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ESTABLISHED IMMUNOGLOBULIN PRODUCING MYELOMA (IgE) AND LYMPHOBLASTOID (IgG) CELL LINES FROM AN IgE MYELOMA PATIENT

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SUMMARY

Two IgEL and one IgGK producing cell lines have been established *in vitro* from peripheral blood and bone marrow of an E myeloma patient. Morphologic and immunologic studies indicate that cells of the IgE producing lines are derived from the same clone of myeloma cells which grew *in vivo*. They have remained essentially unaltered with a stable near-diploid karyotype and continuous production of intact IgE molecules during 18 months in culture. The IgGK producing line has all the characteristics ascribed to permanent lymphoblastoid lines and is considered to be of non-malignant lymphoid origin. The process of establishment is described and it is suggested that lymphoblastoid cells have a selective advantage over myeloma cells. Permanent myeloma cell lines can therefore probably be obtained only if cells capable of forming established lymphoblastoid lines are very few or absent in the original biopsy. The establishment of a permanent cell line continuously producing intact molecules of IgE should permit further studies on the biological role of this class of immunoglobulins.

INTRODUCTION

During the last few years a large number of human immunoglobulin producing cell lines with a lymphoblastoid morphology have been established in different laboratories (for review see Pontén, 1970). Such lines have been derived from normal individuals as well as from patients with different haematopoietic and lymphatic malignancies including multiple myeloma but so far no proofs have been presented that established lymphoblastoid cell lines, regardless of origin, represent cells malignant *in vivo*. It seems likely that lymphoblastoid cell lines can be obtained from almost any adult individual provided that suitable biopsy material, e.g. lymph nodes is grown in an organotypic tissue culture (Nilsson, 1970a).

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In contrast to the relative ease by which lymphoblastoid lines can be established from human lymphoid tissue or peripheral blood only one permanent myeloma cell line of human origin has been reported (Matsuoka *et al.*, 1967). Cells of that line (RPMI no 8226) produce λ-light chains and have a plasmablastoid morphology (Moore & Kitamura, 1968 and Matsuoka *et al.*, 1968). An IgGL myeloma protein was found in the patient's serum and his urine contained a Bence-Jones protein of λ-type. Evidence for the identity between the light chains produced *in vitro* and the Bence-Jones protein type L in the donor's urine was given recently (Matsuoka *et al.*, 1969). Since the myeloma line never produced G heavy chains *in vitro* it was suggested that cells of the RPMI no. 8226 represented a myeloma cell that *in vivo* produced only Bence-Jones protein or that the cells had lost their ability to produce heavy chains during the establishment *in vitro*. We now report successful establishment *in vitro* of one IgGK and two IgEL producing cell lines from peripheral blood and bone marrow of an E myeloma patient. The identity between the IgEL myeloma protein and the immunoglobulin synthesized *in vitro* shows that cells of two permanent lines are the progeny of the donor's malignant cells. These myeloma lines have several morphological and other characteristics which differ from those seen in the IgGK producing lymphoblastoid line derived from non-myeloma cells of the same patient.

MATERIAL AND METHODS

Case history

Peripheral blood samples and several biopsies were obtained from a 53-year-old man (ND). He was hospitalized in 1965 because of suspect myeloma. By the investigations of Johansson & Bennich (1967 and 1968*a*) it was shown that the myeloma protein represented a new immunoglobulin class, IgND, later termed IgE. The myeloma protein was found to represent a complete immunoglobulin (ε_2 λ_2). The patient was intermittently treated with cyclophosphamide, steroids and mercaptopurine. He died in July 1968. The clinical data are given in detail by Killander, Johansson & Bennich (1970).

Culture method

(a) *Grid cultures.* The grid organ culture for establishing lymphoblastoid cell lines described elsewhere (Nilsson, 1970b) was employed. A bone marrow (255 Bm) and a lymph node biopsy (264 C) were brought to the laboratory in sterile tissue culture medium and explanted within 20 min, the bone marrow to four and the lymph node to three grid cultures. Heparinized blood samples (262 Bl and 266 Bl) in test tubes were placed in a vertical position for $1\frac{1}{2}$ hr at room temperature and the erythrocytes were allowed to sediment. The plasma, containing the leucocytes, was then sucked off and centrifuged at 1000 rev/min for 10 min. The leucocytes were resuspended in a small volume of medium and gently pipetted into the gelatin sponge (Spongostan, Ferrosan, Malmö, Sweden) of the grid culture. Leucocytes from 10 ml of blood sufficed for four grid cultures (262 Bl). For 266 Bl a somewhat different technique was used to explant part of the leucocytes from 15 ml blood. Three grid cultures were prepared from half of the resuspended leucocyte pellet as described for 262 Bl. To the remaining leucocytes $1{\cdot}5 \times 10^6$ allogeneic adult skin fibroblasts were added and from that mixture three additional grid cultures were prepared.

Within 3 hr after the patient's death, spleen (268 Sp) and bone marrow specimens (268 Bm) were removed and explanted *in vitro*. Four 268 Sp and four 268 Bm cultures were initiated.

