

PRODUCTION OF MONOCLONAL ANTIBODIES WITH HAEMAGGLUTINATION-INHIBITION ACTIVITY TO THE SKALICA STRAIN FROM THE TICK-BORNE ENCEPHALITIS COMPLEX

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Received April 20, 1982

Summary. — Hybridomas secreting monoclonal antibodies with haemagglutination-inhibition (HI) activity to the Skalica strain of tick-borne encephalitis (TBE) complex were prepared by the fusion of P3-NS1-Ag4-1 myeloma cell line with spleen cells of BALB/c mice immunized with the purified Skalica strain. The highest titres of monoclonal antibodies obtained from the hybridomas S-9, S-15 and S-16 ranged from 512 to 10,240, respectively; the ascitic fluid contained as many as 4.6 mg/ml of monoclonal antibodies. Its analysis by Ouchterlony's double immunodiffusion, agarose electrophoresis, and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) revealed the presence of monoclonal antibodies with μ isotype of the heavy and κ isotype of the light chain. The specificity of the monoclonal antibodies was proved using 11 different antigens from family *Togaviridae* in the HI test.

Key words: monoclonal antibody; Skalica strain from the TBE complex; hybridoma; cell cloning

Introduction

The complex antigenic structure of viruses makes it almost impossible to prepare antibodies with clearly defined specificities to individual viral components (Yewdell and Gerhard, 1981). This problem was overcome by the technology of hybridoma cell lines (Köhler and Milstein, 1975) enabling to obtain monoclonal antibodies to individual antigenic determinants. Hybridomas were used for preparation of monoclonal antibodies to antigens of some enveloped viruses (Koprowski *et al.*, 1977, 1978; Wiktor and Koprowski, 1978; Nowinski *et al.*, 1979; Sethi and Brandis, 1980; Kendal *et al.*, 1981), but no monoclonal antibodies to the viruses of TBE complex have been prepared so far. This paper presents results of production and characterization of hybridomas secreting monoclonal antibodies to the Skalica strain of TBE virus.

Materials and Methods

Virus. Skalice strain of TBE virus was isolated from the organs of bank vole (*Clethrionomys glareolus*) trapped in West Slovakia (Grešíková *et al.*, 1976); it is non-pathogenic for mice after subcutaneous inoculation (Rajčáni and Grešíková, 1982). The virus was grown in primary chick embryo cell cultures (CEC) and purified by differential centrifugation.

Immunization of animals and isolation of splenic lymphocytes. Ten to twelve-week-old females of BALB/c mice were inoculated intraperitoneally (i.p.) with 0.2 ml of purified Skalice strain containing 5,120 haemagglutination (HA) units in Freund's complete adjuvant (FCA, Difco). The same volume of the virus containing 320 HA units was applied intravenously 5 weeks later. Three days after the second immunizing dose, the mice were killed and suspension of splenic lymphocytes was prepared.

Preparation of feeders. Adult BALB/c or C₃H mice males and females were killed and their peritoneal cavity was washed out with 5 ml of 11.6% sucrose solution. In parallel, the mouse spleens were aseptically removed and homogenized. As feeders, the viable peritoneal or spleen cells were grown in Petri dishes (60 mm, Falcon Plastres, Los Angeles, California) or in flat-bottomed microtitre plates (Titertek, Flow Laboratories, Rockville, Maryland) and irradiated 22 hr later (20 Gy, ⁶⁰Co, Chisobalt, Chirana, Prague).

Myeloma cells. The myeloma cell line P3-NS1-Ag4-1 (further designated as NS-1) was kindly supplied by Dr. P. Dráber (Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague). For production of control supernates, the myeloma cell lines X63-Ag8,653 and Sp2/0 were used.

