INDEPENDENT SYNTHESIS OF LIGHT AND HEAVY CHAINS

Quantitation of Light-Chain Production by Mouse Myeloma Variants*

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Plasma cells produce relatively large amounts of immunoglobulin compared to other cell proteins. While little is known about the mechanism responsible for this apparent selective amplification of immunoglobulin synthesis, it must act independently on the production of heavy and light chains since the structural genes for the two polypeptide chains are not linked genetically (1) and the chains are translated from separate messengers (2). On the other hand, the synthesis of the two polypeptide chains is relatively well coordinated since most myeloma tumors and normal lymph node cells synthesize either a moderate excess of light chains or equimolar amounts of heavy and light chains (3–5).

The synthesis of light chains can obviously occur in the absence of heavy chains since many mouse and human myelomas produce only light chains (6, 7). However, it is possible that the loss of heavy-chain synthesis might affect the rate of light-chain synthesis. In order to examine this possibility, we have determined the amounts of light and heavy chains synthesized by two independent MPC-11 mouse myeloma cell lines, and by light-chain-producing variants derived from each of these cell lines.

Materials and Methods

The adaptation of the MPC-11 IgG_{2b}-producing mouse myeloma tumor to continuous culture has been described previously (8). Line I was derived from a spleen clone of the original tumor, while line II was adapted directly from the MPC-11 tumor (8). These lines were maintained in suspension culture either in Petri dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) or spinner bottles by feeding with Dulbecco's medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 20% inactivated horse serum, nonessential amino acids, and glutamine (8). The myeloma cells were cloned in soft agar as described previously (9, 10). Variants which had lost the ability to produce heavy (H) chains were identified and enumerated by "staining" the clones directly on agar plates with specific anti-H-chain antiserum (9, 10).

The doubling time of the cells was calculated from a growth curve. Cells were diluted into fresh medium and the cell counts were made at frequent intervals in a Coulter Counter (Coulter

* This work was supported by grants from the U. S. Public Health Service, the National Institutes of Health (AI-5231, AI-10702), The National Science Foundation (GB 29560), and the American Cancer Society (ACS-VC-33).

Electronics, Inc., Fine Particle Group, Hialeah, Fla.). Total cell protein was determined by a modification of the method of Lowry (11), using crystalline bovine serum albumin (Armour Pharmaceutical Co., Chicago, Ill.) as a standard. Chromosomes were analyzed according to the method of Bunker (12).

The pattern of immunoglobulin production was then determined by incubating cells with radioactive amino acids, making cytoplasmic extracts with the detergent Nonidet P-40 (13), specifically precipitating the intracellular immunoglobulins using indirect immunological precipitation (14), and then analyzing the dissolved immune precipitate in sodium dodecyl sulfate (SDS) containing acrylamide gels (8). To determine the ratio of light (L) and H chains, the cytoplasmic immune precipitates were reduced and alkylated and then electrophoresed on SDS-containing acrylamide gels (8).

In order to determine the relative and absolute amount of gamma globulin synthesized, serial dilutions of cytoplasm derived from cells labeled for 30 min were added to 0.20-ml portions of the rabbit antimouse IgG antiserum. The reaction mixtures were incubated at 4°C for 24 h. The precipitates were washed and dissolved as described above and duplicate 10λ samples were taken from the immune precipitates and the supernate and precipitated with 12.5% TCA. The TCA-precipitated material was collected on millipore filters, washed, and counted in a low background counter (Nuclear-Chicago Corp., Des Plaines, Ill.). By examining dilutions of a given cytoplasm, we were actually carrying out a quantitative precipitin curve and could therefore be sure that the determination was carried out in the presence of excess antibody (14). Controls for the nonspecific precipitation of radioactive material were carried out by adding rabbit anti-λ bacteriophage serum to the cytoplasmic extract together with sheep antirabbit gamma globulin. No more than 1.5% of the acid precipitable radioactivity was precipitated nonspecifically. The relative amount of labeled gamma globulin present in the myeloma cells was calculated by subtracting the amount of acid precipitable radioactivity in the nonspecific precipitate of the cytoplasm from that in the specific precipitate and then dividing by the total amount of TCA precipitable radioactivity in the whole cell.

The relative rate of synthesis of the individual H and L chains was calculated using a ratio of radioactivity in H/L chains of 3/1. This was determined by reducing purified labeled IgG (H_2L_2) and determining the ratio of radioactivity. This 3/1 ratio is higher than that expected from the relative molecular weights of H and L chains and is due to preferential labeling of H chains by the valine, threonine, and leucine mixture, since the H/L ratio of radioactivity in H_2L_2 labeled with mixed amino acids is the expected 2.3/1.

The absolute amount of immunoglobulin synthesized per cell was calculated according to the formula $A = (R \times P)/D$ where: A = the absolute amount of immunoglobulin synthesized expressed as picogram per cell per hour; R = the relative amount of immunoglobulin synthesized (i.e., percent of total TCA precipitable radioactivity); P = protein content (picogram per cell) of the myeloma cells; and D = doubling time (hour) of the cell population in culture. The amount of intracellular immunoglobulin was determined by radial immunodiffusion in agar (15) using purified preparation of MPC-11 myeloma protein to construct a calibration curve.