The culture medium, changed twice a week, was Nutrient medium F-10 (Grand Island Biol. Comp., New York, USA) supplemented with post-natal calf serum (10%), penicillin (100 IE ml), streptomycin (50 μg/ml) and amphotericin B (1·25 μg/ml).

(b) *Maintenance of permanent lines.* Myeloma (266 Bl and 268 Bm) or lymphoblastoid (255 Bm) cells were harvested from the original grid cultures and transferred to 50 ml or 100 ml Erlenmeyer flasks containing 30 and 50 ml medium, respectively. 266 Bl and 268 Bm cells were seeded on top of a feeder layer of allogeneic adult skin fibroblasts while 255 Bm grew in non-stirred suspension without feeder cells. 30% of the medium was changed twice weekly. Original grid cultures were replenished by adding new Spongostan pieces every 1–2 months.

Morphologic observations

Grid cultures were observed twice a week through an inverted Leitz microscope. Cultures or stained cells were photographed by a Leitz Orthomat automatic camera attached to the inverted microscope or to Leitz Orthoplan microscope. Cell smears or mixed fibroblast–plasmablast monolayers were fixed in methanol for 5 min and stained in Giemsa's solution (diluted 1/20) for 10–20 min.

Cell counts

A celloscope (Celloscope 502, Linson Instruments, Stockholm) was used for counting cells. Viability was estimated by the trypan blue exclusion method.

Freezing procedure

Cells of established lines 255 Bm, 266 Bl and 268 Bm were frozen in F-10 medium supplemented with DMSO (20%) and human AB serum (30%) and stored in liquid nitrogen. When fibroblasts were present, they were trypsinized and frozen together with the established cells.

Immunological techniques

Supernatants from the different cultures were concentrated ten-fold and the immunoglobulins produced were qualitatively identified by immunodiffusion in 0·8% agar layers on microscope slides. For further analysis of the IgE synthesized by the myeloma cells gel diffusion according to Ouchterlony using 1·5% agarose in 0·3 M phosphate buffer pH 8·0 as described (Johansson, Högman & Killander, 1968b) was made after a ten-fold concentration of the supernatant by ultrafiltration in Collodion bags (Membran Gesellschaft, Göttingen, West Germany).

Rabbit antisera to Fc- and $F(ab')_2$-fragments of protein ND were used in the gel diffusion analyses. Anti-FcND was absorbed with excess of FabND-fragments and anti-$F(ab')_2$ ND was absorbed with excess of F(ab)ND and FcND-fragments. Both antisera were found to be specific in immunoelectrophoresis and gel diffusion.

The IgE concentrations were determined in unconcentrated supernatants by the radioimmunosorbent test (RIST) as described by Johansson, Bennich & Wide (1968a). By this method it is possible to estimate IgE levels down to 1–5 ng/ml.

E myeloma protein ND was used as the standard and some studies have indicated that the IgE levels given are somewhat low (Johansson & Bennich, unpublished observation). When isolated E proteins from other sources were used a level of approximately double the level given was obtained. The problem of standardization of the IgE levels is under investigation under the supervision of WHO International Reference Centre for Immunoglobulins. The error of estimation, calculated as the standard deviations from the mean value at duplicate analyses, was 10–15%.

The *in vitro* synthesized IgE was compared to E myeloma protein ND by gel filtration on a calibrated column of Sephadex G-150 (3·2 × 92·5 cm) in 0·1 M Tris-HCl, pH 7·7 containing 0·2 M sodium chloride. Three separate experiments were made.

The isolated IgE (ND) as well as the different proteolytic fragments of IgE (ND) used in this study have been described elsewhere (Bennich & Johansson, 1970).

RESULTS

The results of long term cultures at different biopsies are summarized in Table 1. Three lines were established.

Morphology and growth characteristics

255 *Bm*. After having released degenerating bone marrow cells for 2–3 weeks all four original grid cultures started to produce clumps of blastoid cells with the same morphologic characteristics as has been described for established lymphoblastoid cells (Moore, Kitamura & Toshima, 1968). Two of the grid cultures were transferred to allogeneic skin fibroblasts and as soon as the lymphoblasts had attached to these cells a second generation of grid cultures could be prepared from the lymphoblast–fibroblast mixture as has been described before (Nilsson, Pontén & Philipson, 1968).

The original explants eventually produced fibroblastoid cells which attached to the Petri dish bottom. The lymphoblasts, produced simultaneously, attached firmly in clumps on top of or as single cells underneath the fibroblasts (Fig. 1).

The typical morphology of individual 255 Bm cells can be seen in Fig. 2. Cells of line 255 Bm grew well in Erlenmeyer flasks without feeder cells. The doubling time for such cultures was 30–48 hr. Under optimal conditions cell viability was 80–85%. Frozen cells could be revived with a viability of 50%.

262 *Bl*. The grids produced degenerating cells for 3–4 weeks. No lymphoblastoid cells or fibroblasts appeared.

264 *C*. Only few lymphoid cells which disappeared after 3 weeks were observed in cultures of this small lymph node. Fibroblasts showed up in the second week. No cell line was established.