Cell cultivation and freezing. Cells were cultivated in a standard medium RPMI 1640 supplemented by L-glutamine (2 mM), sodium pyruvate (1 mM), 2-mercaptoethanol (0.028 mM), streptomycin (50 µg/ml), penicillin (100 units/ml) and 10% heat-inactivated (56 °C for 30 min) normal horse serum. All cell lines, hybridomas and cloned hybrids were cultivated at 37 °C in humidified atmosphere containing 7.5% CO₂. The selection of hybrid cells was carried out in selective HAT medium (Littlefield, 1964) consisting of the standard medium supplemented by hypoxanthine (0.1 mM), aminopterin (0.004 mM) and thymidine (0.016 mM). For cloning medium served the double concentrated standard medium supplemented with 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane-sulphonic acid). The freezing medium was prepared by mixing a conditioned medium harvested from NS-1 cell cultures in the exponential phase of growth (50%) with heat-inactivated horse serum (40%) and 10% dimethylsulphoxide (DMSO; Koch-Light Laboratories Ltd., Colnbrook, England). The hybrid cells in concentration 1–3 × 10⁶/ml were frozen according to Kennet *et al.* (1980).

Cell fusion and cloning. Immune spleen cells (2 × 10⁷) were fused with HGPR1 (hypoxanthine guanine phosphoribosyl transferase) deficient BALB/c myeloma NS-1 cells (2 × 10⁶) in a standard medium without serum using polyethylene glycol (1,540 m.w.) and DMSO (by the method of Pearson *et al.* (1980). Hybrid cells were selected as described by Kenett *et al.* (1980). Two methods of cell cloning were employed: *a*) limiting dilution technique by Gooding (1980), and *b*) modification of the soft agar method according to Coffino *et al.* (1972). The latter was performed as follows: irradiated feeders in Petri dishes were overlaid with 5 ml of the cloning medium containing 0.5% agar (Bacto-Agar, Difco Laboratories, Detroit, Michigan) and 0.5% Indubiose A 37 (L'Industrie Biologique Francaise), respectively. When the basic layer stiffened, the hybrid cells were added and after 10–12 days cultivation the colonies were transferred in 0.2 ml of the standard medium to 96-well flat-bottomed microtitre plates, in which they were cultivated for additional 5 days. Then the positive clones were transferred to 6-well cultivation plates (M47ARTL, Dynatech 4G, Switzerland) and after further 48 hr to 60 mm Petri dishes. The efficiency of cloning was calculated from the number of colonies revealing a diameter higher than 0.3 mm divided by the whole number of cloned hybrid cells (Coffino *et al.*, 1972).

Production of monoclonal antibodies *in vitro*. Three times recloned hybrid cells were subpassaged as soon as the concentration 4–5 × 10⁵ of the cells/ml had been reached. The resulting culture fluids were collected and 10 × concentrated by ultrafiltration on Amicon 10 TCF.

Production of monoclonal antibodies *in vivo*. The BALB/c mice were inoculated i.p. with 0.5 ml of FCA and 0.5 ml of Pristane (2,6,10,14-tetramethylpentadecane; Koch-Light Laboratories Ltd., Colnbrook, England), respectively. After 10–50 days 10⁶–10⁷ hybrid cells were applied by i.p. route. Seven to 14 days later, when a distinct growth of ascitic tumour was noticed, the mice were killed and their ascitic fluid was harvested.

Labelling of monoclonal antibodies. Hybridoma cultures were labelled overnight with 370 kBq/ml of ^{35}S -methionine (Amersham Radiochemical Centre, England) with the specific activity 53.28 TBq/mM in the medium lacking methionine supplemented with 10% foetal calf serum dialyzed against phosphate buffered saline. Then the cells were removed by centrifugation and labelled monoclonal antibodies were isolated by the use of formalin-killed heat-inactivated *Staphylococcus aureus* loaded with the swine anti-mouse Ig.

