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Chromosomal analysis of cultured skin fibroblasts from patients with infectious mononucleosis

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SUMMARY

In the present study, skin fibroblasts and leukocyte cultures from patients with acute infectious mononucleosis were examined to determine if any *in vitro* marker chromosomes could be detected. No alterations in chromosomes could be found in fibroblasts either from the region of the typical rash or from other areas of skin. This would tend to suggest but does not necessarily prove that this tissue was not extensively involved in the disease process.

Since the observation of herpes-like viral particles in cultured lymphoid cell lines from patients with infectious mononucleosis and Burkitt's lymphoma, there has been much interest in the further study of long-term lymphoid tissue cultures (Henle, Henle & Diehl, 1968; Niederman *et al.* 1968). Cytogenetic alterations have been noted in several cell lines established from Burkitt's lymphomas (Kohn *et al.* 1967; Miles & O'Neill, 1967), and a characteristic marker in the no 10 chromosome (C group) in long-term leukocyte cultures established from patients with acute infectious mononucleosis was recently reported (Kohn *et al.* 1968). The no. 10 marker was absent in short-term cultures (48–72 h) or phytohaemagglutinin-stimulated peripheral leukocytes from patients with infectious mononucleosis. These findings suggest that the cytogenetic alterations noted may be the result of induction by endogenous virus *in vitro*.

Of specific interest to us was the possibility of utilizing the C group chromosomal marker or other cytogenetic alterations as a method of determining latent infection of other tissues in infectious mononucleosis. The purpose of the present study was to examine skin fibroblast and leukocyte cultures from patients with infectious mononucleosis to determine if any *in vitro* marker chromosomes could be detected.

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Skin biopsies and heparinized blood specimens were obtained from three patients with acute infectious mononucleosis and from two healthy controls. In addition, fibroblasts were obtained from three patients with acute infectious mononucleosis specifically from skin regions showing the infectious mononucleosis rash and examined along with three healthy controls. Long-term leukocyte cultures were attempted by a modification of a known reported method (Pope, 1967). Although the cell cultures displayed metabolic activity for extended periods of time, multiplication did not occur and, therefore, chromosomal preparations were not available for study. The skin fibroblast cultures were established in Leighton tubes (Monteleone, Durst & Cherry 1968). Chromosomes were harvested from subcultures following approximately 26 days in culture.

CELL LINES

CGH 52. Skin biopsy was obtained on 6 November 1968 from a 20-year-old woman who had typical clinical symptoms of acute infectious mononucleosis. The serologic test for infectious mononucleosis was positive* and the leukocyte count was 18600/mm^3. Seventy per cent of the leukocytes were lymphocytes and many of these were atypical. Cytogenetic analysis was performed on 3 December 1968.

CGH 53. This cell line was established from fibroblasts obtained on 7 November 1968 from a 22-year-old woman who had typical clinical symptoms of infectious mononucleosis and a positive infectious mononucleosis test.* Peripheral blood smear showed 74% lymphocytes, many of which were classical Downey cells. The total white blood cell count was 15000/mm^3. Cytogenetic analysis was performed on 4 December 1968.

CGH 56. Skin biopsy for fibroblast study was obtained on 21 November 1968 from a 24-year-old woman who had a characteristic clinical picture of acute infectious mononucleosis on 15 October 1967 and a positive heterophile antibody test. The total white blood cell count was 16000/mm^3 and the peripheral blood smear contained 64% lymphocytes, many of which were atypical. Cytogenetic analysis of the cell line was performed on 17 December 1968.

CGH 54. This was a cell line from an apparently healthy 25-year-old woman obtained on 6 November 1968. Cytogenetic analysis was done on 3 December 1968.

CGH 55. Fibroblasts were obtained on 7 November 1968 from a 21-year-old woman who was healthy and well. Chromosomal analysis was performed on 4 December 1968.

CGH 76. Skin fibroblasts were obtained from the region of the rash on a 15 year-old male with the clinical picture of acute infectious mononucleosis on 6 August 1969. Infectious mononucleosis test was positive.* White blood cell count was 17000/mm^3 and peripheral smear contained 79% lymphocytes with many atypical cells. Cytogenetic analysis of the cell line was performed on 3 September 1969.

* Stat test, Wampole Laboratories.

CGH 79. The authors obtained skin biopsies from the region of the rash on a 13-year-old male with the classical clinical features of acute infectious mononucleosis on 15 September 1969 and a positive infectious mononucleosis test.* Total white blood cell count was 16500/mm^3 with 75 % lymphocytes. Chromosomal analysis was done on 14 October 1969.

CGH 94. A 20-year-old female with clinical symptoms of acute infectious mononucleosis and a positive infectious mononucleosis test* was found to have a total white blood cell count of 19000 with 70 % lymphocytes. Skin biopsy from the area of rash was obtained on 13 January 1970 and chromosomal analysis performed 11 February 1970.

CGH 77, 80 and 95. Skin biopsies from three healthy controls, 14, 16, and 21 years of age respectively, were obtained and cytogenetic analysis was done on these cells along with the fibroblasts from the three patients exhibiting the rash of acute infectious mononucleosis (CGH 76, 79 and 94).

Twenty-five metaphases were analysed in each of the 11 cell lines and at least 23 cells in each line were euploid. In those cells which were aneuploid there was no chromosome consistently missing. No abnormal chromosome was found in any metaphase analysed. The C-group chromosomal marker as described by (Kohn *et al.* 1968) represented by a subterminal secondary constriction in the long arms of a no. 10 chromosome was looked for particularly, but was not found in any cell.

The lack of chromosomal alterations in skin fibroblasts cultures from patients with infectious mononucleosis both with and without rash would tend to suggest that this tissue was not extensively involved in the disease process. However, since the chromosomal markers are only manifest following prolonged *in vitro* cultivation, it is possible that fibroblastic cells are also involved but that alterations were not observed because of the limited study methods employed. Likewise, even though no karyotypic abnormalities in the cell line studies were found, this does not necessarily imply the absence of the herpes-like virus.

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REFERENCES

Henle, G., Henle, W. & Diehl, V. (1968). Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. *Proceedings of the National Academy of Science, U.S.A.* **59**, 94–101.

Kohn, G., Diehl, V., Mellman, W., Henle, W. & Henle, G. (1968). C-Group chromosome marker in long-term leukocyte cultures. *Journal of the National Cancer Institute* **41**, 795–804.

Kohn, G., Mellman, W., Moorhead, P., Loftus, J. & Henle, G. (1967). Involvement of C group chromosomes in five Burkitt lymphoma cell lines. *Journal of National Cancer Institute* **38**, 209–222.

Miles, C. & O'Neill, F. (1967). Chromosome studies of 8 *in vitro* lines of Burkitt's lymphoma. *Cancer Research* **27**, 392–402.

* Stat test, Wampole Laboratories.

MONTELEONE, P., DURST, J. & CHERRY, J. (1968). Simplified culture of human skin fibroblasts for chromosomal analysis. *Health Laboratory Science* **5**, 257–258.

NIEDERMAN, J., MCCOLLUM, R., HENLE, G. & HENLE, W. (1968). Infectious mononucleosis. *Journal of the American Medical Association* **203**, 205–209.

POPE, J. (1967). Establishment of cell lines from peripheral leukocytes in infectious mononucleosis. *Nature* **216**, 810–811.