

Involvement of Bacterial Antigens in Immunoglobulin A Nephropathy

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ABSTRACT

To investigate the involvement of bacterial antigens in Immunoglobulin A (IgA) nephropathy, we measured IgA, IgG and IgM antibodies to gram-negative *Escherichia coli* (*E.coli*) and *Haemophilus influenzae* (*H.influenzae*) by ELISA in 24 patients (11 males and 13 females) with IgA nephropathy and 22 normal controls (11 males and 11 females). The titers of IgA and IgM antibodies for *E.coli* and *H.influenzae* were significantly higher in the IgA nephropathy group than in the controls. In addition, IgA and IgM antibody titers for *E.coli* and *H.influenzae* showed a significant positive correlation with serum IgA and IgM levels. These findings suggest that subclinical infection by these bacteria stimulates IgA production and that this may be a factor in the development and progression of IgA nephropathy.

Key words: *Escherichia coli*, *Haemophilus influenzae*, Immunoglobulin A nephropathy

Immunoglobulin A (IgA) nephropathy is the most common form of glomerulonephritis, and since about one third of IgA nephropathy patients progress to chronic renal failure, determining its etiology is an important issue. IgA nephropathy is an immune complex-mediated nephritis, and it has been suggested that a variety of antigens are involved in forming the immune complexes, including those derived from bacteria^{2,3,10}, viruses¹¹) and food^{8,9}). However, the antigens responsible have not yet been identified with any certainty. In the present study, we measured IgA, IgG and IgM antibodies for two common gram-negative bacteria, *Escherichia coli* (*E.coli*) and *Haemophilus influenzae* (*H.influenzae*) in order to determine whether antigens from these microbes were involved in IgA nephropathy.

MATERIALS AND METHODS

Subjects: The subjects comprised 24 patients with IgA nephropathy (11 males and 13 females) and 22 healthy volunteers (11 males and 11 females) as controls. The patients underwent renal biopsy at our hospital and were diagnosed as having IgA nephropathy. Their ages ranged from 13 to 63 years (mean : 36.2 ± 14.5 years), and the period of disease before biopsy was from 3 months to 13 years. None of the patients had received any treatment.

Methods: Bacteria killed in 10% formalin solu-

tion were used as antigen, according to the method of Cost et al¹⁾. *E.coli* and *H.influenzae*, isolated and cultured at the microbiological laboratory of our hospital, were used. Light microscopy showed that the outer membranes of these bacteria had been structurally maintained. One hundred microliters of suspension of each antigen, adjusted to 10^6 microorganisms/ml with carbonated buffer (0.05M, pH 9.6) by McFarland's method⁷⁾, was added to plates for enzyme-linked immunosorbent assay (ELISA) (Becton Dickinson, U.S.A.). The suspensions were allowed to react overnight at 4°C, followed by blocking with 1% PBS-BSA to prevent non-specific binding. Next, 100 μ l each of the serum samples diluted 1:200 and 1:300 for IgA, 1:6000 for IgG and 1:800 for IgM, were added to the plates containing *E.coli* and *H.influenzae*, respectively, and were allowed to react for 2 hours at room temperature. One hundred microliters each of a 1:4000 dilution of peroxidase-labeled anti-human IgA antibody (TAGO, U.S.A.), 1:8000 dilution of peroxidase-labeled anti-human IgG and IgM antibody (TAGO, U.S.A) were added and allowed to react for 1 hour. After color development with O-phenylenediamine, absorbance was determined at 492 nm, and expressed in arbitrary units (a.u.). Blood samples were collected at hospitalization for renal biopsy, and preserved at -30°C until measurement. Informed consent to undergoing this

