Immunological Relationships Between Hexons of Certain Human Adenoviruses

ERLING NORRBY AND GÖRAN WADELL

Department of Virology, Karolinska Institutet, School of Medicine, Stockholm, Sweden.

Received for publication 24 June 1969

Antisera against hexons of serotypes 2, 4, 5, and 6 (subgroup III), and 15 (subgroup II) were absorbed with purified hexons of various serotypes representing the different subgroups of human adenoviruses. Group, subgroup, and type specificities of hexons could be distinguished. The subgroup specificity of type 4 hexons resembled that of hexons of subgroup I members (types 3, 11, and 16). Anti-hexon sera gave a type-specific inhibition of virion-associated hemagglutinin. The inhibiting activity of different sera was found to be inversely related to the length of fibers of the serotype concerned. Virions of serotypes carrying fibers shorter than about 20 nm (types 3, 4, 9, 11, and 15) were readily inhibited, whereas those of serotypes with longer fibers (types 2 and 6) were inhibited only by relatively large amounts of antibody measured in terms of homotypic complement fixation activity. The reciprocal cross-neutralization between serotypes 4 and 16 was studied separately. Hexons of both serotypes each carried a type-specific component and, in addition, a unique antigen specificity common to the two types. This common antigen specificity was interpreted to be available to a larger extent at the surface of virions (and probably also isolated hexons) of type 4 than of type 16. These results suggest an explanation for the predominantly one-sided character of the cross-neutralization between types 4 and 16.

Hexons of mammalian adenoviruses carry a common antigen specificity. In addition to this, they also carry specificities which are unique for each individual serotype (2, 7, 15, 32; Pettersson et al., *personal communication*). This type-specific character has been demonstrated in neutralization, virion-hemagglutination-inhibition (HI), complement-fixation (CF), and gel diffusion tests. Furthermore, absorption of an antiadenovirus type 3 hexon serum with hexons of different heterologous serotypes revealed the presence of antigen specificities, which were common to members of Rosen's subgroup I and, in addition, to serotype 4, the aberrant member of subgroup III (15).

In the present paper, further data elucidating the immunological characteristics of hexons of certain human adenoviruses will be presented. Different problems studied concerned: (i) remaining antibody activities of antisera against hexons of selected members of subgroups II and III, including type 4 of the latter one, after absorption with purified hexons of homologous and heterologous adenovirus serotypes, (ii) immunological characteristics of hexon components demonstrable by use of the virion HI test and the applicability of this test to virions of different

serotypes, and (iii) the nature of the relationship between hexons of serotypes 4 and 16.

MATERIALS AND METHODS

Preparation of virus material. The prototype strains of adenovirus types 2 to 6, 9, 11, 12, 15, and 16 were propagated in Lu 106 or MAS-A cells by techniques previously described (9, 10). At an advanced stage of cytopathic degeneration, the medium was removed and the cell monolayer of one Roux bottle (about 10^8 cells) was scraped off in 5 ml of a phosphate-buffered (0.067 M, pH 7.2) physiological saline. The cell suspension was frozen and thawed five times, after which cell debris was removed by low-speed centrifugation.

Virions were isolated by centrifugation of virus materials on a discontinuous CsCl gradient comprising layers with densities of 1.40, 1.32 and 1.10 g/ml by the technique recently described (19a). Soluble components recovered from the gradients were dialyzed for further separations aiming at the isolation of hexons free from other immunologically active structural components. Anion-exchange chromatography on diethylaminoethyl-Sephadex A25 (Pharmacia Fine Chemicals, Uppsala, Sweden) was used for fractionation of all serotypes. In addition, the following techniques were used: exclusion chromatography on Sephadex G200 for types 2, 5, 6, (28, 31), and 16 (17); erythrocyte adsorption (to remove

dodecons) for types 3 (10), 9, 15 (11, 18), and 11 (12); and zonal centrifugation for type 4 (27). The purified preparations contained neither fibers nor pentons as determined in hemagglutination enhancement (HE) tests, nor vertex capsomers as demonstrated by the hemagglutination enhancement antibody consumption test (15a, 16). The only exception was the preparation of type 12 hexons (15b), which were contaminated with small amounts of vertex capsomers.

