Key words: adenovirus type 2/epitope mapping/fibre, adenovirus

An Antigenic Analysis of the Adenovirus Type 2 Fibre Polypeptide

By G. WATSON,* M. G. BURDON 1 AND W. C. RUSSELL 1

Department of Veterinary Pathology, Royal School of Veterinary Studies, Edinburgh EH9 1QH and 1Department of Biochemistry and Microbiology, Irvine Building, North Street, St Andrews, Fife KY16 9AL, U.K.

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SUMMARY

Twenty-seven monoclonal antisera were generated against the SDS-denatured fibre of adenovirus type 2. The antisera were characterized using radioimmune assay, fluorescent antibody tests, immune precipitation, Western blotting, haemagglutination and neutralization, and formed six groups as follows: A, type-specific neutralizing antisera which exhibited haemagglutination inhibition (Hi+); B, type-specific non-neutralizing antisera which did not exhibit haemagglutination inhibition (Hi-); C, subgroup-specific neutralizing antisera Hi+; D, a subgroup-specific neutralizing antiserum Hi-; E, subgroup-specific non-neutralizing antiserum Hi-; F, a subgroup-specific neutralizing antiserum Hi- which did not react in Western blotting tests. The C-terminal 201 amino acids of the fibre were expressed in Escherichia coli and a total of six antisera from groups A, B and C recognized five epitopes carried on this region which in several models is thought to form the knob of the fibre. At least eight epitopes were expressed by the entire native fibre. The five epitopes of the C-terminal end of the fibre formed three antigenic sites. Two sites each consisted of a neutralizing type-specific and a neutralizing group-specific epitope which overlapped in position. The remaining site consisted of a type-specific, non-neutralizing epitope.

INTRODUCTION

The fibre gene extends from base 31030 to 32778 on the Ad2 genome, has one open reading frame and encodes a single polypeptide with an \( M_r \) of 61925 (Herisse & Galibert, 1981; Herisse et al., 1981). The native fibre has an \( M_r \) of 200000 and may have a dimeric (Green et al., 1983) or trimeric structure (Dorsett & Ginsberg, 1975; Sundquist et al., 1973; Van Oostrum & Burnett, 1985). The model of Green et al. (1983) proposes that the dimeric fibre is 27 nm in length and consists of an N-terminal tail and a C-terminal knob separated by a shaft of 21 nm made up of 22 units of a repeating 15 amino acid motif. Each 15 amino acid repeat forms two short \( \beta \) strands and two \( \beta \) bends. This model is supported by the finding that adenovirus type 3 (Ad3) fibre has only six 15 residue repeats and has the same size tail and knob as the Ad2 fibre but has a considerably shorter shaft (Signas et al., 1985). However, the model of Boudin & Boulanger (1981) based on the comparative carboxypeptidase digests of free and penton base-combined fibres, proposes that the C-terminal end of the fibre is involved in assembly of the penton.

The existence of three antigenic sites on the Ad2 fibre has been described. A \( \gamma \) determinant in the knob is type-specific (Norrby, 1969a) and is the component responsible for interaction with the receptor (Pereira & de Figueiredo, 1962; Valentine & Pereira, 1965; Norrby, 1966b; Petersson et al., 1968; Norrby et al., 1969a). The site can apparently bind two IgG molecules and type-specific antisera against the fibre have been shown, using a variety of techniques, to have
neutralizing activity (Wilcox & Ginsberg, 1963; Banks et al., 1966; Pettersson & Hoglund, 1969; Norrby, 1969a). However Kjellén & Pereira (1968) could not detect such neutralizing activity. A subgroup determinant \( \delta \) is located at the proximal end of the shaft, very close to the junction between the penton base and the fibre (Pettersson & Hoglund, 1969; Wadell & Norrby, 1969; Boudin & Boulanger, 1981), and an intersubgroup-specific determinant has been reported that is common to subgroups C and D (Pettersson et al., 1968; Norrby, 1968, 1969a).

The \( \delta \) or intersubgroup determinant may be involved in the haemagglutination enhancement reaction observed when heterotypic antisera are added to fibres attached to erythrocytes. The antisera are able to bridge the attached fibres through an antigenic site and thereby increase the haemagglutination reaction (Valentine & Pereira, 1965; Pereira & de Figueiredo, 1962; Norrby & Skaare, 1967).

