√									
<i>Bifidobacterium adolescentis</i>	Carboxylesterase aa 25-403	Tg aa 2228-2577	27%, 42%	√	√	√	√	√	√	√	√	√	√
	Hypothetical protein aa 73-101	Tg aa 1034-1062	34%, 44%	-	-	-	-	-	-	-	-	-	-
<i>Bifidobacterium longum</i>	Carboxylesterase aa 26-397	Tg aa 2228-2554	27%, 41%	√	√	√	√	√	√	√	√	√	√
<i>Lactobacillus pentosus</i>	Cell surface protein aa 437-626	TSH-R aa 44-207	25%, 45%	√			√			√	√	√	√
<i>Lactobacillus acidipiscis</i>	Carboxylesterase aa 37-340	Tg aa 2199-2514	29%, 45%	√	√	√	√	√	√	√	√	√	√
<i>Treponema pallidum</i>	DNA helicase II aa 569-669	Tg aa 2384-2478	27%, 41%	√	√	√	√			√		√	√
<i>Candida albicans</i>	Mannan polymerase II complex MNN10 subunit aa 134-196	TPO aa 656-722	27%, 43%			√	√					√	√
Cytomegalovirus (Human herpesvirus 5)	Protein UL150 aa 12-51	TPO aa 133-174	37%, 51%	-	-	-	-	-	-	-	-	-	-

Segments of thyroid autoantigens are typed in boldface on gray background when completely overlapping a known autoepitope, in normal font on gray background when partially overlapping a known autoepitope, in normal font on white background when not overlapping a known autoepitope

To convert the amino acid numbering of the precursor form of thyroid autoantigens, used in this table, into the mature protein numbering, subtract 20, 19 and 19 residues from the numbering appearing in the table for TSHR, Tg and TPO, respectively

In the same paper [37], we also showed that homologous segments of the human and bacterial proteins contained peptide-binding motifs associated to specific HLA-DR molecules that are known as risk factors for AITD (Table 3). This might explain the apparent contradiction between our *in silico* data and epidemiological data published by Völzke et al. [43]. Indeed, not all patients can develop an autoimmune reaction after a given infection, but only those genetically predisposed because they have specific HLA alleles. Völzke et al. [43] admit some limitations of their study. For instance, no information was gathered regarding time and acuteness of infection. They state that “Second, without further diagnostic information, seropositivity to anti-*Borrelia* IgG represents a serum scar after prior exposure to *Borrelia* species rather than an indication of previous Lyme disease. Therefore, we cannot fully rule out that clinically relevant borreliosis may cause an AITD in genetically predisposed persons” [43].

3.2 *Yersinia*

The first reports about a possible link between *Yersinia* and thyroid autoimmunity were published about 40 years ago [44–46]. Subsequent papers found consistent evidence of

cross-reactivity between thyroid autoantigens and *Yersinia* proteins. Yet, the triggering role of this bacterial infection in the pathogenetic mechanism of AITD was not universally accepted for a long time [26]. Starting from the early 90s, some authors investigated the possibility that *Yersinia enterocolitica* plasmid-encoded outer proteins (YOPs) recognized specifically by patients with Graves’ disease could be of potential etiological importance in this disorder [47–49].

Using an *in silico* approach, we found that four segments of human TSHR, of which three overlapping known autoepitopes, are homologous to the *Yersinia* proteins YopM, Ysp, exopolygalacturonase and SpyA [36]. Next, we confirmed and expanded our findings, showing that also two sequences of Tg, two of TPO and 11 of NIS, all overlapping known autoepitopes, had homologies with a total of 19 *Yersinia* proteins. Again this was a restricted phenomenon, because these 19 proteins accounted for only 0.05 % of the 40,964 *Yersinia* proteins deposited in the data bank at that time. Additionally, we found [37, 38] that segments shared by human and bacterial proteins contained binding motifs specific of HLA-DR alleles linked to AITD (Table 3). Experimental findings by Wang et al. [50] showed that the *Yersinia enterocolitica* outer membrane porin F protein (ompF) cross-reacts with a leucine-rich domain of TSHR,

