AN EPIDEMIC OF ACUTE RESPIRATORY TRACT INFECTION AMONG CHILDREN CAUSED BY HA VIRUS: I. SEROLOGY*

VERONICA F. CHAN, B.S.HYG., C.P.H.

and

LOURDES ESPIRITU-CAMPOS, M.D., M.P.H.

Department of Medical Microbiology, Institute of Hygiene
University of the Philippines

In late July through mid-September, 1957, the Children's Memorial Hospital (CMH) in Banawe, Quezon City, recorded a total admission of 142 infants and young children with acute respiratory illness given the diagnosis of acute bronchiolitis. The illness was associated with sudden onset of high fever, restlessness and even sleeplessness. Among younger patients, the manifestation of respiratory distress was observed to be more severe while the febrile reaction was slight. On the other hand, the older patients experienced more severe febrile reaction and less respiratory distress. The frequency of cases was highest in the one-month to one year age group (1). The CMH and the Virus Laboratory, Department of Medical Microbiology of the Institute of Hygiene, embarked on a collaborative effort to study the illness. The plan was to study the disease simultaneously from the clinical as well as the laboratory points of view. A paper on the clinical aspects of the disease has already been published (1).

Workers abroad have associated a number of recognized viruses as responsible for respiratory illnesses among humans. Aside from the various influenza viruses, agents that have already been cited in reports are the adenoidal-pharyngeal-conjunctival (APC) group of viruses (2, 3, 4), hemadsorption (HA) viruses (5), and with less certainty, the croup-associated (CA) virus (6), respiratory syncytial (RS) virus (7,8).

*This study was aided by a grant from the Natural Science Research Center of the University of the Philippines. Research allotment No. 16, year 1958-1959.

Johns Hopkins (JH) virus (9), and 2060 virus (10). With the "backdoor approach" (2) as a guide, work has been done employing some of the viruses mentioned. The availability of seed virus in our laboratory dictated the use of the viruses in our investigation.

The purpose of this paper is to present a preliminary report of the immunological responses of the cases studied. Isolation of virus will form the basis of succeeding reports.

MATERIALS AND METHODS

Serum collection: Blood specimens were extracted aseptically, early in illness and approximately two weeks later, from acute bronchiolitis patients at CMH. In both instances, blood samples were iced soon after extraction and while in transit to the virus laboratory. After allowing the blood clot to retract at 4°C., the sera were separated and stored in a -20°C. freezer.

Out of the 29 patients ill with acute bronchiolitis that were bled during the acute phase of illness, only 17 were subsequently extracted for the convalescent sample. Hence, these 17 paired sera comprised our study group.

Complement fixation (CF) test:

(a) Preparation of antigen: Lyophilized adenovirus type 4 was kindly sent to us by Dr. Garrison Rapmund of the Walter Reed Army Medical Research Center, Washington, D.C., U.S.A. In our laboratory, the adenovirus type 4 was grown in bottles of HeLa cells. When the cells showed sufficient degree of cytopathic changes, the bottles were frozen and thawed alternately, and this procedure was performed twice. Fluids were pooled and centrifuged in a refrigerated International Centrifuge (Model PR-2) at 2,500 rpm for 20 minutes. The clear supernate was ampouled, sealed and shell frozen and stored at -60°C. This preparation served as CF antigen. Uninfected bottles of HeLa cells were similarly treated and served as control.

(b) Immune serum: Rabbits used for the preparation of immune serum were pre-bled. A series of ten biweekly intravenous injections were scheduled and ten days after the last,

the rabbits were bled by cardiac puncture. Serum was stored at -20°C.

(c) Method: Briefly, the method employed 2 units of antigen, 2 full units of complement, 2 units of hemolysin and overnight fixation at 4°C. (11, 12). The highest dilution of serum which exhibited 75% or greater fixation of complement was considered the endpoint. Antibody rise in titer of fourfold or greater was considered significant.

Hemagglutination Inhibition (HI) Test:

(a) Preparation of antigens: HA viruses type I and II were sent to us through the courtesy of Dr. R.M. Chanock of the National Institutes of Health, Bethesda, Maryland, U.S.A. Monkey kidney cells grown in blake bottles prepared at our laboratory according to the technique of Younger *et al.* (13) were used to propagate the viruses. The monkey kidney tissue culture fluids similarly treated as that of the adenovirus type 4 were used as antigens. Uninoculated monkey kidney cells prepared in like manner served as controls.

