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Propagation and Characterization of a Rat Myeloma Cell Line Producing Immunoglobulin E *in Vitro* (40418)

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Mouse myeloma cell cultures have been found to be useful models for the study of immunoglobulin G, M and A synthesis, assembly, and secretion (1–4). Studies of myeloma-myeloma and myeloma-B-cell hybrids and their subclones have provided considerable information about the genetics and regulation of immunoglobulin production (5–6). Similar investigations concerning immunoglobulin E (IgE) have not been possible due to the unavailability of mouse IgE-myelomas and their cell lines. However, the spontaneous myeloma model in Louvain/Wsl rats, developed by H. Bazin and his colleagues (7, 8) does offer a ready source of IgE-producing myelomas. We report here on the establishment of one of these myelomas, IR162, in cell culture, its characterization, and the development of a system for the assay of drug effects on cell growth and IgE production.

Materials and methods. Louvain/Wsl/M rats and the IR162 IgE-secreting tumor were obtained from H. Bazin, University of Louvain, Belgium. The tumor was maintained by subcutaneous passage (7), and ascites passages of the tumor were initiated by intraperitoneal injection of tumor cells. IgE secreting cell cultures were established by a procedure similar to that described by Burtonboy *et al.* (9). Ascites fluid was harvested aseptically; cells were sedimented by centrifugation and resuspended at a density of 3×10^5–3×10^6 cells/ml in RPMI 1640 medium supplemented with penicillin (100 units/ml), streptomycin (0.1 mg/ml) and 20% fetal calf serum, followed by incubation at 37°. Cultures were "fed" by addition of 1 or 2 ml nutrient medium every 24–48 hr depending on the number of cells and the pH of the culture fluid. Culture medium was changed at 7- to 10-day intervals by centrifuging the cells, then resuspending them in fresh medium in the original flask. Adherent cells from the ascites formed a feeder layer to which the tumor cells attached loosely. After 6 to 8 weeks the cell cultures were transferred to medium containing 10% fetal calf serum. Passages were made every 2 or 3 days by breaking up cell clumps with a pipet and diluting the cells 1:5 or 1:10 with fresh medium.

Soft agar cloning of tumor cells was performed in agarose solidified RPMI 1640 medium having a final concentration of 17% fetal calf serum, 5×10^{-5} *M* 2-mercaptoethanol and 5 μg/ml gentamicin. 0.5% agarose was included in the base layer and 0.22% agarose in the cell layer. No cell feeder layer was required.

Cells grown *in vitro* were washed twice in serum-free medium and injected subcutaneously or intraperitoneally into Lou/Wsl/M rats to determine whether they retained tumorigenicity. The animals were observed for a minimum of three months.

A pool of anti-ovalbumin IgE was produced in Lister Hooded rats according to the procedure of Jarrett and Stewart (10). Antibody titer was determined by passive cutaneous anaphylaxis (PCA). The serum titer was the highest dilution of serum that gave a skin reaction with a diameter of at least 5 mm. For inhibition studies, reaginic antibody was diluted 4–8 times and mixed with the substance to be tested.

Cloned cells derived from the IR162 ascites and designated Ta-4 were grown for 72 hr in the presence of various concentrations of 5-bromo-2-deoxyuridine (BUDR) to establish the 50% tissue culture inhibitory dose ($TCID_{50}$) for both cell growth and IgE production. Following this, growth in the presence of the drug at or near its $TCID_{50}$ dose concentration was followed for 72 hr. The Lowry protein assay was performed as adapted for cell culture by Holden *et al.* (11). After removal of the cells for growth analysis, the medium was retained for determination of IgE by quantitative passive hemagglutí-

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nation inhibition (HAI). IgE from ascites fluid was partially purified by the procedure of Isersky *et al.* (12) so that the preparation contained only IgE and IgG. Sheep red cells were labelled with this preparation (13). Anti-rat IgE antiserum (Miles Laboratories, Elkhart, Indiana) was diluted serially in two-fold steps in 96 well Linbro V-well disposable microtiter plates so that each well contained 25 μl of antiserum in HA diluent (0.85% saline supplemented with 1% fetal calf serum and 0.01% sodium azide). The initial antiserum concentration was adjusted so that the endpoint of hemagglutination occurred at well 10. Well 12 contained only diluent. To each row of wells were added 25 μl diluent or cell culture medium, which had been diluted in 0.85% saline so that the final serum concentration was 1% or less to avoid nonspecific interference with hemagglutination. All determinations were done in triplicate. The plate was agitated and incubated at room temperature for 30 minutes. Finally, 50 μl labelled red cells were added to each well; the plate was agitated and incubated overnight at room temperature, and observed for the extent of hemagglutination inhibition.

