

Title	Chromosomal Characteristics of Six Cultured Lymphoblastoid Cell Lines Originating from Marek's Disease Lymphomas
Author(s)	Takagi, Nobuo; Sasaki, Motomichi; Ikuta, Kazuyoshi et al.
Citation	Biken journal : journal of Research Institute for Microbial Diseases. 1977, 20(1), p. 21-28
Version Type	VoR
URL	https://doi.org/10.18910/82601
rights	
Note	

Osaka University Knowledge Archive : OUKA

<https://ir.library.osaka-u.ac.jp/>

Osaka University

CHROMOSOMAL CHARACTERISTICS OF SIX CULTURED LYMPHOBLASTOID CELL LINES ORIGINATING FROM MAREK'S DISEASE LYMPHOMAS

NOBUO TAKAGI and MOTOMICHI SASAKI

Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo, Hokkaido

KAZUYOSHI IKUTA and SHIRO KATO

Department of Pathology, Research Institute for Microbial Diseases, Osaka University,
Yamada-kami, Suita, Osaka

(Received September 25, 1976)

SUMMARY Cytogenetic observations were made on 6 cell lines (MOB-1, MOB-2, MOB-3, MSB-1, HPRS Line 1, HPRS Line 2) originating from Marek's disease lymphomas and 2 clones (1104-B, 1104-X-5) of a cell line established from an avian lymphoid leukosis tumor. The modal chromosome number was within the diploid range in all the lines except HPRS Line 1 and HPRS Line 2, both of which had a mode at about 60. Karyotypes were grossly abnormal in 4 cell lines: trisomy for #1 in MOB-2; the heteromorphic #1 pair in MSB-1, and marker chromosomes derived from rearrangements involving #3 or #5 and unidentified elements in HPRS Lines 1 and 2. The MOB-1 line which had been characterized by cells with an apparently normal karyotype was completely taken over by cells with a heteromorphic #1 pair morphologically similar to the one found in MSB-1 by the 95th day of continuous growth in vitro. BUdR-acridine orange differential staining technique revealed, however, different banding patterns in these abnormal chromosomes.

INTRODUCTION

Marek's disease (MD), a contagious, neoplastic, lymphoproliferative condition affecting the chicken, has several favorable characteristics as an experimental model of lymphoma induced by herpes type virus. Successful long-term cultivation of lymphoblastoid cells from MD tumors (Akiyama et al., 1973) has given an impetus to studies on the fundamental properties of this disease. A number of trials so

far resulted in establishment of 6 permanent cell lines whose immunologic, virologic, morphologic, and biochemical traits were subjects of recent studies (Akiyama and Kato, 1974; Powell et al., 1974, 1975; Kato and Akiyama, 1975; Nazerian and Witter, 1975; Ikuta et al., 1976a, b; Matsuda et al., 1976a, b). The most distinctive characteristics of these 6 MD cell lines are two types of cell surface anti-

genicity which are T cell determinants (Powell et al., 1974; Nazerian and Witter, 1975; Matsuda et al., 1976a, b) and MD tumor-associated surface antigen (Witter et al., 1975; Matsuda et al., 1976b). However, only limited cytogenetic data are available of these cell lines (Akiyama and Kato, 1974; Ikuta et al., 1976b). Chromosome constitution is liable to change during an extended period in culture. Moreover, cytogenetic analysis is inevitably incomplete in avian cells, since 30-40% of the chicken genome comprising nearly 60 microchromosomes can not be examined with certainty. In spite of these drawbacks detailed examination of the chromosome constitution in those cell lines is essential not only for the evaluation of the possible role of the chromosomal alteration in the causation of MD, but for the pursuit of distinguishing characters of each cell line.

MATERIALS AND METHODS

Karyotypes were examined in 6 cell lines (MOB-1, MOB-2, MOB-3, MSB-1, HPRS Line 1, HPRS Line 2) originating from MD lymphomas and 2 clones (1104-B, 1104-X-5) of a cell line established from an avian lymphoid leukosis tumor. Table 1 presents their origins, associated viruses, T- or B-cell determinant and length of in vitro growth at the time of chromosome examination. Details of methods and conditions for in vitro culture were described elsewhere (Akiyama et al., 1973; Powell et al., 1974).

