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Prevalence of anti-toxoplasma antibodies in patients with autoimmune diseases

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ABSTRACT

The identification of etiological factors in the induction of autoimmunity has remained elusive despite an enormous effort at dissection of the molecular structure of the target antigens and effector mechanisms. One characteristic feature of autoantigens is their repetitive structure as well as their conservation and evolution. *Toxoplasma (T.) gondii* is a primitive protozoan. We hypothesized that patients with autoimmune disease would have broad reactions against *Toxoplasma* antigens based on autoantigen conservation.

To address this issue, we assessed serologic evidence of reactivity to *Toxoplasma gondii* along with a large profile of autoantibodies in patients with various autoimmune diseases (AID). We included sera of 1514 patients with 11 different AID collected from referral centers in Europe and Latin America as well as from 437 geographically matched controls, for the prevalence of anti *Toxoplasma* antibodies (ATxA) IgG and IgM and serum autoantibodies utilizing the BioPlex 2200 system (Bio- Rad Laboratories, USA).

Serum ATxA IgG were positive in 42% of patients with AID versus 29% of controls ($p < 0.0001$). Among Europeans, ATxA IgG were associated with anti-phospholipid syndrome (APS; $p < 0.0001$), cryoglobulinemia ($p < 0.0001$), ANCA-associated vasculitides ($p < 0.01$), autoimmune thyroid diseases ($p < 0.0001$), systemic sclerosis (SSc; $p < 0.0001$) and rheumatoid arthritis (RA; $p < 0.0001$). Of note, Latin American RA sera exhibited similar frequency of ATxA IgG as controls. ATxA IgM were more prevalent in European patients with APS ($p < 0.01$), SSc ($p < 0.05$) and inflammatory bowel disease (IBD, $p < 0.05$) than in controls. Further, in AID patients the presence of ATxA correlated with autoantibodies characteristic of APS (anti- cardiolipin, B2GPI, complex of cardiolipin- B2GPI, prothrombin, phosphatydilethanolamine), and of SSc (anti-centromere, Scl-70).

Our findings suggest that *T. gondii* may contribute to the pathogenesis of AID. This interaction may depend on or explain observed geoepidemiological variance in AID.

1. Introduction

The role of infectious agents, mainly viruses and bacteria, in the pathogenesis of autoimmune diseases (AID) has been established in recent years [1] while parasitic infections have been largely overlooked as parasites elicit a complex immunomodulatory effect in the host [2,3]. On the one hand, geoepidemiological as well as experimental evidence may well support protective effect of specific parasitic infections in the susceptibility to autoimmunity [3]. It has been hypothesized, for example, that exposure to malaria renders Africans who live in endemic conditions more resistant than other populations to certain AID [4,5]. In addition, helminths were shown to be protective in several experimental models including type 1 diabetes, autoimmune encephalitis, and ulcerative colitis most likely *via* a Th2 environment as well as induction of regulatory responses [3]. Clinical trials demonstrated that the oral administration of porcine whipworm (*Trichuris suis*)

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eggs improves symptoms in patients with inflammatory bowel disease [6,7].

Conversely, some parasites have been implicated in the evolution of autoimmunity. *Trypanosoma (T.) cruzi* antigens cross-react with host antigens (e.g., ribosomal P, Sm, snRNPs) and Chagas' disease, caused by this parasite, has been considered a paradigm of infection-induced autoimmunity [8]. Finally, sera of animals and humans with malaria, visceral leishmaniasis, schistosomiasis or onchocerciasis were found to bind various autoantibodies including: nuclear antigens (ANA), native DNA, rheumatoid factor, Sm, RNP, SS/A, SS/B, cardiolipin, neutrophil cytoplasmic enzymes (ANCA) and others [9].

Toxoplasma (T.) gondii is an intracellular parasitic protozoan with worldwide infection rates estimated around 30%, despite wide geoepidemiological variance [10]. Indeed, prevalence ranges from as low as 4% in some areas of the Far East, through 10–30% in the US, and in Europe from 10% and up to 60% in regions with high consumption of raw food (e.g., France) [10–12]. Most patients with *Toxoplasmosis* are asymptomatic. The host immune response to *Toxoplasmosis* combines a strong cell mediated response with a Th1 cytokine profile and a humoral response which results in production of specific anti *Toxoplasma* antibodies (ATxA), constituting the standard method for diagnosis [10].