Biological tests. The techniques used for neutralization, HI, HE, and CF tests were already described (15). The only modifications introduced were the use of human diploid lung cells instead of the heteroploid Lu 106 cells in neutralization tests, and the use of the microtechnique of Takatsy (equipment obtained from Flow Laboratories, Irvine, Scotland) instead of the Fulton-Dumbell drop technique for CF.

Preparation of hyperimmune sera. Purified hexons with CF antigen titers ranging between 100 and 1,000 units per 0.025 ml were mixed with Freund's complete adjuvant, and 4 ml of this mixture was injected intramuscularly into rabbits. At 5 weeks later, the animals were given an additional injection of antigen in a volume of 1 to 2 ml intravenously. After another week, the rabbits were exsanguinated, and sera were collected.

Criteria for specificity of hyperimmune sera. Only anti-hexon sera with CF titers in tests with homotypic hexons of 320 units or more per 0.025 ml were employed. It was furthermore required that they did not contain any antibodies against fibers and vertex capsomers at a dilution of 1:4, as determined in HI tests with soluble components and HE tests with erythrocyte-absorbed type 11 material (12, 29) or, in the case of antiadenovirus type 11 sera, with type 3 material (10).

Absorption of hyperimmune sera. Sera were mixed with antigen in an amount corresponding to four times that calculated to be required to bind all antibodies. In some experiments, the absorption was repeated 2 or 3 times to test the significance of antibodies remain-

ing at low titers. When needed, sera were concomitantly mixed with material harvested from uninfected cells to remove antibodies against nonviral components. After incubation for 1 hr at room temperature and in the cold (4°C) overnight, the mixture; were centrifuged at a performance index (Pi) of 16.2 ($81,000 \times g$, R40 Spinco Division, Beckman Instruments Inc., Fullerton, Calif.) for 2 hr, after which the supernatant fluid was collected. Pi is a rating used in the Beckman manuals to express relative performance of rotors, $Pi = (\text{rev/min})^2 / (\log_e R_{\max} - \log_e R_{\min})$ where $R_{\max}$ and $R_{\min}$ represent the distance between axis of rotation and bottom and top of tube, respectively. In most centrifugations, 3-ml tubes fitting special adapters were used.

RESULTS

Effect of absorption of antisera against hexons of adenovirus types 2, 4, 5, 6, and 15 with homotypic and different heterotypic hexons. The technique of removing antibodies present in anti-hexon sera by absorption with excess amounts of homotypic and heterotypic hexon antigen was employed in previous studies of type 3 (15), a subgroup I member, and in the present study of sera against members of subgroups II (type 15) and III (types 2, 4, 5, 6). A minimum of two anti-hexon sera were examined per serotype. The antibody "profiles," i.e., the relative occurrence of antibodies of varying degree of specificity, were markedly constant for sera against one and the same serotype. It should be mentioned that similar antibody "profiles" were demonstrable in studies of sera against both crude virus material and hexons.

The general results obtained in studies of the effect of absorption of anti-hexon sera with homotypic and heterotypic hexons (Tables 1-4) were as follows.

TABLE 1. *Effect of absorption with homotypic and heterotypic hexons on the CF activity of an antiadenovirus type 2 hexon serum*

Serum sample absorbed with hexons of		CF antibody activity in tests with hexons of types									
Type	Subgroup	3	11	16	9	15	2	4	5	6	12
3	I	80	160	160	80	80	1,280	80	320	640	80
		— ^a	—	—	—	—	320	—	160	80	—
11	I	—	—	—	—	—	320	—	80	80	—
16	I	—	—	—	—	—	640	—	160	80	—
9	II	—	—	—	—	—	640	—	160	80	—
15	II	—	—	—	—	—	640	—	160	160	—
2	III	—	—	—	—	—	—	—	—	—	—
4	III	—	—	—	—	—	640	—	160	80	—
5	III	—	—	—	—	—	160	—	—	—	—
6	III	—	—	—	—	—	160	—	—	—	—
12		—	—	—	—	—	640	—	160	160	—

^a Titer of less than 20.

