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CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO ADENOVIRUS TYPE 35 HEXON

É. ÁDÁM, A. LENGYEL, CS. KERÉKGYÁRTÓ[1], J. ERDEI[1], J. FACHET[1], I. NÁSZ

Institute of Microbiology, Semmelweis University Medical School, H-1089 Budapest, and [1]Institute of Pathophysiology, University Medical School, Debrecen, Hungary

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Summary. — Twenty three monoclonal antibody-rich ascitic fluids (MIAFs) to human adenovirus (AV) type 35 hexon were studied by indirect ELISA using various tracer systems, passive haemagglutination (HA) as well as gel diffusion techniques. Eleven different human heterologous hexon types in addition to the homologous one, and two animal adenovirus (AV) hexons were used to determine the reactivity patterns (RPs) of the monoclonal antibodies (MoAbs). Based on the cross-reactivity with the different hexon types, the MoAbs exhibited genus, subgenus and type specificities; furthermore, a variety of intersubgenus and intertype specificities could be found. Fifteen of the MoAbs reacted in ELISA, but not in passive HA, suggesting that certain epitopes on the hexons bound to red blood cells were not available for the MoAbs in question. Four MoAbs were able to form a precipitin line with the hexon antigen in gel diffusion. Two of the four (MoAbs 35H10 and 35H51) formed with the homologous AV35 hexon a single confluent precipitin line only. In spite of the origin of these MoAbs from different hybrid cells (clones) their specificity was probably identical when recognizing the type-specific epitope of the AV35 hexon. The other two MoAbs (35H15 and 35H26) with a broad RPs were able to precipitate not only the homologous but also different heterologous hexon types.

Key words: adenovirus type 35; hexon epitopes; monoclonal antibodies; reactivity patterns

Introduction

The icosahedral protein shell of the adenoviruses consists of 252 capsomers (Valentine and Pereira, 1965) from which 240 are hexons. The hexon has unique structural properties with a highly conserved and stable structure. The top of the hexon is hydrophilic and predominantly negatively charged, whereas the base shows hydrophobic properties (Nermut and Perkins, 1979). This major coat protein of the adenoviruses has been extensively

characterized serologically. The top of the hexons bears the type specific determinants, while the determinants at the hexon-hexon contact regions at the base are genus specific. The use of the MoAbs is very useful in determining the different epitopes on the hexon molecule, and based on the RPs with different hexon types, there was a possibility to recognize the antigenic relationship among the different hexon types studied (Ádám *et al.*, 1986).

AV35 was originally isolated from renal transplant recipients (Myerowitz *et al.*, 1975). This type of adenoviruses belongs to the subgenus B of mammalian adenoviruses (Wadell, 1984).

In the recent communication (Ádám *et al.*, 1987*a*) we described the isolation and partial characterization of 37 MoAbs directed against the AV35 hexon. Six of these clones having different RPs were selected to develop MIAFs in addition to another 17 new hybridoma cell lines producing MoAbs to AV35 hexon. We characterized these MoAbs by determining the common antigenic structures with the help of 12 different human hexon types as well as two AV hexons of animal origin. The precipitin line formation ability of the MoAbs and their immunoglobulin (Ig) classes were also determined. The results of this work are presented.

Materials and Methods

Antigens. AV5 DNA transformed human cell line "293" (Graham *et al.*, 1977) was infected with AV35 and the hexon antigens were prepared partly from the fractions above the virion using ultracentrifugation for separation of soluble proteins and virions of the infected cells, and partly from the media of the infected cells by a precipitation with ammonium sulphate. Purification of the hexon protein was done by repeated anion exchange chromatography on DEAE Sephadex A-50 column (Pharmacia, Sweden). In the case of $(NH_4)_2SO_4$ precipitated hexon the first step of the purification was performed on Phenyl-Sepharose CL-4 (Pharmacia, Sweden) according to the method of Khilko (1988). The purified and concentrated AV35 hexon antigen originating from the infected cells was used for immunization. The crude soluble protein suspensions of AV35 were kindly supplied by dr. Gy. Berencsi (Budapest). Eleven heterologous AV types were cultivated on HEp-2 cells and the hexon antigens were purified as described earlier (Nász *et al.*, 1972). Simian adenovirus SA 7 (SAV16) hexon and bovine adenovirus type 3 (BAV3) were kindly supplied by dr. N. S. Khilko (Moscow) and dr. A. Bartha (Budapest), respectively.