We have produced a series of monoclonal antibodies against the Ad2 fibre to examine the antigenicity of the fibre and we describe here the characterization of six different groups of antisera and the presence of at least eight epitopes on the fibre including four of previously undetected types. We have expressed the C-terminal 201 amino acids of the fibre in *Escherichia coli* and using the monoclonal antisera, have shown that three antigenic sites are expressed in this region, including two sites which each consists of more than one epitope.

**METHODS**

*Viruses and soluble antigens.* Adenovirus type 2, type 5 (Ad5) and type 4 (Ad4) were grown in HeLa suspension cells and were prepared and purified as previously described (Winters & Russell, 1971). Soluble antigens were obtained by three cycles of freezing and thawing adenovirus-infected cells followed by fluorocarbon extraction and caesium chloride density gradient centrifugation (Russell et al., 1967, 1981). Purified virus and soluble antigens were dialysed against phosphate-buffered saline (PBS) immediately before use.

*Fibre purification.* Ad2 fibre was purified from soluble antigen on QAE-Sephadex G-25 as described by Green et al. (1983).

*Immunization.* BALB/c mice were immunized intraperitoneally (i.p.) with 100 µg alum-precipitated, SDS-treated partially purified fibre and boosted i.p. after 1 month with a further 100 µg. After a 1 month interval and 2 to 3 days before the spleen was required, a final boost of antigen in PBS was administered in the tail vein.

Immunized mice were tested for the production of antisera by radioimmune assay (RIA) using purified fibre and serum obtained from tail bleeds.

*Production of monoclonal antibodies.* These were as described previously (Russell et al., 1981) except that after disruption and pelleting of the spleen cells, the cells were resuspended in 5 ml 0·83% NH₄Cl to lyse red blood cells, allowed to stand at room temperature for 3 min and then pelleted through 5 ml newborn calf serum at 200 g for 10 min and washed with PBS (Fazekas de St. Groth & Scheidegger, 1980). They were then fused with SP2/O-Ag-14 cells and dispensed into tissue culture plates at an SP2/O-Ag-14 concentration of 2 × 10⁴ to 4 × 10⁴ cells/well. The hybridomas were fed as appropriate by removal and replacement of the medium.

*Screening of hybridoma cells.* Supernatants from the hybridoma cells were tested for the presence of anti-fibre immunoglobulins by RIA against purified fibre. Positive clones were grown further in HT medium (Russell et al., 1981). The clones were then subcloned and tested as before. Positive cells were then passaged as ascites in mice and stored in liquid nitrogen.

Antibodies were characterized by RIA, fluorescent antibody tests, immune precipitation, Western blotting and competition assays.

*Radioimmune assay.* Nitrocellulose was soaked in 100 µg fibre antigen in 10 ml PBS for 2 h and then washed in PBS for 10 min twice. Supernatants from hybridoma cultures to be tested were dispensed into a 96-well tissue culture plate and the nitrocellulose was clamped between this and a second plate. The apparatus was inverted and incubated at 37 °C for 1 h. The nitrocellulose was then removed, washed for 10 min twice in PBS and incubated with 1 to 2 µCi 125I-labelled Protein A for 1 h, washed for 10 min three or four times in PBS and allowed to dry in air. Autoradiography was as previously described (Russell & Blair, 1977).

*Fluorescence and immune precipitation.* These tests were as described previously (Russell et al., 1981; Hayashi & Russell, 1968; Russell & Blair, 1977).

*Neutralization.* Dilutions of ascitic fluids were incubated at room temperature for 1 h with 1 × 10⁶ p.f.u. of Ad2 in 100 µl medium. HeLa cells (5 × 10⁴) in 100 µl medium were added and incubated for 5 to 7 days at 37 °C. The cells were then fixed in formol saline, stained with 0·1% crystal violet and washed. The titre was taken as the reciprocal of the last dilution of antisera to inhibit plaque formation by 50%.