266 *Bl*. Grid cultures produced large numbers of small, round cells for 2 weeks. The number of cells, dropping down from the grids, gradually declined but after 4 months of cultivation of second phase of round cell production occurred in two of the three grid cultures originally supplied with fibroblast. The cultures consisting exclusively of leucocytes failed to become established.

The cells thrived excellently in the gelatin sponge (Fig. 3). One, 21 months old, original grid is still in culture.

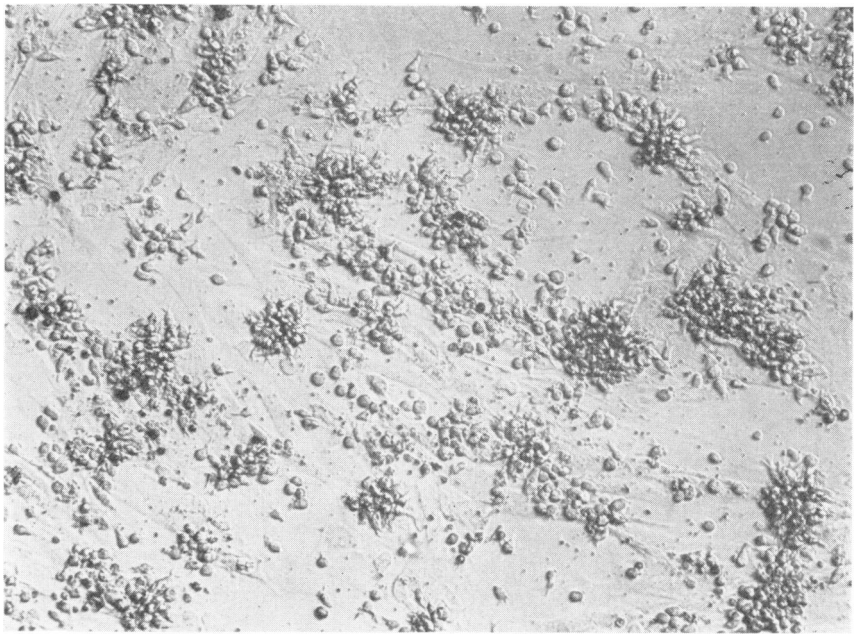

FIG. 1. 255 Bm lymphoblastoid cells. Typical clumping on allogeneic skin fibroblasts. × 110. Inverted microscope.

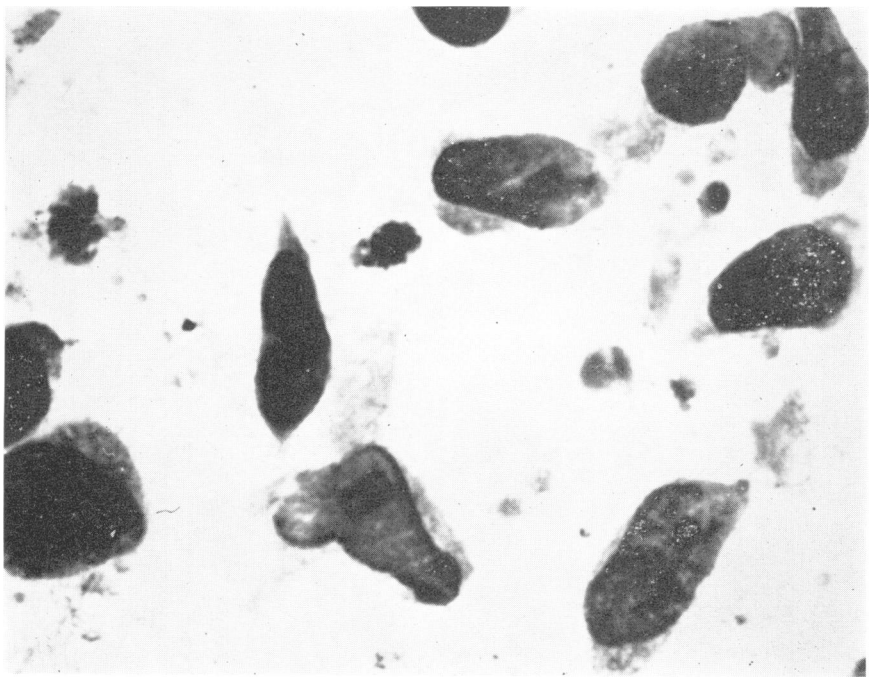

FIG. 2. 255 Bm lymphoblastoid cells. × 1300. Giemsa.

