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Association between polyclonal B cell activation and the presence of autoantibodies in mice infected with *Yersinia enterocolitica* O:3

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Abstract

Eight-week old conventional female Swiss mice were inoculated intravenously with *Yersinia enterocolitica* O:3. A second group of normal mice was used as control. Five mice from each group were bled by heart puncture and their spleens were removed for spleen cell collection on the 3rd, 5th, 7th, 10th, 14th and 21st day after infection. Immunoglobulin-secreting spleen cells were detected by the isotype-specific protein A plaque assay. Total immunoglobulin levels were determined in mouse serum by single radial immunodiffusion and the presence of autoantibodies was determined by ELISA. We observed a marked increase in the total number of cells secreting immunoglobulins of all isotypes as early as on the 3rd day post-infection and the peak of secretion occurred on the 7th day. At the peak of the immunoglobulin response, the total number of secreting cells was 19 times higher than that of control mice and most immunoglobulin-secreting cells were of the IgG_{2a} isotype. On the 10th day post-infection, total serum immunoglobulin values were 2 times higher in infected animals when compared to the control group, and continued at this level up to the 21st day post-infection. Serum absorption with viable *Y. enterocolitica* cells had little effect on antibody levels detected by single radial immunodiffusion. Analysis of serum autoantibody levels revealed that *Y. enterocolitica* infection induced an increase of anti-myosin and anti-myelin immunoglobulins. The sera did not react with collagen. The present study demonstrates that *Y. enterocolitica* O:3 infection induces polyclonal activation of murine B cells which is correlated with the activation of some autoreactive lymphocyte clones.

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Key words
- *Yersinia enterocolitica*
- Polyclonal activation
- Experimental infection
- Autoantibodies

Introduction

Intestinal infections with *Yersinia enterocolitica* in humans can be followed by sequelae that include reactive arthritis and other autoimmune diseases (1). The development of arthritis is mostly determined by genetic susceptibility. Approximately 90% of these patients present the HLA-B27 antigen (2).

The pathogenesis of reactive arthritis continues to be obscure. The expression of the disease is probably multifactorial (3). Structures of the bacterial cell envelope becoming similar to, or possibly modifying HLA-B27 antigens with a subsequent autoimmune response may contribute to the triggering of injury to host cells.

Clinical *Y. enterocolitica* infections are followed by the development of autoantibodies including antibodies against smooth muscle, connective tissue, renal tubular epithelium, and the basement membrane of thyroid epithelial cells (4-6). The production of these autoantibodies may result from polyclonal B lymphocyte activation. Resting B cells with receptors directed against autologous structures may be stimulated as a result of the microorganism-host relationship.

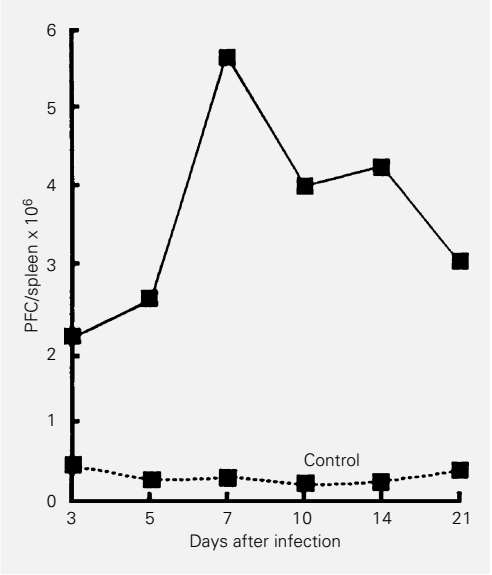

Figure 1 - Number of plaque-forming cells (PFC) of total Ig present in the spleen of control mice and mice infected with 1.2 x 10^8 cells/ml of *Y. enterocolitica* O:3. Results are reported as the average values of plaque-forming cells/spleen for 5 mice in each group. The standard deviation was always less than 25% of the observed values.

The purpose of the present study was to determine the occurrence of polyclonal B cell activation during experimental infection of mice with *Y. enterocolitica* O:3 and to associate this activation with the appearance of autoantibodies in the serum of these animals.

Material and Methods

Yersinia strain

Yersinia enterocolitica biotype 4, serotype O:3, phagotype VIII isolated from human diarrheic feces was studied. This strain harbored the 40-48-MDa virulence plasmid and was positive to virulence tests related to plasmid-mediated gene expression (temperature-dependent autoagglutination, calcium-dependent growth at 37°C and Congo red absorption on CR-MOX agar), and negative to virulence tests related to chromosomal gene expression (pyrazinamidase production, salicin fermentation and esculin hydrolysis).

Experimental infection of mice

Thirty eight-week old conventional female Swiss mice were inoculated intravenously with a 0.5-ml suspension of *Y. enterocolitica* O:3 (1.2 x 10^8 cells/ml). A second group of 30 normal mice was used as control. Five mice from each group were bled by heart puncture and their spleens were removed for spleen cell collection on the 3rd, 5th, 7th, 10th, 14th and 21st day after infection. Sera were separated and stored at -20°C. Spleen cells were utilized immediately in the protein A plaque assay. Cell viability was assessed by Trypan blue exclusion.