TABLE 2. *Effect of absorption with homotypic and heterotypic hexons on the CF activity of an antiadenovirus type 6 hexon serum*

Serum sample absorbed with hexons of		CF antibody activity in tests with hexons of types									
Type	Subgroup	3	11	16	9	15	2	4	5	6	12
3	I	160	320	160	320	640	640	640	640	640	160
11	I	— ^a	20	—	20	20	320	160	320	640	—
16	I	—	—	—	—	—	160	40	160	160	—
9	II	—	—	—	—	—	320	40	160	320	—
15	II	—	—	—	—	—	160	40	320	160	—
2	III	—	—	—	—	—	160	40	160	320	—
4	III	—	—	—	—	—	—	—	—	80	—
5	III	—	—	—	—	—	160	—	160	160	—
6	III	—	—	—	—	—	—	—	—	40	—
12		40	20	—	20	20	160	40	80	320	—

^a Titer less than 20.TABLE 3. *Effect of absorption with homotypic and heterotypic hexons on the CF activity of an antiadenovirus type 4 hexon serum*

Serum sample absorbed with hexons of		CF antibody activity in tests with hexons of types									
Type	Subgroup	3	11	16	9	15	2	4	5	6	12
3	I	1,280	1,280	2,560	1,280	1,280	640	5,120	640	320	640
11	I	— ^a	—	320	—	—	—	1,280	—	—	—
16	I	—	—	320	—	—	—	1,280	—	—	—
9	II	—	—	—	—	—	—	640	—	—	—
15	II	80	80	640	—	—	40	1,280	—	—	—
2	III	40	40	320	—	—	—	1,280	—	—	—
4	III	160	160	640	40	40	—	2,560	—	—	—
5	III	—	—	—	—	—	—	—	—	—	—
6	III	160	80	640	—	—	—	2,560	—	—	—
12		80	80	640	40	40	—	2,560	—	—	—
		—	—	640	—	—	—	2,560	—	—	—

^a Titer less than 40.TABLE 4. *Effect of absorption with homotypic and heterotypic hexons on the CF activity of an antiadenovirus type 15 hexon serum*

Serum sample absorbed with hexons of		CF antibody activity in tests with hexons of types									
Type	Subgroup	3	11	16	9	15	2	4	5	6	12
3	I	320	320	640	640	1,280	640	640	640	640	640
11	I	— ^a	—	80	160	1,280	80	160	80	80	—
16	I	—	—	80	320	1,280	40	80	80	80	—
9	II	—	—	—	40	640	—	—	—	—	—
15	II	—	—	—	—	640	—	—	—	—	—
2	III	—	—	40	160	640	—	80	—	—	—
4	III	—	—	20	80	640	—	—	—	—	—
5	III	—	—	—	160	640	—	20	—	—	—
6	III	—	—	20	160	640	—	20	—	—	—
12		—	—	40	160	1,280	—	40	—	—	—

^a Titer less than 20.

(i) Only homotypic hexons could remove all antibody activity of sera.

(ii) Absorption with hexons of members of the same subgroup (except type 4 in subgroup III, *see below*) removed all antibodies, except some reacting with homotypic hexons. These results demonstrated the presence of type-specific antibodies. The relative concentration of these type-specific antibodies in sera varied in a characteristic way from one serotype to the other. Thus, they occurred in relatively higher concentration in sera against members of subgroups I, as previously described [type 3 (15)], and II [type 15 (Table 4)] and type 4, the aberrant member of subgroup III (Table 3), than in sera against hexons of other members of the latter subgroup [types 2, 6 (Tables 1 and 2), and type 5].

