Anti-streptococcal Cell Membrane and Anti-human Glomerular Basement Membrane Titers in Sera of Patients with Poststreptococcal Acute Glomerulonephritis and Anaphylactoid Purpura

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To clarify the immunological mechanisms in poststreptococcal acute glomerulonephritis (PSAGN) and anaphylactoid purpura (AP), anti-streptococcal cell membrane (anti-SCM) and anti-human glomerular basement membrane (anti-GBM) titers in the sera of patients with PSAGN and AP were determined by passive hemagglutination with chromic chloride-treated sheep erythrocytes. Sodium lauryl sulfate (SLS) soluble SCM and collagenase soluble GBM were used as soluble antigens. Positive anti-SCM titers (> 1:8) were demonstrated in 10 of 14 patients (71.4%) with PSAGN and in 4 of 9 patients (44.4%) with AP with evidence of antecedent streptococcal infection. Two of 4 patients with AP without evidence of antecedent streptococcal infection had positive anti-SCM titers. No correlation was noted between anti-streptolysin O (ASO) titers and anti-SCM titers in patients with PSAGN or AP, but many patients with high ASO titers also had high anti-SCM titers. No positive anti-GBM reactions were detected in patients with PSAGN or AP. No cross-reactions were noted between SLS soluble SCM and collagenase soluble GBM.

The association between Group A streptococcal infection and poststreptococcal acute glomerulonephritis (PSAGN) is well known but the immunological mechanisms are not completely understood. A relationship between anaphylactoid purpura (AP) and an antecedent streptococcal infection has also been suggested by several investigators but the precise pathogenesis of this disease is not known. To clarify the immunological mechanisms involved in PSAGN and AP, we prepared purified Group A streptococcal cell membrane (SCM) by enzymatic disruption and determined the anti-SCM titers in the sera of these patients by passive hemagglutination (PHA) with chromic-chloride treated sheep erythrocytes.

MATERIALS AND METHODS

Strains of Streptococci and Bacteriophage

Group C streptococcal strain 26RP66 (#88) and C 1 phage (#343) for the preparation of phage-associated lysin (PAL) were kindly supplied by Dr. M. K. Wittner, Chicago University. Group A streptococcal strain (Type 12, Tanaka strain) was isolated from a patient with PSAGN.

Preparation of SCM by Treatment with Group C Streptococcal PAL

Crude PAL was prepared by a modification of the procedure reported by Fischetti et al. and

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1. C 1 Phage Preparation
2. Preparation of Crude Lysin
   TH broth + 18hr culture of Group C Streptococcus
   Culture for 2hr at 37°C
   add C 1 phage lysate
   incubate for 13~16 min at 37°C
   quickly centrifuge at 25~30°C
   incubate the cells until lysis occur
   centrifuge at 15,000 rpm for 2hr
   centrifuge at 36,000 rpm for 5hr
3. Preparation of Group A Streptococcal Cell Membrane
   collect Group A Streptococcal cells at exponential phase
   add a suitable volume of Lysin
   incubate for 1hr at 37°C
   add DNase and incubate for 1hr
   incubate in buffer containing DNase and RNase
   tentative determination of Rhamnose concentration
   add an adequate volume of Lysin
   wash several times
   lyophilize
   final determination of Rhamnose concentration
   store at 4°C

Fig. 1. Preparation of group A streptococcal cell membrane

SCM by a modification of Freimer's method
(Fig. 1).

Solubilization of SCM Antigen
SCM antigen for PHA was solubilized by the method of Blue and Lange
SCM was added (0.5% w/v) to a 1% solution of sodium lauryl
sulfate (SLS) and stirred overnight at 37°C.
After centrifugation at 10,000 r.p.m., the supernatant was dialysed against deionized water
changed daily, for 8 days, then centrifuged again
at 10,000 r.p.m. and lyophilized.

Isolation and Solubilization of GBM
Human glomeruli were isolated from the
cortices of necropsy kidneys by sieving meshes
by the method of Spiro. GBM was isolated
from sonicated glomeruli. Solubilization of

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Correlation between Anti-SCM and ASO Titers in Sera of Patients with PSAGN

There was no correlation between these titers in patients with PSAGN, but many patients with high ASO titers also had high anti-SCM titers.

Serial Determination of Anti-SCM Titers in Sera of Patients with PSAGN

As shown in Fig. 2, 10 of 14 cases (71.4%) had positive anti-SCM reactions. There were no apparent differences in clinical or laboratory findings (urinalysis, serum complement, serum BUN and so on) between 10 patients with positive and 4 with negative anti-SCM titers, but all 6 patients with Group A streptococci in throat cultures had positive anti-SCM titers. Figure 3 shows serial anti-SCM and ASO titers in 7 patients with PSAGN who were followed for more than 30 days after onset. In Case 5, for example, there was a good correlation between anti-SCM and ASO titers during the course of the illness. On the other hand, in Case 7 there was no correlation.