*Haemagglutination.* Dilutions of antisera were incubated with Ad2 soluble antigen at 37 °C for 15 min. Lister rat red blood cells at 1·5% cells in PBS were added in an equal volume and incubated at 37 °C for 2 h. All reactions were carried out in a 1/100 dilution of rabbit measles virus antiserum to reduce non-specific reactions.
**Western blotting.** Purified Ad2 virus was denatured and proteins were separated on a 13% denaturing polyacrylamide gel by SDS-PAGE. The proteins were transferred to nitrocellulose by the method of Towbin et al. (1979) and non-specific protein binding sites were blocked as for RIA. Strips of nitrocellulose were incubated with 1/50 dilutions of ascitic fluid or with polyclonal Ad2 antiserum. Binding was detected with iodinated Protein A and further steps were as for RIA.

**Competition assay.** The IgG fraction of the ascitic fluid to be assayed was purified on a Protein A–Sepharose column and labelled with 5 µCi Na\(^{125}\)I (Amersham) using chloramine-T (Hunter, 1978). Serial dilutions of each of the competing monoclonal antibodies were made in 15 µl 3% bovine serum albumin in PBS and \(^{125}\)I-labelled IgG was added at an appropriate dilution. The antisera were then incubated at 37°C for 1 h in an RIA with nitrocellulose-bound soluble antigen. The nitrocellulose was then washed for 10 min three or four times in PBS and autoradiography was as described previously.

**Bacterial strains and plasmid vectors.** The E. coli strains used were all K12 strains. JM101 has the genotype \(\Delta(lac-pro) thi supE F' traD36 proAB lacIqZAM15\) (Messing et al., 1981). The plasmid pUC9 is 2.7 kb in length and encodes ampicillin resistance (Viera & Messing, 1982). JM83 has the genotype ara \(\Delta(lac-pro) thi strA\) \(\phi 80d-lacZAM15\) (Messing, 1979).

The plasmid pSMC1 has the Ad2 Smal fragment C, from base pair coordinate 27569 to 33091 on Ad2, inserted into the EcoRI site of pBR325 using EcoRI linkers.

**Growth media.** L broth was 1% tryptone, 0.5% yeast extract, 1% NaCl pH 7.5 and L agar was 1.5% agar in L broth. Minimal medium was 46 mM-K\(_2\)HPO\(_4\), 33 mM-K\(_2\)HPO\(_4\), 7.5 mM-(NH\(_4\))\(_2\)SO\(_4\), 1.7 mM-NaNO\(_3\), H\(_2\)O, 1 mM-MgSO\(_4\), 0.2% glucose, 0.001% vitamin B. Minimal agar was 1.5% agar in minimal medium.

Ampicillin (Amp) was used at a concentration of 50 µg/µl and isopropyl-\(\beta\)-D-thiogalactopyranoside (IPTG; an inducer for \(\beta\)-galactosidase expression) was used at 0.5 mM in liquid cultures and on agar plates. 5-Bromo-4-chloro-3-indolyl-\(\beta\)-D-galactopyranoside (X-gal; a colour indicator of galactosidase activity) was used at 15 µg/µl.

**Construction of pGW58, pGW60, pGW502 plasmids.** The plasmid pSMC1 was digested with HindIII, the fragments were separated by electrophoresis on a 1% high gelling temperature agarose (Seakem) and the 2.1 kb fragment carrying the 32173 to 33091 bp region of Ad2 was purified using DE81 paper (Dretzen et al., 1983). Minimal agar plates with Amp were used to grow transformed cells and colonies were picked and purified onto minimal plates containing Amp, IPTG and X-gal.

**Induction and extracts of cultures.** Overnight cultures of strains grown in minimal medium with Amp were diluted 1/40 into fresh medium with Amp and grown until the start of the log growth phase (OD\(_{600}\) 0.2). IPTG was added to a final concentration of 0.5 mM and the culture was incubated for 2 h.

Samples of induced cultures were centrifuged (13000 g, 30 s) and resuspended in 1/20 vol. 50 mM-Tris–HCl, 30 mM-NaCl. Lysozyme was added to a concentration of 1 mg/ml and the cells were incubated at 4°C for 30 min. Cells were lysed by three cycles of freezing and thawing followed by sonication to degrade the DNA. This preparation is referred to throughout as sonicated extract.

