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EFFECTS OF CIS-DICHLORO-DIAMMINE-PLATINUM (II) (CIS-DDP) ON EPSTEIN-BARR VIRUS INDUCTION AND CELL DIFFERENTIATION

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Summary. — Morphological changes were induced by cis-dichlorodiammine platinum (II) (cis-DDP) in two Epstein-Barr virus (EBV) transformed cell lines: the productive P3HR-1 and the nonproductive Raji cell line. In P3HR-1 cells cis-DDP induced synthesis of viral antigens, viral particles and morphological changes characteristic for virus replication. In Raji cells, the virus replicative cycle was not induced and virus-specific morphological changes were limited to the sporadic appereance of some very early alterations in cell morphology. However, in Raji cells, but not in P3HR-1 cells, up to 20 per cent of the cell population exhibited differentiation-related changes towards plasma cell morphology. The most advances stage of cell differentiation detected was classified as plasmablast. Cis-DDP also induced some changes associated with the cytostatic effect of the drug. In the treated cell population cytokinesis was inhibited and frequently multinuclear cells appeared; moreover ,extensive degenerative changes were observed.

Key words: Epstein-Barr virus; cis -dichloro-diammine platinum (II); infected-cell ultrastructure

Introduction

Epstein-Barr virus (EBV) is capable of immortalizing B lymphocytes both in vivo and in vitro (Nilsson, 1979; Miller and Lipman, 1973) the cells usually contain multiple copies of the viral genome (Nonoyama and Pagano, 1972) and express latent virus-specific membrane antigen (LMA) (Moss *et al.*, 1981) and the EBV-determined nuclear antigen complex (EBNA) (Reedman and Klein, 1973). Some transformed lymphoblastoid cell lines, the so-called virus producers, are spontaneously capable of expressing antigens of the productive virus cycle in a certain proportion of cells (Nilsson, 1979). In both types of cells, virus nonproducers and virus producers, the synthesis of early antigen (EA) and virus capsid antigen (VCA) can be induced or enhanced by various inducers as e.g. halogenated pyrimidines

(Gerber, 1972; Hampar *et al.*, 1974), 5-azacytidine (Ben Sasson and Klein, 1981), anti-IgM (Tovey *et al.*, 1978), n-butyrate (Luka et al., 1979), 12-o--tetradecanoylphorbol-13-acetate (TPA) (Zur Hausen *et al.*, 1979, 1978) and others.

We have recently shown that n-butyrate, TPA or activated serum factor can affect not only virus-antigen synthesis but also cell differentiation in EBV-positive lymphoma cells (Anisimová *et al.*, 1982, 1984; Roubalová *et al.*, 1985). In the present study we report on the effects of cis-dichlorodiammine-platinum (II) (cis-DDP), a widely used cytostatic on both EBV-antigen induction and cellular morphology. This substance has previously been shown to induce EBV-antigen formation in another Burkitt lymphoma cell line (Anisimová *et al.*, 1974; Vonka *et al.*, 1972).

Materials and Methods

Cells. Virus nonproducer Burkitt lymphoma (BL) cell line Raji (Pulvertaft, 1965) and virus producer BL cell line P3HR-1 were used throughout the experiments. They were cultivated as described elsewhere (Vonka *et al.*, 1972).

Induction of EBV antigens. Cells were grown to a high density, pelleted and resuspended to 5×10^5 viable cells/ml in fresh growth medium. Aliquots of a freshly prepared stock solution of cis-DDP in PBS were added to final cis-DDP concentrations as indicated in the Results section. Cis-DDP was kindly provided by Dr J. Drobník (Institute of Macromolecular Chemistry, Prague); it was stored at +4 °C. At intervals, aliquots were withdrawn for immunofluorescence, autoradiography and electron microscopy.