Chromosomes were examined at the Chromosome Research Unit within 5 days after the receipt of cells from The Research Institute for Microbial Diseases. Each cell line was incubated at 41 C with 5-bromodeoxyuridine (100 μ g/ml) for 9-10 hr including the last 1 hr in the presence of Colcemid (0.1 μ g/ml). Cells were fixed with 3:1 methanol/acetic acid and slides were made according to a routine air-drying techniques. Karyotypes were

TABLE 1. *Origin and chromosomal characteristics of 8 avian lymphoma cell lines*

Cell line	Associated with	T- or B-cell determinant	Original tumor induced by inoculation of	Culture age at examination	Sex chromosome	Chromosome no.	Chromosome aberration
MOB-1 ^a	MDV	T	Blood of chickens infected with BC-1 strain of MDV	85d 95d 166d	ZW ZW	73-80 73-80	— Increase in the length of the short arm of #1
MOB-2 ^b	MDV, ALV	T	MSB-1 line cells	181d	ZW	66-79	Trisomy for #1
MOB-3 ^c	MDV	T	MSB-1 line cells	24d	ZW	68-75	—
MSB-1 ^d	MDV	T	MOB-1 line cells	106d	ZW	69-78	Increase in the length of the short arm of #1
HPRS Line 1 ^e	MDV	T			ZW	52-62	Increase in the length of the short arm of #3 and #5
HPRS Line 2 ^e	MDV	T			ZW	53-60	Increase in the length of the short arm of #5
1104-B ^f	ALV	B	ALV subgroup A		ZZ	67-78	—
1104-X-5 ^f	ALV	B	ALV subgroup A		ZZ	66-79	—

^a Akiyama et al., 1973.

^b Ikuta et al., 1976b.

^c Matsuda et al., 1976b.

^d Akiyama and Kato, 1974.

^e Powell et al., 1974.

^f Hihara et al., 1974.

analysed primarily in metaphases stained with conventional Giemsa. A differential staining technique with acridine orange (Dutrillaux et al., 1973) provided evidence for the identity of individual

chromosomes and chromosomal segments. Exact chromosome numbers were determined on mitoses stained by a C-banding technique (Sumner, 1972).

RESULTS

Since polyploid mitoses were relatively rare in these cell lines, we focused our attention only to cells within the diploid range. The total chromosome number, however, varied considerably within individual cell lines. This may not be specific for the cultured lines, but is also common in normal somatic cells probably because the present technique is inadequate in dealing with microchromosomes.

The total chromosome number varied from high 60s to high 70s in all lines except HPRS Lines 1 and 2. The maximum chromosome count was 62 in the former and 60 in the latter. Decrease in the number of microchromosomes apparently accounted for the low counts. Rearrangements rather than simple losses of chromosomes might be more likely, inasmuch as most microchromosomes including several biarmed ones were relatively large and well-delineated in metaphase spreads compared with those having about 80 chromosomes (Fig. 1, 2.) The C-banding technique demonstrated the W chromosome in most cells from 6 MD cell lines.

So far as the 10 largest pairs were concerned, MOB-1 in early passages, MOB-3, 1104-B, and 1104-X-5 showed no obvious deviation from the normal pattern, but the remaining 4 lines had at least one major karyotypic abnormality (Fig. 3). It should be mentioned, however, that identification of autosomes smaller than #5 was arbitrary especially in HPRS Lines 1 and 2.

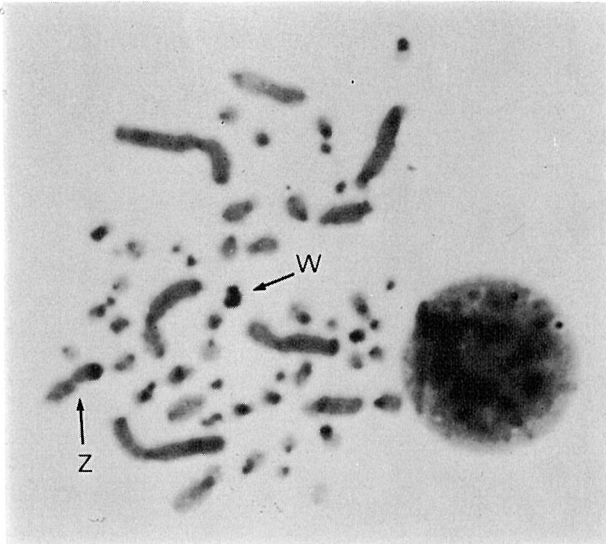


FIGURE 1. Metaphase spreads stained by the C-banding technique. A HPRS Line 1 cell with 57 chromosomes.

