A Latitudinal Gradient Study of Common Anti-Infectious Agent Antibody Prevalence in Italy and Colombia

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Abstract

Background: Infectious agents are important in the pathogenesis of autoimmune disease since they are a major part of the environmental trigger of autoimmunity. A negative relationship between latitude and infectious disease species richness has been suggested.

Objectives: To examine whether their prevalence differs in two latitudinally different populations.

Methods: The prevalence of infections with *Toxoplasma gondii*, rubella virus, cytomegalovirus, Epstein-Barr virus and *Treponema pallidum* was compared between subjects from Italy and Colombia.

Results: We found high titers of antibodies against four of five microorganisms tested, *Toxoplasma gondii* (50.8%), rubella virus (German measles) (75%), cytomegalovirus (86.3%), Epstein-Barr virus (83.3%) and *Treponema pallidum* (6.3%) in completely healthy individuals from a tropical country (Colombia) and a European country (Italy). Differences between two groups of volunteers were noted regarding two infectious agents. The prevalence of immunoglobulin G anti-rubella antibodies was significantly higher among Italian subjects (85% vs. 67.9%, $P = 0.002$), whereas antibodies against CMV were less prevalent among Italian as compared to Colombian subjects (77% vs. 92.9%, $P < 0.001$).

Conclusions: These differences might also result in a different tendency towards development of autoimmune diseases associated with these infectious agents in different populations.

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Infectious agents continue to be among the leading causes of morbidity and mortality worldwide. In addition, they are also implicated in the pathogenesis of indirect consequences such as induction of autoimmune diseases [1-3]. Although observation of high titers of immunoglobulin G to microorganisms associated with diseases is very common in clinical practice, and there are several frequent infectious agents that appear to affect the vast majority of the population, differences in seropositivity towards common infectious agents might explain differences in tendency towards the development of infectious-associated diseases, including autoimmune diseases. In addition, a significant negative relationship between latitude and infectious disease species richness, and a nested spatial organization, i.e., the accumulation of infectious disease species with latitude, have been reported [4]. The aim of this study was to explore serum frequencies of IgG antibodies directed towards five microorganisms commonly associated with disease in healthy people from two different origins – one from Colombia, an equatorial country, and the other from Italy, a northern country. A presumed difference in prevalence of anti-infectious agents (if found) might be a reasonable explanation for differences among various autoimmune phenomena between these two different populations.

Patients and Methods

Subjects

We evaluated 240 healthy volunteers from northwestern Colombia (N=140) and Italy (N=100) who originally served as control groups in the multicenter study "Infection and Autoimmunity." They were apparently healthy at the time of blood collection. Demographic data of volunteers are presented in Table 1.

Antibody assays

All samples were screened for antibodies using five different packs of assays against a different microorganism (T. *pallidum*, rubella, cytomegalovirus, T. *gondii* and Epstein-Barr virus) using the Bio-Rad BioPlex 2200 system® (Hercules, CA, USA). It is a fully automated, random-access immunoassay analyzer built on a synthesis of multiplex, magnetic bead and flow cytometry technologies. At the core of the technology are two different populations of 8 µm magnetic beads that are dyed with two fluorophores for classifications. Each bead is coated with specific recombinant proteins, according to the assay being tested, thus representing a different target antigen. Briefly, colored beads coated with different antigens were mixed together, along with

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CMV = cytomegalovirus

Ig = immunoglobulin

the patient's sample and sample diluent and then incubated for 20 minutes at 37ºC. After a wash cycle, different isotypes of anti-human antibodies conjugated to phycoerythrin were added to the dyed bead and incubated for 10 minutes at 37ºC. After removal of excess conjugate, the bead mixture was passed through the detector that identifies the beads based on the fluorescence of the dyes. The amount of antibody bound to the bead was determined by the fluorescence of phycoerythrin. Raw data were initially measured as the relative fluorescence intensity and then converted to the fluorescence ratio using a pre-dyed internal standard bead. A series of calibrators were analyzed along with the patient samples to convert fluorescence ratio to international units. Two additional control beads were also included in all incubations. A serum verification bead and a blank bead were added to verify the addition of serum to the reaction vessel and the absence of significant non-specific binding respectively.