Results

The relative amount of immunoglobulin produced by clones obtained from two independently adapted MPC-11 cell lines are presented in Table I. Approximately 16% of the protein synthesized by line I and 25% of the protein produced by line II was gamma globulin. The molar ratio of L/H chains produced in both cell lines was approximately 2/1. Since both cell lines could generate variants producing only L chains (16), the number of such variants was monitored throughout these experiments. The contamination of line I increased to between 11 and 15% for different subclones, while the contamination of line II did not exceed 10%. These differences between the lines were not nearly sufficient to

explain the apparent differences in the amounts of H and L chains synthesized by the two cell lines.

When variants producing only L chains were isolated from each cell line, they made the same relative amount of L chains as the parent H plus L chain producer (Table I). The absolute rate of H- and L-chain syntheis was also determined for lines I and II, and a L-chain producer derived from line II (II-L) (Table II). Although each of the numbers used in this calculation (see Materials and Methods) is subject to appreciable error, the results were consistent with those presented in Table I. The absolute size of the intracellular pool of immunoglobulin was also determined for lines I and II using radial immunodiffusion. Cells from line I contained approximately 3.5 pg/cell while line II cells contained approximately 6.0 pg/cell, indicating that the pool size reflected the relative rates of synthesis of immunoglobulin by the two cell lines.

TABLE I

Relative Rate of Gamma Globulin Synthesis

Cell line	Polypeptides produced	Radioactivity specifically precipitated			Molar ratio L/H
		Total	H	L	
		%	%	%	
Cell line I	H,L	16.1	9.7	6.4	2.0
	L	5.3		5.3	
Cell line II	H,L	25.2	15.8	9.4	1.8
	L	9.0		9.0	

Each of the determinations represents the average of 2–4 independent experiments. The difference between experiments was less than 10%.

Discussion

The rate of production of immunoglobulin by these cell lines was comparable to that reported by Nathans et al. (17) and Humphrey and Fahey (18) for a mouse myeloma tumor in vivo. These authors found that 14–19 mg of myeloma protein was produced by each gram of tumor per day, which is equivalent to a production of approximately 30–50 pg/cell/24 h. Nossal and Makela estimated that suspensions of lymphoid cells in vitro also produced similar amounts of antibody (19). A human myeloma cell line produced 18 pg of L chains/cell/day (20). Various human lymphoblastic lines have been shown to produce smaller amounts of immunoglobulin (21). The size of the intracellular pool of gamma globulin has been measured in only a few studies. Berenbaum (22) estimated that normal lymphoid cells contain 0.75 pg/cell, while Salmon and Smith (23) found the myeloma cells contained 1.5 pg/cell. Gitlin and Sasaki (24) examined the immunoglobulin content of human tonsilar lymphoid cells and found between 0.2 and 6.0 pg/cell.

TABLE II

Absolute Amount of Immunoglobulin Synthesized

Cell line	γ-globulin synthesized	H chains synthesized		L chains synthesized	
	pg/cell/h	*pg/cell/h*	*molecule/cell/min*	*pg/cell/h*	*molecule/cell/min*
I	2.5	1.5	280,000	1.0	455,000
II	3.6	2.12	400,000	1.33	605,000
II-L	1.2			1.2	540,000

The amount of immunoglobulin synthesized by each of these cell lines was calculated as described in the Materials and Methods. The doubling time of line I was 22.1 h and its cells contained 346 ± 39 pg/cell. Line II doubled every 20.3 h and contained 327 ± 28 pg/cell. Line II-L doubled every 24.6 h and contained 327 ± 71 pg/cell.

The studies reported here do not provide any explanation for the different amounts of immunoglobulin produced by the two cell lines studied. Both cell lines had a heteroploid modal chromosome number of 65, but detailed karyotypic analysis of mouse chromosomes is not possible without special staining techniques. The two cell lines may represent different populations which were present in the tumor at the time it was adapted to culture, or they may represent variants which arose during the adaptation to culture.

Both cell lines produced excess L chains. However, the L-chain-producing variants derived from lines I and II continued to produce approximately the same amount of L chains as their respective parent cells. This indicates that the amount of L chains produced was not dependent upon the continued synthesis of H chains.

Summary

The rates of immunoglobulin synthesis have been examined in two MPC-11 cell lines which were independently adapted to tissue culture and in light-chain-producing variants derived from each of them. One cell line synthesized 2.5 pg immunoglobulin/cell/h, while the other synthesized 3.6 pg immunoglobulin/cell/h. The ratio of heavy and light chains in the two cell lines was approximately the same, and the size of the intracellular pool of immunoglobulin was proportioned to the rate of synthesis. Variants which had spontaneously lost the ability to produce heavy chains continued to synthesize light chains at approximately the same rate as their parent cell line.

Received for publication 15 July 1974.

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