

Autoantibodies in Nonautoimmune Individuals during Infections

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ABSTRACT: Infections can act as environmental triggers inducing or promoting autoimmune disease in genetically predisposed individuals. Identification of microbial peptides similar to self-tissues may by molecular mimicry, provide the inducing mechanism for an immune response. The aim of this study was to identify autoantibodies (autoAbs) in nonautoimmune individuals during acute bacterial, viral, or parasitic infections. Specific Abs or specific infections with an increased autoAb load may shed insight into the mechanisms of autoimmune disease. Sera from 88 patients with acute infections (41 bacterial, 23 viral, 17 parasitic, and 7 rickettsial) were tested by the ELISA method for antinuclear antibodies (ANA) 8 Pro, and Abs to thyroid peroxidase (TPO),

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thyroglobulin, phospholipids, annexin-V, laminin, anti-*Saccharomyces cerevisiae* (ASCA), and prothrombin, along with 80 normal controls. Elevated titers of Abs to annexin-V and prothrombin were the most prevalent in viral, parasitic, and rickettsial infections and to laminin in viral and parasitic infections. Elevated titers of ASCA and ANA were found in viral and bacterial infections. Antiphospholipid Abs were found in parasitic and Q-fever infections. Thirty-four individuals harbored elevated titers of at least two Abs. An autoAb burden was detected in individuals with hepatitis A, hepatitis B, toxoplasma or Q-fever infections. In nonautoimmune individuals with various (bacterial, viral, parasitic, and rickettsial) infections, elevated titers of Abs to annexin-V, prothrombin, laminin, ASCA, ANA, and phospholipids were most frequently detected.

KEYWORDS: infections; autoantibodies

INTRODUCTION

The relationship between infections and autoimmunity is complex. Current evidence suggests that microbes can initiate and enhance autoimmunity. Infections can act as environmental triggers inducing or promoting autoimmune disease in genetically predisposed individuals.

The relationship between infections and autoimmune disease has been studied extensively over many years. In a current review, the association between bacterial infections (*Klebsiella*, *Mycobacterium tuberculosis*, *Pneumococcus*), viral infections (CMV, EBV, HIV), and the development and/or flare of systemic lupus erythematosus (SLE) were summarized.¹ Recent studies demonstrate that autoreactive B cells, infected by EBV, proliferate and become latently infected memory B cells, which are resistant to apoptosis that occur during normal B cell homeostasis.² In addition, the latent membrane protein 2A (LMP 2A) of EBV shares protein motifs with the B cell receptor, plays a role in B cell receptor signaling, and mimics an activated B cell receptor by providing a survival signal for mature B cells in transgenic mice.^{3,4}

Many clinical studies allude to the importance of infections in the setting of autoimmune diseases. One study describes the clinical characteristics of 100 patients with antiphospholipid syndrome (APS) associated with different infections (skin infection, HIV, pneumonia, hepatitis C, urinary tract infection). APS presenting in the catastrophic form (Asherson's syndrome) is often preceded by an infection.⁵ A mechanism of molecular mimicry was shown by induction of experimental APS through immunization with certain microbial pathogens (*Hemophilus influenzae*, *Neisseria gonorrhoeae*, tetanus toxoid) that share epitope homology with the b2-glycoprotein-I (b2GPI) molecule.⁶ There is a reported association between infections and autoimmune thyroid diseases.^{7,8} All previous studies have investigated the presence of infections in autoimmune individuals. The aim of the present study was to determine

the type and prevalence of several Abs in nonautoimmune individuals during acute infections.