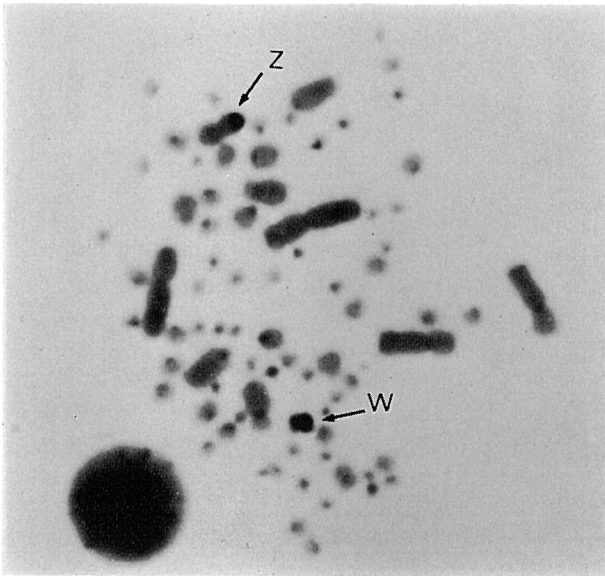


FIGURE 2. Metaphase spreads stained by the C-banding technique. A MOB-1 line cell with 78 chromosomes.