Electrophoretic methods. Monoclonal antibodies were separated by a discontinuous 10% SDS-PAGE (Laemli, 1970). Gels were prepared for fluorography as described (Chamberlain, 1979). The autoradiogram was done on a X-ray film (Medix-Rapid, Prague) at -70°C . Agarose electrophoresis, isolation of antibodies from the agarose gel and densitometric estimation of antibody concentration were described elsewhere (Dráber *et al.*, 1980). Electrophoresis was carried out in the barbital-glycine-Tris buffer, pH 8.8, according to Svendsen, which was diluted 1:1 (v/v) with agarose (Weeke, 1973). Swine IgM and IgG under reduction conditions were used as standards (Zikán, 1980, *b*). Electrophoretic zones in the agarose gel were cut out after staining the non-dried gel with Coomassie Blue R-250 and thawed in the glass tube containing 2% SDS and 5% 2-mercaptoethanol by warming for 5 min in the boiling water bath. The solution obtained was poured on the top of polyacrylamide gel in a glass capillary tube, overlaid with electrophoretic buffer and eventually with 10 μl of 30% glycerine containing 0.1% SDS in 0.1 M Tris-HCl, pH 6.8 and traces of bromphenol blue, respectively.

Double immunodiffusion in agar. For double immunodiffusion agar analysis the following anti-mouse sera were used: rabbit anti IgG1, anti IgG2A, anti IgG2B, anti IgG3, anti α , and anti μ (Bionetics, U.S.A.), and sheep anti IgM, anti IgA, and anti IgG (kindly supplied by Dr. L. Tučková, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague).

The HI test. Supernates from hybridoma cultures were examined for the presence of antibodies to the Skalica strain by the HI test. Supernates from NS-1, or X63-Ag8, 653 myeloma cells as well as the standard and selective (HAT) media served as controls. Specificity of HI activity of monoclonal antibodies was examined with antigens of 11 arboviruses belonging to the family *Togaviridae* (TBE virus, dengue viruses type I, II, III, and IV, West Nile, yellow fever, Western equine encephalitis, Eastern equine encephalitis, Venezuelan encephalitis, and Sindbis viruses). Antigens from viruses tested were prepared by the method of Clarke and Casals (1958).

Electron microscopy. Ultrathin sections from hybridomas were cut on a ultramicrotome (Ultrame III LKB), stained with 2% solution of uranyl acetate and lead citrate, and examined in electron microscope (Philips EM 3,000; 80 kV).

Results

Effect of the cloning procedure and feeder cells on the growth of hybrid clones

For fusion with the NS-1 myeloma cells, spleen cells were selected from BALB/c mice responding with the highest titres of HI antibodies to the immunization with the Skalica strain. After 3 weeks cultivation, hybridomas were obtained from as many as 210 wells (73%) out of 288 wells of 3 microtitre plates each seeded with 5×10^4 fused cells. The HI activity to TBE virus was demonstrated in the supernates of 14 hybridomas. The feeder cells were inevitable for the efficient growth of hybridomas, because in their absence only 5% hybridomas were obtained. This effect of feeder cells on the frequency of hybridomas did not depend on the line of donor mice (BALB/c and C3H, respectively).

Hybrid cells producing HI antibodies to TBE virus were cloned either by the limiting dilution method in microtitre plates or in the soft agar and agarose, respectively. In microtitre plates, for which the cells were diluted so that one cell got into one well, the growth of cloned cells depended on the presence of BALB/c mice irradiated spleen or peritoneal cells used as feeders (Fig. 3). The most efficient were spleen cells stimulating an intense

growth of 60% hybrid clones as early as on day 7, which settled within further 7 days of cultivation and reached the value of 65% clones. The peritoneal cells were less efficient stimulating higher proportion (10–45%) of proliferating clones only after 14 days with no increase in the 3rd week of cultivation.

Similar requirements for feeder cells were found when cloning the hybrids in agar and agarose, respectively. Their addition increased markedly the cloning efficiency, the hybrid cells growing in large macroscopic colonies. Again, more efficient were the spleen cells (in concentration $1-2 \times 10^6/\text{ml}$) and less efficient the peritoneal cells (in concentration $0.1-0.5 \times 10^6/\text{ml}$). An increase in the concentration of peritoneal cells resulted in a depressed growth of hybrid cell colonies; at concentration $2 \times 10^6/\text{ml}$ of peritoneal cells, the hybrid clones did not grow at all (Fig. 4). When comparing the use of agar and agarose on the efficiency of cloning, agarose was found more efficient than agar in the presence of either spleen (42% versus 28%) or peritoneal (28% versus 16%) cells (Fig. 4). In comparison with primary clones, recloned hybrid cells grew better and more rapidly, with an increased proportion of all clones (41–55% in agar and 53–68% in agarose, respectively) as well as of those with a diameter higher than 0.3 mm. After recloning of each positive clone together 20 subclones were obtained, which produced