Correlation between Anti-SCM and ASO Titers in Sera of Patients with AP

Nine cases had some evidence of antecedent streptococcal infection. No correlation between anti-SCM and ASO titers was seen in the sera of patients with AP.

Serial Anti-SCM Titers in Sera of Patients with AP

As shown in Fig. 4, 4 of 9 patients (44.4%) had positive anti-SCM reactions. Figure 5 shows serial anti-SCM and ASO titers, and urinary protein in 4 cases of AP with nephritis followed for more than 2 months. In Cases 1 and 3, ASO titers gradually decreased, but positive anti-SCM titers persisted and massive urinary protein

**RESULTS**

**Human Sera**

Sera were obtained from 14 patients with PSAGN and 13 with AP, at least twice from each patient. Control sera were obtained from 10 healthy children with no evidence of recent streptococcal infection or renal disease. These sera were kept at -20°C until use.

**PHA Tests**

PHA was carried out in "U-type" microtitration plates (DIV Beckton Dickinson & Co., Falcon plastics, USA) by the method of Perucca et al. Each serum sample was incubated with an equal volume of washed sheep erythrocytes for 120 min at 0°C for absorption of heterophilic antibodies. The protein concentration of SCM and GBM antigen was adjusted to 1 mg/ml in PO₄-free saline.

Anti-SCM titers and anti-GBM titers of control sera were ≤ 1:8 and ≤ 1:2, respectively. Therefore, higher titers were regarded as positive.

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continued without improvement. On the other hand, in Cases 2 and 4, anti-SCM titers remained negative and albuminuria gradually decreased. In addition to these 9 cases, another 4 cases of AP with no evidence of antecedent streptococcal infection were examined (not included in the Figures). Anti-SCM reactions were positive in 2 of them.

**Anti-GBM Titers in Sera of Patients with PSAGN or AP**

No positive anti-GBM reactions were seen.

**DISCUSSION**

The routine diagnosis of Group A streptococcal infection is based on positive throat cultures and significant elevations of antibody titers against streptococcal extracellular substances, ASO, ASK or anti-DNase B. However, the early administration of antibiotics prevents these laboratory tests from being positive.

Therefore, other diagnostic procedures are necessary, for instance, streptococcal intracellular substances 24–28.

We prepared purified SCM and attempted to determine the anti-SCM titers in the sera of patients with PSAGN or AP. Clarifying the role of cross-reactivity between SCM and GBM in the pathogenesis of PSAGN was another aim of our present investigation.

The quantitative determination of anti-SCM antibodies in human and monkey sera has been described recently by Banchun et al 29 Using enzyme-linked immunosorbent assay (ELISA) with sodium deoxycholate extracts of SCM, they found that 7 of 27 patients with recent streptococcal infection had positive anti-SCM antibody levels.

When SCM is used as antigen, the most important point is its purity. We prepared SCM by the enzymatic cell disruption method, which is said to be superior to the mechanical disruption method.

Our chemical analytic data 49 were similar to those of other investigators. Transmission electron micrographs of SCM revealed the typical double layer structure of bio-membranes 39. Solubilization was attempted with 1% SLS on the following 3 substances: our SCM, crude streptococcal M-protein (kindly supplied by Dr. Murai, Department of Public Health, Toho University School of Medicine, Tokyo) and streptococcal peptidoglycan (kindly supplied by Dr. Hayama, Department of Microbiology and Immunology, Nippon Medical School, Tokyo). SCM was completely solubilized within several minutes at room temperature, but the latter 2 could not be solubilized. Thus, our SCM appears to be pure enough for further investigations.

In the present study, no patients with PSAGN or AP had positive anti-GBM reactions; thus, cross reactivity between SCM and GBM was not found. Banchun et al 29 have reported that anti-SCM antibody was not present in the sera of 165 monkeys immunized with human GBM. Lange 30 has noted cross reactions between SCM and trypsin or cyanogen bromide soluble GBM antigen, but not collagenase soluble GBM antigen. Thus, the cross-reactivity between SCM and GBM has not as yet been clarified.

In this study, anti-SCM titers were demonstrated in 71.4% of patients with PSAGN and in 44.4% of those with AP with evidence of antecedent streptococcal infection. Although there was no correlation between anti-SCM and ASO titers, all 6 patients with PSAGN who had positive throat cultures for Group A streptococci also had positive anti-SCM titers. Two of 4 patients with AP with no evidence of antecedent streptococcal infection also had positive anti-SCM titers. Moreover, among 4 patients with AP having nephritis, who were followed for more than 2 months, 2 with massive albuminuria who showed no improvement continued to have positive anti-SCM titers.

Although these several interesting results were obtained in the present study, our method of solubilization of SCM is still incomplete. As is well known, SLS, a powerful detergent, solubilizes bio-membrane, but it is also a strong protein denaturant 31. Therefore, non-ionic detergents, such as Triton-X 100, may be better agents for the solubilization of SCM 31. Our study of these points will be reported in the near future.

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