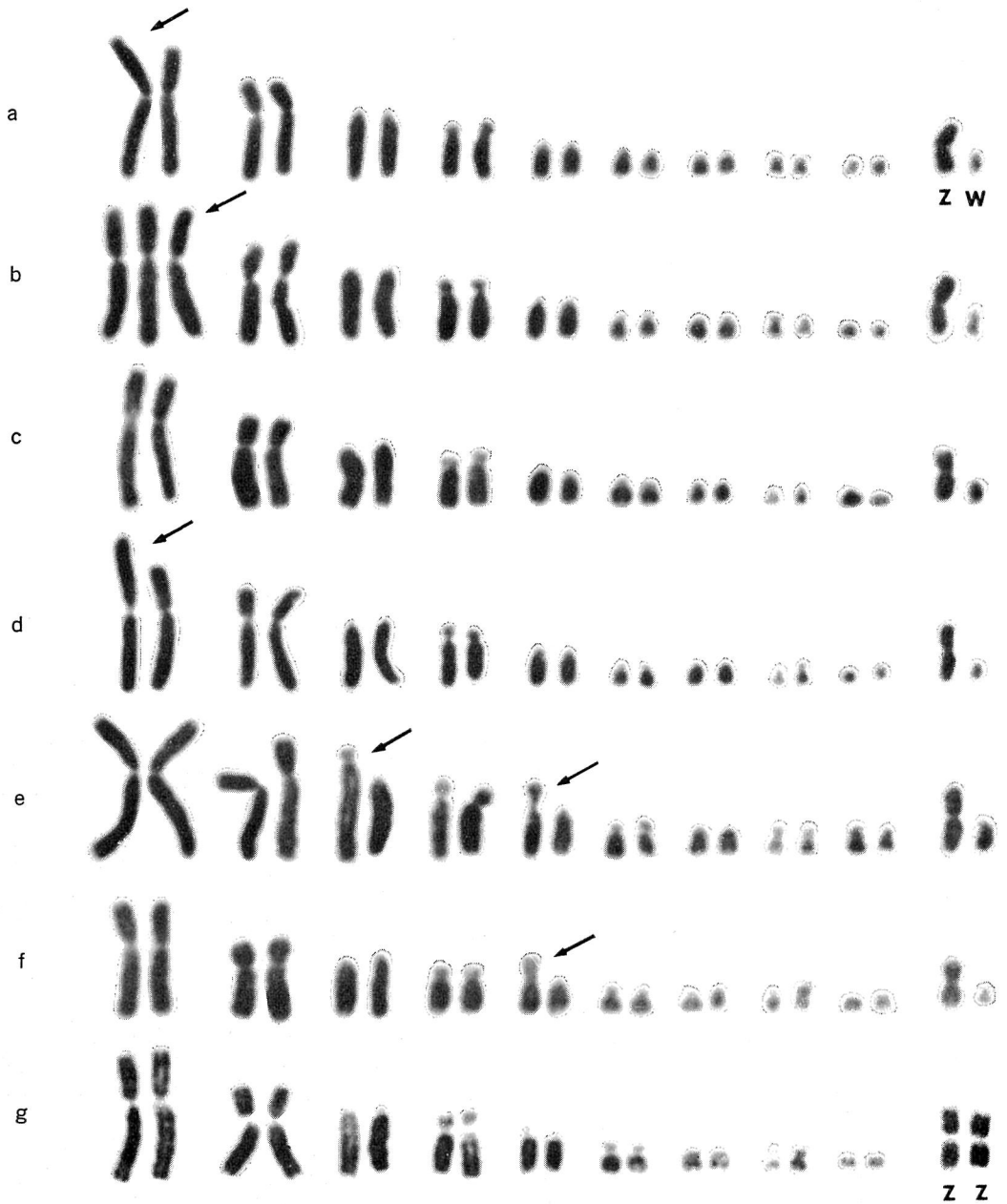


FIGURE 3. Partial karyotypes of 6 cell lines originated from MD lymphomas and a clonal line derived from an avian lymphoid leukemia tumor. Arrows indicate abnormal elements. a) a MOB-1 line cell at 166th day in culture; b) a MOB-2 line cell; c) a MOB-3 line cell; d) a MSB-1 line cell; (e) a HPRS Line 1 cell; f) a HPRS Line 2 cell; g) a 1104-B line cell.

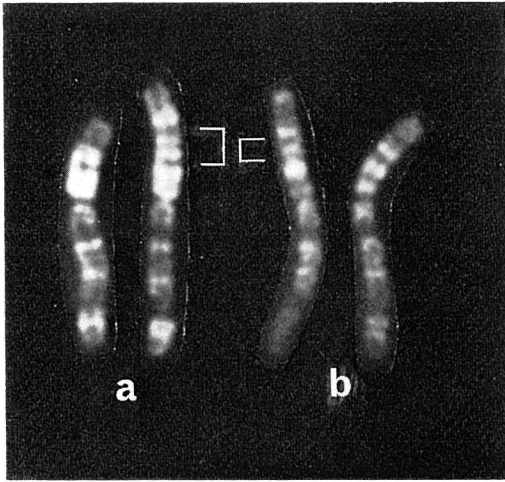


FIGURE 4. Comparison of BUdR-AO banding patterns between marker chromosomes from the MOB-1 (a) and the MSB-1 (b) line cell. Indicated in the photograph are extra segments.

MOB-1: We reported that the karyotype of this line was indistinguishable from that of a normal chicken cell (Akiyama and Kato, 1974) and this was confirmed by examination of recultivated cells from an early culture (85 day-old cultivation) that had been kept frozen. However, a consistent abnormality, a heteromorphic #1, appeared in a culture continuously grown in vitro for 95 days (Fig. 3). The short arm of the abnormal #1 was considerably longer than the normal counterpart, whereas the long arms were comparable in length in both normal and abnormal homologs. Acridine orange banding analysis revealed 2 additional bright bands at the middle of the short arm as tentatively shown in Fig. 4. Inasmuch as the extra segment was not long enough and devoid of distinctive fluorescent pattern, the source of this segment was not determined. Perhaps this might represent partial duplica-