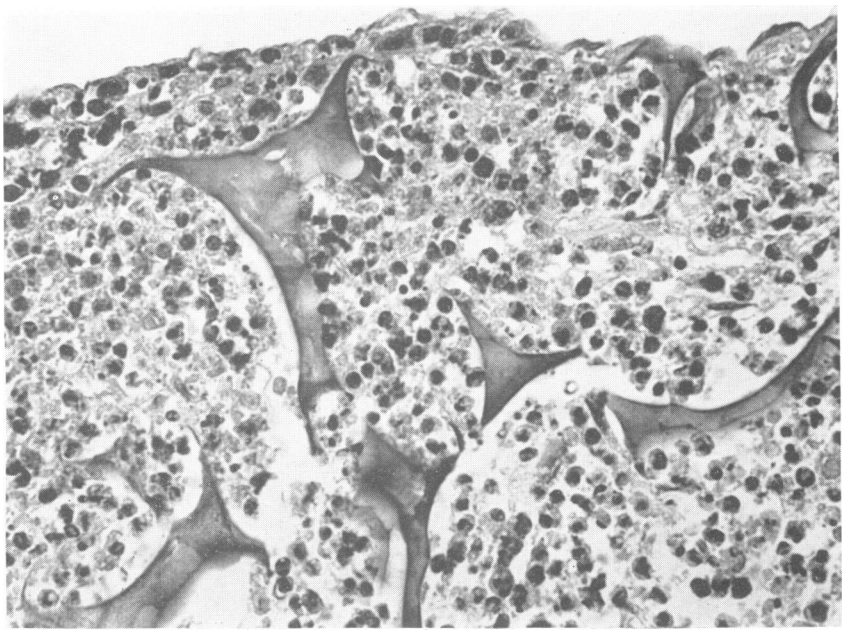

FIG. 3. Histological section of a one year old original Spongostan grid. Between the gelatin structures are found masses of myeloma cells, some of them dead. × 280. Haematoxylin-eosin.

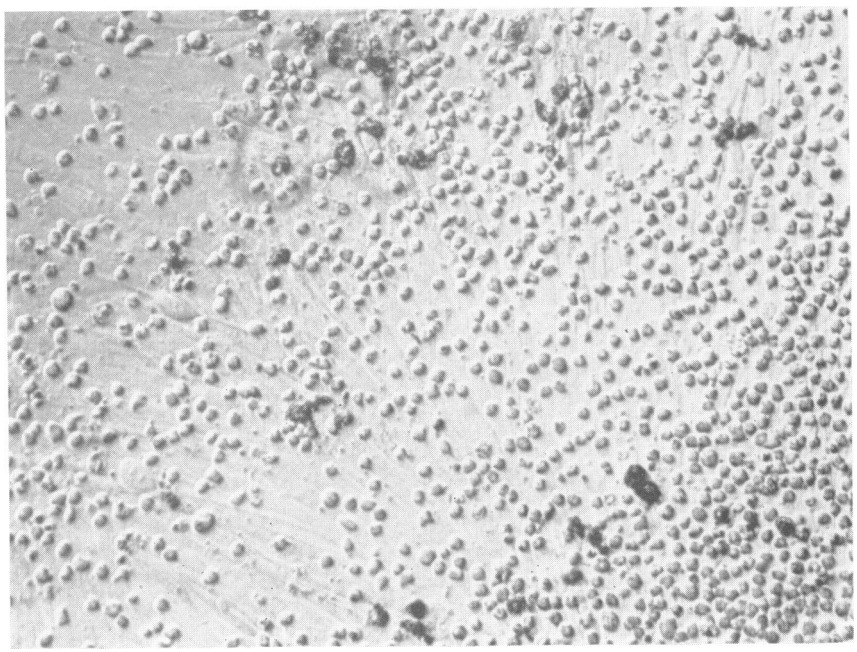

FIG. 4. 266 Bl Myeloma cells on top of a skin fibroblast monolayer. Note absence of clumping. Compare with Fig. 5. × 130. Inverted microscope.

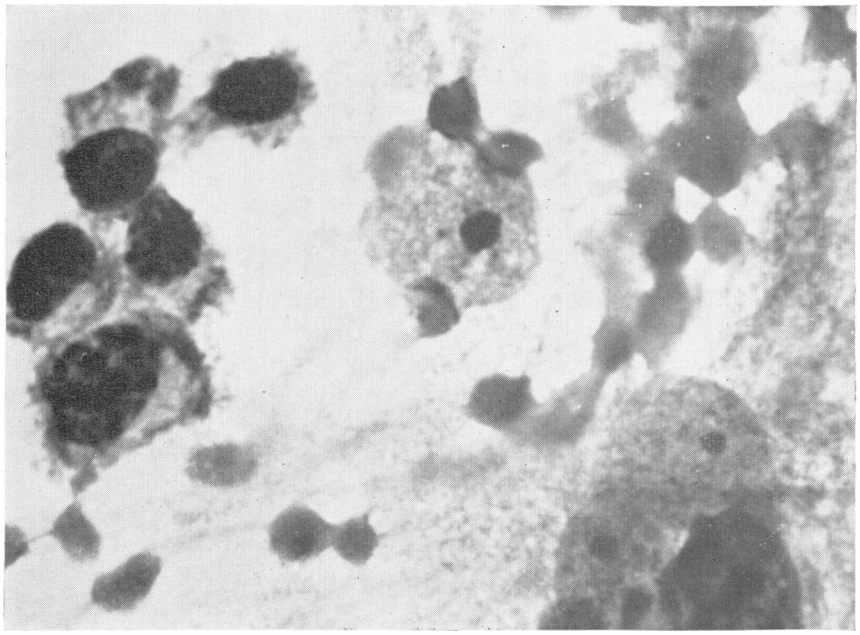

FIG. 5. 266 Bl myeloma cells with plasma cell morphology on a feeder layer of skin fibroblasts. Nuclear reminiscents of dead myeloma cells are located between the fibroblast nuclei. × 1480. Giemsa.