Isotype-specific protein A plaque assay

Immunoglobulin-secreting spleen cells were detected by the protein A plaque assay (7). Rabbit anti-mouse Ig was prepared in our laboratory. Rabbit anti-mouse IgM, IgG_1,

IgG_{2a}, IgG_{2b}, IgG_3 and IgA were purchased from SEROTEC (Oxford, England). Each assay was done in duplicate.

Quantitation of total immunoglobulin levels

Total immunoglobulin levels were determined in mouse serum by single radial immunodiffusion according to the method of Mancini et al. (8). Serum absorption with viable bacteria was performed by the method of Ewing (9).

Autoantigens

The autoantigens used in the present study, myosin, myelin and collagen type I, were prepared at the Department of Microbiology and Immunology, UNICAMP, Campinas, SP. Myosin was isolated from rabbit muscle and prepared according to Offer et al. (10), myelin was isolated from mouse brain according to Diebler et al. (11), and collagen type I from mouse tail tendon according to Miller and Rhodes (12).

Enzyme-immunoassay (ELISA) (13)

Polystyrene microtiter plates were sensitized with 100 µl of the autoantigens (myosin, myelin and collagen type I) diluted with 0.06 M sodium carbonate-bicarbonate buffer, pH 9.6, to a concentration of 20 µg/ml. The plates were then incubated for 18 h at 4°C and washed three times with phosphate-buffered physiological saline containing 0.05% Tween 20 (PBS/T). After washing, 100 µl of a serum sample diluted 1/100 in PBS/T containing 1% bovine serum albumin (PBS/T/BSA) was added and incubated for 2 h at 37°C. The plates were washed and 100 µl of peroxidase-conjugated rabbit anti-mouse immunoglobulin diluted 1/1000 in PBS/T/BSA was added. After 1 h of incubation at 37°C followed by an additional wash, 100 µl of 1 mg/ml o-phenylene diamine in 0.1 M citrate phosphate buffer, pH 5.0, containing 0.03% hydrogen peroxide was added and the plates were incubated at 35°C for 10 min. Finally, the reaction was stopped with 100 µl 3 N hydrochloric acid. Absorbance was read with a spectrophotometer (UNISKAN-II, FLOW) at 492 nm. All serum samples were assayed in duplicate and the results are reported as the average value.

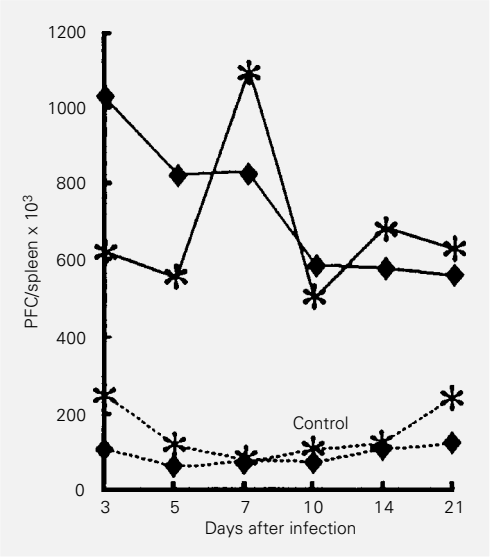

Figure 2 - Number of plaque-forming cells (PFC) of IgM (◆) and IgA (✻) isotypes in the spleen of control mice and mice infected with 1.2 x 10^8 cells/ml of *Y. enterocolitica* O:3. Results are reported as the average values of plaque-forming cells/spleen for 5 mice in each group. The standard deviation was always less than 25% of the observed values.

Results

The determination of the kinetics of immunoglobulin-secreting spleen cells in the mice infected with *Yersinia enterocolitica* O:3 showed a marked increase in the total number of cells secreting immunoglobulins as early as on the 3rd day post-infection and the peak of secretion occurred on the 7th day (Figure 1). At the peak of the immunoglobulin response, the total number of secreting cells was 19 times higher than that of control mice. IgM peaked on the 3rd day and IgA on the 7th day (Figure 2). The cells secreting the IgG subclasses peaked on the 7th day post-infection and most Ig-secreting cells produced the IgG_{2a} isotype (Figure 3).