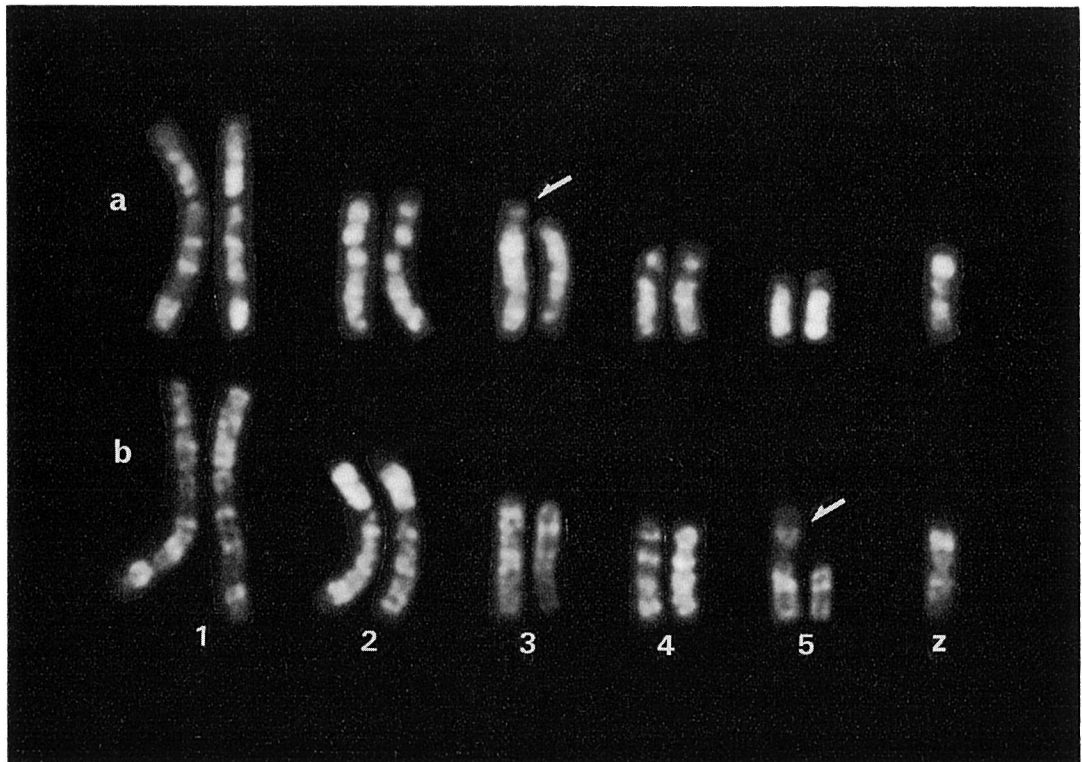


FIGURE 5. BUdR-AO banding patterns of larger chromosomes from HPRS Line 1 (a) and HPRS Line 2 (b).

tion of chromosome #1, since there was no corresponding deletion or any other apparent anomaly in the remaining chromosomes.

MOB-2: The present study fully confirmed the previous observation that chromosome #1 was trisomic (Ikuta et al., 1976b). Chromatid breakages were frequent, but no attempt was made to quantify them.

MSB-1: In agreement with the previous observation (Akiyama and Kato, 1974) the short arm of one of the chromosomes #1 was unusually long. This chromosome like the marker from MOB-1 had an extra segment at the middle of the short arm. However, only one bright band was present in this segment and the fluorescent pattern of the short arm distal to the extra segment appeared slightly different between MOB-1 and MSB-1 (Fig. 4). Therefore, it may be safe to rule out a possible cellular contamination between these lines.

HPRS Line 1: This cell line was karyotypically variable, but one of the chromosomes #3 consistently had a distinct short arm (Fig. 3). Less consistent was a chromosome #5 with a short arm corresponding in length to chromosomes #9-10. Both abnormal elements might have resulted from centric fusion, but the identity of the attached chromosomes remained unknown because of their small sizes and lack of distinct fluorescent characteristics (Fig. 5).

HPRS Line 2: This line was also heterogeneous with variable karyotypes. Nearly all cells had two metacentric chromosomes morphologically similar to the Z (Fig. 3). Banding analysis revealed that the one was in fact the Z, but the other was a product of a rearrangement involving #5 and a chromosome of unknown origin (Fig. 5).