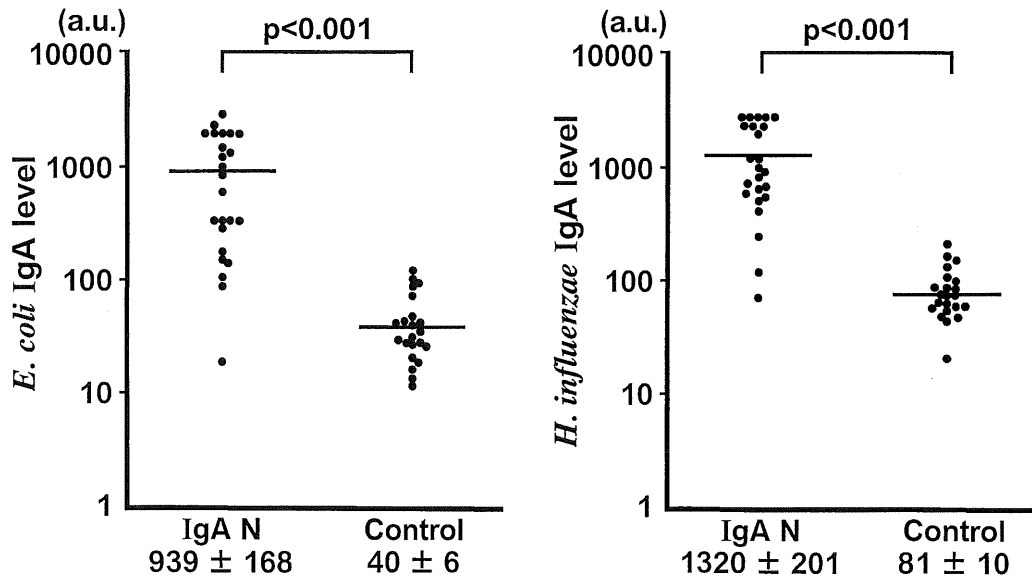


Fig. 1. Serum IgA antibody levels for *E.coli* and *H.influenzae* in the IgA nephropathy and control groups. Both antibody levels were significantly higher in the IgA nephropathy group than in the controls.

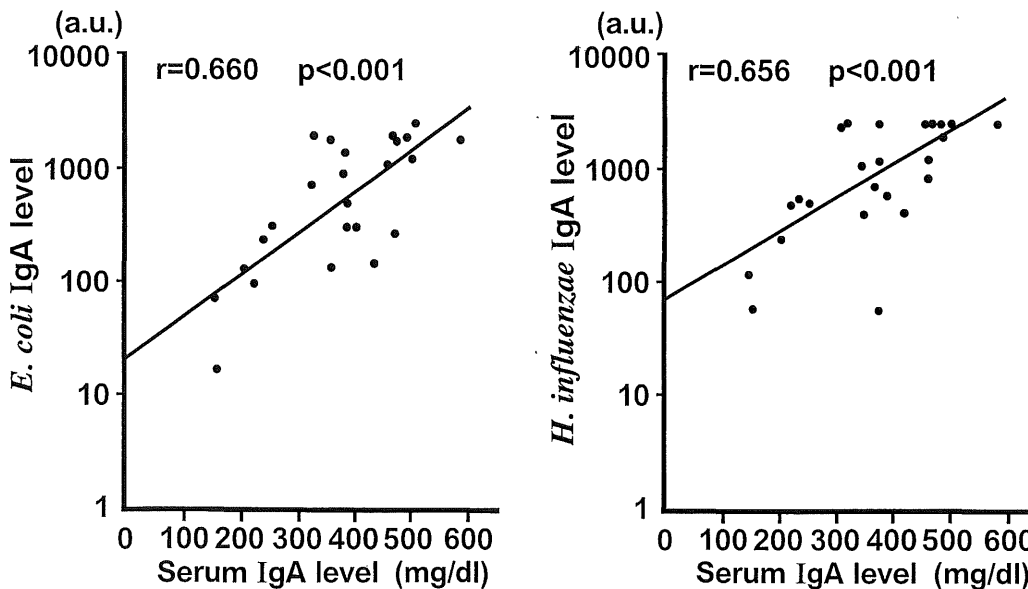


Fig. 2. Relationship of the serum IgA antibody level for *E.coli* and *H.influenzae* to the serum IgA level in IgA nephropathy.