The determination of total immunoglobulins in the serum of infected animals revealed that on the 10th day post-infection total serum immunoglobulin values were 2

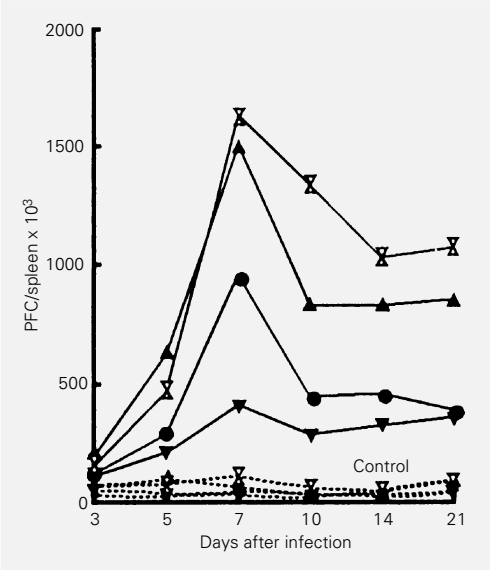

Figure 3 - Number of plaque-forming cells (PFC) of the IgG subclasses IgG_1 (▼), IgG_{2a}, (⧖), IgG_{2b} (●) and IgG_3 (▲) in the spleen of control mice and mice infected with 1.2 x 10^8 cells/ml of *Y. enterocolitica* O:3. Results are reported as the average values of plaque-forming cells/ spleen for 5 mice in each group. The standard deviation was always less than 25% of the observed values.

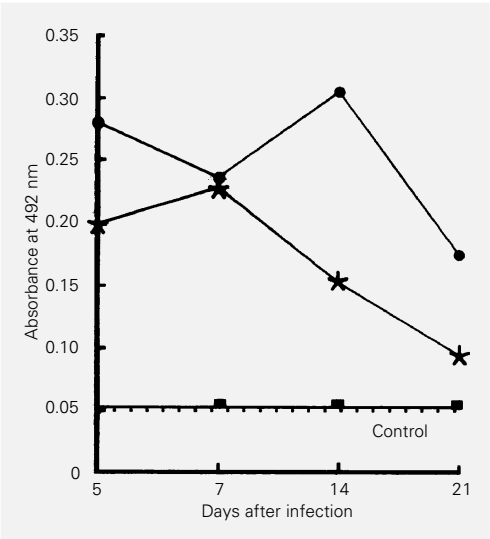

Figure 4 - Kinetics of development of autoantibodies in the sera of mice infected with *Y. enterocolitica* O:3, detected by ELISA. The sera were diluted 1/ 100. The antigens utilized in the sensitization of the plates were myosin (20 µg/ml) (✻), myelin (20 µg/ml) (●) and collagen type I (20 µg/ml) (■). The sera of the control mice were also tested and the average values obtained with the different autoantigens are presented along the dotted line with the origin on the ordinate. The standard deviation was always less than 10% of the observed values.

times higher in infected animals when compared to the control group, and remained at that level up to the 21st day post-infection. When these sera were absorbed with viable *Y. enterocolitica* O:3 cells to remove specific antibodies, we noted that the antibody levels detected by single radial immunodiffusion were 10% lower when compared to the serum not absorbed.

The analysis of autoantibodies revealed that the sera of infected animals reacted with myosin and myelin on the 5th, 7th and 14th day post-infection and the reactivity to myelin was more intense than to myosin. The sera did not react with collagen (Figure 4).

Discussion

The mechanisms by which arthritogenic pathogens interact with the human immune system to produce inflammatory arthritic diseases are not fully understood. These pathogens have the capacity to selectively modulate the immune response of the infected human host and polyclonal lymphocyte stimulation is one of the immunomodulatory mechanisms used by them (14).

In a recent work, Lundgren et al. (15) demonstrated that the outer membrane protein, invasin, of *Y. pseudotuberculosis* is a polyclonal activator of human peripheral B cells.

The results obtained in the present study indicate the occurrence of polyclonal activation of B lymphocytes in animals infected with *Y. enterocolitica* O:3. This activation is not limited to certain isotypes since the number of secretory cells of all isotypes was increased, but the majority of immunoglobulin-secreting cells produced the IgG_{2a} isotype.

The absorption of sera of infected mice with viable *Y. enterocolitica* O:3 cells to remove specific antibodies had little effect on antibody levels detected by single radial immunodiffusion, indicating that infection with the bacterium provoked polyclonal activation of the repertoire of B lymphocytes and not simply of the clones involved in the production of specific anti-*Yersinia* antibodies.

Polyclonal lymphocyte activation induced by bacterial endotoxins and superantigens may contribute to the development of autoimmunity. Bacterial infections may lead to the stimulation of many lymphocytes some of which are autoreactive clones normally anergic to stimulation with autoantigens (16).

Recently, Stuart and Woodward (17) demonstrated that *Y. enterocolitica* serotype O:8 (WA) produces one or more molecules with

properties consistent with those of T cell superantigens. These molecules induce expansion and activation of host T cells and this activation may lead to the expansion of clones of normally suppressed autoreactive T cells and/or favor the production of antibodies by B cells.

Luo et al. (18) reported that immunization of mice with *Y. enterocolitica* O:8 (WA) provoked the development of autoantibodies directed against the thyroid-stimulating hormone receptor (TSHr) and may generate the initial stimulus necessary for the rupture of autotolerance to TSHr, eventually leading to the development of autoimmunity to TSHr.

Although the panel of autoantigens that we used was limited, the present study demonstrates that *Y. enterocolitica* O:3 infection induces polyclonal activation of murine B cells which is correlated with the activation of some autoreactive lymphocyte clones, with the production of autoantibodies for myelin and myosin.

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