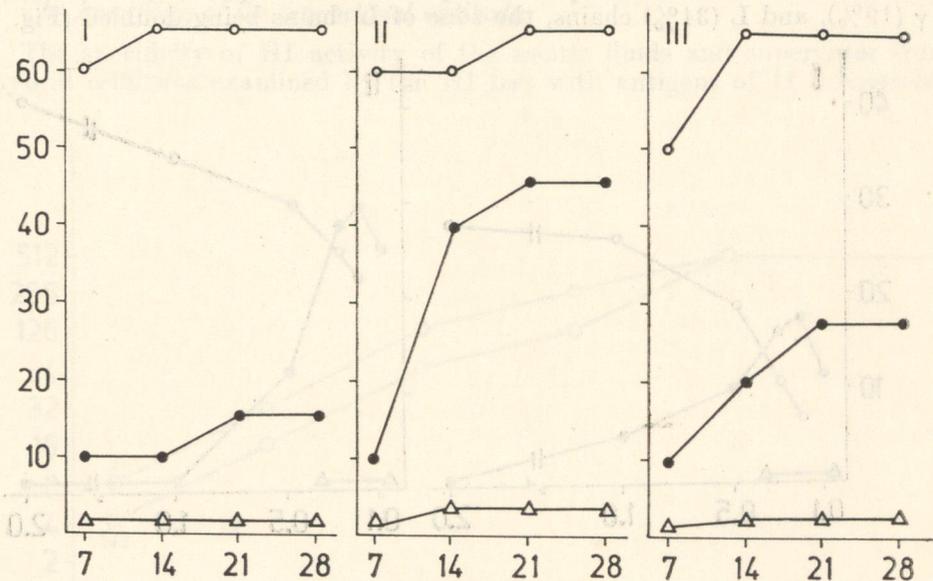


Fig. 3.

Effect of feeder cells on the growth of hybridoma colonies.

One hundred hybrid cells from S-9 (I), S-15 (II), and S-16 (III) hybridomas dispensed into 96 wells of 3 microtitre plates seeded with 0.4×10^6 feeder spleen cells (○—○) or with 0.1×10^5 peritoneal cells (●—●) or without feeder cells (△—△).

Abscissa: days in culture; ordinate: number of wells with hybrid cells (%).

antibodies with HI activity when growing in the standard medium. Electromograms of recloned positive hybridomas are shown in Figs. 1 and 2.

Production of monoclonal antibodies in vitro and in vivo

Supernates of recloned and grown hybrid cells transferred to plastic Petri dishes were examined for the presence of HI antibody activity. A direct dependence of HI antibody titre on the number of hybrid cells in culture was found (Fig. 5). The highest HI antibody titre (512) was reached at the maximum possible density of hybrid cells ($0.5 \times 10^6/\text{ml}$). Ascitic tumours were induced in histocompatible mice 14–21 days after inoculation of 5×10^6 hybrid cells in all mice yielding 2–10 ml of ascitic fluid per mouse with HI antibody titres of 5,120–10,240, irrespective of whether the mice were treated with Pristane or FCA. Application of hybridoma cells without a previous sensitization by Pristan or FCA lead to ascitic tumours only in 20% mice.

Characterization of the antibody synthesized by hybrid cells

Electrophoresis of the ascitic fluids in agarose showed the fast moving zone in the globulin region (Fig. 6). Analysis of the zone under reduction conditions of SDS-PAGE provided products corresponding to μ (47%), γ (19%), and L (34%) chains, the zone of L chains being doubled (Fig. 7).