**DNA isolation.** Plasmid DNA was isolated from bacterial strains by the mini-prep method of Birnboim & Doly (1979).

**RESULTS**

**Immunization and fusion.**

Seven of 11 immunized mice showed detectable levels of Ad2 antibodies when screened in an RIA against fibre antigen. The spleen of a mouse which showed binding at an antiserum dilution of 1 in 640 was used for the fusion.

Twenty-five hybridoma colonies were positive when screened in an RIA against fibre antigen and ascitic fluids were produced from 27 subcloned monoclonal hybridomas derived from them.

**Characterization of monoclonal antibodies.**

The monoclonal antibodies were divided on the basis of their ability to react in fluorescent antibody tests with Ad2-, Ad4- or Ad5-infected HeLa cells. Ad2 and Ad5 are both members of subgroup C and Ad4 is a member of subgroup E, so the results of these tests allowed the division of the monoclonal antibodies into type-specific, subgroup-specific and group-specific classes.
Twelve monoclonal antibodies reacted in a type-specific manner, 15 reacted in a subgroup-specific manner and none reacted in a group-specific manner. Generalized nuclear fluorescence and speckled perinuclear fluorescence were observed with both type-specific and subgroup-specific antisera.

**Immune precipitation**

Extracts of $[^{35}S]$methionine-labelled Ad2- or Ad5-infected cells were incubated with dilutions of each monoclonal antiserum for 1 h at room temperature. Antigen–antibody complexes were then precipitated using fixed *Staphylococcus aureus*, and denatured and separated by SDS–PAGE. Precipitated fibre, penton and hexon antigens were detected by autoradiography. The variation in precipitated antigens probably reflects the formation of complexes with intermediates of virus assembly (Russell *et al.*, 1981). The 12 monoclonal antisera which reacted in a type-specific manner in the fluorescent antibody test precipitated only type 2 antigens and the 15 monoclonal antisera which reacted in a subgroup-specific manner in the fluorescent antibody tests precipitated antigens from both Ad2 and Ad5 virus.

**Western blotting**

Twenty-six monoclonal antibodies were positive against Ad2 and Ad5 virus in a Western blot and bound to the fibre only (Fig. 1). These results provide strong direct evidence that the monoclonal antibodies recognize fibre epitopes that are retained during SDS–PAGE and transfer to nitrocellulose. Monoclonal antibodies known to be type-specific from fluorescent

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**Fig. 1.** Ad2 (lanes 1) or Ad5 (lanes 2) polypeptides were separated by SDS–PAGE on 13% acrylamide gels, transferred to nitrocellulose by the method of Towbin *et al.* (1979) and reacted with: (a) guinea-pig anti-fibre polyclonal antiserum, (b) a type-specific anti-fibre monoclonal antiserum, (c) mouse serum or (d) a subgroup-specific anti-fibre monoclonal antiserum.
Antigenic analysis of Ad2 fibre polypeptide

Table 1. Characterization of monoclonal antisera

<table>
<thead>
<tr>
<th>Monoclonal antisera no.</th>
<th>Fluorescent antibody test*</th>
<th>Antigen precipitated in†</th>
<th>Western blotting in‡</th>
<th>Neutralization titre§</th>
<th>Haemagglutination inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ad2</td>
<td>Ad5</td>
<td>Ad2</td>
<td>Ad5</td>
</tr>
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<td>F</td>
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<td>HBp</td>
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<td>F</td>
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<td>S</td>
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</tr>
<tr>
<td>27</td>
<td>S</td>
<td>HBpF</td>
<td>PBf</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* T, Type-specific; S, subgroup-specific.
† H, Hexon; Pb, penton base; F, fibre.
‡ Neutralization titres are the reciprocal of the last dilution of monoclonal antiserum to inhibit plaque formation by 50%. No neutralization at a titre of $5 \times 10^1$ is represented by -.
§ No, Not done.

antibody and immune precipitation results also demonstrated binding to the Ad5 fibre suggesting that other sites may be exposed due to the denaturation of the fibre polypeptide. One subgroup-specific monoclonal antiserum did not bind to either the Ad2 or Ad5 fibres.