Studies in experimental models of AID and *Toxoplasma gondii* infection reported conflicting results [13–15]. Previous case reports and case-control studies have linked ATxA with polymyositis [16], rheumatoid arthritis (RA) [17–19], autoimmune thyroid diseases [20,21], Crohn's disease [22], anti phospholipid syndrome [23], Wegener's granulomatosis [24] and autoimmune bullous diseases [25]. While ATxA have been previously associated with SLE [26], subsequent studies challenged this association [27,28]. In our recent study conducted in type 1 diabetes mellitus patients, we found a negative association of ATxA with this autoimmune disease [29].

We herein report a large series of patients with defined AID investigated for the prevalence of anti *Toxoplasma* antibodies of both IgG and IgM classes, as well as for associations with a large profile of autoantibodies. We further sought to unravel geoepidemiological variance by investigating the link of this parasite with autoimmunity in subjects from distinct geographical regions.

2. Material and methods

2.1. Serum samples

We included serum samples from 1514 patients with 11 different AID collected from tertiary referral centers in Europe and Latin America. All patients fulfilled the diagnostic criteria for each specific autoimmune disease [30]. The European group included 1062 patients with anti-phospholipid syndrome (APS; $n = 159$), cryoglobulinemia ($n = 117$), ANCA-associated vasculitides ($n = 68$), autoimmune thyroid diseases (AITD; $n = 120$), systemic sclerosis (SSc; $n = 80$), inflammatory bowel disease (IBD; $n = 115$), polymyositis ($n = 99$), systemic lupus erythematosus (SLE; $n = 169$), rheumatoid arthritis (RA; $n = 35$) and multiple sclerosis (MS; $n = 100$). The Latin American group included 452 patients with SLE ($n = 120$), RA ($n = 152$), Sjogren's syndrome ($n = 82$), and MS ($n = 98$). All sera from patients with autoimmune diseases were collected prior to the initiation of any medical treatment and samples were kept at −20'C until used for analysis. Control sera were obtained from 437 healthy subjects of similar age and sex distribution (297 from Europe and 140 from Latin America). The study received approval of the local ethics committees and fulfilled the ethical guidelines of the most recent declaration of Helsinki.

2.2. Testing of serum markers of T. gondii infection and autoantibodies reactivity

Exposure to *T. gondii* was assessed by testing anti *T. gondii* antibodies (ATxA) IgG and IgM utilizing the BioPlex 2200 system (Bio-Rad Laboratories, Hercules, CA), according to the manufacturer's protocol, as previously described and evaluated [31,32]. In parallel, a large profile belonging to 4 groups of autoantibodies was tested in all sera, using the same platform. The autoantibodies included: anti nuclear antibodies (ANA IgG: Anti- dsDNA, Sm, chromatin, ribosomal-P, RNP, SmRNP, Ro/SSA, La/SSB, centromere, Scl-70, Jo-1), vasculitis associated (IgG: Anti- glomerular basement membrane [GBM], proteinase 3 [PR3], myeloperoxidase [MPO]), gastrointestinal associated (IgG, IgA: Anti - *Saccharomyces cerevisiae* [ASCA], gliadin [AGA], tissue transglutaminase [tTg]), and thrombophilia associated (IgG, IgM: Anti- cardiolipin [CL], B2 glycoprotein 1 [B2GP1], complex of both [CL–B2], phosphatydilserine- B2GP1 complex [PS–B2], phosphatydilethanolamine [PE], prothrombin [PT], phosphatydilserine–prothrombin complex [PS–PT]).

2.3. Statistical analysis

For analysis the StatSoft-STATISTICA v8.0 software was utilized. Two-tailed Fisher's exact tests or Chi-square tests were used as appropriate for comparison of categorical variables between groups. P values<0.05 were considered as statistically significant following Bonferroni correction for multiple testing.