(iii) After absorption with hexons of members not belonging to the same subgroup, antibodies of both type-specific and subgroup-specific nature in most cases remained in the sera. This demonstrated the occurrence of antigen specificities in hexons other than those with type and group characteristics. These antigen specificities in most cases appeared to be restricted to members of one subgroup, but some results indicated the occurrence of specificities common to a larger number of serotypes. For example, absorption of anti-hexon sera of type 15 with hexons of types 3 and 11 demonstrated the presence of antibodies reacting with antigen specificities of members of both subgroups II and III. Furthermore, absorption with hexons of subgroup III members left some antibodies reacting with hexons not only of subgroup II members, but also of types 16 (subgroup I) and 4 (subgroup III). Repeated absorptions with excess amounts of antigen did not remove additional antibodies, confirming that the differences encountered were due to qualitative and not to quantitative variations in the composition of antigenic specificities of hexons.

The behavior of hexons of types 4 and 12 deserve some special comments. The finding that absorption of antiadenovirus type 6 hexon sera with hexons of members of subgroup I and II left a small amount of antibodies reacting with type 4 hexons (Table 2) suggested some relationship of type 4 to other members of subgroup III, also on the level of hexons. However, most other results indicated a strong subgroup relationship between hexons of types 4 and members of subgroup I. Absorption of sera against hexons of adenovirus types 2, 5, and 6 with type 4 hexons could not remove subgroup-specific antibodies present in these sera (Tables 1 and 2). Furthermore, absorption of antiadenovirus type 4 hexon sera with hexons of subgroups II and III left antibodies reacting with homotypic antigen as

well as hexons of members of subgroup I (Table 3). The relationship between hexons of type 4 and those of one of the members of subgroup I, type 16, was particularly intimate (Table 3).

The hemagglutinating characteristics of adenovirus type 12 indicate a relationship of this type to members of subgroup III (15*b*, 24). In spite of this, preparations of hexons of type 12 did not display a capacity to remove anti-hexon antibodies similar to that of hexons belonging to members of this subgroup. A comparison of results (Tables 1 and 3) suggests that the antigenic makeup of type 12 hexons may be more similar to that of hexons of subgroup I than of subgroup III. However, the degree of relatedness is relatively small and type 12 cannot, on the basis of immunological cross-relationships on the level of hexons, be associated with any one of the three different subgroups of Rosen.

Capacity of antisera against hexons of different adenovirus serotypes to inhibit the hemagglutinating activity of homotypic and heterotypic virions. In previous studies of adenovirus type 3, it was found that anti-hexon sera could inhibit the hemagglutinating activity of virions, but not of soluble HA (15). To analyze the immunological specificity and other characteristics of this reaction, antisera against hexons of types 3, 16 (subgroup I), 9, 15 (subgroup II), and 4, 6, 2 (subgroup III) were tested in HI tests against virions of all these different serotypes. The absolute titer values (Table 5) are directly comparable, since all

TABLE 5. *Effect of antisera against adenovirus hexons on the hemagglutinating activity of virions of different representative members of Rosen's subgroups*

Anti-hexon serum of ^a		HI antibody titers in tests with virions of types						
Type	Sub-group	3	16	9	15	4	6	2
3	I	1,600	— ^b	—	—	—	—	—
16	I	—	800	—	—	100	—	—
9	II	—	—	3,200	—	—	—	—
15	II	—	—	—	1,600	—	—	—
4	III	—	—	—	—	200	—	—
6	III	—	—	—	—	—	(16) ^c	—
2	III	—	—	—	—	—	—	(8)

^a Prior to testing, sera were diluted to contain corresponding amounts of homotypic hexon CF activity (640 units per 0.02 ml). All sera analyzed contained HI antibodies reacting with soluble HA in a titer of less than 8.

^b Titer of less than 8.

^c Parentheses indicate that traces of agglutination remained in all tubes counted as negative.

sera were diluted to contain the corresponding amount of homotypic hexon CF antibody activity (640 units per 0.025 ml). The capacity of different sera to inhibit virion HA activity was strictly type-specific. Only antiadenovirus type 16 hexon sera displayed a deviating behavior. They reacted not only with type 16 virions, but also with type 4 virions on a somewhat lower titer level. Sera against hexons of types 3, 16, 9, and 15 contained type-specific HI antibody titers, which were of a similar order of magnitude. The corresponding titer of type 4 sera was somewhat lower. Antisera against type 2 and 6 hexons carried a capacity to inhibit homologous virions, which was only barely detectable.