Neutralization

Twenty-seven monoclonal antibodies were tested for neutralization of Ad2 virus and 16 antisera neutralized to titres of at least 1 in 50. Seven neutralizing monoclonal antisera were directed against type-specific epitopes and nine neutralizing monoclonal antisera recognized subgroup-specific epitopes. Five type-specific and six further subgroup-specific monoclonal antisera did not show neutralizing activity (Table 1).

The collective results described above were used to assign the monoclonal antibodies to the groups shown in Table 2.

Haemagglutination

The ability of an antiserum to neutralize virus appears to be related to its ability to inhibit the haemagglutination of red blood cells. Therefore haemagglutination inhibition assays were carried out on representative samples from each group of monoclonal antisera described in Table 2. All neutralizing monoclonal antisera with the exception of 15 and 27 demonstrated haemagglutination inhibition, suggesting that these two antisera recognize a different epitope...
Table 2. Designation of monoclonal antisera into groups according to results of characterization

<table>
<thead>
<tr>
<th>Monoclonal antiserum no.</th>
<th>Characteristics of monoclonal antiserum*</th>
<th>Group designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3, 4, 5</td>
<td>Type-specific neutralizing Hi⁺</td>
<td>A</td>
</tr>
<tr>
<td>6, 7, 8, 9, 10, 11, 12</td>
<td>Type-specific non-neutralizing Hi⁻</td>
<td>B</td>
</tr>
<tr>
<td>13, 14, 16, 17, 18, 19, 20</td>
<td>Subgroup-specific neutralizing Hi⁺</td>
<td>C</td>
</tr>
<tr>
<td>15</td>
<td>Subgroup-specific neutralizing Hi⁻</td>
<td>D</td>
</tr>
<tr>
<td>21, 22, 23, 24, 25, 26</td>
<td>Subgroup-specific neutralizing W⁻ Hi⁻</td>
<td>E</td>
</tr>
<tr>
<td>27</td>
<td>Subgroup-specific neutralizing W⁻</td>
<td>F</td>
</tr>
</tbody>
</table>

* Hi⁻, Does not exhibit haemagglutination inhibition; Hi⁺, exhibits haemagglutination inhibition; W⁻, does not react in a Western blotting test.

from other neutralizing antisera. Furthermore as monoclonal antiserum 27 does not recognize its epitope in Western blotting experiments whereas antiserum 15 binds the fibre antigen in such tests, the two epitopes recognized by antisera 15 and 27 are probably distinct from one another.

None of the non-neutralizing groups of antisera demonstrated haemagglutination inhibition.

Expression of the C-terminal 201 amino acids of the fibre

Plasmids that expressed the C-terminal 201 amino acids of the fibre, e.g. pGW55 and pGW58, were produced as described and used to transform JM83, in which there is constitutive expression of the β-galactosidase gene and its insert. Plasmids were also produced in which the fibre gene fragment was cloned in the opposite orientation to that which produced expression of the C-terminal end of the fibre, e.g. pGW60.

Sonicated extracts from JM83, JM83(pUC9), JM83(pGW55), JM83(pGW58) and JM83(pGW60) were tested in an RIA with Ad2 polyclonal antiserum. No binding occurred with extracts from the host strain, the host strain carrying the vector or JM83(pGW60) which carried the plasmid with an insert in one orientation. However, extracts from strains JM83(pGW55) and JM83(pGW58), carrying the insert in the other orientation and expressing the C-terminal 201 amino acids of the fibre, bound the Ad2 antiserum intensely. Restriction enzyme digests of DNA from pGW55 and pGW58 showed that the cloned fragment was in the correct orientation for expression in these plasmids (Fig. 2).

The plasmid pGW502 (Fig. 2) expressed the same region of the fibre polypeptide as pGW55 and pGW58, but expression of the polypeptide was inducible allowing direct comparison of Ad2 antiserum binding to the expressed polypeptide. Sonicated extracts of induced and non-induced cultures of JM101(pGW502) were bound to nitrocellulose and used to perform RIAs with 27 monoclonal antisera, each at a dilution of 1 in 50. Six monoclonal antisera bound to induced cell extracts and no monoclonal antiserum bound to non-induced cell extracts (Fig. 3). The six positive monoclonal antisera (1, 5, 11, 17, 18 and 19) therefore recognize epitopes carried by the C-terminal 201 amino acids of the fibre, which according to the model of Green et al. (1983) form the knob and 21 amino acids of the shaft.