3. Results

ATxA IgG were found in 42% (637/1514) of patients with AID while detected in 29% (127/437) of control subjects ($p < 0.0001$). When analyzing by geographical region, 45% (478/1062) of European patients with AID manifested ATxA–IgG positivity compared with 26% (77/297) of geographically matched controls ($p < 0.0001$). Conversely, ATxA–IgG positivity rates were similar in Latin American patients with AID (35%, 159/452) and their geographically matched controls (36%, 50/140).

The prevalence of serum ATxA–IgG was significantly higher compared to controls in 6/11 autoimmune diseases (Table 1). Among Europeans, higher prevalence of ATxA–IgG were found in

Table 1
Prevalence of anti *Toxoplasma gondii* IgG antibodies (ATxA) in 1514 serum samples of patients with different autoimmune diseases.

Disease	ATxA–IgG positive	Geographical region	*P* value[c]
Antiphospholipid syndrome[a]	82/159 (52%)	Europe	**<0.0001**
Cryoglobulinemia[b]	65/117 (56%)	Europe	**<0.0001**
ANCA vasculitis	45/68 (66%)	Europe	**<0.01**
Autoimmune thyroid diseases	68/120 (57%)	Europe	**<0.0001**
Systemic sclerosis	46/80 (58%)	Europe	**<0.0001**
Inflammatory bowel disease	38/115 (33%)	Europe	NS
Polymyositis	31/99 (31%)	Europe	NS
Sjogren's syndrome	33/82 (40%)	Latin America	NS
Systemic lupus erythematosus	54/169 (32%)	Europe	NS
Systemic lupus erythematosus	42/120 (35%)	Latin America	NS
Rheumatoid arthritis	27/35 (77%)	Europe	**<0.0001**
Rheumatoid arthritis	55/152 (36%)	Latin America	NS
Multiple sclerosis	22/100 (22%)	Europe	NS
Multiple sclerosis	29/98 (30%)	Latin America	NS

NS non-significant.
[a] Including primary and secondary APS.
[b] Including cryoglobulinemia and mixed cryoglobulinemia.
[c] *P* value for the comparison with matched healthy controls of the same geographical region.

patients with APS, cryoglobulinemia, ANCA-associated vasculitides, AITD, and SSc compared to matched controls. The association between ATxA–IgG positivity and RA was observed only among the patients and controls from Europe while the Latin American group failed to reach significant differences.

ATxA IgM were detected in 5% (64/1238) of patients with AID compared to 2% (5/238) of healthy controls ($p < 0.05$). Among Europeans, ATxA–IgM were more prevalent in patients with APS (10%, 16/160; $p < 0.01$ *vs.* controls), SSc (8%, 6/78; $p < 0.05$ *vs.* controls) and IBD (8%, 9/119; $p < 0.05$ *vs.* controls), when compared with geographically matched controls (1%, 1/98). All the remaining groups of patients with AID originating either from Europe or Latin America had similar prevalence of ATxA–IgM.

ATxA–IgG antibodies were directly associated with the presence of serum anti-centromere and anti-Scl-70 autoantibodies ($p < 0.05$ and $p < 0.02$, respectively) and inversely correlated with IgG anti gliadin anditbodies ($p < 0.01$) (Table 2). Similarly, ATxA–IgM antibodies were associated with the presence of anti CL-B2 (IgG, $p < 0.05$), anti-CL (IgM, $p < 0.01$), anti B2GPI (IgM, $p < 0.0001$), anti CL-B2 (IgM, $p = 0.01$), anti PE (IgM, $p = 0.01$), and anti PT (IgM, $p < 0.0001$) (Table 3).

4. Discussion

The association between parasitic infections and AID remains elusive [3] despite growing evidence on the role of helminths and *Trypanosoma cruzi*. In particular, there is limited data on the effects of *T. gondii*. In the present study we investigated a large number of sera from patients with different AID from different geographical areas and matched controls. We demonstrate that patients with AID manifest a higher prevalence of ATxA–IgG antibodies than controls, among the latter the prevalence rates were relatively comparable to previously reported studies [10].

ATxA–IgG antibodies are also associated with specific AID, in some cases consistent with previous reports [20,22–25]. Nevertheless, the association between APS and SSc with ATxA antibodies of both Ig subclasses is novel and further supported by the significant association of this serum reactivity with serum autoantibodies specific for APS (anti-CL, B2GPI, CL-B2, PT, PE), and SSc (anti-centromere, Scl-70).