DISCUSSION

MD tumors can be passaged by inoculating blood and tissues from chickens infected with MD virus (MDV). Tumors thus passaged are, however, primary and not produced by the

proliferation of inoculated tumor cells, since the sex chromosome constitution of the tumor cells always corresponds to the phenotypic sex of the host chicken (Owen et al., 1966; Bloom, 1970). Consequently, virus infection rather than multiplication of inoculated tumor cells seems important in the growth of MD tumors as suggested in avian Rous sarcomas by Ponten (1964). The situation may scarcely be different when tumors are induced by inoculating cultured cells: present cytogenetic findings strongly suggested that multiplication of MSB-1 with an abnormal #1 had not directly contributed to the progressive growth of tumors from which MOB-2 and MOB-3 derived.

Owen et al. (1966) and Bloom (1970) showed that experimentally induced primary MD tumors had a normal diploid number of chromosomes without any morphologically abnormal element as in avian Rous sarcomas and erythroleukemias (Ponten, 1963). It is thus probable that the overwhelming majority of cells comprising original tumors from which the present cultured lines derived would have been characterized by karyotypes indistinguishable from the normal chicken somatic complex. Emergence of a new marker chromosome in the 95 day-old MOB-1 line suggests that certain karyotypic changes observed have arisen during *in vitro* passages. It is unknown, however, whether all of the karyotypic deviations from euploidy in the remaining 4 MD cell lines had originated *in vitro*. Chromosomally aberrant cells present in predominantly diploid tumors might have overgrown the culture. Clones of such cells, if any, should have been too small in size to be detected cytogenetically in original tumors in view of the possible mode of tumor growth discussed above.

The present study failed to detect any gross chromosomal change common to 6 MD cell lines as reported in such neoplastic conditions as chronic myelogenous leukemias (Rowley, 1973) and African-type Burkitt's lymphomas (Zech et al., 1976) in man, thymomas in AKR mice (Dofuku et al., 1975), and DMBA-induced

leukemias in rats (Sugiyama et al., 1967). It is worth mentioning that chromosome #1 was involved in karyotypic change in 3 out of 5 MD cell lines, whereas two clones of an avian lymphoid leukemia cell line independently maintained for more than two years showed no gross chromosomal aberration. Any conclusion must await fuller cytological studies, but it is tempting to postulate that MDV infection was responsible for the observed aberrations. The finding of Owen et al. (1966) that chromatid breaks were confined to chromosomes #1 and #2 in primary MD tumors induced by HPRS-20 strain of MDV is indeed intriguing. Recently Yoon et al. (1976) obtained similar findings in peripheral blood and bone marrow cells of chicks shortly after infection with JM-V, a lymphoblastic leukemia virus, derived from the JM strain of MDV.

The observed marker chromosomes and abnormal karyotypes provide invaluable means of distinguishing one culture from another.

REFERENCES

- Akiyama, Y., N. Iwa, and S. Kato. 1973. Continuous cell culture from lymphoma of Marek's disease. *Biken J.* 16: 177-179.
- Akiyama, Y., and S. Kato. 1974. Two cell lines from lymphomas of Marek's disease. *Biken J.* 17: 105-116.
- Bloom, S. E. 1970. Marek's disease: Chromosome studies of resistant and susceptible strains. *Avian Dis.* 14: 478-490.
- Dofuku, R., J. L. Biedler, B. A. Spengler, and L. J. Old. 1975. Trisomy for chromosome 15 in spontaneous leukemia of AKR mice. *Proc. Natl. Acad. Sci. USA* 72: 1515-1517.
- Dutrillaux, B., C. Laurent, J. Couturier, and J. Lejeune. 1973. Coloration des chromosomes humains par l'acridine orange apres traitement par le 5-bromodeoxyuridine. *C. R. Acad. Sci. [D] (Paris)* 276: 3179-3181.
- Hihara, H., T. Shimizu, and H. Yamamoto. 1974. Establishment of tumor cell lines cultured from chickens with avian lymphoid leukemia. *Natl. Inst. Anim. Health Q. (Tokyo)* 14: 163-173.
- Ikuta, K., S. Kato, T. Ando, and T. Konobe. 1976a. Lack of expression of endogenous (gs, chick help-

Chromosome examination may be the only easy and reliable way to detect cellular contamination occurring between different cell lines. Abnormal karyotypes are constantly produced in human lymphoblastoid cell lines harboring Epstein-Barr virus (EBV) (Takagi and Sandberg, 1969), and probably the same holds in cultured MD cell lines. Routine cytogenetic monitoring will not only make identification of individual cell lines certain but help clarify the causal relationship between MDV infection and karyotypic alterations.