Immunofluorescence test. Percentages of EA and VCA-positive cells were determined by the indirect-immunofluorescence technique of Henle and Henle (1966) using serum from a patient with nasopharyngeal carcinoma that contained antibodies against EA and VCA and fluorescein-isothiocyanate-conjugated goat anti-human IgG (Hyland). The two antigen complexes concerned, EA and VCA, were not distingu ished in the present experiments. At least 1000 cells were counted for each determination.

Autoradiography. Autoradiography was done on acetic acid-methanol (3 : 1) — fixed smears of cells that had been prelabelled with ^{3}H- thymidine (0.56 MBq/ml) at indicated intervals. The smears were dehydrated in increasing ethanol concentrations and covered with ORWO autoradiographic emulsion. They were then air-dried in the dark and exposed for 4—5 days at 4 °C. After development they were counterstained with Giemsa solution.

Electron microscopy (EM). Preparation of specimens for EM and the EM examination techniques employed were the same as described earlier (Anisimová, E. *et al.*, 1977). At least 1000 profiles were examined for morphological characterization.

Results

Morphological characterization of cells

Untreated Raji cells displayed the morphology of typical poorly differentiated lymphoblasts (Fig. 1). They were relatively large and contained a rounded or lobular nucleus with one or two nucleoli. The cytoplasm contained a well-developed Golgi apparatus, a relatively small number of mitochondria and weakly expressed rough endoplasmic reticulum (RER). Cells at various stages of mitosis were frequently detected.

Untreated P3HR-1 cells, when not producing virus, had a morphology similar to Raji cells. However, owing to spontaneous virus activation in

Table 1. Induction of EBV antigens[1]) in P3HR-1 and Raji cells by cis-DDP

Cells	Time after drug addition (hrs)	Per cent of EA + VCA[1])-positive cells in response to cis-DDP concentration			
		0	1 μg/ml	5 μg/ml	10 μg/ml
P3HR-1	72	2.7	6.3	6.0	3.9
	120	2.6	9.6	6.5	4.0
Raji	72	0.1	0.1	0.1	0.1
	120	0.1	0.1	0.1	0.1

[1]) Early antigen (EA) and viral capsid antigen (VCA) were not distinguished.

P3HR-1, about 2.3% of the cell population showed virus-specific changes in cellular morphology and 0.3 per cent contained virus particles (see below, Table 2).

Effect of cis-DDP on cell mitosis and size

After the addition of cis-DDP, mitoses were inhibited and cell division stopped. However, the growth of individual cells continued. As a result, the size of treated cells doubled in comparison with untreated controls. Some of the cells contained two or more nuclei, as evidenced by autoradiography (Fig. 2), and occasionally large multinuclear cells were detected (Fig. 3). Such changes were found in the presence of any concentration of cis-DDP and reached the highest frequency on day 3 after the addition of the drug. The changes were observed in both cell lines used but were somewhat more pronounced in Raji cells.

Table 2. Induction of virus particles and virus-specific changes in cellular morphology in P3HR-1 and Raji cells by cis-DDP

Cells	Time after drug addition (hrs)	Per cent of cells with virus-specific changes at cis-DDP concentrations				Per cent of cells with virus particles at cis-DDP concentrations			
		0	1 μg/ml	5 μg/ml	10 μg/ml	0	1 μg/ml	5 μg/ml	10 μg/ml
P3HR-1	72	2.3	5.9	4.8	3.6	0.3	3.1	2.7	1.4
	120	2.2	4.5	4.5	3.9	0.3	3.0	2.3	1.0
Raji	72	0	0.3[1])	0	0	0	0	0	0
	120	0	0	0	0.4[1])	0	0	0	0

[1]) Occasionally rare Raji cells were detected containing virus-associated morphological changes that had previously been suggested to precede the synthesis of early antigen (Anisimová *et al.*, 1984; Seigneurin *et al.*, 1977).