Both antibody levels showed a significant positive correlation with serum IgA level.

test was obtained at the time of blood collection.

Statistical analysis: Results are shown as the mean \pm S.E. The significance of differences was determined by the Mann-Whitney U-test, and linear regression analysis was performed.

RESULTS

As shown in Fig. 1, the IgA antibody titers to *E.coli* and *H.influenzae* were significantly higher in the IgA nephropathy group than in the healthy controls. In addition, there was a signifi-

cant positive correlation between the IgA antibody titers to either *E.coli* or *H.influenzae* and the serum IgA level (Fig. 2). The IgM antibody titers to *E.coli* and *H.influenzae* showed the same outcome (Figs. 3, 4). Nevertheless, the IgG antibody titers to *E.coli* and *H.influenzae* showed no difference between the IgA nephropathy group and the healthy controls (Figs. 5, 6).

DISCUSSION

To investigate the relationship between IgA

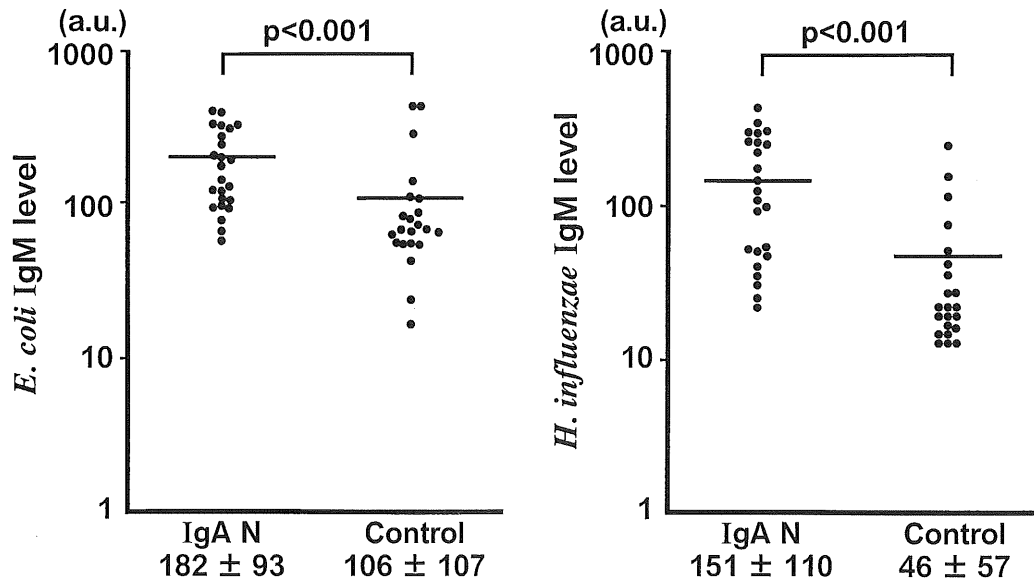


Fig. 3. Serum IgM antibody levels for *E.coli* and *H.influenzae* in the IgA nephropathy and control groups. Both antibody levels were significantly higher in the IgA nephropathy group than in the controls.