Results. The Ta-4 cell line was derived by propagation of tumor cells from an IR162 ascites for 47 passages in cell culture, intraperitoneal injection of tumor cells into a rat, and reinitiation of a cell culture followed by cloning in agarose solidified medium. The cloning efficiency was approximately 20%. This Ta-4 line continued to produce IgE in cell culture and tumors in animals after 140 *in vitro* passages and two years in culture.

Tumors could be induced by the injection of as few as 10^3 cells ip or 10^5 cells sc and were capable of extended passage in rats. Tumor bearing animals contained large amounts of IgE in their sera and ascites fluid. Examination of the tumors indicated they were macroscopically and microscopically indistinguishable from the original animal-passed IR162 tumor and from two other IgG producing rat myelomas from the Notre Dame Louvain/Wsl colony.

Ta-4 cells growing in culture and in the ascites fluid of tumor-bearing animals had large nuclei, multiple nucleoli, frequent mitotic figures, basophilic cytoplasm, and prominent Golgi apparatus. They grew in small clumps in stationary suspension cultures or spinner flasks. These clumps were readily dispersed by gently pipetting and viability remained high after pipetting.

Figure 1 illustrates the growth and IgE production of Ta-4 cells in culture. Three experiments provided similar results and the standard deviations of protein determinations were consistently less than 4 μg/ml. The initial rapid increase in IgE content of the medium and the continued secretion of IgE after cell growth ceased were consistent findings with an additional 6 cell lines developed from IR162. The IgE was quite stable in cell culture medium. There was no detectable decrease in IgE titer in medium for 5 days at 37°. An HAI assay similar to that used for IgE and capable of detecting 0.5 μg IgG/ml failed to detect any rat IgG in media in which Ta-4 cells were grown.

To establish that the myeloma produced *in vitro* was capable of binding for long periods to rat skin mast cells, i.e., characteristic of IgE, it was mixed with rat reagin in passive cutaneous anaphylaxis tests. Table I presents the results of such tests when anti-ovalbumin reagin with an initial titer of 1:320 was injected at a dilution of 1:40 and 1:80 in the presence of ascites fluid and spent culture

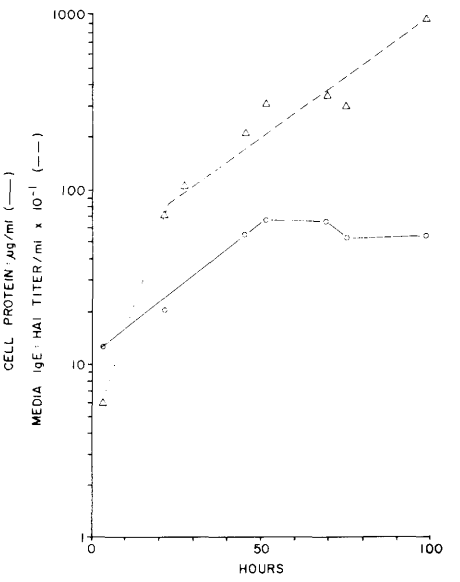

FIG. 1. Growth and IgE Production of Cell Culture Line Ta-4 at Passage 55 *vs* Time. —○— Cell Protein, μg/ml. —△— Medium IgE, HAI Titer/ml.

medium. Ascites fluid containing myeloma IgG and IgM had no effect on the PCA reaction, while IR162 ascites fluid was inhibitory. Likewise, cell culture medium was not effective but medium (concentrated 20× by lyophilization and unconcentrated) in which Ta-4 cells had grown inhibited the PCA reactions completely.

BUDR was examined over a wide range of concentrations *in vitro* to see if the system could be used to evaluate drug effects. The 50% inhibitory dose (ID_{50}) for growth (cell protein) was approximately 0.1 μg/ml while the ID_{50} for IgE secretion was 2.0 μg/ml.

In three experiments 72-hr growth and IgE production were evaluated in the presence and abscence of BUDR. Table II provides data from one of these experiments. A difference in cell growth and IgE synthesis was not considered significant unless the gain in IgE synthesis was at least 4 times the gain in cell protein.

Discussion. The Ta-4 cell line of IR162 has been in culture for more than 2 years and continues to produce rat IgE *in vitro* and *in vivo* with no evidence of contaminating IgG. It thus provides a source of IgE uncontaminated by other rat immunoglobulins. This has also been shown by establishing the tumor in germfree Lou/M rats. These rats have the usual low serum IgG content but high levels of myeloma IgE. The ability of the Ta-4 myeloma protein to competitively inhibit rat PCA reactions is additional evidence of its nature.