Table 3. Effect of cis-DDP on Raji cell differentiation[2]

Time after drug addition (hrs)	Per cent of differentiated cells at cis-DDP concentrations 0	1 μg/ml	5 μg/ml	10 μg/ml
0	0	0	0	0
24[1])	0	9.7	6.6	5.0
48	0	11.0	7.2	5.7
72	0	20.4	17.5	9.7
120	0	20.2	17.3	10.0

[1]) 24 hrs after addition of the drug only cells at initial stages of differentiation were detected. Later, frequent plasmablasts appeared.
[2]) A concentration of 10 μg cis-DDP/ml was highly toxic for cells.

Induction of EBV antigens

As shown in Table 1, about 2.6 per cent of untreated P3HR-1 cells spontaneously expressed antigens of the productive virus cycle. The number of EA and VCA-positive cells in cis-DDP-treated cultures was 2—3 times higher than in untreated cells. This increase was most marked on day 5 after the induction and attained maximal values at a cis-DDP concentration of 1 μg/ml, which exhibited relatively strong toxicity for the cells; with higher cis-DDP concentrations the rate of antigen induction was lowered. On the other hand, no induction of EBV antigens was noted in Raji cells.

Induction of viral particles and virus-specific alterations in cellular morphology

In cis-DDP-treated P3HR-1 cells, an increased number of cells expressing virus-specific cellular alterations and containing virus particles was noted Table 2). The EBV-associated changes in cellular morphology were as follows: granular structures in the nuclei, reduplicated or disintegrated nuclear membrane, changed appearance of mitochondria, and the formation of tubular structures in the cytoplasm (Figs 4-a,-b). The largest proportion of such cells was detected at 1 μg cis-DDP/ml, on day 3 after drug addition. At a concentration 5 μg/ml of cis-DDP the induction was less effective, and a further decrease was observed with a 10 μg cis-DDP/ml concentration, which was strongly toxic for the cells.

The number of P3HR-1 cells containing viral particles on day 3 was about one-half of those expressing virus-associated morphological changes (see Table 2). The particles observed represented various stages of virus maturation: empty capsids and nucleocapsids with complete or incomplete nucleoids were seen. In the great majority of cases the particles lacked the envelope (Fig. 4-c).

Interestigly, the expression of some virus-specific cellular alterations was sporadically found also in some preparations of Raji cells (see Table 2). These cells never formed viral particles, however.

Induction of cell differentiation

Cis-DDP-treated P3HR-1 cells did not differentiate. On the other hand Raji cells, at all the cis -DDP concentrations employed, exhibited a strong differentiative response towards plasma cells (Table 3). This response was most pronounced at 1 μg cis-DDP/ml and decreased with increasing cis--DDP concentrations. It peaked on day 3—5 after the addition of the drug. Already on the first day the amount of rough endoplasmic reticulum (RER) and the number of mitochondria increased (Fig. 5-a). The cisternae of RER were flat and contained ribosomes on their outer membranes. Such alterations had been designated earlier (Roubalová *et al.*, 1985) as the initial stages of cell differentiation. By the third day after induction the amount of RER increased further and its compartments were mainly localized in ring-like structures around the nucleus. The mitochondria formed clusters. The localization of the nucleus, its size and the distribution of chromatin, remained unchanged (Fig. 5-b). Cells with such morphology were classified as plasmablasts (Roubalová *et al.*, 1985). Cells with signs characteristic of plasma cells were not found. Interestingly, some large, multinuclear cells also possessed an increased amount of RER (Fig. 3).

Cis-DDP-induced degenerative changes

Besides the alterations described above, cis-DDP induced frequent, concentration-dependent degenerative changes in both nucleus and cytoplasm in the two cell lines. These changes involved margination of chromatin, formation of granular elements in the nucleoplasm, and, at later time intervals, segregation of nucleoli. The cytoplasm vacuolized strongly, with lipid vacuoles appearing (Fig. 6-a). Remarkable changes were observed in mitochondrial structure in both cell lines. In some mitochondria the cristae were destroyed, this leading so to the appearance of electron-transparent vacuole-like structures (Fig. 6-a). In the other mitochondria membranous bodies were detected in the matrix. These bodies resulted from mitochondrial membrane proliferation (Fig. 6-b). They resembled myelin structures and were heterogeneous in size and shape. Various stages of their development could be observed (Fig. 6-c).