The EBV IgG pack incorporated three different populations of beads screening for nuclear antigen-1, viral capsid antigen and early antigen-D. The Syphilis IgG pack used a group of recombinant proteins to T. *pallidum* that detect three analytes (TPr15, TPr17 and TPr47, numbered according to their size in kilo Daltons) that help improve sensitivity and specificity of the assay. Results for Syphilis IgG can be interpreted with the highest titer of the three parameters. TORC packs IgG screen for antibodies against three target analytes: *Toxoplasma gondii*, rubella (German measles) and cytomegalovirus. The *Toxoplasma* antigen used is a disrupted cell lysate from tachyzoites grown in murine ascites fluid.

Variable definition

The antibody titers were dichotomized according to published prevalence for the Italian general population. For rubella we used the prevalence of 84% [5], which generated a cutoff level of 65 IU; for toxoplasmosis the prevalence was 41% and the cutoff level > 1 IU [6]; for CMV the prevalence used was 91.7% and the cutoff level was > 0.2 UI [7]; for EBV a prevalence of 81.9% [8] and three cutoff levels; and for syphilis 0.2 IU was considered the lowest titration possible.

Statistical analysis

For comparison of age differences between Italian and Colombian subjects and differences between IgG reactivities the chi-square test was used. Correlations between IgG reactivities were calculated using the phi correlation coefficient. Statistical analysis was performed using SPSS® software. Since this was an exploratory study, a statistically significant difference was considered if $P < 0.01$.

Results

The demographic information presented in Table 1 indicates that the Colombian women were significantly older than the Italian women ($P < 0.001$) in our cohort, whereas the male volunteers showed no differences although this group was of a smaller size in both nationalities. The detailed frequencies of IgG titers for the five microorganisms measured in both populations considering the cutoff levels defined above are presented in Table 2. The general frequencies were: anti-*Toxoplasma gondii* antibodies 50.8%, anti-rubella virus antibodies 75%, anti-CMV antibodies 86.3%, anti-EBV antibodies 83.3% and anti-*Treponema pallidum* 6.3%. Differences between the two groups of volunteers were noted regarding two infectious agents. The prevalence of IgG anti-rubella antibodies was significantly higher among Italian subjects (85% vs. 67.9%, $P = 0.002$), whereas antibodies against CMV were less prevalent among Italian compared to Colombian subjects (77% vs. 92.9%, $P < 0.001$). As shown in Table 3, no significant correlations among the various IgG anti-infectious agents' reactivity and the age groups could be found in either of the volunteer groups tested.

Table 1. Demographic data, nationality, stratified age and gender of study subjects

	Males				Females			
	Italians		Colombians		Italians		Colombians	
Age (yrs)	n	%	N	%	n	%	n	%
18–44	22	64.7	8	80.0	61	92.4	89	68.5
45–63	12	35.3	2	20.0	5	7.6	41	31.5
Total	34	100.0	10	100.0	66	100.0	130	100.0
Mean (SD)	41.3 (8.8)		35.1 (10.9)		29.2 (8.2)		39.4 (10.0)	
P value of chi-square test	0.361				< 0.001			

Table 2. IgG reactivity in two volunteer groups according to microorganism

	Total		Volunteers				
			Italians		Colombians		
IgG	n	%	n	%	n	%	*P*
Toxoplasmosis							
< 1.0	118	49.2	52	52.0	66	47.1	0.458
> 1.0	122	50.8	48	48.0	74	52.9	
Rubella							
< 65	60	25.0	15	15.0	45	32.1	0.002
> 65	180	75.0	85	85.0	95	67.9	
Cytomegalovirus							
< 0.2	33	13.8	23	23.0	10	7.1	<
> 0.2	207	86.3	77	77.0	130	92.9	0.001
Syphilis							
< 0.2	225	93.8	93	93.0	132	94.3	0.685
> 0.2	15	6.3	7	7.0	8	5.7	
Epstein-Barr virus (CA)							
< 3	40	16.7	17	17.0	23	16.5	0.926
> 3	199	83.3	83	83.0	116	83.5	