identified the epitope involved and demonstrated thyroid-stimulating antibody (TSAb) activity of the ompF antibody. They also observed a statistically significant direct correlation between anti-ompF antibodies and TSAb. As underlined in our second paper [38], this work [50] independently confirmed our previous data which suggested the potential role of outer membrane proteins, namely ompM [37]. Moreover, we showed that, based on a homogeneous bioinformatics comparison (which was not possible in our 2006 article, because ompF was not present yet in Entrez Protein), the homology of ompM protein with the human TSHR autoepitope is numerically superior to that of ompF [38]. In the same paper, search for HLA binding motifs suggested that ompF can trigger anti-TSHR autoimmunity more frequently than ompM in subjects bearing HLA-DR8, while the opposite is true for those bearing HLA-DR4 and HLA-DR9, and the probability should be identical in HLA-DR3-positive subjects [38].

Two very recent papers [51, 52] further explain the involvement of *Yersinia enterocolitica* in the generation of TSHR-stimulating antibodies and the consequent phenomena, typical of Graves' disease. Hargreaves et al. [51] demonstrated cross-reactivity of monoclonal TSAbs with ompA, ompC and ompF, and also showed that “early precursor B cells are expanded by *Yersinia enterocolitica* porins to undergo somatic hypermutation to acquire a cross-reactive pathogenic response to TSHR”. Giménez-Barcons et al. [52] demonstrated the expression of functional TSHR in maturing thymocytes and the possibility of stimulation of such cells by TSAbs. In view of these findings, they suggested that this mechanism could explain the thymic hyperplasia associated to Graves' disease, and hypothesized that “the continuous stimulation of thymocytes by TSAbs could lead to a vicious cycle of iterative improvement of the affinity”, thus “stimulating capability of initially low-affinity antibacterial (e.g., *Yersinia*) Abs cross-reactive with TSHR, eventually leading to TSAbs”.

In contrast, two northern European studies [53, 54] suggest that *Yersinia enterocolitica* infection has no relationship with AITD. Hansen et al. [53] evaluated IgA and IgG antibodies to YOPs in 147 Danish twins who were thyroid antibody-positive (cases) and in 147 age- and sex-matched twins who were thyroid antibody-negative (controls). They found a lower prevalence of anti-YOP antibodies in cases than in controls, and the odds ratio between the two groups was not significant. Effraimidis et al. [54] evaluated prospectively the *Yersinia enterocolitica* serological status of 790 euthyroid women who developed overt hypothyroidism or overt hyperthyroidism over 5 years and matched controls. At baseline, there was an equal frequency of subjects positive for anti-YOP IgG or IgA among patients and controls. One year before the development of overt hypo- or hyperthyroidism, the proportion of subjects with YOP IgG was not different between cases and controls, but YOP IgA were less prevalent in cases. They also followed for four years a group of 388 euthyroid women

without thyroid antibodies at baseline, and compared the *Yersinia enterocolitica* serological status of those who developed anti-TPO and/or anti-Tg antibodies with the status of those who remained negative. They found that the occurrence of thyroid autoantibodies did not differ in the two groups at baseline, and that the *de novo* occurrence of anti-YOP antibodies correlated with thyroid autoimmunity. As for *Borrelia burgdorferi*, the HLA genotype of patients was not considered in both studies [53, 54].

