Human Myeloma Cell Line Carrying a Philadelphia Chromosome

Abstract. *A new human plasmacytoma cell line (Karpas 707) has been established from a myeloma patient. The cultured cells are negative for Epstein-Barr viral nuclear antigen and free of mycoplasma. They are similar to plasma cells and secrete only lambda light chains. The cells are hypodiploid and contain the Philadelphia chromosome and other abnormalities. This cell line may be suitable for the production of human monoclonal antibodies.*

We describe the unusual properties of the first human myeloma cell line since the immunoglobulin E (IgE) lambda (λ)–producing line (U266) was established in 1968 (*1*). Normal human hemic cell lines have been developed only for B cells infected with Epstein-Barr virus (EBV). A relatively small number of EBV-negative leukemic cell lines have been established from leukemic patients. These lines include B- and T-cell lymphoblasts as well as malignant cells of the myeloid series (*2*). Among the various human hematological malignancies, myeloma is probably one of the most difficult from which to establish hemic cell lines in continuous proliferation in vitro. The availability of a human myeloma cell line is of great interest for the study of the biology and etiology of the disease. Furthermore, such a line could be used for the development of human monoclonal antibodies. The technique for the production of monoclonal antibodies in vitro was developed in 1975 with cultured mouse myeloma cells (*3*). In the past few years this has proven to have an enormous potential for research and the development of diagnostic reagents. However, unlike the easily available mouse myeloma, which can be induced at will in vivo and which proliferate readily in vitro and therefore enabled rapid progress in this field, no real progress has been made in the development of human monoclonal antibodies, mainly because of the unavailability of a suitable human myeloma cell line.

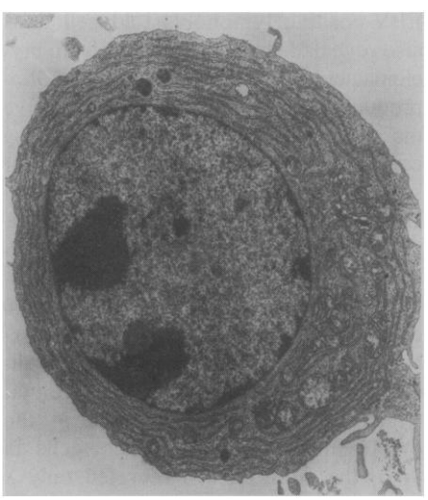

Fig. 1. Ultrastructure of a myeloma cell after 5 months in culture. The nucleus shows prominent nucleoli, and the cytoplasm contains large amounts of rough endoplasmic reticulum arranged in parallel concentric fashion (×5000).

The cells described in this report originated from a 53-year-old Caucasian male diagnosed in September 1980 as having multiple myeloma. His bone marrow aspirate revealed that 64 percent of the nucleated cells were plasma cells. Serum electrophoresis showed an immunoglobulin G (IgG) paraprotein (6.5 g/liter) with severe depression of immunoglobulin A (IgA) (0.2 g/liter) and immunoglobulin M (IgM) (0.2 g/liter). Immunoelectrophoresis of the serum showed that the paraprotein was IgG with large amounts of free λ light chains, while immunoelectrophoresis of the urine revealed only free λ light chains. Initially the patient was treated with cyclophosphamide, Adriamycin, vincristine, and prednisolone, then with melphalan, vincristine, and prednisolone. Although he responded initially to the treatment, abnormal plasma cells persisted in his bone marrow. He relapsed quickly and his condition deteriorated, with an increase in the plasma cell count in the peripheral blood of up to 70×10^9 per liter. An irreversible renal failure was the cause of death in July 1981.

Bone marrow aspirate, obtained on 3 April 1981, and peripheral blood, obtained on 5 June 1981, were used to initiate the cell culture. The suspension of bone marrow cells was placed on a Ficoll-Triosil solution and centrifuged at 2000 rev/min for 15 minutes. The interphase, which contained the white blood cells, was suspended in growth medium (RPMI 1640 supplemented with 10 percent fetal bovine serum and antibiotics) and seeded in Erlenmeyer flasks under the culture condition described for the establishment of leukemia-derived cell lines (*4*). Buffy coat was used to initiate the blood culture. For cytological and cytochemical studies, cytocentrifuge smears were prepared and stained with Leishman's stain, for morphological examination, and with the cytochemical stains used in the study of our other cultured cell lines (*4*).

