

Serum antibody response in patients with mild Typhoid fever from southern Vietnam

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Abstrak

Penelitian ini bertujuan untuk melihat tanggapan kebal terhadap antigen LPS, flagelin dan Vi Salmonella typhi (*S. typhi*) pada pasien-pasien demam tifoid ringan di daerah endemik. Serum akut diperoleh dari 164 penderita dengan hasil kultur darah positif demam tifoid, dan dilakukan pemeriksaan kadar antibodi anti-LPS (total imunoglobulin, IgA, IgG dan IgM), anti-flagella (total Ig, IgG) dan anti-Vi (total Ig, IgG) dalam serum. Variasi antar individu sangat tinggi pada semua kombinasi antigen-antibodi yang diperiksa. Analisis korelasi menunjukkan bahwa hal ini antara lain disebabkan oleh perbedaan umur dan lama demam. Beberapa penderita, khususnya anak-anak, mempunyai tingkat antibodi yang relatif rendah meskipun telah lama sakit. Hasil penelitian kami menunjukkan bahwa uji diagnosis secara serologis dengan antigen yang digunakan pada penelitian ini mungkin memiliki keterbatasan dalam sensitivitas, khususnya pada anak-anak kecil, pada penderita yang belum lama sakit, dan pada orang-orang yang tinggal di daerah endemis.

Abstract

The aim of this study was to describe the antibody response to *Salmonella typhi* (*S. typhi*) antigens (lipopolysaccharide (LPS), flagella, Vi) in patients with non-severe typhoid fever from an endemic region. Acute serum samples were obtained from 164 patients with blood culture proven typhoid fever. Serum levels of anti-LPS (total Immunoglobulin (Ig), IgA, IgG, IgM), anti-flagella (total Ig, IgG) and anti-Vi (total Ig, IgG) antibodies were determined. Inter-individual variation was high for all antibody-antigen combinations investigated. Correlation analyses showed this was due, in part, to differences in age and duration of fever. Some patients had relatively low antibody levels despite a long history of illness, particularly children. Our results suggest that serological diagnostic tests based on the antigens used in this study may have limited sensitivity, particularly in young children, in patient with a short history of illness, and in those living in endemic areas.

INTRODUCTION

Exposure to *S. typhi* can induce a protective immune response, however, this immunity can break down and second infections can occur, particularly if the re-challenge dose is high^{1,2}. The protective components of the immune response are not well defined, but are thought to include both cellular and humoral responses³⁻⁸. The aim of this study was to describe the total immunoglobulin and class-specific antibody responses to the *S. typhi* antigens LPS, flagella, and Vi.

MATERIALS AND METHODS

The study was performed at the Centre for Tropical Disease, a 500 bed infectious diseases hospital in HCMC Vietnam. Patients are referred from the city and the surrounding provinces, including those of the Mekong Delta. Antibodies against *S. typhi* LPS, flagella and Vi were measured in serum samples from 164 Vietnamese patients with bacteriologically confirmed non-severe typhoid fever. Ethical permission was obtained from the hospital and informed consent from the patients or guardians.

LPS was purified from BRD985, a Vi negative an *ompR* mutant of BRD691 (a gift from Medeva). Flagella were purified from BRD691, an *aroC aroD* mutant of Ty2 (kind gift of Medeva). Tyramine-Vi antigen was a kind gift from M.M. Levine.

Standard ELISA protocols were used to detect serum antibodies to LPS, flagella and Vi antigen. Bound immunoglobulin was detected by alkaline phosphatase conjugated detecting antibody and levels interpolated from the standard curve and expressed in arbitrary

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units (i.e. the reciprocal of the $-\log_{10}$ of the corresponding standard dilution). Spearman's correlation co-efficients were used to determine the associations between antibody levels, age and duration of illness.

RESULTS AND DISCUSSION

The median (IQR, range) age of the patients in the study was 18 (11-26, 1-63) years and the median (IQR, range) duration of illness was 12 (9-17, 3-33) days. There was no correlation between age and duration of illness ($r=0.05$, $p>0.05$).

Anti-flagella and anti-Vi total immunoglobulin were found to increase with age ($r=0.36$, $p<0.001$ and $r=0.17$, $p=0.03$, respectively) but there was no correlation between anti-LPS total immunoglobulin and age ($r=0.04$, $p>0.05$).

We were able to detect IgG against all three antigens but were unable to detect IgA and IgM to flagella or Vi. Anti-LPS IgM antibodies were found to decrease with age ($r=-0.36$, $p<0.001$) while anti-LPS IgG was found to increase with age ($r=0.17$, $p=0.03$). There was no correlation between IgA and age ($r=0.04$, $p>0.05$).

Anti-flagella IgG was found to increase with age ($r=0.42$, $p<0.001$) but there was no correlation between anti-Vi IgG and age, however, IgG levels were only determined in adults (>14 years) for Vi antigen.

The results are similar to those from healthy individuals from a typhoid endemic area where anti-LPS agglutinating antibodies, presumably IgM and IgG, were detected in sera of subjects of all ages whereas there was an age related increase in anti-flagella antibodies⁹. The switch in the type of anti-LPS response and the increase in flagella and Vi antibody response suggests that there is an acquisition of some immunity with age.

Significant correlations were found between duration of illness and anti-LPS total immunoglobulin and IgG ($r=0.24$, $p=0.002$), anti-flagella total immunoglobulin and IgG ($r=0.25$, $p=0.001$), and anti-Vi total immunoglobulin and IgG ($r=0.35$, $p<0.001$ and $r=0.31$, $p=0.002$, respectively). When the data were plotted by week of illness, we found total immunoglobulin, for all three antigens, to peak after the third week of illness.

There was no significant correlation between anti-LPS IgM or IgA and duration of illness ($r=0.05$ and 0.1 , respectively, $p>0.05$). It is likely that IgM levels are raised before patients are admitted to hospital and that no further increase is observed. Serum IgA may not be a good indicator of the IgA response which is predominantly found at mucosal sites.

Many patients had relatively low antibody levels despite a long duration of illness. Indeed, the high-interindividual variation and lack of response in some patients suggest that serological tests based on these antigens will have limited sensitivity. Test to detect anti-flagella or anti-Vi antibodies will have greater sensitivity in adults than children, and be of greater use later in the disease than early. Anti-LPS antibodies may be of more use than anti-flagella or anti-Vi antibodies for the diagnosis of typhoid fever.

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