3.3 *Clostridium botulinum*

We observed a woman with Hashimoto's thyroiditis, under replacement therapy with L-T4, who repeatedly experienced, over 10 years, elevations of serum TSH after cosmetic eyelid injections of *Clostridium botulinum* neurotoxin A (Btx) [39]. Transient elevation of serum TSH followed each Btx injection, with a peak between the second and the third month post-injection. TSH elevation was variable (lowest peak =10 mU/L; highest peak =45 mU/L). Both log₁₀-transformed serum TSH levels and log₁₀-transformed TSHR Ab correlated negatively at highly significant levels with the time (in log₁₀-transformed months) elapsed after each preceding Btx injection (TSH, $r = -0.611$, $P = 0.007$; TSHR Ab, $r = -0.999$, $P = 0.0017$). This pattern suggested that changes of TSH and TSHR Ab over the 10-year-follow-up were not random fluctuations. Particularly, TSHR Ab were elevated when assayed shortly (2 months) after Btx injections and within normal limits when assayed longer (5 months) after Btx injection. Evident was the positive correlation with serum TSH: the highest TSHR Ab value coincided with a TSH peak, while the lowest with a low TSH level, and the intermediate with an intermediate TSH level.

Searching for a possible link, we found [39] multiple homologies between Btx and thyroid autoantigens, some of which were located in known autoepitopic segments and contained mainly binding motifs specific of HLA-DR3 and/or HLA-DR7 (Table 3). This is relevant because the HLA-DR3 confers genetic susceptibility for AITD [for refs. see 53]. After local injection, Btx undergoes systemic diffusion, with subsequent detection of anti-Btx serum antibodies, which may be of neutralizing nature in approximately 50 % of treated persons [for refs. see 53]. Owing to molecular mimicry between Btx and TSHR, some anti-Btx antibodies can recognize and bind to TSHR. Thus, transient elevations of TSH in blood that follow each Btx injection might be due to elicitation of TSHR Ab that “neutralize” the TSHR signaling (TSHR-blocking Ab) to some degree. The 1–3 month duration of each serum TSH elevation coincides with the half-life of circulating antibodies of the IgG class, such as TSHR-blocking Ab. In sum, there would be an acquired, transient TSH resistance that overlaps the biochemical picture of increased serum TSH coexisting with normal serum free thyroxine observed in the

heterozygotes with genetic TSH resistance, viz. impaired TSHR signaling due to a monoallelic inactivating mutation of TSHR.

Because of the frequently subclinical nature of AITD, because AITD occurs preferentially in women (who are the fundamental users of the Btx treatments), we suspect that Btx-triggered AITD is underestimated.

3.4 *Rickettsia prowazekii*

Together with another researcher, we recently published one patient with Graves' disease following infection by *Rickettsia prowazekii* [40]. The Australian colleague contacted one of us (S.B.) to consult about the possibility of the infection being one stressful, negative life event, as our group had published on triggering of either the onset or relapses of Graves' disease by stressful events, including infections [55, 56]. However, we wished to ascertain whether thyrotoxicosis could have been triggered by the bacterial infection via molecular mimicry. We found that only one of the 13,899 proteins of *Rickettsia prowazekii* known at the time of our search (0.007 %) had significant homology with TSHR, namely with a segment of this autoantigen which completely overlapped a known autoepitope [40]. Moreover, the patient possessed the HLA-DR3 allele, and the homologous segments of the human and the rickettsial protein contained 3 and 7 HLA-DR3 binding motifs, respectively (Table 3) [40]. As said above, HLA-DR3 is a known risk factor for AITD.

3.5 *Helicobacter pylori*

A significantly increased prevalence of *Helicobacter pylori* infection in patients affected by autoimmune atrophic thyroiditis was reported in 1998 by de Luis et al. [57], and, one year later, by Figura et al. [58]. Since then, the topic has been a matter of debate [59–69], but with a clear chronological tendency towards confirming the correlation initially suspected by de Luis et al. [57]. Some authors [70, 71], based on epidemiological data, have also proposed a possible link between *Helicobacter pylori* infection, autoimmune thyroiditis and type 1 diabetes mellitus.