Most of the cultured cells were mononuclear, but there were numerous bi- and multinucleated giant cells. In the culture derived from the peripheral blood, approximately 1 percent of the cells had more than one nucleus, and in the bone marrow, approximately 5 percent of the cells were multinucleated. Many of the multinucleated cells were more than ten times the size of mononuclear cells, and some contained nuclear fragments of various sizes and shapes. In the mononuclear cells, the nuclei appeared eccentric, and the cytoplasm was stained dark blue as in typical plasma cells. Ultrastructural examination of the cells revealed that many of the cultured cells, even after 6 months in culture, resembled normal plasma cells with a single round nucleus. The nuclei contained

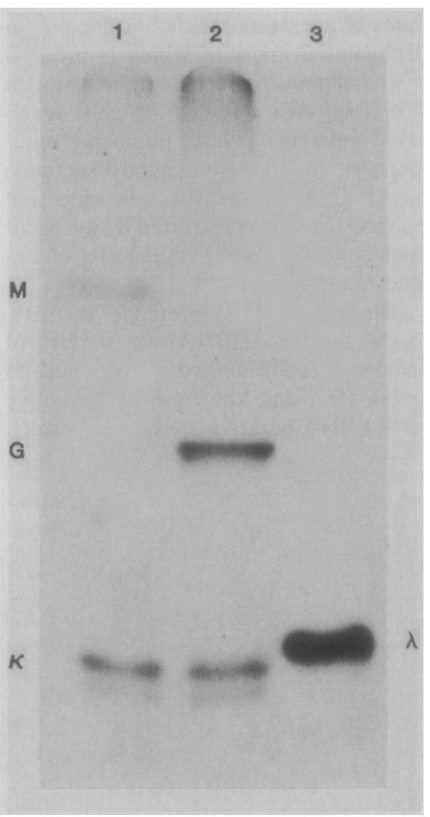

Fig. 2. Analysis of immunoglobulin secreted by the human myeloma line (Karpas 707). Cells (10^6) were incubated with lysine-free medium containing 10 percent fetal bovine serum dialyzed (against water) and [1-^{14}C]-lysine (5 μCi). Incubation continued for 18 hours at 37°C in a humidified CO_2 (5 percent) incubator. After incubation, the cell suspension was centrifuged at 1000*g* for 10 minutes. The supernatant was analyzed by sodium dodecyl sulfate–polyacrylamide (15 percent) gel electrophoresis after total reduction. For control and markers ^{14}C-labeled IgM kappa (κ) (lane 1) and IgG κ (lane 2) produced by rat hybridoma cell lines were used. Lane 3 contains the human immunoglobulin.

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Table 1. Summary of properties of the Karpas 707 human myeloma cell line. Only major changes of the karyotype are listed. Epstein-Barr viral nuclear antigen was determined by the method of Reedman and Klein (*5*).

Morphology	Plasma cell
Secreted immunoglobulin	λ light chain
Karyotype	45 XY, Ph+, 4p+, +6p, −6, −12, −16, +t(6q;7q)
Histocompatibility antigen profile	A1, A2, B8, Bw22, Cw3
EBNA	Negative
Enzymes present	Acid phosphatase
	Chloroacetate esterase

very prominent nucleoli and had the typical peripheral chromatin condensation. In the cytoplasm of all the cells examined, large amounts of rough endoplasmic reticulum (RER) were prominent (Fig. 1). The arrangement and the degree of dilation of the cisternae of the RER varied from cell to cell. In many cells, the RER was arranged in parallel or concentric fashion. In some cells, the RER has been distended to such an extent that it resembled sacs. The contents of the sacs appeared as fine granular material, most of which is presumably immunoglobulin. Sections through the Golgi area revealed a highly developed apparatus. A large number of mitochondria could be seen in most sections; many appeared irregular in size and shape, with others distorted by the accumulation of amorphous material of unknown composition.

The test for the presence of EBV nuclear antigen (EBNA) was performed according to the method of Reedman and Klein (*5*) using known EBNA-positive and EBNA-negative cells as controls. The fresh cells as well as the cells that had been growing in vitro for 5 months were EBNA-negative. The fresh cells from another nine myeloma patients were also EBNA-negative. The cultured cells were examined for the presence of mycoplasma, but the microorganism could not be isolated. Tests for the secretion of immunoglobulin revealed that the cultured cells secreted only λ light chains (Fig. 2).

Cytogenetic analysis was performed on the fresh peripheral blood cells and of the lines derived from the bone marrow and peripheral blood after 3 months in culture. Dividing cells were hypodiploid, the stem line being 45. Giemsa banding revealed similar abnormalities in the karyotypes of both fresh and cultured cells. All metaphases had a Philadelphia chromosome (Ph+) with the breakpoint at q1.1. Other consistent abnormalities included loss of one of the chromosomes 6, 12, and 16 and the presence of a marker chromosome made up of the long arm of chromosomes 6 and 7 t(6q;7q). A second small marker could represent the short arm of 6 (6q−). Additional material seemed to be present at the end of the short arm of chromosome 4 (4p+). In all metaphases, there was an abnormal banding pattern on the long arm of one chromosome 11 and, in most spreads, of one chromosome 17 (Fig. 3). The occurrence of Ph+ chromosome in human myeloma has been documented (*6*); our cell line is the first reported Ph+ myeloma cell line.