Further studies on the relationship between hexons of adenovirus types 4 and 16. As was described above, findings both in CF tests with sera absorbed with heterotypic hexons and in virion HI tests indicated a close relationship between hexons of types 4 and 16. These results, taken together with the previously demonstrated reciprocal cross-neutralization between these two serotypes (cf. reference 25), prompted further studies on the qualitative and quantitative character of this relatedness. It is known that serotypes 4 and 16 display no cross-reactivity in HI tests with soluble components, i.e., their fibers carry different antigenic specificities [cf. Stevens et al. (25)]. For this reason, only anti-hexon sera were studied. Sera of this kind were absorbed with hexons of types 3, 4, and 16 and tested in neutralization and HI tests with type 4 and 16 virions and in CF tests with hexons of types 3, 4, and 16.

With the high-titer antiserum employed in the experiment presented in Table 6, it was found that antiadenovirus type 4 hexon serum can cause some inhibition of the HA activity of type 16 virions (cf. Table 5). Absorption with type 3

hexons removed the CF antibody activity against this kind of hexon in both types of sera. Furthermore, it eliminated all capacity of antiadenovirus type 16 hexon sera to interact in CF tests with type 4 hexons, whereas, after absorption of antiadenovirus type 4 hexon sera, high CF antibody titers remained in tests with both type 4 and 16 hexons. Absorption of antiadenovirus type 4 hexon serum with homotypic hexons abrogated all demonstrable activities, as expected. In contrast, absorption of type 4 serum with type 16 hexons did not change the neutralizing activity against homotypic virions. The CF antibody titer against homotypic CF antigen displayed an eightfold reduction. All activities in different tests with type 16 antigens were removed. Absorption of antiadenovirus type 16 hexon serum with type 4 hexons removed activities in neutralization, virion-HI, and CF tests, with type 4 antigens. In addition, there was a twofold reduction in the neutralization and HI tests with type 16 virions. This reduction was repeatedly encountered, but remains within the acceptable error of the tests. The reduction in CF activity with type 16 hexons was fourfold. Absorption of type 16 anti-hexon serum with homotypic components finally abrogated all demonstrable antibody activities.

DISCUSSION

Hexons carry a group-specific component [denoted α (20)], shared among all mammalian adenoviruses. In addition, a type-specific component [denoted ϵ (13)] can be distinguished in neutralization tests (7, 32) and in CF tests by using anti-hexon sera absorbed with heterotypic hexons, as demonstrated previously in studies of type 3 hexons (15) and of hexons of types 2, 4, 5, 6, and 15, as described above. The finding that

TABLE 6. *Effect of absorption with hexons of types 3, 4, and 16 on the neutralizing, HI, and CF activities of antisera against hexons of types 4 and 16*

Antiserum against hexons of type	Absorbed with hexons of type	Neutralizing activity (units/0.1 ml) in tests with virions of types		HI activity (units/0.4 ml) against virions of types		CF ₁ activity (units/0.02 ml) against hexons of types		
		4	16	4	16	3	4	16
4		20,480	1,280	1,280	40	1,280	5,120	2,560
	3	20,480	1,280	1,280	— ^a	<80	2,560	1,280
	4	< 80	<80	<80	— ^a	<80	<80	<80
	16	20,480	<80	1,280	— ^a	<80	640	<80
16		640	2,560	160	640	1,280	1,280	1,280
	3	640	2,560	160	640	<40	<40	320
	4	<40	1,280	<40	320	<40	<40	320
	16	<40	<40	<40	<40	<40	<40	<40

^a Not tested because of the high dilution (1:80) obtained after absorption.