Four monoclonal antisera, 3, 11, 17 and 19, that recognized the expressed fibre antigen were labelled with ¹²⁵I and tested in competition assays with each of the six antisera that recognized the expressed fibre antigen (Fig. 4). The results are shown in Table 3.

The monoclonal antisera 17 and 18 both belong to group C (Table 2) and are therefore both directed against the same kind of epitope. In competition assays (Table 3) these two antisera compete for a binding site on Ad2 at a titre of 5120 and it can therefore be concluded that these antisera recognize the same epitope. However, monoclonal antiserum 19 which also belongs to group C (19C) did not compete with either 17C or 18C and competed in a different way from 17C and 18C in assays with other monoclonal antisera. It can therefore be concluded that 19C recognizes a different epitope from 17C and 18C. Similarly 1A and 5A represent different epitopes.

Monoclonal antisera 1A and 17C, although of different types and therefore recognizing different epitopes, exhibit competition for binding to Ad2 at a titre of 1280 and therefore appear
Antigenic analysis of Ad2 fibre polypeptide

Fig. 2. Relevant restriction enzyme sites are shown for (a) the entire fibre gene in the plasmid pSMC1, (b) a HindIII fragment carrying the 3’ terminus of the fibre gene cloned into the HindIII site of pUC9, in the plasmids pGW55 and pGW58 and (c) a HindIII/MspI fragment carrying the 3’ terminus of the fibre gene cloned into the HindIII/Accl sites of pUC9, in the plasmid pGW502. Values under the maps represent the position in bp from the start of the Ad2 sequence.

Table 3. Competition assays among monoclonal antisera which exhibit binding to the C-terminal 201 amino acids of fibre

<table>
<thead>
<tr>
<th>Group designation of monoclonal antiserum</th>
<th>Unlabelled monoclonal antiserum no.</th>
<th>(^{125}\text{I}-\text{labelled monoclonal antiserum no.})</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>1280*</td>
</tr>
<tr>
<td>C</td>
<td>17</td>
<td>5120</td>
</tr>
<tr>
<td>C</td>
<td>18</td>
<td>5120</td>
</tr>
<tr>
<td>C</td>
<td>19</td>
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</tr>
<tr>
<td>B</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
<td>–</td>
</tr>
</tbody>
</table>

* Figures are the reciprocal of the last dilution of unlabelled antiserum which competes for binding with labelled antiserum. –, No competition at a 1/20 dilution of unlabelled antiserum.
Fig. 3. Sonicated E. coli cell extracts from (a) non-induced or (b) induced cultures of cells expressing the C-terminal 201 amino acids of the fibre, were bound to nitrocellulose and reacted with monoclonal antisera at dilutions of 1 in 50. Positions A10 and E10 are non-immunized mouse serum. Positions C10 and G10 are anti-fibre mouse polyclonal serum. Monoclonal antisera binding to sonicated cell extract from induced cultures at positions E2, E6, F9 and G9 are monoclonal antisera 1, 17, 5 and 19 respectively.

Fig. 4. Competition assays of $^{125}$I-labelled monoclonal antiserum 17 with unlabelled monoclonal antisera (a) 1, (b) 17, (c) 18, (d) 19, (e) 5, (f) 11 and (g) non-immunized mouse serum. Competition for binding to Ad2 was assayed at 1/4 serial dilutions of unlabelled monoclonal antiserum from an initial dilution of 1/20 (line 1) to a final dilution of 1/81920 (line 7).

Fig. 5. The arrangement of epitopes within the C-terminal 201 amino acids of the fibre is shown according to the results of competition assays. The epitope recognized by monoclonal antiserum 5, for example, overlaps with that recognized by monoclonal antiserum 19, but not with those recognized by monoclonal antiserum 1, 11, 17 or 18.

to represent overlapping epitopes. Similarly 19C and 5A represent overlapping epitopes of different types (Fig. 5).