PATIENTS AND METHODS

Patients and Sera

Sera were collected from 88 unselected patients with acute infection determined by blood cultures or appropriate serology collected from two medical centers. These patients were not known to harbor autoimmune diseases. Blood samples were drawn before the initiation of therapy when indicated. There were 23 patients with viral infections (hepatitis A [HAV] [$n = 10$], hepatitis B [HBV] [$n = 5$], and hepatitis C [HCV] [$n = 8$]); 17 patients with parasitic infections (echinococcus [$n = 1$], cysticercosis [$n = 3$], amoebiasis [$n = 2$], malaria [$n = 2$], toxoplasmosis [$n = 7$], schistosoma [$n = 2$]); 41 patients with bacterial infections (*Helicobacter pylori* [$n = 7$], *Staphylococcus aureus* coagulase positive [$n = 5$], *S. aureus* coagulase negative [$n = 2$], methicillin-resistant *S. aureus* (MRSA) [$n = 2$], enterobacter [$n = 2$], *S. pneumonia* [$n = 4$], *S. pyogenus* [$n = 1$], *S. viridans* [$n = 1$], *Stomatococcus* [$n = 1$], *Escherichia coli* [$n = 11$], *Klebsiella pneumonia* [$n = 2$], *Legionella* [$n = 1$], *H. influenzae* [$n = 1$], *Leuconostoc* [$n = 1$]); 7 patients with rickettsial infections (*Rickettsia conorii* [$n = 3$], *Coxiella burnetii* [$n = 4$]); and 80 healthy blood donors from the Israel National Blood Bank.

Detection of Autoantibodies (AutoAbs) Employing the ELISA Method

The panel of the autoAbs analyzed were directed to nuclear components (antinuclear antibodies [ANA]) utilizing the ANA 8 Pro (IgG for the Hep2 positives) (SSA, SSB, Sm, U1RNP, Sm/RNP, centromer, JO-1, Scl-70), thyroid peroxidase (TPO), thyroglobulin (TG), phospholipids (PLs) [cardiolipin (CL), CL in the presence of b2GPI, phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI), sphingomyelin], annexin-V, laminin (LMN), anti-*Saccharomyces cerevisiae* (ASCA) (IgA, IgG), and prothrombin (PT) (IgM, IgG). Briefly, ELISA plates (Aesku Diagnostics, Wendelsheim, Germany) were coated with the autoantigens and blocked with BSA. The sera were added at dilution of 1:100 for 30 min at room temperature. The binding of the specific immunoglobulin was probed by antihuman IgG and antihuman IgM conjugated to peroxidase, incubated for 30 min at room temperature. Appropriate substrate was added and color reaction were detected by an ELISA reader at 450 nm.

Statistical Methods

Associations between categorical variables were examined by a Pearson chi-square test or Fisher's exact test as appropriate. Statistical significance was set at the 5% level. Analyses were performed using SPSS 11.5 software (SPSS Inc., Chicago, IL).

RESULTS

Anti-annexin-V Abs were detected in 60.9% patients with viral infection, in 47.1% of sera from patients with parasitic infection, in 57.1% sera from patients with rickettsial infection ($P < 0.001$), and in 17.1% patients with bacterial infection ($P < 0.03$). Anti-LMN Abs were detected in 30.4% of patients with viral infection ($P < 0.001$), and in 23.5% of patients with parasitic infection ($P < 0.003$). They were also found in 14.3% of Q-fever infection (not significant statistically). Anti-PT Abs were found in 69.6% of individuals with viral infections ($P < 0.001$). In 23.5% of patients with parasitic infection, elevated titers of aPT were detected ($P < 0.003$). Anti-PL Abs were detected in 28.6% of patients with rickettsial infection and 17.6% patients with parasitic infection ($P < 0.036$). None of the patients with bacterial infection had detectable levels of Abs to PL or PS, PC, PE, PI, or sphingomyelin. ASCA IgA and IgG Abs were found in 26.1% of patients with viral infection ($P < 0.014$). ASCA IgA and IgG Abs were detected in 22.0% of patients with bacterial infection ($P < 0.016$). Among the patients with parasitic infection, 17.6% of the sera were positive for ASCA. The sera of 6.3% of blood donors were positive for ASCA. ANA were detected in 21.7% of patients with viral infection ($P < 0.013$) and in 20% of patients with bacterial infection ($P < 0.006$). Three patients with HAV and one with HBV were positive for all eight antigens tested in ANA 8 Pro, whereas another patient with HBV was positive for Sm, U1RNP, Sm/RNP, centromer Abs. ANA were found in 17.6% of patients with parasitic infection but without any statistical significance. None of the patients with rickettsial infection had detectable levels of ANA. Anti-TPO Abs were detected more frequently in healthy blood donors than in those with viral or bacterial infections. None of the patients with parasitic and rickettsial infections had detectable levels of anti-TPO Abs. Anti-TG Abs were not elevated significantly in any of the infectious diseases (TABLE 1). Thirty-four individuals harbored more than one Ab: 16 patients had two Abs, 7 had three Abs, 8 had four Abs, 2 had five Abs, and 1 patient was positive to six Abs.