Infected chorioallantoic fluids of 12-day old embryonated eggs were sources of antigens for mumps virus and the influenza viruses: A'/FMI/47, A/PHIL/57, A/PR8/34, A/Swine-15 1976/31, A/Denver/57, B/Lee/40 and D/Sendai/52.

(b) Immune serum: Immune sera were prepared in big roosters that were pre-bled. Ten days after a series of intravenous injections, the roosters were test-bled. If satisfactory titers were obtained, the roosters were exsanguinated by cardiac puncture. Sera were stored frozen at -20°C. without preservative.

(c) Method: The sera were pre-treated with trypsin in phosphate buffer pH 8.2 and inactivated at 56°C. for 30 minutes (14). Guinea pig erythrocytes were used in the HI test of the HA viruses allowing the erythrocytes to sediment at 4°C. and the test read by the pattern method after 2 hours (5). With the influenza viruses, fowl erythrocytes were employed, sedimentation taking place at room temperature and the test read by the pattern method after one hour. As in the CF test, a fourfold or greater rise in antibody titer was considered significant.

RESULTS

Complement Fixation

Paired sera obtained from 17 children with acute bronchiolitis were tested for development of complement fixing antibodies against adenovirus type 4. Results are summarized in Table 1. Only one out of 17 cases showed a significant rise in CF antibody titer.

Hemagglutination Inhibition:

For the purpose of economy on serum samples, only the convalescent phase of the 17 paired sera were tested by HI test against the following influenza viruses: A'/FM1/47, A/PR8/34, A/Swine-15 1976/31, A/Denver/57, and B/Lee/40. In no single instance was an antibody titer greater than 1:8 of serum dilution.

A/Phil/57, an Asian influenza strain isolated in our laboratory and found to be closely related to A/Jap 305/57 was used in HI test with the 17 paired sera. Table 2 presents the data of HI tests performed with the 17 paired sera. In all instances, appreciable antibody titers were shown to exist starting at a serum dilution of 1:16. Out of the 17 pairs tested, 1 case showed significant increase in HI antibody titer.

HI antibody levels against HA-I (para-influenza 3) and HA-II (para-influenza 1) (15) were determined from the 17 cases of acute bronchiolitis. Because of the relation that exists between the HA viruses and Sendai and or mumps virus (5), the latter viruses have also been included in the tests. Table 2 summarizes the data. As may be seen from Table 2, out of 17 cases, 3 showed a significant rise in HI antibody titer against HA-I, and another against HA-II. Of interest to note is the presence of a significant rise in HI antibody titer against A/Phil/57 and HA-II in the same patient (No. 10 C.R.). Very low antibody levels have been exhibited in all cases against mumps and Sendai viruses.

DISCUSSION

With the adenoviruses sharing extensively complement fixing antigens (16) the CF test employing only one type of adenovirus as an antigen proves a serologic test of value in the detection of the existence of an infection caused by any of the other known types (2). Studies made by Heubner *et al.* (2) showed that more than 70% of persons infected with any given strain showed fourfold heterotypic rises against other antigenic types. The likelihood that a type of an adenovirus is the probable etiologic agent of this epidemic of acute bronchiolitis is not great since only one out of 17 cases manifested a significant antibody rise.

It must be mentioned that cases of acute bronchiolitis appeared at a time when the new A/Asian influenza/57 strain was circulating. One patient (No. 10) with significant antibody rise against the Asian strain may very well have been a true case of influenza. The HI antibody titers of all cases studied reflect recent exposure to the Asian strain of influenza epidemic of 1957 which occurred before the outbreak of the acute bronchiolitis epidemic.

Five out of 17 cases showed a significant rise in antibody titer against the HA viruses, one of the 5 showing a significant rise to both Asian strain and HA-II and is probably a case of double infection. Chanock *et al.* (5) in a recent publication, incriminated the HA viruses, with HA-I significantly more prevalent among infants and young children with respiratory illnesses such as febrile pharyngitis, acute bronchiolitis and pneumonias, inasmuch as half of the cases studied yielded HA-I virus in their throat swabs. In reexamining the paired sera of cases obtained, we found that not all were ideal specimens for testing as far as time of collection is concerned. In some of the cases, there is tendency to manifest a significant antibody rise but this probably did not become evident because the acute phase sera were extracted too late in the course of the disease. Because of this limitation, the authors with calculated optimism entertain the possible relation of the HA-I virus as etiologic agent in this epidemic of acute bronchiolitis.