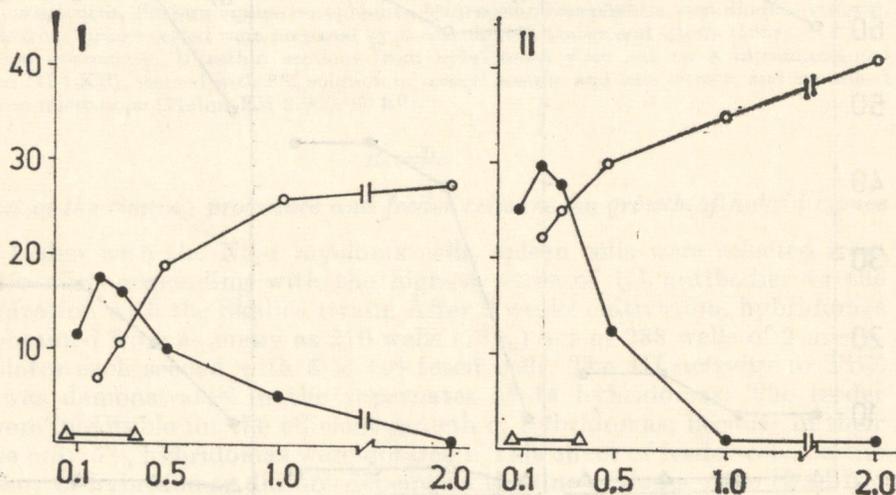


Fig. 4.

Effect of feeder cells on the growth of hybrid cell colonies in soft agar and agarose. Three hundred hybrid cells in RPMI-1640 with 0.25% agar (I) and agarose (II), respectively (the whole volume 0.8 ml), were layered on 0.5% agar containing different amounts of peritoneal (●—●) or spleen (○—○) cells or lacking feeder cells (△—△). Numbers of colonies represent mean values from 3 parallel clonings as evaluated on day 14 of cultivation. Abscissa: number of feeder cells; ordinate: number of hybrid colonies (%).

Further analysis of the isolated fast moving zone by double immunodiffusion in agar using antibodies to mouse isotypes revealed strong precipitation lines with anti-IgM and anti- α serum, but markedly less distinct lines with other sera. According to densitometric analysis of the fast moving zone in the same gel with different amounts of the standard IgM, the protein corresponding to this zone has the concentration of 4.6 mg/ml of the ascitic fluid.

Supernates from 3 times clones hybrid cells were $10\times$ concentrated by ultrafiltration and analyzed by Ouchterlony's immunodiffusion test with the use of rabbit (anti-IgG1, IgG2A, IgG2B, IgG3, λ , and λ and sheep (anti-IgM, IgA, and IgG) anti-mouse sera. Precipitation lines of supernates from S-9, S-15, and S-16 hybrid clones were found only with anti-IgM and anti- α sera. Besides examination and analysis of the culture and ascitic fluids, the nature of secreted monoclonal antibody was further investigated by radioactive labelling of hybrid cells with ^{35}S -methionine for 12 hr. When analyzing radioactive secreted proteins in SDS-PAGE, only two lines, one with mobility of μ isotype of the heavy chain, another with mobility of α isotype of the light chain were seen (Fig. 8). No line corresponding to the myeloma NS-1 light chain was detected indicating that clones S-9, S-15, and S-16 produced only specific heavy and light chains of monoclonal antibody. As a standard monoclonal IgG 3 antibody was employed.

The specificity of the monoclonal antibody

The specificity of HI activity of the ascitic fluids and supernates from hybrid cells was examined by the HI test with antigens of 11 arboviruses