Immunization and cell fusion. Balb/c mice were given 3 injections of the purified AV35 hexon antigen. Three days after the final booster injection, spleen cells were fused to myeloma cells by the technique of Köhler and Milstein (1975), with PEG 6000 as fusing agent. Hybrid cells were obtained by growing the cells in a selective medium containing hypoxanthine, aminopterine, and thymidine (HAT). The hybridoma culture fluids were screened for their reactivity with AV35 hexon by an indirect enzyme-linked immunosorbent assay (ELISA). The selected hybridoma cells were cloned and injected intraperitoneally into pristane-primed Balb/c mice and the MIAFs were collected and kept at −20 °C.

Indirect ELISA. For screening the antibody secreting clones the enzyme tracer was horse radish peroxidase (HRPO) coupled to rabbit antimouse immunoglobulins by the method of Nakane and Kawaoi (1974) in our laboratory. The coating was done by incubation of 50 µl of different purified hexon antigens (diluted in the coating buffer) at 37 °C, overnight. After washing, wells were blocked with PBS containing 0.5% BSA and 0.5 mol/l NaCl. After washings, 50 µl of the diluted supernates of the hybrid cells were applied to each well for 2 hr at 37 °C. For the determination of specific antibody titres of the MIAFs (because of the high background of the more concentrated specimens) serial twofold dilutions were prepared from 1 : 100 dilution and applied to the antigen coated wells. After incubation and washings, substrate (3 mg/ml ortho-phenylene diamine) and H_2O_2 were added and the reaction was stopped by adding 50 µl

of 4N H_2SO_4. The developed colour reaction was evaluated at 492 nm using a Multiskan Titertek (Flow Laboratories) automatic plate analyser. In a part of the experiments HRPO-labelled Staphylococcus protein A (SpA) was used (HUMAN Institute for Serobacterial Products and Research, Budapest, Hungary). Other enzyme tracers were urease coupled to rabbit antimouse IgG and to SpA (Allelix, Canada) using UREASE substrate. In these cases the colour changes from yellow to purple were evaluated visually.

Indirect sandwich ELISA was used for the determination of class and subclass specificity of the MoAbs. Wells were coated with AV35 hexon, then 1 : 100 dilution of MIAFs were applied. After 1 hr incubation, class and subclass (isotype) specific rabbit antimouse immunoglobulins (Miles Laboratories, England) were added and the bound second antibodies were detected with the help of HRPO-labelled goat antirabbit immunoglobulin (HUMAN Institute for Serobacterial Products and Research, Budapest, Hungary).

Passive haemagglutination (HA). Tannic acid-treated sheep erythrocytes were coated with the respective highly purified antigens in PBS (pH 6.4) containing 125—200 μg hexon protein per ml; haemagglutination was performed with the MoAbs as previously described (Lengyel *et al.*, 1985).

Gel diffusion experiments. One per cent agar gel (Difco, Noble) containing 2 or 4% PEG 4000 was used. The antigens were used in about 0.5 μg/ml conc. and the MIAFs in a dilution optimal for precipitation. Biclonal mixtures of two MoAbs were studied as well (MoAbs 35H15 and H12).