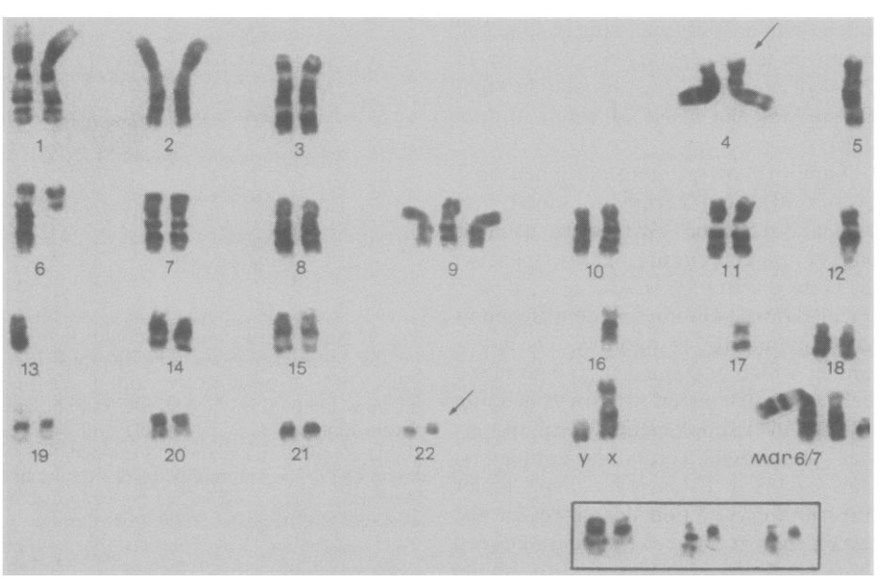

Fig. 3. Karyotype of a metaphase from the line Karpas 707. Changes present in every metaphase analyzed from fresh and cultured cells were Ph+, monosomy 6, 12, and 16, 4p+, +6p, and 11q. This karyotype also had monosomy 5, 13, and 17 and trisomy 9. Two chromosomes to the right of marker 6/7 could not be identified. (Inset) Higher magnification from three metaphases showing chromosome 22 with Ph+.

During the past few years a large number of human immunoglobulin-producing cell lines have been established. These lines have been derived from the peripheral blood, lymph nodes, or bone marrow of patients with hematological malignancies, as well as from normal individuals. A study of the various cell lines revealed that most of the immunoglobulin-producing lines are lymphoblasts that are infected with EBV. Normal hemic cells and leukemic cells, whether lymphoid or myeloid, are EBV-negative, but since the majority of the population have had an infection with EBV, most if not all individuals affected by this virus remain latent carriers of the EBV in some of their B lymphoblasts. Therefore even a few EBV-positive cells are often sufficient to outgrow leukemic or normal hemic cells in vitro. Like the leukemia-derived EBV-positive lymphoblasts growing in vitro, normal B cells can be immortalized in vitro, but only after they are infected with EBV (*7*). Such cells also produce immunoglobulin. A study of some of our lymphoblastoid cell lines for immunoglobulin expression and synthesis revealed that production of immunoglobulin by lymphoblastoid cells was many times lower than that by myeloma cells (*8*). Ultrastructural studies of EBV-positive lymphoblastoid cell lines also revealed sharp differences from myeloma cultures. The immunoglobulin-producing lymphoblasts had a primitive morphological appearance, displaying considerable pleomorphism in nuclear size and shape and in cytoplasmic organelles (*4*).

In contrast, the newly established myeloma cell line resembles plasma cells. Ultrathin sections from more than 100 cells were examined, and, without exception, the cytoplasm of all had a well-developed RER and Golgi apparatus, which together occupied most of the cytoplasm. Like the myeloma cells in vivo, the cultured cell secreted only λ light chains (Fig. 2). Analysis of the secretion of immunoglobulin revealed that our human cell line produces at least as much light chain as is produced by the rat and mouse (X63) plasmacytoma cell lines. This was shown by using as mark-

er the incorporation of ^{14}C-labeled amino acid lysine into newly synthesized immunoglobulin. Since the murine lines secrete 10 to 50 μg of immunoglobulin per milliliter (*9*), it is reasonable to assume that our human myeloma cells secrete similar quantities (*10*).

An intensive effort has been made to develop human myeloma cell lines for the production of monoclonal antibodies. Although the availability of mouse myeloma cell lines enabled the development of monoclonal antibodies against a wide range of antigens, the use of rodent antibodies for human immunotherapy is limited. It is probably impossible to obtain stable interspecies hybridomas, such as mouse-human, that secrete human antibodies, because the human chromosomes are lost in such hybridomas (*11*).