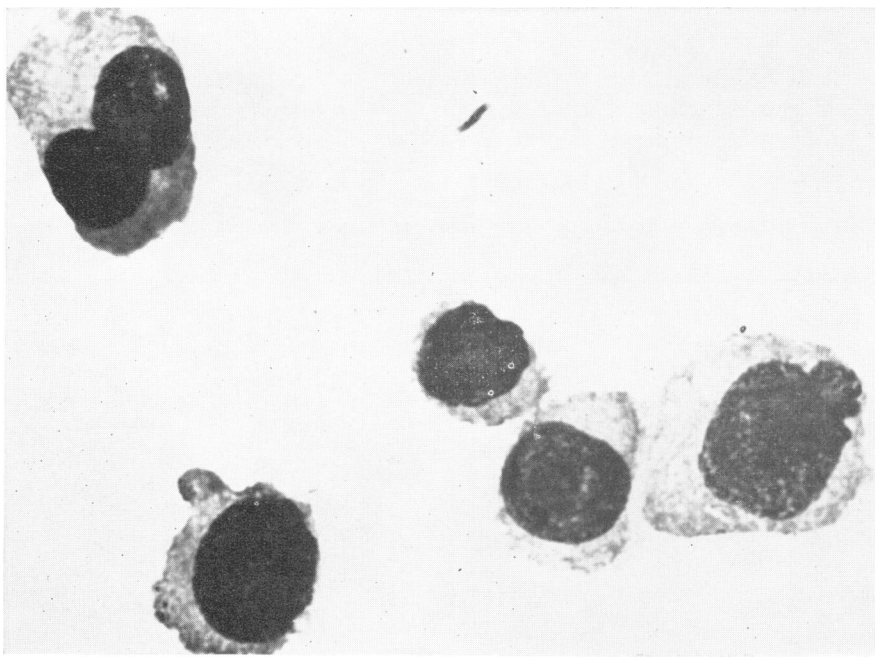

FIG. 6. 266 Bl myeloma cells with immature morphology. × 1300. Giemsa.

The second crop referred to as myeloma cells differed morphologically from lymphoblastoid cells. No cell clumps and no pear-shaped cells were recognized. The cells attached poorly to the feeder layer and did not move around the fibroblast surfaces with thin membrane projections typical of lymphoblasts or fibroblasts (peripolesis) (Fig. 4).

In higher magnifications cells resembling mature plasma cells (Fig. 5) as well as immature blast cells (Fig. 6) were seen. The mature cell type has a peripherally located nucleus with clumped chromatin and not demonstrable or indistinct nucleoli. The cytoplasm is strongly basophilic and has a juxtanuclear clear area. The immature cell is larger and in approximately 1% bi- or multinucleated. The nucleus with its finely granular chromatin is centrally placed. Distinct nucleoli can be observed in some of the cells. The nucleocytoplasmic ratio is higher than for the mature cell type.

266 Bl cells have been maintained in long term culture by the grid culture method (one original grid is still producing viable myeloma cells) or in Erlenmeyer flasks only in the presence of feeder fibroblasts, preferentially slow growing ones. The doubling time was 7–10 days with a pronounced cell death among the myeloma cells. Thawed myeloma cells showed even better recovery than lymphoblastoid cells.

268 *Sp.* Cultures produced lympoid cells, myeloma cells and macrophages for 4 weeks and stroma fibroblasts from day 10. No cell line was established.

268 *Bm.* These bone marrow explants resembled 255 Bm during the first 2–3 weeks. Later no lymphoblastoid cells but fibroblasts appeared. After 3 months, myeloma cells with the same characteristics as 266 Bl were produced.

At present (April 1970) three lines, 255 Bm, with a lymphoblastoid morphology, 266 Bl and 268 Bm with characteristics of myeloma cells have been in culture for 21, 18 and 17 months, respectively.

Immunological studies

By immuno-diffusion in agar IgE was identified in the supernatants from 255 Bm for 2 months after explantation. Later tests were negative for this class of immunoglobulins. IgGK, also identified from the start, is, in contrast, still being produced (Table 1).

TABLE 1. Derivation of cell lines from lymphoid tissue and peripheral blood of an IgE myeloma patient (N.D.)

Designation of biopsy	Established cell line	Morphology of established cell line	Type of immunoglobulin produced
255 Bm	yes	lymphoblastoid	IgGK*
262 Bl	no	—	—
264 C	no	—	—
266 Bl	yes	mainly plasmablastoid	IgEL
268 Bm	yes	mainly plasmablastoid	IgEL
268 Sp	no	—	—

*Produced IgEL during the first 2 months after explantation.