It is noteworthy that in P3HR-1, but not in Raji cells, an additional type of alteration of mitochondria was detected. Some of them contained electron-dense granular inclusions in the central part of the matrix, where cristae were missing (Fig. 7). In the close proximity of these mitochondria accumulations of dense granules were seen that resembled glycogen granules in size and shape. In differentiating Raji cells the degenerative changes were less pronounced. However, some of them exhibited some of the changes described above, vacuolization and cytoplasmic multilamelar bodies in particular.

Discussion

Cis-DDP induced in virus-producer P3HR-1 cells the synthesis of EBV antigens, the appearance of virus-specific changes in cellular morphology, and formation of virus particles. This was in line with our previous observations made in other EBV-transformed cell lines (Anisimová *et al.*, 1974; Vonka *et al.*, 1972). Similar findings had been made when other EBV-inducers, n-butyrate or TPA, were used (Anisimová *et al.*, 1984). Cis-DDP did not produce these effects in the non-producer Raji cells, which are only abortively inducible also by various other inducers (Bauer *et al.*, 1982; Luka *et al.*, 1979). Only occasionally did a very small proportion of Raji cells (0.4 per cent) exhibit some virus-specific cellular alterations that had previously been suggested to precede the appearance of early antigen (Anisimová *et al.*, 1984; Seigneurin *et al.*, 1977).

On the other hand, up to 20 per cent of Raji cells exhibited morphological signs of cell differentiation towards plasma cells. The most advanced stage of differentiation reached was classified as plasmablast. Earlier we had observed a similar response to the other EBV-inducers, n-butyrate (Anisimová *et al.*, 1982) and TPA (Anisimová *et al.*, 1984), or when using pH shock-activated calf serum (Roubalová *et al.*, 1985) or a chemically purified activated serum factor (Anisimová *et al.*, 1987). Cell differentiation was not observed either in this or in our previous studies in P3HR-1 cells (Anisimová *et al.*, 1982); this may somehow be related to the cytopathogenicity of the particular virus strain, which can switch off host macromolecular synthesis (Gergely *et al.*, 1971), thus leading to a state incompatibile with cell differentiation.

Cis-DDP, a widely used cytostatic, also exhibited various effects on the cells used. It rapidly inhibited cytokinesis, but the growth of the cells and nuclear division continued for some time. In agreement with this, incorporation of ^{3}H-thymidine into DNA was inhibited by cis-DDP after some delay (Nováková, unpublished observation). As a consequence, large-sized multinuclear cells appeared in the treated cultures. Similar effects have been described in other mammalian cells by Aggarwal and Sodhi (1973), Aggarwal (1974), and Vates *et al.* (1986).

Cis-DDP also induced extensive degenerative changes in both nucleus and cytoplasm of treated cells. They were similar to those described by others (Aggarwal and Sodhi, 1973; Daskal *et al.*, 1980). Mitochondria were particularly sensitive to cis-DDP action. Similar defects as those described in the present paper have been detected in them after treating the cells with various other agents (Daskal *et al.*, 1975, 1980; Hwang *et al.*, 1974; Markand and Agostino, 1971). One can assume that the changes described may be associated with aberrant regulation of the intracellular concentration of Ca^{2+} ions, that may be deleterious to the formation of the mitotic spindle (Aggarwal, 1974; Aggarwal *et al.*, 1980).

The inhibition of cell division and the induction of the degenerative changes in treated cells undoubtedly belong to the cytostatic mechanisms of cis-DDP action. Here we report that also the induction of cell differen-

tiation towards relatively advanced stages resulting in reduced proliferative capacity could contribute to this activity.