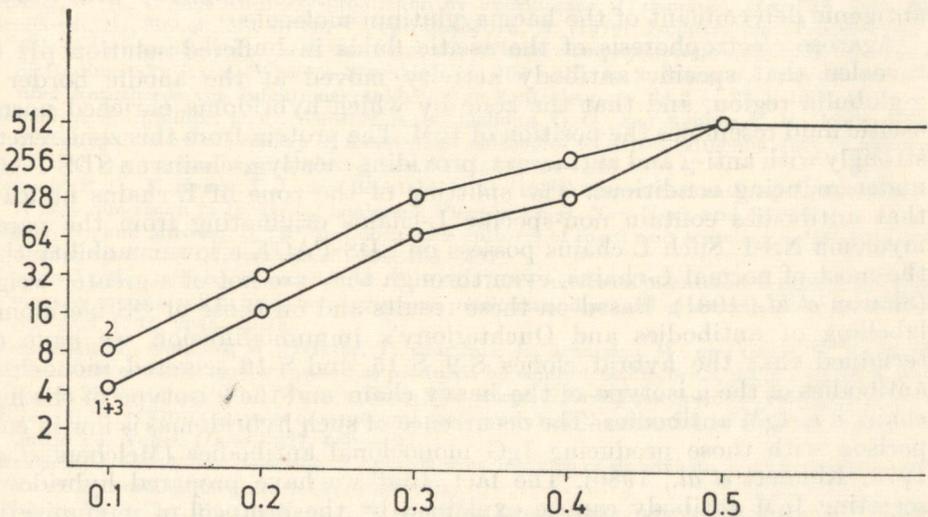


Fig. 5.

Effect of number of hybrid cells on the amount of monoclonal antibody secretion
Abscissa: number of hybrid cells; ordinate: medium dilution reciprocals in the HI test.

belonging to the family *Togaviridae*. Monoclonal antibodies produced by clones S-8, S-15, and S-16 possessed the HI activity reacting exclusively with the haemagglutinin of the Skalica strain.

Discussion

Though a great attention has been paid recently to the preparation of monoclonal antibodies to some arboviruses (Gentry *et al.*, 1981; Roehrog *et al.*, 1981; Schmaljohn *et al.*, 1982) we were not aware of any paper dealing with production of monoclonal antibodies to viruses of the TBE complex.* Because of a great public health importance of TBE virus and inability to differentiate it from viruses causing louping-ill and Russian spring-summer encephalitis, respectively, by common serologic methods, we tried to prepare specific monoclonal antibodies which would allow a differentiation. The virus used for immunization was the strain Skalica (Grešíková *et al.*, 1976), which is non-pathogenic for adult white mice after subcutaneous inoculation (Rajčáni and Grešíková, 1982) so that it does not require any inactivation before immunization.

We succeeded in production of hybridomas secreting specific monoclonal antibodies with HI activity to the Skalica strain. The specificity of both supernates from hybrid cultures and ascitic fluids was proved by the HI test, in which 11 togaviruses were tested. Thus, the supernates from hybrid cultures as well as the ascitic fluids can replace conventional antisera in serologic tests with TBE virus which require monospecific HI antibodies. Moreover, the permanent proliferation of hybrid cultures enables the production of a great amount of homogeneous antibody reacting only with a single antigenic determinant of the haemagglutinin molecule.

Agarose electrophoresis of the ascitic fluids in buffered solution pH 8.6 revealed that specific antibody activity moved at the anodic border of γ -globulin region, and that the zone by which hybridoma enriched normal ascitic fluid resembles the position of IgM. The protein from this zone reacted strongly with anti- μ and anti- α sera, providing mostly μ -chains on SDS-PAGE under reducing conditions. The splitting of the zone of L-chains indicates that antibodies contain non-specific L-chains originating from the parent myeloma NS-1. Such L-chains possess on SDS-PAGE a lower mobility than the most of normal L-chains, even through they are not of a greater weight (Sharon *et al.*, 1981). Based on these results and on those of ^{35}S -methionine labelling of antibodies and Ouchterlony's immunodiffusion we have determined that the hybrid clones S-9, S-15, and S-16 secreted monoclonal antibodies of the μ isotype of the heavy chain and the α isotype of the light chain, i. e. IgM antibodies. The occurrence of such hybridomas is low in comparison with those producing IgG monoclonal antibodies (Melchers *et al.*, 1978; Kennett *et al.*, 1980). The fact, that we have prepared hybridomas secreting IgM antibody can be explained by the protocol of immunization

* The paper by Heinz, F. X. *et al.* (*Infect. Immun.* 37, 869, 1982) appeared after this manuscript had been submitted to press.

and by an interval of 69 hr of the harvest of spleen cells after the secondary intravenous dose of immunizing virus.