Further and more extensive controlled studies to corroborate our findings regarding the HA viruses will be the subject of a future work.

SUMMARY

A rough immunological screening was performed on 17 acute bronchiolitis cases.

Tests employed were CF against adenovirus type 4 and HI against the various influenza viruses: viz., A'/FM1/47, A/PR8/34, A/Swine-15 1976/31, A/Phil./57, B/Lee/40 and D/Sendai/52; the mumps virus, HA-I and HA-II.

The likelihood that a type of an adenovirus as the probable viral etiologic agent of this epidemic of acute bronchiolitis is not great since only one of 17 cases gave a significant antibody rise in titer.

The possible role of the various influenza viruses have been eliminated.

The possible etiologic relation between the HA-I virus and acute bronchiolitis is suspected based on the observation that four (4) of the seventeen (17) cases tested or about one-fourth showed positive results, but further and extensive controlled study is needed.

ACKNOWLEDGMENTS

Grateful acknowledgment is due to Dr. Fe del Mundo, Director, Children's Memorial Hospital, who made possible the collection of clinical specimens. The authors wish to thank Dr. Potenciano R. Aragon, Head, Department of Medical Microbiology, Institute of Hygiene, for his suggestions in writing this paper. The technical assistance of Mrs. Gorgonia T. Ocampo is greatly appreciated.

REFERENCES

1. MUNDO, F. del, SORIANO, L., AFRICA-JULIANO, L., MATAWARAN, A., and SIAZON P.: An Epidemic of Bronchiolitis in Infants in 1957, Jour. Phil. Med. Ass., 12:729-735, Dec. 1958.

2. HEUBNER, R.J., ROWE, W.P., WARD, T.G., PARROTT, R.H., and BELL, J.A.: Adenoidal-Pharyngeal-Conjunctival Agents, a Newly Recognized Group of Common Respiratory System Viruses, New England Jour. Med., 251:1077-1086, 1954.

3. PARROTT, R.H., ROWE, W.P., HEUBNER, R.J., BERNTON, H.W., and McCULLOUGH, N.B.: Outbreak of Febrile Pharyngitis and Conjunctivitis Associated with Type 3 APC Virus Infection, New England Jour. Med., 251:1087-1090, 1954.

4. HILLEMAN, M.R., and WERNER, J.H.: Recovery of New Agent from Patients with Acute Respiratory Illness, Pro. Soc. Exper. Biol. and Med., 85:183-188, 1954.

5. CHANOCK, R.M., PARROTT, R.H., COOK, K., ANDREWS, B.E., BELL, J.A., REICHELDERFER, T., KAPIKIAN, A.Z., MASTROTA, F.M., and HEUBNER, R.J.: Newly Recognized Myxoviruses from Children with Respiratory Diseases, The New England Jour. Med., 5:207-213, Jan. 30, 1958.

6. CHANOCK, R.M.: Association of a New Type of Cytopathogenic Myxovirus with Infantile Group, The Jour. Exper. Med., 4:555-575, Oct. 11, 1956.

7. CHANOCK, R.M., REIZMAN, B., and MYERS, R.: Recovery from Infants with Respiratory Illness of a Virus related to Chimpanzee coryza Agent (CCA) I. Isolation Properties and Characterization, Am. Jour. Hyg., 63:281-290, Nov. 1957.

8. CHANOCK, R.M., and FINBERG, L.: Recovery from Infants with Respiratory Illness of a Virus related to Chimpanzee Coryza Agent (CCA). II. Epidemiologic aspects of Infection in Infants and Young Children, Am. Jour. Hyg., 63:291-300, Nov. 1957.

9. PRICE, W.H.: The Isolation of a New Virus Associated with Respiratory Clinical Disease in Humans, Proc. Nat. Acad. Sci. 42:892-896, 1956.

10. PELON, W. MOGABGAB, W. J., PHILIPS, I.A., and PIERCE, W.E.: A Cytopathogenic Agent Isolated From Naval Recruits with Mild Respiratory Illnesses, Proc. Soc. Exper. Biol. and Med., 94:262-267, 1957.

11. BENGTSON, I.A.: Complement Fixation in the Rickettsial Diseases Technique of the Test, Pub. Health Rep., 59:402-405, 1944.