TABLE 2. Quantitative estimation of *in vitro* synthesized IgE/grid culture (ng/ml) during the establishment

Date	Biopsy No.			Medium + 10% calf serum
	255 Bm	266 Bl	268 Bm	
4 May 1968	1325			1–5
9 July 1968	135			1–5
6 Aug. 1968	205	15800		1–5
20 Aug. 1968	16;15			1–5
23 Sept. 1968	24;22			1–5
29 Nov. 1968			295	1–5
2 Dec. 1968		190		1–5
14 Feb. 1969		1550	1900	1–5
3 Mar. 1969	1·4			

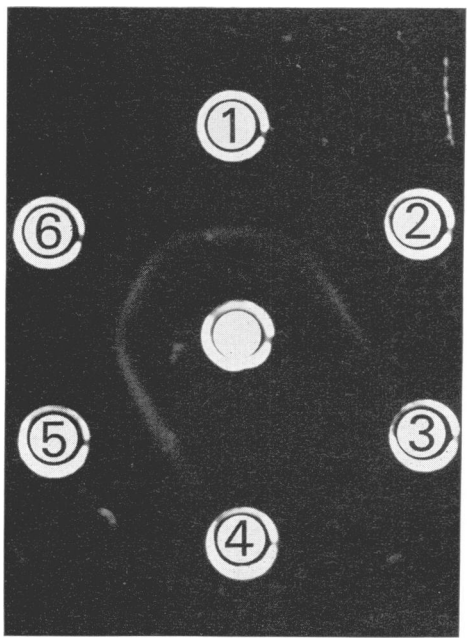

FIG. 7. Gel diffusion analyses of IgE synthesized by cell line 266 Bl (concentrated supernatant) (1); IgE isolated by immunosorbent from plasma of an allergic patient (2); Bence-Jones protein, isolated from patient ND, 0·1 mg/ml (3); Fab-fragments from IgE (ND) 1·0 mg/ml (4); $F(ab')_2$-fragment from IgE (ND) 0·1 mg/ml (5); E myeloma protein ND 1·0 mg/ml (6). The antiserum in the centre well is a specific rabbit anti-Fc(ND) absorbed with 0·1 mg Fab (ND) per ml of antiserum.

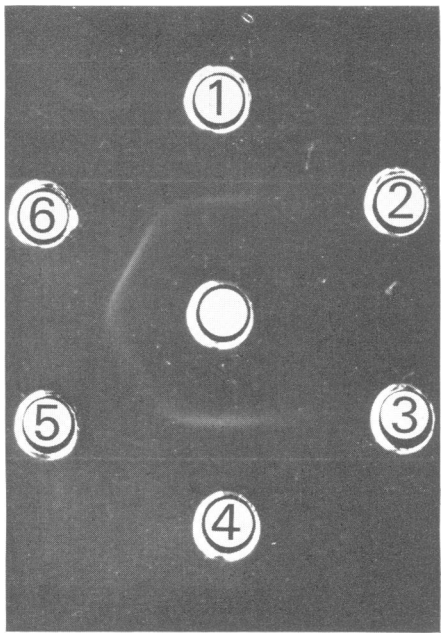

FIG. 8. Gel diffusion analyses of IgE synthesized by cell line 266 Bl (concentrated supernatant) (1); IgE from an allergic individual (2); Bence-Jones protein (ND) 0·1 mg/ml (3); $F(ab')_2$(ND) 0·5 mg/ml (4); Fab (ND) 0·1 mg/ml (5); IgE (ND) 0·5 mg/ml (6). The antiserum in the centre well is a rabbit anti-$F(ab')_2$ND absorbed with 0·1 mg Bence-Jones lambda ND and 0·1 mg of Fc(ND) per ml antiserum.

In supernatants of 266 Bl and 268 Bm IgEL was analysed only when production of myeloma cells had started in the cultures, 4 and 3 months after explantation, respectively.

The RIST determination of IgE in the culture supernatants at different times are given in Table 2. Initially the concentrations were high. They later went down but again increased in lines 266 Bl and 268 Bm.

To quantitate the amount of IgE produced by 266 Bl myeloma cells five experiments were made. In each $1{\cdot}5 \times 10^6$ viable cells were incubated in 30 ml medium in a 50 ml Erlenmeyer flask. The IgE concentrations in the supernatant at the start and after 48 hr were determined by the RIST. The production was found to be 2–7 μg of IgE per 10^6 viable cells per 48 hr.

In gel diffusion the synthesized IgE was found to carry the E class determinants (Fig. 7). A precipitin line of identity was obtained between E protein ND and IgE from allergic sera with high levels of reagins by using an anti IgE (FcND). When an antiserum specific for the individual (clone)—specific antigenic determinants of E protein ND was used, no reaction was obtained with IgE from allergic sera. A precipitin line of identity was, however, found between the synthesized IgE and E protein ND (Fig. 8). Thus, the gel diffusion data show that the synthesized IgE carries both the IgE class determinants and the individual antigenic determinants of E protein ND.