Whether any of the steps leading to these changes are also crucial for the induction of EBV, or whether any other of the pleiotropic cis-DDP effects are involved in this process, remains to be determined.

References

Aggarwal, S. (1974): Inhibition of cytokinesis in mammalian cells by cis-dichloro-diammine-platinum (II). *Cytobiol.* **8**, 395–402.

Aggarwal, S., and Sodhi, A. (1973): Cytotoxic effects of cis-dichloro-diammine-platinum (II) on the mammalian cells in vitro: A fine structural study. *Cytobiol.* **7**, 366–374.

Aggarwal, S., Whitehouse, M., and Ramachandran, C. (1980): Ultrastructural effects of cisplatin, pp. 79–111. In Prestayk A, (Ed.): *Cisplatin Current Status and New Development*, Academic Press, New York.

Anisimová, E., Prachová, K., Roubal, J., and Vonka, V. (1984): Effects of n-butyrate and phorbol-ester (TPA) on induction of Epstein-Barr virus antigens and cell differentiation. *Arch. Virol.* **81**, 223–237.

Anisimová, E., Roubalová, K., Bauer, G., and Roubal, J. (1987): Chemically purified serum factor can induce both Epstein-Barr virus antigen synthesis and cell differentiation. *Arch. Virol.* **94**, 309–314.

Anisimová, E., Roubal, J., Vlčková, I., Kutinová, L., and Vonka, V. (1974): Electron-microscopic findings in EB-3 lymphoblastoid cell line exposed to cis-dichloro-diamine-platinum (II). *Acta virol.* **18**, 203–209.

Anisimová, E., Saemundsen, A., Roubal, J., Vonka, V., and Klein, G. (1982): Effect of n-butyrate on Epstein-Barr virus carrying lymphoma lines. *J. gen. Virol.* **58**, 163–171.

Anisimová, E., Tučková, E., Vonka, V., and Závadová, H. (1977): Ultrastructural changes induced by influenza virus in permissive and nonpermissive cells. *Virology* **77**, 330–336.

Bauer, G., Höfler, P., and zur Hausen, H. (1982): Epstein-Barr virus induction by a serum factor I: Induction and cooperation with additional inducers. *Virology* **121**, 184–194.

Ben Sasson, S. A., and Klein, G. (1981): Acivation of Epstein-Barr virus genome by 5-azacytidine in latently infected human lymphoid lines. *Int. J. Cancer* **28**, 131–135.

Daskal, Y., Merski, J., Hughes, J., and Busch, H. (1975): The effects of cycloheximide on the ultrastructure of rat liver cells. *Exper. Cell Res.* **93**, 395–401.

Daskal, Y., Prestayko, A., and Crooke, S. (1980): Morphological manifestations of cisplatin analogs in rats: an ultrastructural study, pp. 249–269. In Prestayko A. (Ed.): *Cisplatin Current Status and New Development*, Academic Prass, New York.

Gerber, P. (1972): Activation of Epstein-Barr virus by 5-bromodeoxyuridine in "virus free" human cells. *Proc. natn. Acad. Sci. U.S.A.* **69**, 83–85.

Gergely, L., Klein, G., and Ernberg, I. (1971): Host cell macromolecular synthesis in cells containing EBV-induced early antigens, studied by continued immunofluorescence and radioautography. *Virology* **45**, 22–29.

Hampar, B., Derge, J. G., Nonoyama, M., Chang, S. Y., Tagamets, M. A., and Showalter, S. D. 1974): Programming of events in Epstein-Barr virus-inactivated cells induced by 5-iododeoxyuridine. *Virology* **62**, 71–89.

Henle, G., and Henle, W. (1966): Immunofluorescence in cells derived from Burkitt's lymphoma. *J. Bacteriol.* **91**, 1248–1256.

