

6. Boggs, D. R., Cartwright, G. E., Wintrobe, M. M., *Am. J. Physiol.*, 1966, v211, 51.
7. Sheagren, J. N., Wolff, S. M., Shulman, N. R., *ibid.*, 1967, v212, 884.
8. Olitzki, L., *Acta Med. Orient.*, 1959, v8, 103.
9. Farr, R. S., LeQuire, V. S., *Proc. Soc. Exp. Biol. & Med.*, 1950, v75, 661.
10. Smith, W. W., Alderman, I. M., Cornfield, J., *Am. J. Physiol.*, 1961, v301, 396.
11. Wolff, S. M., Rubenstein, M., Mulholland, J. H., *Blood*, 1965, v26, 190.
12. Mechanic, R. C., Frei, Emil III, Landy, Maurice, Smith, W. W., *J. Clin. Invest.*, 1962, v41, 196.
13. Chervenick, P. A., Boggs, D. R., Marsh, J. C., Cartwright, G. E., Wintrobe, M. M., submitted to *Am. J. Physiol.*, 1967.
14. Gagon, T. E., Athens, J. W., Boggs, D. R., Cartwright, G. E., *Am. J. Clin. Path.*, 1966, v36, 684.
15. Gordon, A. S., Handler, E. S., Siegel, C. D., Dornfest, B. S., LoBue, J., *Ann. N. Y. Acad. Sci.*, 1963, v113, 766.
16. Fukuda, T., Oide, H., Miyesada, A., *Nature*, 1960, v188, 860.
17. Boggs, D. R., Marsh, J. C., Cartwright, G. E., *J. Clin. Invest.*, 1966, v45, 988.
18. Fukuda, T., Murata, K., *Jap. J. Physiol.*, 1965, v15, 169.
19. Brunning, R. D., Woolfrey, B. F., Schrader, W. H., *Am. J. Path.*, 1964, v44, 401.
20. Kerby, G., *Proc. Soc. Exp. Biol. & Med.*, 1952, v81, 381.

Received August 30, 1967. P.S.E.B.M., 1967, v126.

Heterotypic Antibody Responses in Man Following Vaccination with Adenovirus Type 1 Hexon Antigen. (32599)

JULIUS A. KASEL, DAVID W. ALLING, PHILIP R. GLADE AND PETER A. BANKS
U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Clinical Investigation, Bethesda, Maryland 20014

Recent evidence suggests that a rise in serum neutralizing antibody following immunization with either fiber or hexon antigens of adenovirus types 1 and 4 is protective(1,2). Furthermore, volunteers vaccinated with fiber antigen of type 1 virus showed a high relative frequency of neutralizing and hemagglutination-inhibition (HI) antibody rises to other adenovirus serotypes(3). An analysis of these responses according to the immunologic classification of adenoviruses and the status of pre-existing antibody revealed that all rises in heterotypic antibody were confined to group 3, the group to which the vaccine type belonged. This report will describe the heterotypic antibody responses to adenoviruses that developed in adult volunteers after administration of an adenovirus type 1 hexon antigen vaccine.

Materials and methods. Vaccine preparation and immunization schedule. Procedures for the preparation, assay and safety-testing of the adenovirus type 1 hexon antigen vaccine administered to the participants in this study have been previously described(4). In brief,

the hexon antigen was obtained by fractionation of virus suspensions by column chromatography with DEAE-cellulose. The fraction containing the bulk of the antigen was rechromatographed twice.

The volunteers received the hexon antigen preparation as follows: 8 received 1.0 ml intramuscularly on the 1st and 8th day; 2 received a single injection of 2.0 ml on the 1st day; one man received 2 ml initially and 1 ml on the 8th day.

Source of serum specimens. Paired serum specimens from 11 adult male volunteers who were immunized with an adenovirus type 1 hexon antigen vaccine preparation were used in this study(4). The 1st serum specimens were obtained before vaccine administration and the 2nd specimens were collected 28 days after vaccination. All of the individuals were antibody negative (<1:4) to type 1 adenovirus before vaccination but developed a 4-fold or greater rise in serum neutralizing antibody titer by 28 days.

