INFECTION AND IMMUNITY, June 1977, p. 1024-1026
Vol. 16, No. 3

Printed in U.S.A.

NOTES

Detection of Anti-Exfoliatin Antibodies in Healthy Adults and Children by the Passive Hemagglutination Test

YASUNAGA SARAI,[1]* HIDEOMI NAKAHARA,[2] TOMOAKI ISHIKAWA,[3] ISAMU KONDO, SHOHEI FUTAKI, AND KAORU HIRAYAMA

Departments of Microbiology and Dermatology, The Jikei University School of Medicine, Tokyo 105, Japan*

Received for publication 10 December 1976

Sera collected from healthy adults and children were examined by the passive hemagglutination test for the presence of antibodies to exfoliatin A and B.

Clinical manifestations of the staphylococcal scalded-skin syndrome (SSS) have been known to be caused by the production of exfoliatin in vivo by certain strains of *Staphylococcus aureus* (2, 5, 6, 9). It is interesting to note that this syndrome occurs mainly in young children and very rarely in adults (1, 4). The presence of humoral antibodies, which can neutralize the action of exfoliatin, may play a role in this age-related factor.

Recently, in this respect, Wiley et al. (11) studied these anti-exfoliatin antibodies in sera collected from 64 adults and children selected at random. By the radioimmunological assay, they revealed that 73% of these individuals tested had some antibodies to exfoliatin in their sera. However, they did not find a specific antibody titer distribution according to age, nor did they find evidence of increasing titer with age.

We have uncovered a new type of exfoliatin (exfoliatin B), which has different physicochemical and serological properties from the known type (exfoliatin A) (7), and we have also isolated staphylococcal strains that produce both types of exfoliatins concurrently (8).

With these two types of exfoliatin as sensitizing antigens, we conducted a passive hemagglutination (PHA) test with the sera collected from 400 healthy Japanese. Purified preparations of exfoliatin A and B were obtained by the method previously described (6, 7), and the purity was assessed by immunodiffusion and immunoelectrophoresis. We closely followed the original procedure of Boyden for PHA tests (3) with the microtiter technique. The antibody titer is expressed as the reciprocal number of the maximum serum dilution showing complete hemagglutination. Rabbit anti-exfoliatin A and B sera were used as positive controls. The titers of two control antisera to exfoliatin A and B were 16,000 and 64,000, respectively. The details of the immunization of rabbits for this purpose are described elsewhere (6, 7).

The sera for this series of tests were supplied by the Blood Bank of Jikei University Hospital. Sera were obtained from 200 children, 1 to 5 years of age, the most susceptible ages to SSS (10), and 200 adults, 20 to 50 years of age. In selecting the samples, we tried to eliminate all individuals who had had a history of SSS; however, there is always the difficulty of ascertaining what is "normal" with respect to staphylococcal infections.

The distribution of exfoliatin A and B antibody titers, as determined by the PHA test in our series of 400 normal human sera, is shown in Fig. 1. The adult's and children's distribution patterns for both anti-exfoliatin A and anti-exfoliatin B show significant similarities, in particular, the co-occurence of two antibody titer peaks, one at less than 4 and another between 64 and 256. The results indicate that serum with an antibody titer of more than 64 should be regarded as positive and that of less than 4 should be considered as negative.

In the preliminary examination, sera from four convalescing SSS patients, from whom exfoliatin A-producing staphylococci were isolated, showed anti-exfoliatin A titers of 64, 256, 1,024, and 4,096, respectively. Therefore, an antibody titer of 64 or more is considered as positive.

The number and percentage of cases with a positive PHA test for both exfoliatin A and B are shown in Table 1. With exfoliatin A, the

[1] Present address: Department of Pediatrics, National Medical Center Hospital of Japan, 1, Toyamacho, Shinjuku-ku, Tokyo 162, Japan.

[2] Present address: Department of Hygiene, The Jikei University School of Medicine, Tokyo 105, Japan.

[3] Present address: Department of Internal Medicine, The Third Hospital, The Jikei University School of Medicine, 106, Izumi, Komae, Tokyo, Japan.

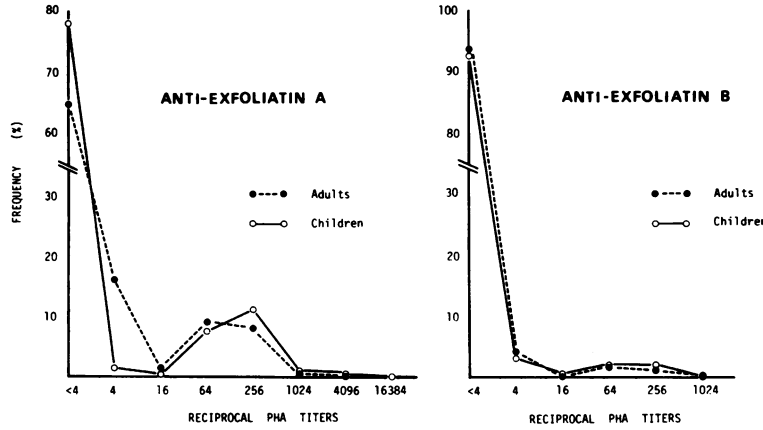

FIG. 1. *Distribution of PHA titers for anti-exfoliatin A and B in healthy human sera. Sera from 200 adults and 200 children were used. The same individuals were subjected to the PHA test for both types of exfoliatin.*