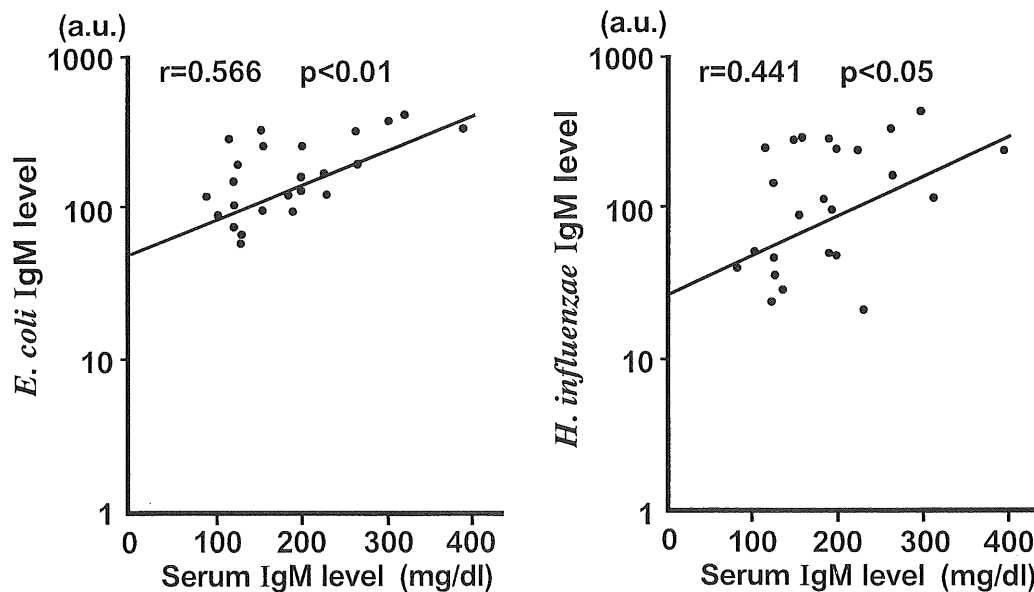


Fig. 4. Relationship of the serum IgM antibody level for *E.coli* and *H.influenzae* to the serum IgM level in IgA nephropathy.

Both antibody levels showed a significant positive correlation with serum IgM level.

nephropathy and bacterial antigens, Isaacs et al⁶) and the present authors⁵) have created models of this disease in mice by intraperitoneal injection or oral administration of dextran, respectively.

Subsequently Endo et al⁴) created a model in mice using gram-negative bacteria. In clinical studies of IgA nephropathy, Drew et al³) found an increase of IgA antibodies to pneumococcal polysaccharides, and Davin et al²) found an increase of IgA antibodies to the α -galactosyl group shared by *Mycoplasma pneumoniae* and *E.coli*. More re-

cently, Suzuki et al¹⁰) found an increase of IgA antibodies to *Haemophilus parainfluenzae* outer membrane (OMHP). *E.coli* is part of the normal intestinal flora, while *H.influenzae* is both a major respiratory tract pathogen and part of the normal flora of the mouth and nasopharynx.

The results of the present study suggest that even when there is no clinically evident infection, chronic stimulation of lymphoid tissues (tonsils, bronchus-associated lymphoid tissue, gut-associated lymphoid tissue, etc.) by these gram-