In a preliminary report Bennich *et al.* have also described the establishment of IR162 cells in culture (14). They too noted a parallel between cell number and IgE production and the ability of IR162 cells to continue to produce IgE *in vitro*. However, in contrast to our experience, they were unable to propagate the IR162 cells without a fibroblast feeder layer or establish long term cloned lines. We noted the ascites fluid contained sufficient adherent cells to act as a feeder layer until the IR162 cells began to grow well (after about 6–8 weeks). The IR2 line, another IgE myeloma, did grow without a feeder layer also after about a 20-day lag, but these cells failed to produce IgE. We are unable, at this time, to explain this difference between the two cell lines.

We wished to determine whether the constitutive IgE production of these tumor cells could be modulated by an external agent. To rule out simple toxic effects on cell growth we looked at increases in cell protein (cell growth) and compared it to secretion of IgE into culture medium. Because of the parallel

TABLE I. EFFECT OF MYELOMA PROTEINS AND CELL CULTURE MEDIUM ON THE PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) REACTION IN RATS.

Material injected intradermally			Results (PCA)
Test sample	Dilution	Reagin dilution[a]	Number positive/ Total number tested
Ascites fluid from tumor bearing rats			
IgE myeloma IR162	1:100	—	0/2
	1:100	1:40	0/2
IgG myeloma SP2	1:100	—	0/2
	1:100	1:40	2/2
IgG myeloma SP4	1:100	—	0/2
	1:100	1:40	2/2
IgM myeloma M	1:100	—	0/2
	1:100	1:40	2/2
Cell culture medium			
Nutrient medium, no cells		1:40	2/2
Nutrient medium, Ta[b]		—	0/2
Nutrient medium, Ta (20×)		1:40	0/4
Nutrient medium, no cells		1:80	2/2
Nutrient medium, Ta		1:80	0/2
Nutrient medium, Ta (20×)		1:80	0/4

[a] The test dilution of an antiovalbumin IgE having an initial titer of 1:320.

[b] Nutrient medium, Ta is spent culture medium from the growth of Ta-line myeloma cells. 20× is the same medium concentrated 20-fold.

TABLE II. EFFECTS OF BUDR IN IgE SYNTHESIS AND Ta-4 CELL GROWTH DURING 72 HR IN CULTURE.

Concentration of BUDR	Net IgE synthesis fraction of control	Net protein synthesis fraction of control	Ratio IgE/protein
0	1.00	1.00	1.00
0.05	1.15	0.86	1.34
0.1	0.71	0.63	1.13
0.2	0.71	0.44	1.61
0.3	0.71	0.12	5.62[a]
0.5	0.57	0.12	4.75[a]
1.0	0.44	0	>10[a]
5.0	0.35	0.02	>10[a]
10.0	0.29	0	>10[a]

[a] Denotes a differential effect on cell growth and IgE synthesis.

of cell growth and IgE secretion we initially thought that IgE synthesis might be irreversibly linked to cell division. However, the IgE content of medium continued to increase after cell increase stopped. To rule out cell lysis as the mechanism for this effect, we lysed cells and analyzed the IgE released. Very little IgE was released in this fashion and the amounts did not correlate to IgE increases in stationary growth phase.

We then looked at the nucleic acid inhibitor BUDR for its effect on IgE secretion. The differential effects of BUDR on growth and IgE synthesis is probably due to its ability to inhibit cell division. The effect is similar to that of X-irradiation which stops cell growth but permits continued IgE secretion (unpublished data) and to the continued IgE secretion after cessation of cell growth in stationary phase. Continuing work with other cytotoxic drugs should allow us to determine whether factors which can prevent cell division without causing cell death allow these tumor cells to become more like their more differentiated normal counterparts and put a larger proportion of their energy into immunoglobulin production. The simultaneous determination of changes in total cell protein and IgE secretion provides an assay for the *in vitro* effects of drugs on growth and IgE synthesis in rat myeloma cells.

Summary. Propagating stationary suspension cultures of a cell line (Ta-4) derived from the IR162 IgE secreting rat myeloma produced high concentrations of IgE, formed colonies in agarose solidified medium, and when injected into rats induced tumors similar to the original myeloma. The *in vitro* produced IgE was capable of binding to rat skin mast cells. A system was developed to assay the effects of drugs on IgE synthesis and results with 5-bromo-2′-deoxyuridine indicate that IgE synthesis can continue at high levels in the absence of cell replication.

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