Based on our cross-sectional data we may hypothesize the mechanisms by which ATxA–IgG antibodies may be correlated with AID. First, we should note that it is not uncommon for adult sera to manifest naturally occurring antibodies against *T. gondii* [33] and the presence of these natural antibodies could reflect the cross-reactivity of parasite antigens with antigens from the host [27]. This is well supported by the observation that ATxA from healthy individuals cross-react with malignant cervical tissue antigens [34]. Second, an association between ATxA–IgG antibodies and anti-thyorid antibodies (TPOAb) was observed in sera from 1591 pregnant women [21]. Thus supporting, along with our data on AITD, the hypothesis that *Toxoplasma* can initiate a pathogenic process that may eventually result in clinically overt autoimmunity. Similarly, our group previously suggested mimicry between *T. gondii* antigens and phospholipids based on a strong homology found between B2GPI-related peptides (i.e., target epitope for anti-B2GPI

Table 2
Autoantibody prevalence in sera from patients with autoimmune disease arrayed according to the anti *Toxoplasma gondii* (ATxA) IgG status.

Autoantibodies	ATxA–IgG Positive ($N = 637$)	ATxA–IgG Negative ($N = 877$)	*P* value
ANA (IgG)			
Anti-dsDNA	18%	21%	NS
Anti Sm	7%	8%	NS
Anti Chromatin	23%	26%	NS
Anti Ribosomal P	3%	4%	NS
Anti RNP	10%	9.5%	NS
Anti SmRNP	9%	10%	NS
Anti Ro/SSA	17%	17%	NS
Anti La/SSB	8.3%	6%	NS
Anti Centromere	7%	4%	**<0.05**
Anti Scl-70	5%	3%	**<0.02**
Anti Jo-1	<1%	1.5%	NS
Gastrointestinal associated			
Anti tTG (IgG)	4.2%	3%	NS
Anti tTG (IgA)	<1%	<1%	NS
Anti gliadin (IgG)	6%	10%	**<0.01**
Anti gliadin (IgA)	3%	3%	NS
Anti *Saccharomyces cerevisiae* (IgG)	6%	5%	NS
Anti *Saccharomyces cerevisiae* (IgA)	3%	4%	NS
Thrombophilia associated			
Anti CL (IgG)	11%	11%	NS
Anti B2GPI (IgG)	14%	11%	NS
Anti CL-B2 (IgG)	5%	4%	NS
Anti PE (IgG)	2%	2%	NS
Anti PT (IgG)	7%	6%	NS
Anti CL (IgM)	8%	9%	NS
Anti B2GPI (IgM)	28%	24%	NS
Anti CL-B2 (IgM)	14%	18%	NS
Anti PS-B2 (IgM)	24%	21%	NS
Anti PE (IgM)	3%	5%	NS
Anti PT (IgM)	35%	34%	NS

NS, non-significant. Bold values represent the significant *P* values ($P < 0.05$).

Table 3
Autoantibody prevalence in sera from patients with autoimmune disease arrayed according to the anti *Toxoplasma gondii* (ATxA) IgM status.

Autoantibodies	ATxA–IgM Positive ($N = 64$)	ATxA–IgM Negative ($N = 1174$)	*P* value
ANA (IgG)			
Anti-dsDNA	22%	18%	NS
Anti Sm	3%	5.8%	NS
Anti Chromatin	33%	25%	NS
Anti Ribosomal P	1.5%	2%	NS
Anti RNP	5%	9%	NS
Anti SmRNP	11%	8%	NS
Anti Ro/SSA	12.5%	17%	NS
Anti La/SSB	4.6%	7%	NS
Anti Centromere	6.3%	6%	NS
Anti Scl-70	8%	4%	NS
Anti Jo-1	5%	1.5%	NS
Gastrointestinal associated			
Anti tTG (IgG)	5%	4.5%	NS
Anti tTG (IgA)	3%	<1%	NS
Anti gliadin (IgG)	8%	10%	NS
Anti gliadin (IgA)	3%	3.7%	NS
Anti *Saccharomyces cerevisiae* (IgG)	5%	6.6%	NS
Anti *Saccharomyces cerevisiae* (IgA)	5%	4%	NS
Thrombophilia associated			
Anti CL (IgG)	13%	11.5%	NS
Anti B2GPI (IgG)	19%	12.5%	NS
Anti CL-B2 (IgG)	9.5%	4%	**<0.05**
Anti PE (IgG)	1.5%	2%	NS
Anti PT (IgG)	6.5%	6.3%	NS
Anti PS-PT (IgG)	5%	2.5%	NS
Anti CL (IgM)	19%	8%	**<0.01**
Anti B2GPI (IgM)	49%	25%	**<0.0001**
Anti CL-B2 (IgM)	27%	15%	**=0.01**
Anti PE (IgM)	11%	4%	**=0.01**
Anti PT (IgM)	57%	33%	**<0.0001**