homotypic antihexon sera can inhibit the hemagglutinating activity of virions, as first observed in studies of type 3 (15) and more extensively analyzed in the experiments described above, gives further evidence for the presence of type specificities in hexons. The basis for this virion HI reaction is the availability of predominantly type-specific parts of hexons at the surface of virions (19). Two different basic mechanisms might be responsible for the virion-HI activity of antihexon sera. These are (a) an agglutination of virions and (b) an attachment of antibodies to paravertex hexons, whereby the former, because of steric interference, can prevent fibers from interacting with receptors on red cells. A comparison of the length of extended immunoglobulin G antibodies and adenovirus fibers (cf. 1, 3, 14, 15a, 19a, 26) indicates that most likely only the HA activity of virions of members of subgroups I and II and type 4 might be inhibited via mechanism b. As a consequence, it could be hypothesized that the low virion HI titers of sera against hexons of types 2 and 6 could be due to the absence of any steric interference between immunoglobulin G antibodies and the long fibers carried by these types. However, electron microscopy demonstrated that the antisera against hexons of type 2 and 6, employed in the present study, contain relatively few antibodies reacting with the surface of homotypic virions (Norrby, *unpublished data*). The reasons for the relative absence of this kind of antibody in sera against members of subgroup III, except type 4, is at present being subjected to further studies.

A possible occurrence of a subgroup specificity in hexons of members of subgroup III was previously suggested from the character of heterotypic antibody responses after vaccination with type 1 hexons (6), heterologous neutralization described by Kjellén and Pereira (7), and by the character of CF curves obtained in tests with sera against homotypic and heterotypic purified hexons (2). Additional evidence for the presence of subgroup antigen specificities was indicated by results of previous antibody absorption studies with type 3 (15) and with additional types as presented above. It should be pointed out that findings made by use of this technique are limited by the extent to which the antigenic integrity of hexons used both for immunizations and absorptions remains unaltered. However, the facts that hexon antibody profiles of sera against whole virus material and hexons (in some cases prepared by different techniques) of the same serotype were similar and that antibody activities remaining after absorption with

both purified and nonpurified components were comparable, make it less likely that antigen modifications of this kind may have influenced the present results. It is possible that an extension of the present kind of analysis to include an immunological characterization also of hexons of other serotypes of human adenoviruses might allow a classification by a factor formula similar to that developed by Kauffman-White for *Salmonella* bacteria.

Although the HA activity of serotypes 4 (22) and 12 (15b, 24) suggests that they should be incorporated in subgroup III, the relationship between their hexons and those of other members (types 2, 5, and 6) of this subgroup suggests only traces of cross-reactions other than those caused by the presence of anti- α (group) antibodies. The immunological characteristics of hexons of type 4, as found in previous studies (15) and as described above, instead suggested a relatively close relationship to hexons of subgroup I members. It can further be mentioned that, with regard also to immunological characteristics of vertex capsomers, adenovirus type 4 is much more closely related to members of subgroup I than to members of subgroup III (29). The occurrence of biological characteristics relating type 4 to subgroup I or subgroup III or both has been established by a number of different studies (13, 23, 30).

The characteristics of type 12 hexons indicate that this serotype should be grouped separately. The immunological characteristics of its hexons, which in the experiments described above only were studied in one-way tests, suggested that they contain rather few antigen specificities, particularly relating them to hexons of members of any special subgroup. The same picture has emerged from studies of the immunological complexity of vertex capsomers (29). Since type 12, furthermore, displays rather unique characteristics as regards occurrence of different soluble components (Norrby and Ankerst, *J. Gen. Virol.*, *in press*), guanine-cytosine content of its deoxyribonucleic acid (21), genetic relatedness to other serotypes (8), and oncogenic behavior (5), a separate subgrouping of this serotype (and, therefore, most likely also types 18 and 31) is indicated. This was, in fact, already done in the classification with reference to oncogenic characteristics, which was proposed by Huebner (5).