Results

Reactivity of the MoAbs

Twenty three MIAFs were analysed for their titres and reactivity patterns (RPs) using two hexon types of animal origin beside the 12 different hexon types of human adenoviruses belonging to 5 subgenera. In indirect ELISA experiments six different types of RPs were shown (Table 1). MoAb 35H15 has the broadest RP, it has reacted not only with the hexon of different human adenovirus types, but also with the two hexon types of animal adenoviruses (SAV16 and BAV3). The RP of MoAb 35H26 differs from the RP of MoAb 35H15 only in the absence of the reaction with BAV3 hexon. There are three other MoAbs (35H49, 35H43, and 35H50), which show similar RPs to each other, the only difference being the absence of positivity with BAV3 in the case of the latter two MoAbs. This type of RP can be intersubgenus specific, because of the negativity with hexon types of subgenus C. More than the half of the MIAFs examined, showed subgenus specific RP, i.e. fifteen of the 23 MoAbs reacted only with the types of subgenus B (AV7 and AV35). Three MoAbs (35H10, 35H30, and 35H51) could probably recognize the type specific epitope on the AV35 hexon based on their RP.

Titres and RPs of the MIAFs were tested in HA experiments, as well. The hexon antigens were the same as in indirect ELISA with the exception of BAV3 hexon (Table 2). Six RPs were observed in HA, but this means only 8 MIAFs of the 23. Fifteen of the MoAbs reacted subgenus specifically in ELISA (Table 1) but have not reacted in HA with any of the hexon types studied. Comparing the RPs determined in indirect ELISA and HA experiments, MoAbs 35H15 and 35H26 reacted similarly with the hexons of human adenoviruses and with the hexon of SAV16, but based on the ELISA results, they recognize different epitopes (Table 1). Comparing the

Table 1. RPs of the MoAbs directed against AV35 hexon in the MIAFs determined by indirect ELISA

MoAbs	Human adenovirus types according to subgenera												Animal adenoviruses	
	A	B		C				D				E		
	12	7	35	1	2	5	6	8	9	10	13	4	SAV16	BAV3
35H15*	12[a]	12	10	7	11	11	10	12	12	12	11	6	12	2
35H26	9	11	9	9	11	11	10	11	11	11	11	5	10	—[b]
35H49	8	11	10	—	—	—	—	9	9	9	8	9	11	1
35H43*	11	11	10	—	—	—	—	11	10	10	10	5	11	—
35H50	3	5	6	—	—	—	—	2	3	3	2	2	2	—
35H18*	—	12	11	—	—	—	—	—	—	—	—	—	—	—
35H53	—	4	5	—	—	—	—	—	—	—	—	—	—	—
35H54	—	8	7	—	—	—	—	—	—	—	—	—	—	—
35H55	—	12	10	—	—	—	—	—	—	—	—	—	—	—
35H56	—	9	8	—	—	—	—	—	—	—	—	—	—	—
35H57	—	10	8	—	—	—	—	—	—	—	—	—	—	—
35H58	—	11	8	—	—	—	—	—	—	—	—	—	—	—
35H59	—	9	5	—	—	—	—	—	—	—	—	—	—	—
35H61	—	9	7	—	—	—	—	—	—	—	—	—	—	—
35H62	—	10	7	—	—	—	—	—	—	—	—	—	—	—
35H63	—	9	7	—	—	—	—	—	—	—	—	—	—	—
35H64	—	9	4	—	—	—	—	—	—	—	—	—	—	—
35H65	—	5	2	—	—	—	—	—	—	—	—	—	—	—
35H66	—	6	6	—	—	—	—	—	—	—	—	—	—	—
35H1*	—	7	7	—	—	—	—	—	—	—	—	—	—	—
35H10*	—	—	5	—	—	—	—	—	—	—	—	—	—	—
35H30*	—	—	5	—	—	—	—	—	—	—	—	—	—	—
35H51	—	—	12	—	—	—	—	—	—	—	—	—	—	—

[a] titres are expressed as $\log_2$ (reciprocal dilution of MIAF $\times$ 10^{-2})
[b] negative reaction in 1 : 100 dilution of the MIAF
* RPs of the marked MoAbs were determined in the supernatants, too (Ádám *et al.*, 1987*a*)