As follows from other studies (Dráber, personal communication, 1979; Goding, 1980), an early primary cloning and frequent recloning are the most important requirements for obtaining the stable active clone. We have found that the presence of feeder cells was decisive during the primary cloning, efficiency of which was increased further by 14% (with spleen cells) and by 12% (with peritoneal cells), respectively, in the favour of agarose, when agarose and agar were compared.

Acknowledgements. Precious technical assistance of M. Strmeňová and V. Bieliková is greatly acknowledged. We are indebted further to Dr. P. Dráberr (Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague) for his suggestions for preparation of monoclonal antibodies, and to Dr. V. Laginová for kind help with irradiation of the cells.

References

- Clarke, D. H., and Casals, J. (1958): Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Am. J. trop. Med. Hyg.* **7**, 561—573.
- Chamberlain, J. P. (1979): Fluorographic detection of radioactivity in polyacrylamide gels with the water-soluble fluor, sodium salicylate. *Anal. Biochem.* **98**, 132—135.
- Coffino, P., Baumal, R., and Scharff, M. D. (1972): Cloning of mouse myeloma cells and detection of rare variants. *J. Cell Physiol.* **79**, 429—440.
- Dráber, P., Zikán, J., and Vojtíšková, M. (1980): Establishment and characterization of permanent murine hybridomas secreting monoclonal anti-Thy-1 antibodies. *J. Immunogen.* **7**, 455—474.
- Gentry, M. K., Henchal, E. A., McCown, J. M., and Brandt, W. E. (1981): Development of monoclonal antibodies for identification of dengue virus serotypes. *Arthropod-Borne Virus Inform. Exch.* **41**, 13.
- Goding, J. W. (1980): Antibody production by hybridomas. *J. immunol. Meth.* **39**, 285—308.
- Grešíková, M., Mrciak, M., Brtek, V., and Sekeyová, M. (1976): Isolation and identification of tick-borne encephalitis virus from a bank vole (*Clethrionomys glareolus*) trapped in the vicinity of Radimovsky forest (Western Slovakia), pp. 105—110. In 2. Internat. Arbeitskolloquium über Naturherde von Infektionskrankheiten in Zentraleuropa, 25.2 — 28.2. 1976, Graz.
- Kendal, A. P., Phillips, D. J., Webster, R. G., Galland, G. G., and Reimer, C. B. (1981): Effect of test system on the ability of monoclonal antibodies to detect antigenic drift in influenza A (H1N1) virus haemagglutinins. *J. gen. Virol.* **54**, 253—261.
- Kennett, R. G., McKearn, T. J., and Bechtold, K. B. (1980): Monoclonal antibodies (Hybridomas: A new dimension in biological analyses). Plenum Press, New York and London.
- Köhler, G., and Milstein, C. (1975): Continuous cultures of fused cells secreting antibody of pre-defined specificity. *Nature (Lond.)* **256**, 495—497.
- Koprowski, H., Gerhard, W., and Crose, C. M. (1977): Production of antibodies against influenza virus by somatic cell hybrids between mouse myeloma and primed spleen cells. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2985—2988.
- Koprowski, H., Gerhard, W., Wiktor, T., Martinis, J., Schander, M., and Croce, C. M. (1978): Anti-viral and anti-tumor antibodies produced by somatic cell hybrids, pp. 8—19. In F. Melchels, M. Potter and N. L. Warner (Eds): *Lymphocyte Hybridomas*. Springer-Verlag, New York.
- Laemli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* **227**, 680—685.
- Littlefield, J. W. (1964): Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants. *Science* **145**, 709—710.
- Melchers, F., Potter, M., and Varner, N. L. (1978): Lymphocyte hybridomas. *Curr. Top. Microbiol. Immunol.* **81**, 1—246.
- Nowinski, R., Lostram, M. R., Tam, M., Stone, M. R., and Burnette, W. N. (1979): The isolation of hybrid cell lines producing monoclonal antibodies against the p15(E) protein of ecotropic murine leukemia viruses. *Virology* **93**, 111—126.