NS, non-significant. Bold values represent the significant *P* values ($P < 0.05$).

Abs) and the parasite [14]. The present data support this view of APS pathogenesis by demonstrating an ATxA association with aPL antibodies and the anti-phospholipids syndrome.

Third, we may hypothesize that *T. gondii* infection induces a bystander effect *via* activation of toll-like receptors (TLR) [21], as previously demonstrated for multiple TLR [35], and may thus lead to the expansion of autoantibodies under aberrant conditions (e.g., excessive and/or chronic TLR activation) [21,36].

Additionally, a direct inflammatory insult may be caused by *T. gondii* infection as illustrated by a mouse model of IBD. *T. gondii* induced massive necrosis of the villi and digestive mucosal cells in the ileum of C57BL/6 mice, with a CD4+ T cell immunopathology characterized by a robust Th1-mediated increase in pro-inflammatory mediators [10,13]. Consistent with these experimental findings, we report herein an association between signs of ongoing infection such as ATxA–IgM class and IBD.

Our data did not report an association between *T. gondii* infection and other specific AID, nor with most serum autoantibodies. This is in agreement with the complex and multifactorial origin of autoimmunity manifested by the different outcomes of *T. gondii* infection. As an example of this latter feature, the lupus experimental model NZB/NZWXF1 mouse infected with *T. gondii* develop a milder renal disease and a prolonged survival when compared to non-infected mice thus suggesting a putative protective effect [15]. Moreover, our results do not support the view that patients with specific AID, as in the case of SLE, may be prone to immune dysregulation and opportunistic infections.

There is a broader interpretation of our data when considering the recent evidence of a geoepidemiology of AID worldwide [4,5,37]. Geoepidemiology demonstrates that genetic individual susceptibility interacts with lifestyle and environmental factors, which include socioeconomic status, nutritional habits, environmental pollutants, ultraviolet radiation exposure, and infections (in each case as triggering or protective agents) to determine the risk of developing autoimmunity [4,5]. For this reason it becomes of critical importance to perform parallel comparisons of groups of patients and controls from different geographical and ethnical backgrounds such as Europe and Latin America. In this study geographical specificity was demonstrated for RA, as an association with ATxA prevalence was significant only for Europeans. RA has been previously linked with *Toxoplasma* in Egypt [17,18], yet different effects of exposure to this parasite in other geographically distinct populations remain unknown [38]. Nevertheless, we are aware that the number of cases included in our comparison may prove insufficient to draw solid conclusions.

5. Conclusion

In conclusion, although it would be intriguing to postulate a link between past infection by *T. gondii* and several AID in two geographically distinct case-control cohorts, further studies would be needed. There has been significant effort in using epidemiology to understand the etiological factors of autoimmune disease and we cite a recent symposium which discusses these in depth [39–85].

We emphasize the conservation of autoantigens and it is more likely that reactivity is based upon this phenomenon. The cross-sectional design of this study does not allow discriminating a causal relationship. There are however interesting associations between the parasite response and specific autoantibodies. We propose that further comparative immunology be performed to identify the mechanisms involved in this relationship and whether the data herein is primarily an epiphenomenon or whether it reflects a direct association with either *toxoplasma* or a related infection on either the initiation or exacerbation of autoimmune disease.

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