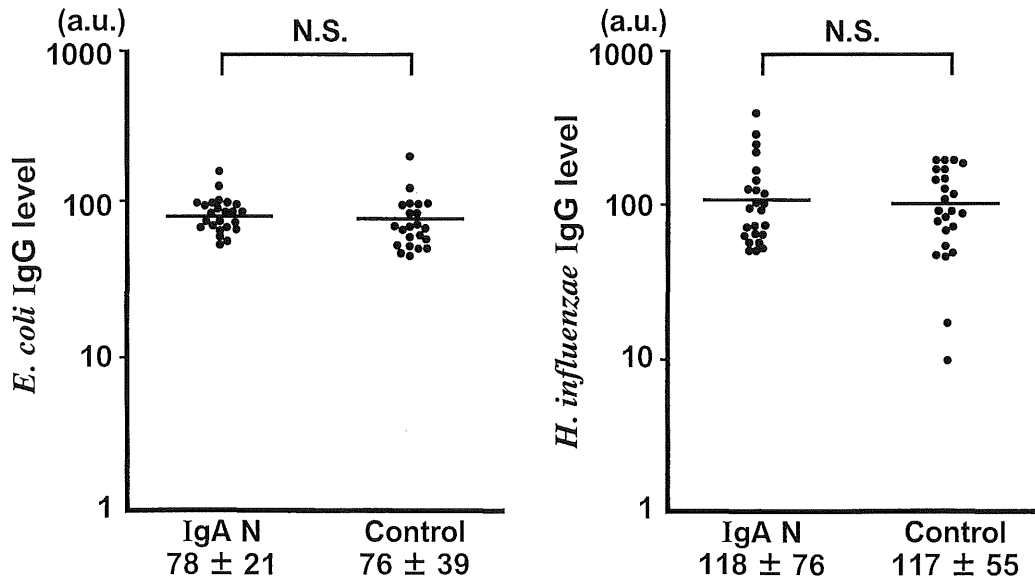


Fig. 5. Serum IgG antibody levels for *E.coli* and *H.influenzae* in the IgA nephropathy and control groups. Neither antibody level showed any a difference between the two groups.

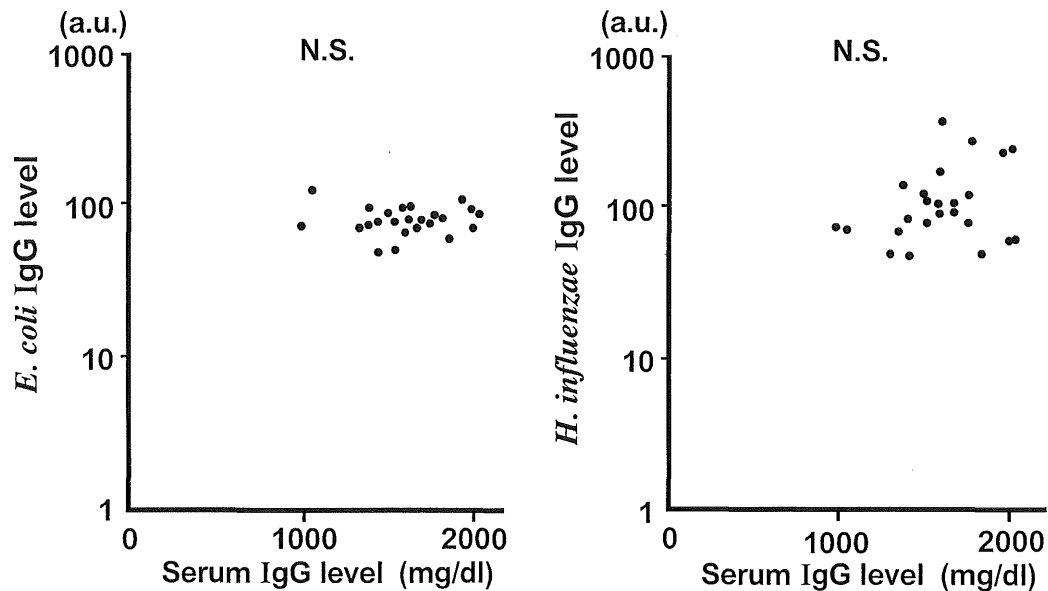


Fig. 6. Relationship of the serum IgG antibody level for *E.coli* and *H.influenzae* to the serum IgG level in IgA nephropathy.

Neither antibody level showed any a correlation with serum IgG level.

negative bacteria may occur in IgA nephropathy patients. This may lead to the overproduction of IgA antibodies to certain bacterial antigens, and precipitate the development of IgA nephropathy.

As for the increase of IgM antibody, it was suggested that a systemic immune response (acute phase) to gram-negative bacteria may occur more easily in the IgA nephropathy group than in the healthy controls. Since we used crude antigens in

the present study, future studies need to determine which bacterial constituents (lipopolysaccharide, capsular polysaccharide, lipoprotein, porin, etc.) are related to the production of IgA antibodies.

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