Table 2. RPs of the MoAbs directed against AV35 hexon in the MIAFs determined by HA

MoAbs	Human adenovirus types according to subgenera												Animal adenovirus
	A	B		C				D				E	
	12	7	35	1	2	5	6	8	9	10	13	4	SAV16
35H15*	9[a]	8	14	8	9	8	9	9	12	13	15	12	7
35H26	10	10	14	12	10	4	6	8	4	6	6	12	7
35H49	12	12	14	14	—[b]	—	—	9	6	9	6	14	14
35H43*	—	10	15	12	—	—	—	—	—	—	—	5	11
35H50	5	5	10	8	—	—	—	—	—	—	—	—	7
35H18*													
35H53—													
35H59	—	—	—	—	—	—	—	—	—	—	—	—	—
35H61—													
35H66													
35H1*													
35H10*	—	—	6	—	—	—	—	—	—	—	—	—	—
35H30*	—	—	9	—	—	—	—	—	—	—	—	—	—
35H51	—	—	12	—	—	—	—	—	—	—	—	—	—

[a] titres are expressed as $\log_2$ (reciprocal dilution of MIAF $\times$ 10^{-2})
[b] negative reaction in 1 : 100 dilution of the MIAF
* RPs of the marked MoAbs were determined in the supernatants, too (Ádám *et al.*, 1987a)

Table 3. Detection of specifically bound MoAbs with different tracer systems

MoAbs	Tracer systems[a]				Immunoglobulin class and subclass of the MoAbs
	Ig-HRPO	IgG-urease	SpA-HRPO	SpA-urease	
35H15, 35H26, 35H49, 35H43, 35H50, 35H18, 35H54, 35H58, 35H62, 35H1, 35H30, 35H51	+	+	−	−	IgG1
35H57, 35H59, 35H63	+	+	+	+	IgG2b
35H53, 35H61, 35H64, 35H65, 35H66, 35H10	+	−	−	−	IgM
35H55, 35H56	+	−	+	+	IgG3

[a] see text for details

RPs of the MoAbs 35H49, 35H43, and 35H50 by two methods (Tables 1 and 2), all three MoAbs reacted with AV1 hexon of subgenus C in HA but did not react with the other types of this subgenus, while in ELISA they did not react even with the AV1 hexon. Another difference is the negativity for the hexon types of subgenus D (MoAbs 35H43 and 35H50). MoAb 35H43 failed to react with AV12 hexon, too. Three of the MoAbs studied showed type specificity with both methods (35H10, 35H30, and 35H51). RPs of the supernatants (Ádám *et al.*, 1987 *a*) and of the MIAFs were compared (MoAbs indicated by asterisks in Tables 1 and 2). In the case of three MoAbs (35H15, 35H43, and 35H30) the RPs determined by indirect ELISA were the same for both specimens. The others (MoAbs 35H18 and 35H10) exhibited narrower RPs in the MIAFs than in the supernatans of the given hybrid cells. The greatest difference was shown by the MoAb 35H1, which reacted with all hexon types when the undiluted supernatant was studied, but it gave positive reaction only with types 7 and 35 (subgenus B) if the MIAF originating from this cell line was investigated. Comparing the data of HA experiments with these six MoAbs originating from the supernatants and from the MIAFs, five of the six failed to react identically in both specimens. In general, the RPs exhibited by the supernatants were broader, than that of the MIAFs. Only MoAb 35H15 showed a broader RP when studied in the MIAF. MoAb 35H18 failed to react in HA experiments.

