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An Immunologic Classification of Heterotypic Antibody Responses to Adenoviruses in Man. (30404)

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When human beings are infected or immunized with an adenovirus, neutralizing and hemagglutination-inhibition (HI) antibody responses to other adenovirus serotypes frequently occur(1-12). Antigenic relationships between several serotypes have been inferred from the fact that previous exposure with one type is followed by a significantly increased frequency of responses to other types; namely, between types 3, 7, 11 and 14(8,9), and between types 26 and 27(11). More commonly, however, the responses are distributed in an apparently random fashion among the various serotypes. The term "recall" has been used to indicate that such heterotypic responses may represent a non-specific evocation of antibody to serotypes with which the host has had prior contact(9).

Recently, studies in volunteers in this laboratory have suggested an explanation for heterotypic antibody responses to adenovirus type 26 and type 27 infections in man (13). The results presented in that study and the findings reported herein indicate that these responses are specific and reflect the hitherto unrecognized existence of 3 major immunologic groups among the adenoviruses. These groups were delineated when it was noted that heterotypic antibody responses in man separate into 3 groups which coincide with an earlier classification of human adenoviruses by Rosen which is based on the agglutination of rat and rhesus monkey erythrocytes(14).

The present report will describe the studies which relate the proposed immunologic classification of human adenoviruses to the hemagglutination (HA) groups and will assess the

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significance of this classification to the epidemiology of adenovirus infection in man.

Materials and methods. Source of sera. Paired sera were obtained from 61 adult male volunteers during the course of study with adenoviruses at the Clinical Center, National Institutes of Health. Serum specimens were selected on the basis of absence of neutralizing antibody to the homotypic virus and evidence of virus infection or serologic conversion after experimental procedure. Except where noted, sera were collected prior to and 20 to 28 days after viral administration.

Ten men were given type 7 virus by conjunctival inoculation.† Seven of these individuals had been previously immunized by feeding of virus in an enteric-coated capsule (15). Initial serum specimens from the latter volunteers were obtained prior to administration of the capsule and the second was obtained after conjunctival challenge.

Three individuals received a conjunctival inoculation of adenovirus type 16(16).

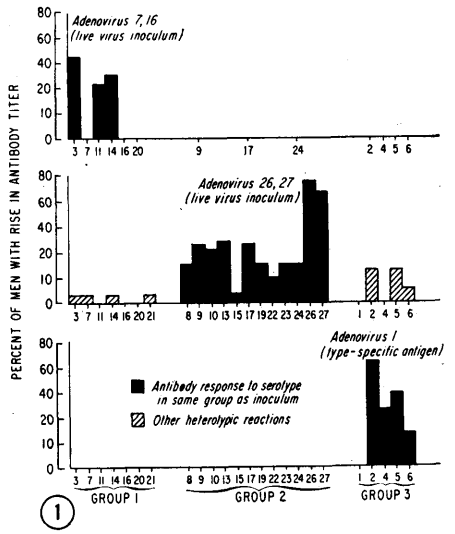
Thirty-two subjects were given either type 26 or 27 adenovirus by nasal or conjunctival route; 17 persons were given the former serotype and 15 the latter(17).

Sixteen men had been vaccinated with the type-specific soluble antigen prepared from an adenovirus type 1 suspension(18,19). This antigen had been previously referred to as C antigen(20) and E antigen(21).

Hemagglutination-inhibition test. The HI tests were done with prototype strains as previously described(14). A rise was considered significant if the titer increased from $<1:10$ to 1:10 or greater or when any initially measurable titer increased 4-fold.

Serum neutralization test. A modification of the neutralization test (procedure 2) as described by Rowe *et al*(1), was used to determine antibody titers. Final endpoints were recorded when titration indicated that the virus dilution used in the test contained 32 to 100 $TCID_{50}$. Sera were tested at an initial dilution of 1:8.

Results and discussion. The relationship of the HA groups to the immunologic classification is shown in Fig. 1. The results in the type 7 and type 16 and in the type 26 and type 27 volunteers were combined since the former inocula belong to HA group 1 and the latter inocula belong to HA group 2 and since the patterns of response were quite similar in the respective groups. Heterotypic antibody responses in the 3 volunteer groups were determined against the indicated serotypes from each HA group. In 13 volunteers inoculated with 2 serotypes of Group 1 (types 7 and 16), a high relative frequency of heterotypic neutraliz-



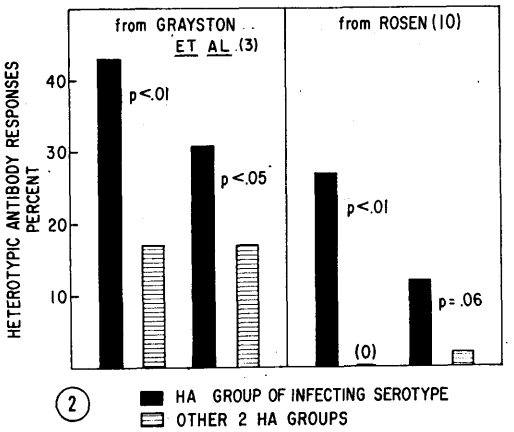


FIG. 1. Relative frequencies of heterotypic antibody rises according to serotype and type of adenoviral inoculum.

FIG. 2. Relative frequencies of heterotypic antibody responses according to the immunologic classification in 4 population groups.

† Sera from these volunteers were generously supplied by Dr. Robert M. Chanock, Laboratory of Infectious Diseases, Nat. Inst. of Allergy and Infect. Dis., Bethesda, Md.