We analyzed the topic from a bioinformatics point of view in 2006 [72]. Our results indicated that 10 bacterial proteins shared local amino acid sequence similarity with TSHR, one with TPO and three with NIS (Table 3). These 14 proteins represent 0.10 % of the 13,574 proteins of *Helicobacter pylori* that were deposited in the Entrez Protein database at that time. The segments of TSHR (with one exception), TPO and NIS which showed homology to *Helicobacter pylori* proteins contained, partly or completely, at least one autoepitope. Also, we found several HLA-DR binding motifs in the human peptides and in their bacterial counterparts. Of interest, HLA-DR3 binding motifs were present in all cases where TSHR was involved. This finding agrees well with data reported by

Larizza et al. [73], which suggest that the copresence of HLA-DR3 and *Helicobacter pylori* infection might favor the development of AITD. In detail, they evaluated retrospectively anti-*Helicobacter pylori* antibodies in 90 children with AITD and 70 age- and sex-matched healthy controls. The AITD group had a significantly higher frequency of positive *Helicobacter pylori* serology, as well as a significant interaction between HLA-DRB1*0301 (HLA-DR3) and *Helicobacter pylori* infection [73].

3.6 *Toxoplasma gondii*

Serum concentrations of IgG against *Toxoplasma gondii* significantly higher in AITD patients than in controls were observed using proteomic technology [74]. This initial observation was confirmed in a large study on 1514 patients from Europe and Latin America [75]. Two other studies [76, 77], performed in the USA and Czech Republic on 1591 and 1248 women, showed that previous infection with *Toxoplasma gondii* was associated significantly with elevation of TPO Ab.

Very interestingly, our *in silico* analysis matches perfectly these literature data, as it shows that of the 102,206 proteins of this bacterium deposited in the Entrez Protein as of March 2016, only one (~0.001 %) has significant amino acid sequence homology with TPO. In detail, four segments of the bacterial calcium binding egf domain-containing protein are homologous to TPO, and in two segments the mimicked parts of TPO partially overlap known autoepitopes (Table 4). HLA-DR binding motifs present in the segment 754–896 of TPO as well as in the segment 27–146 of the bacterial protein are those of HLA-DR1, -DR2, -DR3, -DR4, -DR8 and -DR9. Segment 756–835 of TPO and 119–184 of its bacterial counterpart shared binding motifs of HLA-DR3, -DR4, -DR8 and -DR9 (Table 4). HLA-DR4 and -DR5 are associated with Hashimoto's thyroiditis, while HLA-DR8 and DR-9 confer genetic risk for AITD in Asians (for refs, see [25, 37]).

In addition, we found that two other proteins of *Toxoplasma gondii* are homologous to human Tg. One protein shows homology with a Tg segment which overlaps a T-cell and a B-cell autoepitope completely and three other B-cell epitopes partially. The matched peptides contain HLA-DR1, -DR2, -DR3, -DR4, -DR7, -DR8 and -DR9 binding motifs (Table 4). The second microbial protein is homologous to a Tg segment partially overlapping a known B-cell autoepitope, and only HLA-DR1 binding motifs are present on the homologous peptides (Table 4).

3.7 *Bifidobacterium* and *Lactobacillus*

The importance of microbiota, particularly gut microbiota, as a modulator of immune response and, consequently, its potential role as a risk/protective factor for autoimmune diseases has recently become a “hot topic” in medical research.

Few groups have published data on a possible correlation between gut microbiota and thyroid autoimmunity via molecular mimicry. In 2011, Kisaleva et al. [78] found that molecules from some strains of *Bifidobacterium* and *Lactobacillus* (namely, *Bifidobacterium bifidum* 791, *Bifidobacterium adolescentis* 94 BIM, *Bifidobacterium longum* B379M and *Lactobacillus plantarum* B-01) have structural homologies with human TPO and Tg, as they selectively bind human TPO and Tg antibodies, and compete with natural antigens for the binding of the same autoantibodies. Additionally, they found that sera containing TPO Ab are significantly more likely to bind antigens of *Lactobacillus plantarum* and *Bifidobacterium bifidum* than control sera. Thus, they suggested that microorganisms of the genera *Bifidobacterium* and *Lactobacillus* could trigger AITD via molecular mimicry [78].