Immunoglobulin classes and subclasses of the MoAbs

Four different tracer systems were used to determine the specifically bound MoAbs in indirect ELISA. Only one of these which recognize different immunoglobulin classes (rabbit antimouse Ig coupled to HRPO in our laboratory), was able to detect all the 23 MoAbs used in the experiments. Urease-labelled antimouse IgG gave positive reaction with fifteen MoAbs bound to the hexon antigen. Out of these three MoAbs reacted also with the HRPO- and urease-labelled SpA. Two MoAbs failed to react with urease-labelled IgG, while six MoAbs reacted with our HRPO-conjugate only (Table 3). As the HRPO-labelled antimouse Ig was able to detect both immunoglobulin classes IgM and IgG, MoAbs bound to the hexon and reacting only with this type of tracer system should be IgM. According to the data of Kornvall *et al.*, (1970) mouse IgG subclasses could be distinguished by the binding properties to SpA. Our results suggest that three MoAbs should be IgG2a or 2b. More than the half of the MoAbs failed to react with the labelled SpA preparations suggesting that the immunoglobulin subclass of these MoAbs should be IgG1. Two of the MoAbs reacting with the HRPO-conjugate prepared in our laboratory as well as with the two SpA systems proved to be IgG3 as determined with rabbit antimouse IgG3 specific immune serum. Using immunoglobulin class and subclass specific rabbit antimouse sera for the determination of the Ig properties, all suggested specificities proved to be correct.

Gel diffusion analysis of the MoAbs

All the 23 MIAFs were studied in gel diffusion experiments with different hexon antigens. Different PEG concentrations were used and the MIAFs were applied in neat or diluted forms. Four of the 23 MIAFs were able to precipitate the homologous AV35 hexon (MoAbs 35H15, 35H26, 35H10, and 35H51). Two of these (MoAbs 35H10 and 35H51) were able to precipitate only the homologous AV35 hexon with a line formation of complete identity (Fig. 1). The remaining two MoAbs of broad RPs (Tables 1 and 2) were able to precipitate not only the homologous, but different heterologous hexon types in gel diffusion experiments. The precipitin line formation ability of the MoAbs 35H15 and 35H26 varied with the PEG concentration in the gel. At 2% PEG, precipitin lines formed "bird formation" where the diffusion areas of the two MoAbs and the hexon antigen intersected, but not between the MoAb 35H15 and hexon wells or between the 35H26 and the antigen wells (Fig. 2). Increasing the PEG concentration (4%) precipitin line appeared between the wells of MoAb 35H15 and the antigen (Fig. 3) with a "bird" formation in the intersecting area.

MoAb 35H15 (to AV35 hexon) and MoAb 1A3 (to AV1 hexon), as well as MoAb 35H26 (to AV35 hexon) and MoAb H12 (to AV1 hexon) show similar RP to each other. Fig. 4 shows that MoAbs 35H15 and 1A3 formed a line of complete identity with the hexon antigen. MoAbs with different RPs (35H15 and H12) and directed against different hexon types were

studied in a three well pattern and double spur formation was observed (Fig. 5). Mixtures of these two MoAbs were tested in six doubling dilution with AV1 hexon antigen, and the biclonal mixture formed double lines at 1 : 2, 1 : 4, 1 : 8 and 1 : 16 dilutions of MIAF 35H15 in MoAb H12 (Fig. 6).

Discussion

Twenty three MoAbs directed against AV35 hexon were investigated by indirect ELISA and passive HA for determining the RPs. Six RPs were found by both methods, but these RPs were different from each other when determined by ELISA and HA (Tables 1 and 2). In ELISA experiments all the 23 MIAFs reacted with one or more hexon types studied showing probably type, subgenus or genus specificity, and several variety of intertype and intersubgenus specific RPs. In contrast to ELISA experiments, the six RPs determined in HA were exhibited only by 8 MoAbs of the 23, the remaining fifteen MIAFs failed to react with any hexon types. All the latters were found to be specific for subgenus B in ELISA. This phenomenon could be due to the difference of the adsorption behaviour of the hexon to the erythrocytes and to the plates, causing sterical blocking of some epitopes, and therefore certain epitopes i.e. the subgenus B specific ones are not available for the MoAbs on the hexons bound to the red blood cells.