The interesting relationship between hexons of types 4 and 16 deserves some concluding comments. The results of cross-absorption tests on different biological activities of antihexon sera most readily can be interpreted in the following way. Both type 4 and type 16 hexons carry type-

specific components demonstrable in neutralization, virion HI, and CF tests. In addition to these specificities of hexons, there occurs another antigen specificity, which is uniquely shared by hexons of the two serotypes. This latter specificity, which is responsible for the interaction with cross-neutralizing antibodies, probably is available to a considerable extent at the surface of virions of type 4, but occurs only in relatively small amounts at the surface of virions of type 16. This hypothesis could explain why the ratio between homotypic and heterotypic neutralization titer is much higher in type 16 antisera (a 2- to 4-fold difference) than in type 4 antisera (a 16- to 32-fold difference), as was already described (25) and confirmed in the present study. The behavior of virion-HI antibodies, which parallels those of neutralizing antibodies, could be explained in the same way. To explain the results in CF tests after absorptions with different hexons, it is necessary to postulate further that type 4 hexons carry (or display) larger amounts of the antigen specificity common to the two types than type 16 hexons. This could explain why antiadenovirus 4 hexon sera contain relatively higher antibody titers against this specificity (cf. the effect of absorption with type 3 hexons). It is possible that the quantitative occurrence of common antigen specificities as well as their topographical distribution may provide a general explanation for more or less one-sided immunological cross-reactions occurring among different members of the same virus group.

ACKNOWLEDGMENTS

This investigation was supported by grants from the Swedish Medical Research Council (Project B69-16X-548-05A) and the Swedish Cancer Society (Project 171-K69-02X).

The excellent technical assistance of Ylva Gollmar, Marianne Peterson, Margareta Jurstrand, and Ulla Carlson is gratefully acknowledged.

LITERATURE CITED

- Almeida, J. D., B. Cinander, and D. Naylor. 1965. Univalent fragments of antibody: A study by electron microscopy. *Immunochemistry* 2:169-176.
- Döhner, L. 1968. Untersuchungen zum Nachweis von serologischen Unterschieden zwischen den Gruppenantigenen aus verschiedenen Adenovirustypen. II. Demonstration von Antigenunterschieden mit der KBR in der Isofixationstechnik. *Z. Immunforsch.* 136:451-456.
- Elek, S. D., B. V. K. Smith, and W. Highman. 1964. The interaction of antigen and antibody in agglutination. A study by electron microscopy. *Immunology* 7:570-585.
- Freeman, A. E., P. H. Black, E. A. Vanderpool, P. H. Henry, J. B. Austin, and R. J. Huebner. 1967. Transformation of primary rat embryo cells by adenovirus type 2. *Proc. Nat. Acad. Sci. (U.S.A.)* 58:1205-1212.
- Huebner, R. J. 1967. The problem of oncogenicity of adenoviruses, p. 73-80. *In Proceedings of the first international conference on vaccines against viral and rickettsial diseases of man*, Pan American Health Organization publication 147.
- Kasel, J. A., D. W. Alling, P. R. Glade, and P. A. Banks. 1967. Heterotypic antibody responses in man following vaccination with adenovirus type 1 hexon antigen. *Proc. Soc. Exp. Biol. Med.* 126:895-896.
- Kjellén, L., and H. G. Pereira. 1968. Role of adenovirus antigens in the induction of virus neutralizing antibody. *J. Gen. Virol.* 2:177-185.
- Lacy, S. S., and M. Green. 1967. The mechanism of viral carcinogenesis by DNA mammalian viruses: DNA-DNA homology relationships among the "weakly" oncogenic human adenoviruses. *J. Gen. Virol.* 1:413-418.
- Norrby, E. 1966. The relationship between the soluble antigens and the virion of adenovirus type 3. I. Morphological characteristics. *Virology* 28:236-248.
- Norrby, E. 1966. The relationship between the soluble antigens and the virion of adenovirus type 3. II. Identification and characterization of an incomplete hemagglutinin. *Virology* 30:608-617.
- Norrby, E. 1968. Comparative studies on the soluble components of adenovirus types 9 and 15 and the intermediate strain 9-15. *J. Virol.* 2:1200-1210.
- Norrby, E. 1968. Identification of soluble components of adenovirus type 11. *J. Gen. Virol.* 2:123-133.
- Norrby, E. 1968. Biological significance of structural adenovirus components. *Curr. Top. Microbiol. Immunol.* 43:1-43.
- Norrby, E. 1968. Comparison of soluble components of adenovirus types 3 and 11. *J. Gen. Virol.* 2:135-142.
- Norrby, E. 1969. The relationship between the soluble antigens and the virion of adenovirus type 3. IV. Immunological complexity of soluble components. *Virology* 37:565-576.
- Norrby, E. 1969. The structural and functional diversity of adenovirus capsid components. *J. Gen. Virol.* 5:221-236.
- Norrby, E., and J. Ankerst. 1969. Biological characterization of structural adenovirus type 12 components. *J. Gen. Virol.* 5:183-194.
- Norrby, E., and P. Skaaret. 1967. The relationship between the soluble antigens and the virion of adenovirus type 3. III. Immunological identification of fiber antigen and isolated vertex capsomer antigen. *Virology* 32:489-502.
- Norrby, E., and P. Skaaret. 1968. Comparison between soluble components of adenovirus types 3 and 16 and of the intermediate strain 3-16 (the San Carlos agent). *Virology* 36:201-211.
- Norrby, E., B. Nyberg, P. Skaaret, and A. Lengyel. 1967. Separation and characterization of soluble adenovirus type 9 components. *J. Virol.* 1:1101-1108.
- Norrby, E., H. Marusyk, and M. L. Hammarskjöld. 1969. The relationship between the soluble antigens and the virion of adenovirus type 3. V. Identification of antigens available at the surface of virions. *Virology* 38:477-482.
- Norrby, E., G. Wadell, and H. Marusyk. 1969. Fiber-associated incomplete and complete hemagglutinins of adenovirus type 6. *Arch. Gesamte Virusforsch.* 28:239-244.
- Pereira, H. G. 1960. Antigenic structure of noninfectious adenovirus materials. *Nature (London)* 186:571-572.
- Pina, M., and M. Green. 1965. Biochemical studies on adenovirus multiplication. IX. Chemical and base composition analysis of 28 human adenoviruses. *Proc. Nat. Acad. Sci. U.S.A.* 54:547-551.
- Rosen, L. 1960. A hemagglutination-inhibition technique for typing adenoviruses. *Amer. J. Hyg.* 71:120-128.
- Schlesinger, R. W. 1969. Adenoviruses: The nature of the virion and of controlling factors in productive or abortive infection and tumorigenesis. *Advan. Virus Res.* 14:1-61.
- Schmidt, N. J., C. J. King, and E. H. Lennette. 1965. Hemagglutination and hemagglutination-inhibition with adenovirus type 12. *Proc. Soc. Exp. Biol. Med.* 118:208-211.
- Stevens, D. A., M. Schaeffer, J. P. Fox, C. D. Brandt, and M. Romano. 1967. Standardization and certification of refer-