Table 4 shows the results of our search for amino acid sequence homologies between thyroid autoantigens and bacteria of the two genera mentioned above. *In silico* data match with experimental evidence concerning the lack of protein antigens of *Bifidobacterium bifidum* that are potentially cross-reactive with human Tg, TPO or TSHR. However, they suggest that the situation could be different when considering other species of *Bifidobacterium*. Indeed, two proteins of *Bifidobacterium adolescentis* and one of *Bifidobacterium longum* are homologous to human Tg. In two of these three cases (carboxylesterase of both species), the homology could be important for the triggering of thyroid autoimmunity, because the Tg segment involved contains known T and B-cell epitopes. Moreover, binding motifs for all the HLA-DR examined are present in the involved segments of Tg and in their bacterial counterparts (Table 4).

We also found that carboxylesterases of several species of the *Lactobacillus* genus display significant homology with a segment of human Tg that largely coincides with the one mentioned above. Table 4 shows the best homology we found ($E = 6 \times 10^{-22}$), between human Tg and carboxylase of *Lactobacillus acidipiscis*, a species phylogenetically related to *Lactobacillus salivarius* [79]. As for *Bifidobacteria*, both the homologous bacterial and human segments possess binding motifs for all HLA-DR molecules. The other homologous carboxylases were, in decreasing order of statistical significance (E values between 10^{-20} and 3×10^{-6}), those of *Lactobacillus ultunensis*, *Lactobacillus ginsenosidimutans*, *Lactobacillus heilongjiangensis*, *Lactobacillus nodensis*, *Lactobacillus koreensis*, *Lactobacillus paucivorans*, *Lactobacillus nagelii*, *Lactobacillus xiangfangensis*, *Lactobacillus senmaizukei*, *Lactobacillus parabrevis*, *Lactobacillus tuccei*.

3.8 *Treponema pallidum*

The occurrence of seroreactivity against *Treponema pallidum* in AITD patients has been evaluated in a single paper, and it was found to be not significantly higher than in controls [74].

Among the 18,259 treponemic proteins, we found that only DNA helicase II is homologous to human Tg, and that the homologous segment partially overlaps a known autoepitope. We also found that both the bacterial and the human protein contained binding motifs of all HLA-DR molecules, except HLA-DR5 and -DR7. These data would suggest the possible induction of AITD by *Treponema pallidum* by molecular mimicry. Currently, we have no explanation for this discrepancy between our *in silico* data and the paper mentioned above [74].

3.9 *Candida albicans*

Immunological cross-reactivity between thyroid autoantigens and *Candida albicans* proteins has been investigated by a single group [80]. These authors showed that when sera containing high levels of anti-*Candida* antibodies are mixed with thyroid antigens, antibody titers decrease by 10–15 %. Vice versa, when thyroid antibody positive sera are incubated with *Candida albicans*, a similar reduction occurs in thyroid antibody levels [80]. Our *in silico* investigation provides a possible explanation. As shown in Table 4, significant similarity exists between one of the 183,825 fungal proteins (~ 0.0005 %), namely the mannan polymerase II complex MNN10 subunit, and a segment of human TPO partially overlapping with an autoepitope. The two homologous segments contain HLA-DR3, -DR4, -DR8 and -DR9 binding motifs.

3.10 *Cytomegalovirus (Human herpesvirus 5)*

The possible involvement of cytomegalovirus in the pathogenesis of AITD was initially suspected on the basis of its ability to induce *in vitro* HLA-DR expression on thyroid follicular cells [81]. However, evidence from the three subsequent studies on this topic [74, 76, 82] was against such hypothesis. Our *in silico* data show that only one of the 41,627 proteins of cytomegalovirus is homologous to a segment of TPO, and that this segment is not part of any known autoepitope (Table 4). Such data are against a possible triggering role of cytomegalovirus in AITD by molecular mimicry, in full agreement with literature.