Two of the MoAbs (35H15 and 35H26) have the broadest RPs reacting with all human hexon types studied, as well as with SAV16 hexon. The only difference was the absence of the reaction with BAV3 hexon in indirect ELISA (MoAb 35H26). This minimal difference means however different epitope on the hexon molecule. In gel diffusion experiments MoAb 35H15 seems to recognize the same epitope as MoAb 1A3 directed against AV1 hexon forming a line of complete identity (Fig. 3). This epitope is present on the different adenovirus hexon types and could be the genus specific determinant. MoAbs 1A3 and H12 directed against AV1 hexon belonged to different antibody groups (Ádám *et al.*, 1985) and according to the competition experiments (Ádám *et al.*, 1987*b*) they recognize epitopes on different antigenic sites of the hexon. MoAbs 35H15 and H12 formed double spur in a three well pattern with the hexon antigen (Fig. 4) and double line in the biclonal mixture of these MoAbs (Fig. 5) suggesting the differences of the epitopes recognized. These results are in good accordance with the results of Molinaro and Eby (1984), i.e. one antigen is able to form two precipitin lines and two spurs in gel diffusion experiments using two MoAbs, if the MoAbs are specific for two, sterically distinct epitopes.

MoAbs 35H49, 35H43, and 35H50 reacting both in ELISA and HA could be characterized as intersubgenus and intertype specific ones. MoAbs 35H10 35H30, and 35H51 reacted only with the homologous AV35 hexon in indirect ELISA and HA (Tables 1 and 2) suggesting that they are directed against the type specific epitope of AV35 hexon. Two of them (35H10, and 35H51) gave a line of complete identity in gel diffusion assay (Fig. 1), while MoAb 35H30 failed to precipitate the antigen preparation.

The distribution of the immunoglobulin classes of the MoAbs showed an interesting pattern. Immunoglobulin class M and three isotypes of IgG were represented among the MoAbs. Isotypes of the immunoglobulins could be predicted on the basis of the different types of reactions with the different tracer systems applied, first of all on the basis of different SpA affinity for IgG subclasses (Kornvall *et al.*, 1970; Goding, 1978).

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References

Ádám, É., Erdei, J., Lengyel, A., Fejér, Gy., Fachet, J., and Nász, I. (1987a): Monoclonal antibodies to adenovirus type 35 hexon. *Intervirology* **27**, 9—16.

Ádám, É., Lengyel, A., Takács, M., Erdei, J., Fachet, J., and Nász, I. (1986): Grouping of monoclonal antibodies to adenovirus hexon by their cross-reactivity. *Arch. Virol.* **87**, 61—71.

Ádám, É., Nász, I., Lengyel, A., Erdei, J., and Fachet, J. (1985): Differentiation of adenovirus hexon epitopes with monoclonal antibodies by gel diffusion assays. *Molec. Immun.* **22**, 967—971.

Ádám, É., Nász, I., Lengyel, A., Erdei, J., and Fachet, J. (1987b): Determination of different antigenic sites on the adenovirus hexon using monoclonal antibodies. *Arch. Virol.* **93**, 261—271.

Goding, J. W. (1978): Use of *Staphylococcus* protein A as an immunological reagent *J. immunol. Meth.* **20**, 241—253.

Graham, F. L., Smiley, J., Russel, W. C., and Nairu, R. (1977): Characterization of human cell line transformed by DNA from human adenovirus type 5. *J. gen. Virol.* **30**, 59—72.

Khilko, S. N. (1988): A simplified procedure to purify the adenovirus hexon protein. *J. virol. Meth.* (in press).

Kornvall, G., Grey, H. M., and Williams, R. C. (1970): Protein A reactivity with mouse immunoglobulins (structural relationship between some mouse and human immunoglobulins). *J. Immunol.* **105**, 1116—1123.

Köhler, G., and Milstein, C. (1975): Continuous culture of fused cells secreting antibody of predefined specificity. *Nature (London)* **256**, 269—270.