Hwang, K., Yang, L., Carrico, C., Schultz, R., R., Schenkman, J., and Sartorelli, A. (1974): Production of membrane whorls in rat liver by some inhibitors of protein synthesis. *J. Cell Biol.* **62**, 20–30.

Luka, J., Kallin, B., and Klein, G. (1979): Induction of the Epstein-Barr virus (EBV) cycle in latently infected cell by n- butyrate. *Virology* **94**, 228–231.

Markand, O., and Agostino, A. (1971): Ultrastructural changes in skelet muscle induced by colchicine. *Arch. Neurol.* **24**, 70–81.

Miller, G., and Lipman, M. (1973): Release of infectious EBV by marmoset transformed leucocytes. *Proc. natn. Acad. Sci. U.S.A.* **70**, 190–194.

Moss, D., Rickinson, A., Wallace, L., and Epstein, M. (1981): Sequential appearance of Epstein-Barr virus nuclear antigen and lymphocyte-determined membrane antigen in B-cell transformation. *Nature (London)* **291**, 664—666.

Nilsson, K. (1979): The nature of lymphoid cell lines and their relationship to the virus, pp. 225—281. In Epstein M. A., Achong B. G. (Ed.): *The Epstein-Barr virus*, Springer, Berlin — Heidelberg — New York.

Nonoyama, M., and Pagano, J. C. (1972): Separation of Epstein-Barr virus DNA from large chromosomal DNA in nonvirus-producing cells. *Nature (London)* **238**, 169—171.

Pulvertaft, R. (1965): A study of malignant tumor in Nigeria by short-term tissue culture. *J. clin. Pathol.* **18**, 261—273.

Reedman, B., and Klein, G. (1973): Cellular localization of an Epstein-Barr virus (EBV) - associated complement fixing antigen in producer and non-producer lymphoblastoid cell lines. *Int. J. Cancer* **11**, 499—520.

Roubalová, K., Anisimová, E., and Roubal, J. (1985): Effects of activated serum factor on the induction of Epstein-Barr virus antigens and cell differentiation. *Arch. Virol.* **85**, 85—94.

Seigneurin, J., Vuillaume, M., Lenoir, G., and De Thé, G. (1977): Replication of Epstein-Barr virus ultrastructural and immunofluorescent studies of P3HR 1-superinfected Raji cells. *J. Virol.* **24**, 836—845.

Tovey, M. G., Lenoir, G., and Begon-Lours, J. (1978): Activation of latent Epstein-Barr virus by antibody to human IgM. *Nature (London)* **276**, 270—272.

Vates, T., Brake, S., Mayumdar, S., and Fergusen, G. (1986): Response of murine erythroleukemia cells to cis-and trans-diammine-dichloro-platinum (II). *In Vitro Cell Development Biol.* **22**, 305—310.

Vonka, V., Kutinová, L., Drobník, J., and Bräuerová, J. (1972): Increase of Epstein-Barr-virus-positive cells in EB 3 cultures after treatment with cis-dichloro-diammine-platinum (II). *J. natn. Cancer Inst.* **48**, 1277—1281.

Zur Hausen, H., Bornkamm, G. W., Schmidt, R., and Hecker, E. (1979): Tumor initiators and promotors in the induction of Epstein-Barr virus. *Proc. natn. Acad. Sci U.S.A.* **76**, 782—785.

Zur Hausen, H., O'Neill, F. J., Freese, U. K., and Hecker, E. (1978): Persisting oncogenic herpesvirus induced by the tumor promotor TPA. *Nature (London)* **272**, 373—375.

Legends to the Figures (Plates LXVI—LXX)

Fig. 1. Electron micrograph of untreated Raji cells with characteristic signs of large, poorly differentiated lymphoblasts containing a lobular, irregular nucleus (N). The cytoplasm contains few mitochondria (m), the Golgi apparatus (g), and a weakly pronounced rough endoplasmic reticulum (arrows). Magnification × 6,700.