Serologic tests. Neutralization and hemagglutination-inhibition (HI) tests were done

TABLE I. Comparison of Heterotypic Neutralizing Antibody Responses in Adult Volunteers Following Vaccination with Adenovirus Type 1 Hexon Antigen.

Immuno- logic group	Serotypes tested	Pre-existing antibody						
		Absent			Present			
		No. tests	No. rises	Per- cent		No. tests	No. rises	Per- cent
1	3, 7, 11, 14, 16, 20, 21, 25, 28	69	0	0	} $\chi^2=20.2$ $p=<.001$	30	0	0
3	2, 4-6	25	8	32		19	7	37
2	8-10, 13, 15, 17	110	3	3	} $\chi^2=19.6$ $p=<.001$	22	1	5

with prototype strains as previously described (4,5). A rise was considered significant if the titer increased at least 4-fold.

Results and discussion. In Table I is shown the frequency of heterotypic serum neutralizing antibody responses to serotypes in each of the 3 immunologic groups. Responses were classified according to the absence ($<1:8$) or presence ($\geq 1:8$) of neutralizing antibody titer prior to vaccination. It can be seen that the preponderance of rises in heterotypic antibody in both antibody groups occurred to serotypes in group 3, the group to which the hexon antigen belonged. The relative frequency of responses in group 3 was significantly greater in all cases, the level ranging from $<.001$ to $<.05$. Moreover, it is noteworthy that in 52 instances where pre-existing antibody was present to serotypes in groups 1 and 2, only one rise occurred. On the other hand, the relative frequencies of heterotypic neutralizing antibody responses to serotypes of group 3 were: 64%, type 2; 45%, type 4, 9%, type 5 and 18%, type 6. These per cent responses are similar to those observed in serum specimens obtained from adult volunteers following vaccination with type 1 fiber antigen.

Paired serum specimens from 8 volunteers were examined in HI tests against types 3, 7, 11; 14, 16, 20 and 21 of group 1, types 8-10, 13, 15, 17, 22-24, 26 and 27 of group 2 and types 1, 2, 4, 5 and 6 of group 3. In no instance was a rise in antibody titer detected to a serotype in any of the 3 groups. This finding was not unexpected since it has been

shown that the hexon antigen of type 1 adenovirus lacks hemagglutinating activity(6).

These results demonstrate the capacity of the hexon antigen of type 1 virus to stimulate heterotypic neutralizing antibody in adult volunteers in addition to homotypic antibody. Since available data suggests that heterotypically-induced antibody affords some protection against adenoviral disease(3), it is important to determine the degree of protection conferred when such antibody is induced by the hexon antigen.

Summary. Vaccination of 11 adult male volunteers with adenovirus type 1 hexon antigen resulted in the formation of heterotypic as well as homotypic serum neutralizing antibody responses. The relative frequency of responses to serotypes belonging to immunologic group 3, which includes type 1, was significantly higher than those which occurred to serotypes in group 1 or group 2.

1. Kasel, J. A., Huber, M., Loda, F., Banks, P. A., Knight, V., Proc. Soc. Exp. Biol. & Med., 1964, v117, 186.

2. Kasel, J. A., In Vacc. against Viral and Rickettsial Dis., Man, PAHO, Sc. Pub., 1967, v147, 81.

3. Kasel, J. A., Banks, P. A., Wigand, R., Knight, V., Alling, D. W., Proc. Soc. Exp. Biol. & Med., 1965, v119, 1162.

4. Banks, P. A., Kasel, J. A., Huber, M., Alford, R. H., Knight, V., *ibid.*, 1966, v121, 240.

5. Rosen, L., Am. J. Hyg., 1960, v71, 120.

6. Pereira, H. G., deFigueiredo, M. V. T., Virol., 1962, v18, 1.

Received September 5, 1967. P.S.E.B.M., 1967, v126.