Lengyel, A., Ádám, É., Nász, I., Erdei, J., and Fachet, J. (1985): A sensitive method for detecting polyclonal and monoclonal antibodies directed against adenovirus hexon. *Acta virol.* **29**, 362—372.

Molinaro, G. A., and Eby, W. C. (1984): One antigen may form two precipitin lines and two spurs when tested with two monoclonal antibodies by gel diffusion assays. *Molec. Immun.* **21**, 181—184.

Myerowitz, R. L., Oxman, M. N., Levin, M. J., Moore, M., Leith, J. D., Gantz, N. M., Pellegrini, J., and Hierholzer, J. C. (1975): Fatal disseminated adenovirus infection on a renal transplant recipient. *Am. J. Med.* **59**, 591—598.

Nakane, P. K., and Kawaoi, A. (1974): Peroxidase-label of antibody: a new method for conjugation. *J. Histochem. Cytochem.* **22**, 1084—1091.

Nász, I., Lengyel, A., and Cserba, I. (1972): Comparative studies of adenovirus hexon antigens. *Arch. ges. Virusforsch.* **36**, 80—92.

Nermut, M. V., and Perkins, W. J. (1979): Consideration of the three dimensional structures of the adenovirus hexon from electron microscopy and computer modelling. *Micron* **10**, 247—266.

Valentine, R. C., and Pereira, H. G. (1965): Antigens and structure of the adenoviruses. *J. mol. Biol.* **13**, 13—20.

Wadell, G. (1984): Molecular epidemiology of human adenoviruses. *Curr. Top. Microbiol. Immunol.* **110**, 191—220.

Legends to Figures (Plates LIX)

Fig. 1. Gel diffusion test of the type specific MoAbs 35H51 (upper left) and 35H10 (upper right) with the homologous AV35 hexon (bottom).

Figs. 2—3. Gel diffusion tests of MoAbs 35H15 (upper left) and 35H26 (upper right) with AV35 hexon antigen (bottom) at PEG conc. of 2% (2) and 4% (3).
Fig. 4. Gel diffusion test of MoAb 35H15 (upper left) directed against AV35 hexon and MoAb 1A3 (upper right) directed against AV1 hexon with AV1 hexon antigen (bottom).
Fig. 5. Gel diffusion assay of MoAb 35H15 (upper left) directed against AV35 hexon and MoAb H12 (upper right) directed against AV1 hexon with AV1 hexon antigen (bottom).
Fig. 6. Gel diffusion analysis of biclonal mixtures of MoAbs. Serial twofold dilutions of MoAb 35H15 in MoAb H12 (outer wells) were tested with AV1 hexon antigen (centre). First dilution of MoAb 35H15 is 1 : 2 in the well at upper left (wells were filled clockwise).

Book Review

The Molecular Biology of Baculoviruses

W. Doerfler, and F. Böhm (Eds): The Molecular Biology of Baculoviruses; Current Topics in Microbiology and Immunology, vol. 131, VIII + 168 pp., Springer-Verlag, Berlin—Heidelberg—New York, 1986; price DM 134.—

Baculoviruses represent a family of viruses infecting insects. The introduction of insect cell cultures in the seventies made possible their study at the molecular biological level. The present book represents the first concentrated treatise of the molecular biology of baculoviruses. By selecting appropriate specialists as authors of the individual chapters, the editors (W. Doerfler and F. Böhm) succeeded in securing a high professional standard of the contributions. The book is not a general encyclopaedia on baculoviruses, but an excellent treatise of the actual trends in their molecular biology. Attention has been paid to the structure and expression of the viral genome, the use of baculoviruses as expression vectors of eukaryotic and prokaryotic genes in insect cells, persistent and productive infections and the structure of baculovirus-induced proteins. The lists of references are really up-to-date, they include papers published up to 1986.

For virologists interested in baculoviruses, in particular their molecular biology, the book offers a lot of important informations, including methodical details. But it may also be recommended to all those interested in general virology and gene technology.

K. Marcinka, Bratislava