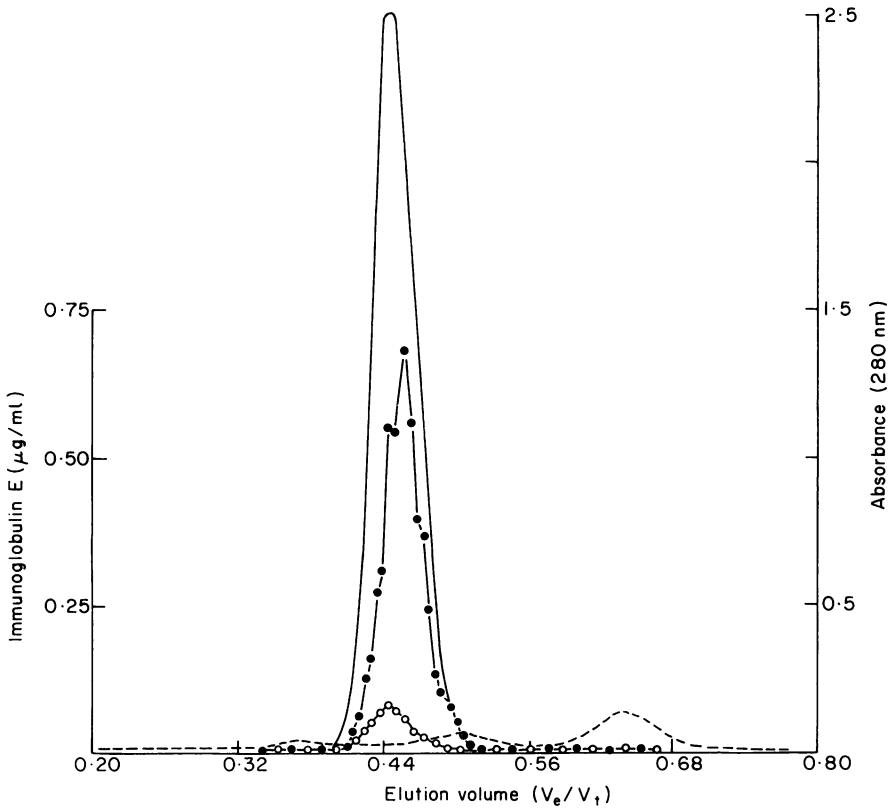

FIG. 9. Gel filtration patterns of IgE synthesized *in vitro* and E myeloma protein ND. Results of three separate experiments on a calibrated column of Sephadex G-150 (3·2 × 92·5 cm) in 0·1 M Tris-HCl, pH 7·7 containing 0·2 M sodium chloride. ○ = IgE synthesized by 266 Bl cells cultured in medium supplemented by 10% calf serum. ● = IgE synthesized by cell line 266 Bl cultured in serum free medium. Quantitative determinations of IgE were made by a radioimmunosorbent tests (RIST) as described (Johansson *et al.*, 1968). ——— = Purified E myeloma protein ND; absorbency 280 nm. - - - = Calf serum; absorbency 280 nm.

DISCUSSION

Most if not all lines established from human lymphoid tissue produce immunoglobulins *in vitro*. These are often oligo- or monoclonal and the immunoglobulin producing cells therefore superficially resemble myeloma cells. Proof of an origin from a myeloma cell population requires that an identity can be established between the myeloma protein produced *in vivo* and the immunoglobulin synthesized *in vitro*. This has only recently been accomplished in a single line described by Matsuoka *et al.* (1967), Moore & Kitamura (1968) and Matsuoka *et al.* (1968 and 1969), which, however, produced only light chains identical to the Bence-Jones protein found in the patient's urine. No heavy chain was ever produced by that line. Repeated analyses of its chromosomes revealed the unstable character of these myeloma cells (Huang, Imamura & Moore, 1969). The chromosome pattern changed from

a bimodal distribution with peaks in the hypodiploid and the hyperdiploid regions to a modal number in the triploid region with considerable mixoploidy. The IgEL producing cell lines described here (266 Bl and 268 Bm) can be considered true myeloma cell lines since they produce an immunoglobulin with antigenic determinants in its Fab-region inseparable by immunodiffusion from those of myeloma protein ND. The elution pattern on a Sephadex G-150 column revealed a complete IgE molecule identical to that of IgE (ND) and our 266 Bl and 268 Bm therefore seem to be the first human myeloma lines cultivated *in vitro* which produce intact immunoglobulins. The morphology, growth characteristics, rate of IgE synthesis and the near diploid chromosome pattern (Nilsson, unpublished observation) of the cells have remained unchanged throughout the 18 months *in vitro*. Because of this