TABLE 1. *Prevalence of antibodies to exfoliatin A and B in sera from healthy adults and children*

Type of exfoliatin	Subject[a]	No. (%) of sera giving a positive[b] PHA test	
A	Children	40[c]	(20)
	Adults	35[d]	(17.5)
B	Children	8[c]	(4)
	Adults	5[d]	(2.5)

[a] Adults: 200, children: 200.
[b] PHA antibody titer of 64 or greater was regarded as positive.
[c] $P < 0.01$.
[d] $P < 0.01$.

percentages of positive cases were 20% for children and 17.5% for adults. With exfoliatin B, the percentages of positive cases were 4% for children and 2.5% for adults. These results showed that the distribution of antibody titers in normal adults and children was almost identical and that nearly 80% of the samples were negative.

It is not yet clear why the PHA test detected fewer positive sera (2.5 to 20%) for exfoliatin antibodies, whereas the radioimmunobinding assay detected more positive sera (73%) for the "same" antibodies. These discrepancies might be caused by differences in the antigens used, in the ethnic origin of the subjects (Japanese versus American), and in the technical details.

In adults and children, respectively, the markedly higher percentage of anti-exfoliatin A antibodies than anti-exfoliatin B antibodies is statistically significant ($P < 0.01$) (Table 1). Infrequent infections by the exfoliatin B-producing staphylococci and/or a lower antigenicity of exfoliatin B than exfoliatin A are considered to be possible causes for this difference in percentage.

Also, we performed an in vivo toxin neutralization test (6, 7) to examine the specificity of this PHA test. All the sera with antibody titers of 250 or more in the PHA test neutralized the action of homologous types of exfoliatin, but the sera with antibody titers of 16 or less did not have this neutralizing effect. However, among sera with titers of 64, both the ability to neutralize in some cases and the inability to neutralize in others was observed.

From these results, the PHA test can be considered as a sensitive method to detect exfoliatin antibodies. Wiley et al. (11) also showed that the neutralization test was less sensitive than their radioimmunobinding assay.

Because our results showed that the exfoliatin antibody titers in adults and children were similar, we were unable to explain, by a humoral antibody alone, the susceptibility of children to SSS. Therefore, it may be considered that additional complicated factors, such as cell-mediated immunity and other humoral factors, influence the manifestation of SSS.

In the hope of resolving the unsolved questions mentioned above, we are conducting further studies on the exfoliatin antibodies in the sera from SSS patients, with this PHA test.

We are grateful to Akio Kobayashi, professor of the Department of Parasitology, for helpful discussions and valuable suggestions.

LITERATURE CITED

1. **Achten, G., G. De Dobbeleer, N. Moriamé, P. Gottignies, R. Kahn, and P. Potvliege.** 1975. Staphylococcal scalded skin syndrome in an adult. Dermatologica 151:342-348.
2. **Arbuthnott, J. P., J. Kent, A. Lyell, and C. G. Gemmell.** 1971. Toxic epidermal necrolysis produced by an extracellular product of *Staphylococcus aureus*. Br. J. Dermatol. 85:145-149.
3. **Boyden, S. V.** 1951. The adsorption of proteins on eryth-

rocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. J. Exp. Med. 93:107–120.

4. **Epstein, E. H., P. Flynn, and R. S. Davis.** 1974. Adult toxin epidermal necrolusis with fatal staphylococcal septicemia. J. Am. Med. Assoc. 229:425–427.
5. **Kapral, F. A., and M. M. Miller.** 1971. Product of *Staphylococcus aureus* responsible for the scalded-skin syndrome. Infect. Immun. 4:541–545.
6. **Kondo, I., S. Sakurai, and Y. Sarai.** 1973. Purification of exfoliatin produced by *Staphylococcus aureus* of bacteriophage group II and its physicochemical properties. Infect. Immun. 8:156–164.
7. **Kondo, I., S. Sakurai, and Y. Sarai.** 1974. New type of exfoliatin obtained from staphylococcal strains, belonging to phage groups other than group II, isolated from patients with Impetigo and Ritter's disease. Infect. Immun. 10:851–861.
8. **Kondo, I., S. Sakurai, Y. Sarai, and S. Futaki.** 1975. Two serotypes of exfoliatin and their distribution in staphylococcal strains isolated from patients with scalded skin syndrome. J. Clin. Microbiol. 1:397–400.
9. **Melish, M. E., L. A. Glasgow, and M. D. Turner.** 1972. The staphylococcal scalded-skin syndrome: isolation and partial characterization of the exfoliative toxin. J. Infect. Dis. 125:129–140.
10. **Rook, A., and S. O. B. Roberts.** 1972. Bacterial infections, p. 475–537. *In* A. Rook, D. S. Wilkinson, and F. J. G. Ebling (ed.), Textbook of dermatology, 2nd ed., vol. 1. Blackwell Scientific, Oxford.
11. **Wiley, B. B., L. A. Glasgow, and M. Rogolsky.** 1976. Staphylococcal scalded-skin syndrome: development of a primary binding assay for human antibody to the exfoliative toxin. Infect. Immun. 13:513–520.