- Rajčáni, J., and Grešíková, M. (1982): Pathogenicity of the Skalica strain (from the tick-borne encephalitis complex) for white mice. *Acta virol.* **26**, 264—269.
- Roehrog, J. T., Mathews, J. H., Konney, R. M., and Day, J. W. (1981): Biochemical and biological analysis of Venezuelan equine encephalomyelitis virus and its disease process using monoclonal antibodies. *Arthropod-Borne Virus Inform. Exch.* **41**, 35.
- Schmaljohn, A. L., Johnson, E. D., Dalrymple, J. M., and Cole, G. A. (1982): Non-neutralizing monoclonal antibodies can prevent lethal alphavirus encephalitis. *Nature (Lond.)* **297**, 70—72.
- Sethi, K. K., and Brandis, H. (1980): Production of neutralizing antibody to rabies and vesicular stomatitis viruses by hybrid cell lines. *Cell. Immunol.* **49**, 260—265.
- Sharon, J., Kabat, E. A., and Morrison, S. L. (1981): Studies on mouse hybridomas secreting IgM or IgA antibodies to α (1-6)-linked dextran. *Mol. Immunol.* **18**, 831—846.
- Weeke, B. (1973): Quantitative immunoelectrophoresis. General remarks on principles, equipment, reagents and procedures. *Scand. J. Immunol. Suppl. No. 1*, 15—83.
- Wiktor, T. J., and Koprowski, H. (1978): Monoclonal antibodies against rabies virus produced by somatic cell hybridization: Detection of antigenic variants. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3938—3942.
- Yedwell, J. W., and Gerhard, W. (1981): Antigenic characterization of viruses by monoclonal antibodies. *Ann. Rev. Microbiol.* **35**, 185—206.
- Zikán, J. (1980a): Interactions of pig Fab γ fragments with protein A from *Staphylococcus aureus*. *Folia microbiol. (Praha)* **25**, 246—253.
- Zikán, J., (1980b): Interactions of pig Fab μ and Fab γ fragments with protein A from *Staphylococcus aureus*. *Folia microbiol.* **25**, 254—258.

Explanation of Figures (Plates VI—VIII):

Fig. 1.

Polynuclear hybrid cell with small round nuclei and dense chromatine occupying large part of the nucleus. The cytoplasm contains numerous dilated chanel of rough encoplasmic reticulum with retrovirus particles in its cisternae. ($\times 22,000$).

Fig. 2.

The hybrid cell after fusion of nuclei. Well-preserved ultrastructure of the nucleus with more dense chromatine and granular nucleolus. The cytoplasm contains individual membrane vacuoles, short parts of rough endoplasmic reticulum, numerous free ribosomes, and mitochondriae with well-preserved substructure. ($\times 12,000$).

Fig. 6.

Agarose electrophoresis of the ascitic fluids from hybridomas (A₁, A₂) and of normal serum (S). Arrow indicates the zone by which hybridoma enriches the ascitic fluid.

Fig. 7.

SDS-PAGE of the hybridoma product isolated from the ascitic fluid by agarose electrophoresis (10% gel under reduction conditions). Marked are the positions of stanrd immunoglobulin chains (1 — μ , 2 — γ , 3 — L).

Fig. 8.

SDS-PAGE of ³⁵S-methionine-labelled monoclonal antibodies produced by hybridomas S-9 and S-16. Monoclonal IgG₃ antibodies were used as standards.

Samples (left to right) were prepared from supernates by precipitation with *Staphylococcus aureus* loaded with swine anti-mouse Ig:

- No 1 — supernate from hybridoma producing IgG₃ (control)
- No 2 — supernate from hybridoma S-9 producing IgM
- No 3 — supernate from NS-1 myeloma cells (control)
- No 4 — supernate from hybridoma S-16 producing IgM
- No 5 — supernate from Sp2/0 myeloma cells (control)
- No 6 — supernate from hybridoma producing IgG₃ (control).