Five epitopes therefore exist on the C-terminal 201 amino acids of the fibre and these are exemplified by the monoclonal antisera 1A, 5A, 11B, 17C/18C, and 19C. These monoclonal antisera are all members of three groups of antisera described in Table 2. A further three groups have been described, representing at least a further three epitopes on the fibre. A minimum of eight epitopes are therefore expressed on the fibre polypeptide.

**DISCUSSION**

The use of a series of monoclonal antibodies to study the antigenicity of the Ad2 fibre has revealed the existence of several previously undetected epitopes. Eight epitopes of six epitope types exist on the fibre: neutralizing and non-neutralizing type-specific epitopes, neutralizing
and non-neutralizing subgroup-specific epitopes, one neutralizing subgroup-specific epitope that does not inhibit haemagglutination but is retained during transfer to nitrocellulose and Western blotting, and a further neutralizing subgroup-specific epitope that does not inhibit haemagglutination and is not retained during transfer to nitrocellulose. In Western blotting experiments, antisera that are known to be type-specific from immune precipitation and fluorescent antibody tests will bind to Ad5 fibre which has been subjected to denaturation, SDS-PAGE and transferred to nitrocellulose. This suggests that further small epitopes are revealed by monoclonal antisera against denatured fibre that are not normally available in the native form. With the exception of Western blotting, all experiments in this study were based on the binding of monoclonal antisera to the native form of the fibre.

Conflicting results have been reported regarding the neutralization of Ad2 by type-specific anti-fibre serum. Several groups have reported type-specific antisera raised against the fibre that have neutralizing activity (Wilcox & Ginsberg, 1963; Banks et al., 1966; Pettersson & Hoglund, 1969; Norrby, 1969a). Others have reported similar antisera that have no neutralizing activity (Kjellén & Pereira, 1968). This study has demonstrated that both neutralizing and non-neutralizing type-specific epitopes exist on the fibre. The neutralizing type-specific antisera reported here also inhibit haemagglutination of rat erythrocytes by Ad2 soluble antigen. The mechanism of neutralization is as yet unknown but may involve the prevention of the recognition or attachment of the viral attachment protein to the cell receptor unit.

This study also indicates that subgroup-specific neutralizing epitopes exist on the fibre. Nine subgroup-specific neutralizing monoclonal antisera of three different types were produced. Seven of these antisera inhibit haemagglutination of rat erythrocytes by soluble antigen. From competition assay results, these antisera are known to represent at least two epitopes. The other two subgroup-specific neutralizing antisera do not inhibit haemagglutination and behave in different ways in Western blotting tests. Neutralization and haemagglutination are generally considered to be type-specific properties and are used for the typing of adenoviruses. These results indicate that subgroup-specific epitopes may exhibit neutralization and haemagglutination properties. Therefore, care should be taken to ensure that any neutralizing monoclonal antiserum is type-specific before use in typing assays.

The C-terminal 201 amino acids which, according to the model of Green et al. (1983), form the knob and 21 amino acids of the shaft in the native fibre were expressed in E. coli and specifically bound six monoclonal antisera from three groups. We have demonstrated that these monoclonal antisera represent five epitopes. The epitopes recognized by the monoclonal antisera 1 and 1/18, which are type-specific neutralizing and subgroup-specific neutralizing antisera respectively, overlap in position. These are distinct from the overlapping epitopes recognized by antisera 9 and 5 which are also type-specific neutralizing and subgroup-specific neutralizing antisera respectively. The remaining epitope expressed by the C-terminal 201 amino acids of the fibre polypeptide is a type-specific epitope which will not neutralize Ad2. These results demonstrate that three antigenic sites are expressed on the C-terminal end of the fibre and that two of these sites consist of more than one epitope. We are currently determining the locations and limits of each of the five epitopes on the C-terminal 201 amino acids in order to produce an antigenic map of this region. It is possible that some of the 21 monoclonal antisera which did not bind to the expressed C-terminal fibre polypeptide will recognize the epitopes expressed by this region with a lower affinity than the six monoclonal antisera described above. However, it is predicted that further investigations currently being undertaken will reveal the presence of several different epitopes on the shaft of the fibre.

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REFERENCES


Antigenic analysis of Ad2 fibre polypeptide


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