TABLE I. Comparison of Heterotypic Antibody Responses.

HA group	Adeno-virus	Pre-existing antibody: Absent — HA group	No. tests	No. rises	%		Present — No. tests	No. rises	%	
1	7, 16	1	54	9	17	$\chi^2 = 8.1$, $p < .01$	16	4	25	$\chi^2 = 6.4$, $p < .02$
		2, 3	56	0	0		35	0	0	
2	26, 27	2	264	37	14	$\chi^2 = 18.6$, $p < .01$	24	10	42	$\chi^2 = 13.9$, $p < .01$
		1, 3	351	14	4		33	0	0	
3	1	3	53	16	30	$\chi^2 = 8.0$, $p < .001$	11	6	55	Exact test, $p = .004$
		1, 2	271	0	0		15	0	0	

ing antibody responses occurred to types 3, 11 and 14 of Group 1, but none to 7 other serotypes in the 2 other HA groups. Thirty-two individuals administered types 26 or 27 (Group 2 viruses) exhibited HI antibody responses predominantly to viruses in the group to which the inocula belonged. A few responses developed to serotypes of the other 2 groups. All of the HI antibody responses in 16 men vaccinated with a purified non-infectious soluble antigen of adenovirus type 1, a Group 3 virus, occurred to serotypes in the same group as the vaccine. Sera demonstrating antibody rises by the HI test were confirmed in serum neutralization tests.

Data from Fig. 1 is summarized in Table I according to the presence or absence of antibody before inoculation or immunization. The great majority of rises in heterotypic antibody occurred to serotypes belonging to the same HA group as the inoculating or immunizing serotype whether or not pre-existing antibody was present. The excess of responses in the same HA group as the stimulating serotype was significant in all cases, the level ranging from $<.001$ to $<.02$. Of particular interest is the lack of responses to serotypes not belonging to the same HA group as the inocula in 83 instances where pre-existing antibody was present.

These data, therefore, reveal a preponderance of heterotypic antibody responses in HA groups to which the inoculating or immunizing serotype belonged, and presumably represent intragroup antigenic relationships.

Further studies supporting the proposed immunologic classification were sought from epidemiologic studies of natural illness. In Fig. 2 are summarized the heterotypic antibody responses in 4 population groups reported from 2 laboratories(3,10) according to the immunologic classification described here. The heterotypic responses observed in their studies are presented as 4 series of 2 bar graphs. Responses to serotypes in the same HA group as the infecting virus are represented in black and those to serotypes of the other 2 HA groups by bars with horizontal lines.

In the study of Grayston *et al*(3), it was found that in 28 military recruits following infection with group 1 viruses (types 3 and 7), neutralizing antibody responses against serotypes in the same group as the infecting virus occurred more frequently than against serotypes of the other 2 groups, 43 *vs* 17%, a highly significant difference (bars 1 and 2). The findings were similar following infection of 25 men in the same type of population by type 4, a group 3 serotype, 31 *vs* 17%, (bars 3 and 4).

In Rosen's study(10), 17 adults who experienced natural infection with a group 3 virus (type 4) showed a greater preponderance of HI antibody responses to serotypes in the same group, 27 *vs* 0% (bars 5 and 6). Furthermore, 27 children infected with group 3 viruses (types 1, 2 or 5) demonstrated a similar pattern of response (bars 7 and 8).

The division of human adenoviruses into 3 immunologic groups invites a further re-examination of the epidemiology of these infections in man. One subject of importance is the degree of protection afforded by heterotypically-induced antibody. Recently, it has been reported that volunteers with heterotypic antibody to type 26 virus induced by prior infection with type 27 infection, experienced significantly less infection and illness responses following challenge with type

26 virus(17). Similar results were noted in a reciprocal virus challenge experiment. Another instance of protection is described from studies in children by Bell(22). He noted that infection with types 1, 3 or 5 markedly reduced the risk of subsequent infection and illness with type 2 virus. Similarly, prior infection with virus types 2, 3 and 5 reduced subsequent risk of infections with type 1. Type 3 is not a group 1 serotype and thus might not be expected to contribute to immunity for this group. It seems possible that further study of the data might show the presence of type 3 to be unessential. In contrast, prior infections with types 1, 2 or 5 did not appear to influence risk to subsequent infections or illnesses with type 3. In addition, in a serum survey for infection with group 2 serotypes carried out among children by Rosen *et al*(23), multiple antibody rises to group 2 serotypes under study occurred with unusual frequency which could not be accounted for on the basis of multiple infections. The antigenic relationships defined in the present classification, therefore, suggest the possible protective effect of heterotypic antibody under natural circumstances.

Finally, there is the possibility that one or more broadly antigenic serotypes can be selected from each immunologic group for preparation of a polyvalent vaccine which may be protective against infection with a large majority of human adenoviruses.

Summary. Studies of heterotypic antibody responses in volunteers infected or immunized with adenoviruses indicate the presence of 3 distinct immunologic groups of serotypes. These groups correspond to an earlier classification of adenoviruses by Rosen which was based on varying patterns of agglutination of rat and rhesus monkey erythrocytes. Review of published epidemiologic studies reveals a strong association of heterotypic antibody responses after natural illness with the classification described. Some interpretations of the role of this classification in the epidemiology of adenoviruses is offered and the possibility of a broadly effective vaccine prepared from serotypes representing the three immunologic groups is suggested.

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