DISCUSSION

Our study confirmed a high prevalence of serological markers of autoimmunity among nonautoimmune individuals with different infections.

TABLE 1. The prevalence of Abs in nonautoimmune individuals with acute infections

Infection/ autoantibody	<i>P</i> - value	Rickettsial <i>n</i> = 7	<i>P</i> - value	Parasitic <i>n</i> = 17	<i>P</i> - value	Bacterial <i>n</i> = 41	<i>P</i> - value	Viral <i>n</i> = 23	Control <i>n</i> = 80
Annexin-V	0.001	4 (57.1%)	0.001	8 (47.1%)	0.030	7 (17.1%)	0.001	14 (60.9%)	3 (3.8%)
LMN	0.155	1 (14.3%)	0.003	4 (23.5%)	1.000	1 (2.4%)	0.001	7 (30.4%)	1 (1.3%)
aPT	0.016	2 (28.6%)	0.003	4 (23.5%)	0.112	3 (7.3%)	0.001	16 (69.6%)	1 (1.3%)
ASCA	0.405	1 (14.3%)	0.143	3 (17.6%)	0.016	9 (22.0%)	0.014	6 (26.1%)	5 (6.3%)
aPL	0.031	2 (28.6%)	0.036	3 (17.6%)	NR	0	0.215	2 (8.7%)	2 (2.5%)
ANA	NR	0	0.065	3 (17.6)	0.006	8 (20%)	0.013	5 (21.7%)	3 (3.8%)
TG	NR	0	0.443	1 (5.9%)	1.000	1 (2.4%)	NR	0	2 (2.5%)
TPO	NR	0	NR	0	0.162	1 (2.4%)	0.451	1 (4.3%)	9 (11.3%)

LMN = antilaminin antibodies; TPO = antithyroid peroxidase antibodies; TG = antithyroglobulin antibodies; ASCA = anti-*Saccharomyces cerevisiae* antibodies; aPT = antiprothrombin antibodies; aPL = antiphospholipid antibodies; ANA = antinuclear antibodies; NR = not relevant.

We demonstrated the presence of anti-annexin-V Abs at a high frequency in patients with variable infections. Interestingly, we detected anti-annexin-V Abs in 80% of patients with hepatitis A. In addition, a correlation was found between anti-annexin-V Abs and hepatitis B, hepatitis C, malaria, toxoplasmosis, cysticercosis, amoebiasis, *E. coli*, *S. pneumonia*, *Staphylococcus* coagulase negative, *Stomatococcus*, and Q fever. Annexins belong to a family of proteins that are able to bind to negatively charged PLs and membrane bilayers through calcium-dependent interaction.⁹⁻¹¹ Anti-annexin-V Abs, primarily detected in sera of patients with RA, have been shown to display lupus anticoagulant (LAC) activity,¹² and are associated with the occurrence of thrombotic events and/or recurrent abortions in patients with SLE and APS as well as digital ischemia in patients with systemic sclerosis (SSc).^{13,14} There is only one report of the presence of anti-annexin-V Abs in patients with infectious diseases. In this study, sera from 46 patients with infectious mononucleosis (IM), 18 with SLE, 21 with primary APS, 50 with *H. pylori* infection, and 30 healthy blood donors were tested for anti-annexin-V, anti-protein S, anti-PT, and anti-b2GPI Abs. Elevated titers of aCL Abs were detected in IM concomitantly with anti-cofactor protein Abs including anti-annexin-V Ab. However, these Abs seem to occur at a lower frequency in IM than in primary APS.¹⁵ Titers of anti-LMN Abs were elevated in patients with viral and parasitic infections. Serum Abs against LMN have been detected in patients with SSc, Raynaud's phenomena, and the MRL-lpr-mouse strain.¹⁶⁻¹⁸ In a recent study, pathogenic murine lupus Abs reacted with a 21-mer peptide located in the globular part of the alpha-chain of LMN. Treatment of MRL/lpr/lpr mice with these peptides prevented Ab deposition in the kidneys, ameliorated renal disease, and prolonged survival of the peptide-treated mice.¹⁹ The relationship between anti-LMN Abs and infection was observed in some studies. In one study, Abs specific for several uropathogenic strains (*E. coli*, *Pseudomonas* sp., and *Klebsiella* sp.) as well as anti-LMN, anti-PL, and anti-b2GPI Abs analyzed in the sera of 20 patients with long-lasting uncomplicated recurrent infections of the lower urinary tract led to a significant rise in IgG specific for PLs, b2GPI, and mouse LMN.²⁰ In an additional study, sera of 413 patients with inner ear disorders of unknown etiology were tested for anti-LMN Abs and found in 68% of patients with sensorineural hearing loss (SNHL).²¹ Anti-LMN Abs have also been found in Chagas' disease,²² but the anti-LMN-Abs that are present in filariasis have different specificities from those spontaneously produced by individuals with SLE.²³ We discovered that the occurrence of anti-PT Abs was significantly higher in patients with HAV infection compared to other patients. In a meta-analysis, 17 studies on 2339 patients and 613 controls concluded that no clear association with thrombosis was found for aPT Ab, irrespective of isotype, site and type of event, and SLE.²⁴ In some studies, aPS/PT Abs strongly correlated with the clinical manifestations of APS and with the presence of LAC.²⁵ The presence of aPT Abs has not been studied in infections to any degree, and it is only recently that their prevalence in some infections has begun