One group of investigators reported the development of human monoclonal antibodies (to dinitrochlorobenzene) using the IgE λ myeloma cell line U266 (*12*); the failure to reproduce these results has been blamed on a latent infection of these myeloma cells by mycoplasma (*13*). Another group reported the production of human monoclonal antibodies by use of the GM1500 line, which had been derived from a patient with multiple myeloma (*14*); these results have not been reproduced, probably because the cell line, rather than being a myeloma, is a lymphoblastoid line that is EBNA-positive (*15*). Like other EBNA-positive B lymphoblast cell lines, they probably produce only small amounts of immunoglobulin. Furthermore because of their latent infection with EBV, such lines may be unstable. Therefore, it appears that so far a human counterpart to the mouse and rat (*16*) myeloma cell lines does not exist. Cells of our unique line should provide an opportunity for detailed study of the cytogenetics of myeloma, as well as an ideal fusion partner for the production of human monoclonal antibodies.

ABRAHAM KARPAS
Department of Haematological Medicine, Cambridge University Clinical School, Cambridge CB2 2QL, United Kingdom

PATRICIA FISCHER
Department of Haematological Medicine, Cambridge University Clinical School, Cambridge CB2 2QL, United Kingdom, and *Cancer Institute, University of Vienna, Vienna, Austria*

DAVID SWIRSKY
Department of Haematological Medicine, Cambridge University Clinical School, Cambridge CB2 2QL, United Kingdom

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Ribosomal Crystalline Arrays of Large Subunits from *Escherichia coli*

Abstract. *Crystalline sheets of the 50S ribosomal subunits of* Escherichia coli *have been formed in vitro. Electron micrographs of these arrays diffract to 35-angstrom resolution. The lattice parameters of the crystals are* a = *330 ± 20 angstroms,* b = *330 ± 30 angstroms, and* α = *123° ± 5°, and the space group is most likely p21. These arrays of ribosomal subunits are sufficiently ordered to resolve such known features of the large ribosomal subunit as the L7/L12 stalk and the central protuberance.*

One of the principal requirements for understanding the molecular events that occur during protein synthesis is the determination of the three-dimensional structure of the ribosome. In this report, we describe conditions for the growth in vitro of crystalline arrays of large ribosomal subunits from *Escherichia coli.* These microcrystals of ribosomes are sufficiently well ordered to allow the calculation of a density map with 40-Å resolution by three-dimensional reconstruction from electron micrographs. Crystalline arrays formed in vivo in eukaryotes have been isolated and analyzed by three-dimensional reconstruction (*1, 2*) and crystalline arrays of prokaryotic ribosomes have been grown in vitro (*3–5*). However, the microcrystals we grew diffract to a considerably higher resolution than any previously reported. Their symmetry and unit cell dimensions have been characterized. Since they are grown in vitro from *E. coli* subunits, they offer the optimal opportunities for correlating biochemical and functional information with structural studies. The arrays are nearly ideal for analysis by three-dimensional reconstruction.

When solutions of 50*S* ribosomal subunits are maintained in vapor diffusion wells in the presence of ethanol (see legend to Fig. 1 for buffer conditions), a particulate suspension forms at the bottom of the wells within 2 weeks. After 1 to 2 months, the suspension, when examined by electron microscopy, contains closed tubes (Fig. 1A) and crystalline sheets one or more layers thick (Fig. 1B). Optical and computer-calculated diffraction patterns indicate that the unit cell dimensions, symmetry, and packing are the same in the sheets and the tubes.

Tubes, when negatively stained, flatten on the carbon support films used for electron microscopy to produce "two-sided" images [for example, see (*6*)]. The flattened tubes are 1750 Å in diameter and of variable length (4000 to 40,000 Å). In the approximately meridionally symmetric optical diffraction pattern shown in Fig. 1C, reflections from one side of the tube have been circled. These reflections index on a primitive lattice with parameters $a = 330 \pm 20$ Å, $b = 330 \pm 30$ Å, and $\alpha = 123° \pm 5°$. Optical and computer-filtered images of tubes (Fig. 1E) suggest that the symmetry, in projection, is p2, corresponding to space group p21 (*7*). The projected structure can also be referred to a centered lattice with approximate symmetry cmm corresponding to space group c222. Analysis of the calculated three-dimensional Fourier transform, however, favors a three-dimensional symmetry of p21, but does not completely exclude c222 (Fig. 2). In addition, by using the lower symmetry space group (p21), we do not rule out the additional symmetry elements contained in c222.

Present in the backgrounds of electron

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