- ence antigens and antisera for 30 human adenovirus serotypes. *Amer. J. Epidemiol.* 56:617-633.
26. Valentine, R. C., and N. M. Green. 1967. Electron microscopy of antibody-hapten complex. *J. Mol. Biol.* 27:615-617.
27. Wadell, G., and P. Skaaret. 1967. Separation of adenovirus type 4 components. *Arch. Gesamte Virusforsch.* 21:366-382.
28. Wadell, G., and E. Norrby. 1969. The soluble hemagglutinins of adenoviruses belonging to Rosen's subgroup III. II. The slowly sedimenting hemagglutinin. *Arch. Gesamte Virusforsch.* 26:53-62.
29. Wadell, G., and E. Norrby. 1969. Immunological and other biological characteristics of pentons of human adenoviruses. *J. Virol.* 4:671-680.
30. Wadell, G., E. Norrby, and U. Schöning. 1967. Ultrastructure of soluble antigens and the virion of adenovirus type 4. *Arch. Gesamte Virusforsch.* 21:234-242.
31. Wadell, G., E. Norrby, and P. Skaaret. 1969. The soluble hemagglutinins of adenoviruses belonging to Rosen's subgroup III. I. The rapidly sedimenting hemagglutinin. *Arch. Gesamte Virusforsch.* 26:33-52.
32. Wilcox, W. C., and H. S. Ginsberg. 1963. Production of specific neutralizing antibody with soluble antigens of type 5 adenovirus. *Proc. Soc. Exp. Biol. Med.* 114:37-42.