to emerge. aPT Abs were found in patients with HIV,^{5,26,27} HCV,²⁷ EBV,²⁸ leprosy,^{28–30} malaria,²⁷ and syphilis,^{27,28} but was not associated with the development of thrombosis and/or thrombocytopenia. In another study the prevalence of aPT Ab in chronic hepatitis C infection was not significantly different from controls at either time point and did not support a role for HCV infection in the pathogenesis of the APS.³¹ We found the presence of ASCA in patients with viral and bacterial infections. ASCA, recently proposed as a serological marker of Crohn's disease, have also been detected in other autoimmune disorders, including ankylosing spondylitis, psoriatic arthritis,³² undifferentiated spondyloarthropathy,³² primary biliary cirrhosis,³³ primary sclerosing cholangitis,³³ and Behcet's disease.³⁴ The relationship between infections and ASCA has not been investigated. In our study we found an association between aPL Ab with parasitic (amoebiasis, cysticercosis, toxoplasmosis) and Q-fever infections. One study reviews the relationship between viral infections (HCV, Varicella, parvovirus 19, CMV, HTLV-1, HIV), bacterial infections (typhus, leprosy, TB, mycoplasma pneumonia, *S. aureus*, *Streptococcus*, *Salmonella*, *E. coli*, ornithosis, Q fever, leptospirosis, Lyme disease) and the presence of aPL.³⁵ We found a high prevalence of ANA in patients with HAV and HBV patients. It is interesting that all patients with HAV and one with HBV had Abs to all 8 of the antigens in the ANA 9 Pro. ANA were also found in patients with bacterial infections but none of them were positive for ANA 8 Pro. ANA have been detected in a large variety of infectious diseases, including HCV, HAV, HBV, CMV, *Bartonella vinsonii*, *Ehrlichia canis*, *Leishmania infantum*, malaria, *Chlamydia trachomatis*, schistosomiasis (reviewed in Ref. 1). In one study of 156 patients with acute viral hepatitis, 20.5% of patients were ANA positive, but during convalescence only 6.4% of patients showed ANA positivity.³⁶ In a variety of autoimmune diseases, including rheumatoid arthritis,³⁷ SLE,³⁸ type I diabetes mellitus,³⁹ and inflammatory bowel disease,³⁸ the appearance of specific serum Abs may precede the onset of clinical disease by many years. Predictive Abs may not be directly responsible for specific manifestations of the disease, but are markers of future disease in presently healthy individuals.³⁶ In our study, sera were tested at one time point, and hence we do not know if these Abs are persistent and hence of pathogenic nature or play a role in the development of autoimmunity.

In conclusion, in nonautoimmune individuals with various (bacterial, viral, parasitic, and rickettsial) infections, elevated titers of Abs to annexin-V, PT, LMN, ASCA, ANA, and PLs were the most prevalent. Further investigation is warranted in the predictive value of infection and the development of autoimmune disease.

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