CA = capsid antigen

Discussion

In this study we report the prevalence of high titers of anti-infectious agents among patients from different geographic and cultural origins (*Toxoplasma gondii*, rubella virus, CMV, EBV and *Treponema pallidum*). To the best of our knowledge, no

EBV = Epstein-Barr virus

Table 3. Spearman correlations among the antibody titrations towards microorganisms studied plus age

Spearman correlation		Age	Toxo	Rubella	CMV	Syphilis	EBV-CA
Age	Correlation	1	0.205	-0.034	0.058	-0.124	-0.079
	P value		0.041	0.740	0.569	0.218	0.437
Toxoplasmosis (Toxo)	Correlation	0.039	1	-0.213	0.097	0.129	0.062
	P value	0.644		0.033	0.337	0.202	0.541
Rubella	Correlation	-0.072	0.177	1	-0.163	0.115	-0.116
	P value	0.396	0.036		0.105	0.254	0.252
Cytomegalovirus	Correlation	-0.056	0.127	0.165	1	-0.129	0.069
	P value	0.512	0.135	0.051		0.199	0.495
Syphilis	Correlation	-0.164	0.048	-0.028	0.068	1	0.020
	P value	0.053	0.577	0.740	0.423		0.845
Epstein-Barr virus	Correlation	0.089	0.003	-0.060	0.026	0.027	1
Capsid antigen (EBV-CA)	*P* value	0.300	0.971	0.484	0.762	0.753	

The shaded area refers to Italians (n=100) and the unshaded area to Colombians (n=140).

study has compared so many microorganisms in such a large sample. The patients exhibited high titers of IgG to most of the microorganisms tested, except for T. *pallidum*. With regard to reliability of results, other authors in southern Italy who were also studying healthy children and teenagers found a frequency of 76% of anti-rubella antibodies, providing similar results to our findings [9]. In a recent paper describing the frequency of IgG to rubella and CMV in 33 healthy people (15 women, mean age 44 ± 25 years) in Turkey, the authors found high titers for rubella (96.9%) and also for CMV (93.9%) [10]. In another study evaluating healthy people from southern Italy, the authors found a prevalence rate of 91.7% for anti-CMV antibodies among 10–15 year old volunteers [11]. These high rates of anti-CMV antibodies were not detected in other regions, since of the 22,260 healthy donors from Germany (12,015 women, mean age 36.4 years) a total prevalence of only 45.8% of anti-CMV antibodies was reported [11]. Ossa et al. examined 129 children from northwest Colombia and found a seroprevalence of 94% and 98% for EBV and CMV, respectively, regardless of gender, age and number of first-degree relatives with these disorders [12]. With respect to T. *gondii*, our results are similar to those described in the literature. Studying a large population in Italy (28,247 serum samples), the authors found a prevalence of 48%, while in another study of 146 healthy volunteers (115 women, mean age 44 years) from the United States 59% of the subjects had these antibodies [13]. Regarding EBV, the seroprevalence of anti-EBV antibodies among children aged 8–10 years was 81.9% in southern Italy [8], whereas in Sweden the same antibodies were found only in 62% of the children tested [14].

The present comparison of five different infectious agents between two different populations, Italian and Colombian subjects, disclosed significant differences in the prevalence of two very common infectious agents: rubella and CMV. The Italian volunteers exhibited a higher prevalence of positive titers to rubella (85%) whereas the Colombians displayed a higher prevalence of titers to CMV (92.9%). This difference in positivity rate might be incidental, yet as described above, various populations differ in their seropositivity rate. It is also possible that this difference may be due to the existence of a relationship between these infectious agents and latitude [4]. Considering that autoimmune diseases may result from both genetic exposure and environmental triggers [15], these differences might influence the prevalence of a given autoimmune disease in an entire population. Nonetheless, it is possible that these differences are also a result of a vaccination strategy in the case of rubella, and moreover, the role of IgM antibodies to these infectious agents and their prevalence were not considered here [16].

The data presented here support the notion that asymptomatic infection or healthy carriage is a common state when considering healthy populations. Our findings might serve as reference data for other studies aiming to evaluate the role of those microorganisms in autoimmune diseases, and highlight the fact that not only genetic factors differ among various ethnic and geographical populations, but also the environmental trigger should be considered as an important variable determinant in the induction of autoimmunity.

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