ACKNOWLEDGMENTS

We are indebted to Drs. P. C. Powell and H. Hihara who kindly provided the HPRS Lines 1 and 2, and 1104-B and 1104-X-5 respectively. This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture.

- er factor) and exogenous avian RNA tumor viruses in the MOB-1 and MSB-1 lines derived from Marek's disease lymphomas. *Biken J.* 19: 39-42.
- Ikuta, K., Y. Akiyama, T. Konobe, and S. Kato. 1976b. A lymphoblastoid cell line dually infected with Marek's disease virus and avian leukemia virus. *Biken J.* 19: 33-37.
- Kato, S., and Y. Akiyama. 1975. Continuous cell culture from lymphomas of Marek's disease. *Oncogenesis and Herpesviruses II part 2, IARC, Lyon*, p. 101-108.
- Matsuda, H., K. Ikuta, and S. Kato. 1976a. Detection of T-cell surface determinants in three Marek's disease lymphoblastoid cell lines. *Biken J.* 19: 29-32.
- Matsuda, H., K. Ikuta, H. Miyamoto, and S. Kato. 1976b. Demonstration of a Marek's Disease tumor-associated surface antigen (MATSA) on six Marek's lymphoma-derived cell lines. *Biken J.* 19: 119-123.
- Nazerian, K., and R. L. Witter. 1975. Properties of a chicken lymphoblastoid cell line from Marek's disease tumor. *J. Natl. Cancer Inst.* 54: 453-458.

- Owen, J. J. T., M. A. S. Moore, and P. M. Biggs. 1966. Chromosome studies in Marek's disease. *J. Natl. Cancer Inst.* 37: 199-209.
- Ponten, J. 1963. Chromosome analysis of three virus-associated chicken tumors: Rous sarcoma, erythroleukemia and RPL 12 lymphoid tumor. *J. Natl. Cancer Inst.* 30: 897-921.
- Ponten, J. 1964. The in vivo growth mechanism of avian Rous sarcoma. *J. Natl. Cancer Inst. Monogr.* 17: 131-142.
- Powell, P. C., L. N. Payne, J. A. Frazier, and M. Rennie. 1974. T lymphoblastoid cell lines from Marek's disease lymphomas. *Nature* 251: 79-80.
- Powell, P. C., L. N. Payne, J. A. Frazier, and M. Rennie. 1975. T-lymphoblastoid cell lines from Marek's disease lymphomas. *Oncogenesis and Herpesviruses II part 2*, IARC, Lyon, p. 89-99.
- Rowley, J. D. 1973. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 243: 290-293.
- Sugiyama, T., Y. Kurita, and Y. Nishizuka. 1967. Chromosome abnormality in rat leukemia induced by 7,12-dimethylbenz(a) anthracene. *Science* 158: 1058-1059.
- Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* 75: 304-306.
- Takagi, N., and A. A. Sandberg. 1969. Cytogenetics of hematopoietic cells in long-term culture, p. 27-57. *In* G. L. Tritsch [ed.] *Axenic Mammalian Cell Reactions*. Marcel Dekker, New York/London.
- Witter, R. L., E. N. Stephens, J. M. Sharma, and K. Nazerian. 1975. Demonstration of a tumor associated surface antigen in Marek's disease. *J. Immunol.* 115: 177-183.
- Yoon, J. W., S. N. Kim, E. C. Hahn, and A. J. Kenyon. 1976. Lymphoproliferative disease of fowl: Chromosome breaks caused in lymphocytes by JM-V herpesvirus. *J. Natl. Cancer Inst.* 56: 757-762.
- Zech, L., U. Hagland, K. Nilsson, and G. Klein. 1976. Characteristic chromosomal abnormalities in biopsies and lymphoid-cell lines from patients with Burkitt and non-Burkitt lymphomas. *Int. J. Cancer* 17: 47-56.