Fig. 2. Autoradiograph of a multinuclear P3HR-1 cell 120 hrs after addition of cis-DDP (1μg/ml). The cells were labelled with ^{3}H-thymidine for 5 hrs before they were harvested for autoradiography. Magnification × 375.

Fig. 3. Fragment of a giant multinuclear Raji cell pretreated for 72 hrs with cis-DDP. Magnification × 6,700.

Fig. 4. Electron micrograph of P3HR-1 cells 72 hrs after addition of cis-DDP. a) The nucleus (N) contains an accumulation of small electron-dense granules (g) and exhibits margination of chromatin and disintegration of the nuclear membrane (arrow). The cristae and matrix of the mitochondria are replaced by headed or clubbed electron-dense material (m). Typical immature EBV particles can be seen in the cytoplasm (small arrows). b) The cytoplasm of some cells contains randomly distributed tubular structures (arrows). c) The nucleoplasm contains EBV particles at various stages of maturation. Magnifications × 15,000; × 40,000; × 84,000, respectively.

Fig. 5. Fragment of a Raji cell treated with cis -DDP for 24 hrs. a) The cytoplasm contains more rough endoplasmic reticulum (arrows) and mitochondria (m), than do lymphoblasts. Magnification × 12,300. b) Fragment of a Raji cell treated with cis-DDP for 72 hours. A cell at the plasmablast stage of differentiation is shown: a relatively abundant endoplasmic reticulum (arrow) is localized in ring-like structure around the nucleus (N). Clusters of mitochondria (m), a well-developed Golgi apparatus (G) and lipid vacuoles (V) are visible. Magnification × 12,300.

Fig. 6. Electron micrograph of Raji cells 72 hrs after induction by cis-DDP. a) Cells with degenerative changes exhibit margination of chromatin and contain small granular elements in the

nucleoplasm (e), a strongly vacuolized cytoplasm (v) and electron-transparent mitochondria (arrow). Disintegration of a cell is shown in the bottom right-hand corner. Magnification × 4,900. b) Thin section throughout a mitochondrion. In its central part the formation of a membranous body begins (arrow). Magnification × 40,000. c) Membranous bodies at various stages of development (arrows) Magnification × 25,000.

Fig. 7. A fragment of cis-DDP-treated P3HR-1 cell showing dense mitochondrial inclusion (arrow). Dense granules in its surroundings are visible (g). Magnification × 18,700.

Book Review

Arenaviruses

A. Clarke, R. W. Compans (series Eds): Current Topics in Microbiology and Immunology, vol. 133. Oldstone (Ed.): Arenaviruses, 39 Figs, 116 pp. Springer Verl., New York—Berlin—Heidelberg—London—Paris—Tokyo, 1987, price DM 94.—

The 133rd issue of Current Topics in Microbiology and Immunology contains the most recent description of the Arenaviruses group. Valuable data on the diseases they may cause as well as their biology, chemistry, genetics and molecular biology are presented by well known specialists from U.S.A., United Kingdom and France.

Though the Arenaviruses like Machupo virus, Junin virus or Lassa virus cause mostly local economic and health problems in W. Africa or S. and Latin America, owing to increasing travelling activities they create new problems for health care personnel all over the world. LCMV problematic is also largely discussed, especially its viral genome persistence in animals and cultured cells. Using LCMV in animal model the first data on the virus-induced immunopathologic disease, T cell-mediated killing and its mechanisms were discovered.

The six individual chapters of the book cover: arenavirus gene structure and organization, sequence comparison, protein structure and expression among arenaviruses, mapping arenavirus genes causing virulence, state of viral genome and proteins during persistent lymphocytic choriomeningitis virus infection and finally pathology and pathogenesis of arenavirus infections.

This volume may be recomended for all proffessional virologists, clinicians, pathologists as well as for pre- and post-graduate students of medicine or natural sciences.

D. Stanček, Bratislava