3.11 *Hepatitis C virus (HCV)*

Extrahepatic complications, mainly of autoimmune nature, are observed in 40–74 % of patients with hepatitis C [83]. AITD are among the most frequent HCV-related extrahepatic diseases. Such association has been confirmed in adults and in children [84]. Additionally, mixed cryoglobulinemia (MC) can be an important extrahepatic HCV-related disease, and a very recent study has shown that patients with HCV-related MC have a high incidence of AITD [85]. If AITD are absent initially, they may occur after interferon-alpha treatment

[86–88], because this molecule has immune stimulatory and direct toxic effects on the thyroid [89]. HCV interferes with multiple steps of immune response, because it has a direct cytolytic effect on thyrocytes, lowers the B-cell activation threshold, infects lymphocytes modifying their response to apoptotic and regulatory signals as well as their pattern of cytokine production [86]. Moreover, as suggested by some authors, HCV can induce autoreactivity through molecular mimicry [86].

In a study on 348 Italian patients with chronic hepatitis C [87], seven homologous and cross-reactive linear epitopes shared by the HCV polyprotein, CYP2D6 (also known as liver/kidney microsomal antibody type 1) and TPO were reported. Cross-reactivity was found in 86 % of HCV patients with AITD, but not in those without AITD, nor in controls (HCV-negative patients with autoimmune hepatitis or subjects with AITD not associated to liver disease).

Short homologies (8–10 amino acids) have been reported between the HCV polyprotein and six segments of Tg, three of NIS, one of TSHR, one of TPO, and one of pendrin [90]. The authors concluded that “further studies are necessary in order to evaluate the clinical relevance of the presence of the molecular mimicry between the HCV and the thyroid antigens in the progression of autoimmune disease” [90].

An authoritative review [88] suggests that, in the light of currently available data, bystander activation appears more likely than molecular mimicry as a triggering mechanism of AITD. In the bystander activation model, viral infection of a tissue can induce local, low level inflammation, with subsequent activation of resident autoreactive T-cell clones, which are usually suppressed by peripheral tolerance mechanisms [88]. Indeed, the presence of CD81, the HCV receptor, on the membranes of thyroid cells, as well as the activation of IL-8 production after binding of the viral envelope glycoprotein E2 to human thyrocytes, seem to support this hypothesis [91].

We were unable to find amino acid sequence homologies between HCV proteins and TSHR, Tg or TPO using our criteria of bioinformatics search. The reason of the discrepancy between our *in silico* analysis and previous papers [87, 90] is probably the small size of the homologous peptides reported in literature, which prevents them from being revealed by the BLAST algorithms when performing comparisons between the entire amino acid sequence of proteins.

4 Conclusions

Forty years after the innovative intuition of Shapiro and colleagues [10], molecular mimicry is no longer a hypothesis, but a well consolidated fact, as confirmed by abundant experimental evidence. Yet, the biological importance of molecular mimicry, in physiological as well as in pathological processes,

appears still underestimated and not completely explored. This is particularly true for autoimmune diseases, including AITD.

Data in this review show not only the current contribution of molecular mimicry to a better understanding of AITD, but also the potential of future research in this field, as suggested by the new regions of homology presented here. Moreover, the data show the consistency between *in silico* prediction of cross-reactivity and experimental results, with interesting implications: bioinformatics tools are useful to precisely identify possible cross-reacting molecules (or at least narrow down their number), their epitopes, and may even suggest the individual genetic risk linked to specific HLA-DR haplotypes. The *in silico* approach used here has currently some limitations, particularly for which concerns the identification of small, non-peptidic or non-linear cross reactive epitopes. However, new techniques are being developed, which exploit the increasing processing power of computers to achieve a better simulation of molecular interactions and improve precision of predictions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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