12. BEEMAN, E.A., HEUBNER, R.S., and COLE, R.M.: Studies of Coxsackie Viruses. Laboratory Aspects of Group A Viruses, Am. Jour. Hyg., 55:83-107, 1952.

13. YOUNGER, J.S.: Monolayer Tissue Cultures. I. Preparation and Standardization of Suspensions of Trypsin-Dispersed Monkey Kidney Cells, Proc. Soc. Exper. Biol. and Med., 85:202-205, 1954.

14. KEITH, E.J.: "Influenza", American Public Health Diagnostic Procedures for Viruses and Rickettsial Diseases, 2nd ed. New York, 1956.

15. ANDREWES, C.H., BANG, F.B., CHANOCK, R.M. and ZHDANOV, V.M.: Para-Influenza Viruses 1, 2, and 3: Suggested Names for Recently Described Myxoviruses, Virology, 8:129-130, 1959.

16. ROWE, W.P., HEUBNER, R.J., HARTLEY, J.W., WARD, T.G. and PARROTT, R.H.: Studies on the Adenoidal-Pharyngeal-Conjunctival (APC) Group of Viruses, Am. Jour. Hyg., 61:197-218, March 1955.

TABLE 1

RESULTS OF CF TESTS WITH 17 BRONCHIOLITIS CASES TO ADENOVIRUS TYPE 4

Patient	Age (Months)	Date Onset of Illness	Days Serum taken following Onset of Illness	CF Antibody Titer
1. J. C. (Male)	5	9-4-57	6 20	0 0
2. G. C. (Male)	23	8-25-57	5 15	0 0
3. H. C. (Female)	1	9- 1-57	8 13	0 0
4. C. C. (Female)	11	x	x	0 0
5. J. C. (Male)	30	9- 9-57	1 5	0 0
6. L. H. (Female)	11	8-20-57	13 18	0 0
7. A. J. (Male)	12	8-27-57	6 11	1:256 1:256
8. M.L. Jr. (Male)	11	9- 1-57	8 13	0 1:64
9. L. R. (Female)	4	8-27-57	3 10	0 0
10. C. R. (Female)	12	8-31-57	2 7	0 1:8
11. J. S. (Male)	9	8-24-57	10 19	0 0
12. E. SJ. (Female)	2	9- 1-57	8 15	0 0
13. L. SJ. (Female)	2	8-30-57	4 11	0 0
14. B. S. (Male)	11	8-28-57	2 9	1:16 1:8
15. JM. S. (Male)	4	8-25-57	5 15	0 0
16. BB. V. (Male)	3	8-21-57	9 18	0 0
17. D. V. (Male)	4	8-25-57	5 12	0 0

0 — Less than 1:8 dilution.

x — The patient was first admitted at the CMH June 15, 1957, and re-admitted with the same complaints September 4, 1957, at which date the patient was bled for 1st blood sample. On September 15, 1957, a second blood sample was withdrawn.

TABLE 2

HI ANTIBODY TITERS TO INFLUENZA ASIAN STRAIN A/PHIL/57, HA VIRUS TYPE I AND HA VIRUS TYPE II

Patient No.	A/Phil/57	HA-I	HA-II
1	1:256	0	1:8
	1:256	0	1:16
2	1:16	1:32	1:16
	1:16	1:32	1:16
3	1:32	1:64	1:32
	1:32	1:64	1:16
4	1:256	0	0
	1:128	1:32	0
5	1:64	1:128	0
	1:128	1:512	0
6	1:32	0	0
	1:32	0	0
7	1:256	1:64	1:32
	1:128	1:256	1:32
8	1:128	0	1:128
	1:256	0	1:128
9	1:16	1:8	1:16
	1:16	1:8	1:16
10	0	0	0
	1:32	0	1:64
11	1:256	1:64	1:32
	1:128	1:32	1:32
12	1:64	1:128	1:32
	1:64	1:128	1:64
13	1:64	1:8	1:16
	1:32	1:8	1:16
14	1:32	1:8	1:8
	1:32	1:128	0
15	1:64	1:8	1:8
	1:64	1:8	1:8
16	1:64	1:128	1:32
	1:64	1:128	1:32
17	1:64	1:32	1:16
	1:64	1:16	1:16

0 — Less than 1:8 dilution.