TABLE 3. Clinical status of patient ND on different biopsy occasions

Biopsy No.	Date of biopsy (1968)	Clinical data: General condition	Total No. of leucocytes/mm³ of blood	Total No. of myeloma cells/ mm³ of blood	IgE (N.D.) conc. in serum	Treatment
255 Bm	5 May	Remission	5,300	No detectable	1/g/100 ml	None
262 Bl	17 July	Relapse	20,000	16,000	Approx. 4 g/ 100 ml	Mercaptopurin 50 mg × 3 Prednisolone 20 mg × 3
264 C	23 July	Relapse	73,000	69,000	Approx. 4 g/ 100 ml	
266 Bl	24 July	Moribund	110,000	105,000	Approx. 4 g/ 100 ml	
268 Bm	26 July	Dead	151,000 (25 July)	148,000 (25 July)	Approx. 4 g/ 100 ml	—
268 Sp	26 July	Dead				

stability the cell lines would lend themselves to studies of multiple myeloma *in vitro*. From the patient ND a third line was also established, which produced apparently monoclonal IgGK. A corresponding M-component was not detected in the patients' serum. The IgGK line was morphologically similar to all other lymphoblastoid lines established in our laboratory including several examples from normal individuals. The morphology was distinct from the plasmablastoid aspect of the IgEL producing line. It is therefore concluded that lymphoblastoid lines, although they may only produce a single species of immunoglobulin, are not equivalent to myeloma cells. The biological significance of the autonomously multiplying monoclonal lymphoblastoid cell lines remains unknown.

Table 3 shows that patient ND was in remission and had no circulating myeloma cells when the biopsy which produced lymphoblasts *in vitro* (255 Bm) was aspirated. When peripheral blood, 266 Bl and bone marrow 268 Bm was explanted *in vitro* almost all peripheral leucocytes were found to be myeloma cells. It seems that, if at the time of explanation the biopsy material contains lymphoblasts, even in small numbers, these cells and not the myeloma cells have the best chance to become predominant. The production *in vitro* for 2 months of both IgG and IgE in 255 Bm and unpublished experiments *in vitro* where myeloma

cells were mixed with lymphoblasts are compatible with that assumption. In these instances myeloma cells eventually disappeared even if feeder cells were present. Myeloma cells seem to be handicapped by their slow growth rate and their need for feeder cells. The successful establishment of lymphoblastoid cell lines and no myeloma lines from the bone marrows of two of three other myeloma patients supports the relative difficulty entailed in the establishment of myeloma cells as permanent lines (Nilsson, unpublished results).

The quantitative estimation that the myeloma cells produced 2–7 of IgE $\mu g/10^6$ viable cells/48 hr corresponds to a rapid synthesis of immunoglobulin.

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REFERENCES

BENNICH, H. & JOHANSSON, S.G.O. (1971) Structure and biological function of human IgE. *Advanc. Immunol.* **13.** In press.

HUANG, C.C., IMAMURA, T. & MOORE, G.E. (1969) Chromosomes and cloning efficiencies of hematopoietic cell lines derived from patients with leukemia, melanoma, myeloma and Burkitt lymphoma. *J. Nat. Cancer Inst.* **43,** 1129.

JOHANSSON, S.G.O. & BENNICH, H. (1967) Immunological studies of an atypical (myeloma) immunoglobulin. *Immunology*, **13,** 381.

JOHANSSON, S.G.O., BENNICH, H. & WIDE, L. (1968a) A new class of immunoglobulin in human serum. *Immunology*, **14,** 265.

JOHANSSON, S.G.O., HÖGMAN, C. & KILLANDER, J. (1968b) Quantitative immunoglobulin determination. *Acta path. microbiol. scand.* **74,** 519.

KILLANDER, A., JOHANSSON, S.G.O. & BENNICH, H. (1970) Manuscript. To be published.

MATSUOKA, Y., MOORE, G.E., YAGI, Y. & PRESSMAN, D. (1967) Production of free light chains of immunoglobulin by a hematopoietic cell line derived from a patient with multiple myeloma. *Proc. Soc. exp. Biol. (N.Y.).* **125,** 1246.

MATSUOKA, Y., TAKAHASHI, M., YAGI, Y., MOORE, C.E. & PRESSMAN, D. (1968) Synthesis and secretion of immunoglobulins by established cell lines of human haematopoietic origin. *J. Immunol.* **101,** 1111.

MATSUOKA, Y., YAGI, Y., MOORE, G.E. & PRESSMAN, D. (1969) Isolation and characterization of a free λ chain of immunoglobulin produced by an established cell line of human myeloma cell origin. 1. λ chain in culture medium. *J. Immunol.* **102,** 1136.

MOORE, G.E. & KITAMURA, H. (1968) Cell line derived from patient with myeloma. *N.Y. State J. Med.* **68,** 2054.

MOORE, G.E., KITAMURA, H. & TOSHIMA, S. (1968) Morphology of cultured haematopoietic cells. *Cancer*, **22,** 245.

NILSSON, K., PONTÉN, J. & PHILIPSON, L. (1968) Development of immunocytes and immunoglobulin production in long-term cultures from normal and malignant human. *Int. J. Cancer*, **3,** 183.

NILLSON, K. (1970a) High frequency establishment of immunoglobulin producing lymphoblastoid cell lines from normal and malignant lymphoid tissue and peripheral blood. To be published.

NILSSON, K. (1970b). Histological changes in long term explants of human lymph nodes during lymphoblastoid transformation. To be published.

PONTÉN, J. (1970) Spontaneous and virus induced transformation in cell culture. *Handbook of Virus Research*, Springer Verlag, Wien-New York.