fatty acids (*5*) and of ketone bodies (*6*). However, ours is the first demonstration of the existence of thiolase in prenatal tissues.

After birth, the development of thiolase activity reflects the nutritional environment of the young rats whose nourishment for the better part of the first 3 weeks of life is maternal milk, which is high in fats. Evidence for the expected increased breakdown of fatty acids and ketogenesis by the liver was provided by its thiolase activity which increased threefold between birth and weaning. During the same period, brain thiolase activity was maximum. The fact that the kidney can utilize as well as produce ketone bodies is reflected in a pattern of thiolase development intermediate between that of liver and brain.

Another and similar index of the nutritional environment of the growing rats is D-(−)-β-hydroxybutyrate dehydrogenase which has been shown by Klee and Sokoloff (*7*) to rise immediately after birth in rat brain, to reach a maximum at age 30 days, and to fall to low levels in adulthood. This enzyme could then be reinduced by starvation (*8*) or by a diet high in fat (*9*). To test whether thiolase would exhibit a similar behavior, we subjected adult rats to starvation for periods up to 72 hours and determined the thiolase activity in brain and liver (Table 2). Although the rats became ketonemic after 24 hours and also showed increased cholesterol in the serum, brain thiolase activity was not elevated significantly and the increase of liver thiolase activity seen temporarily at 48 hours of starvation could not be correlated with concentrations of ketone bodies or cholesterol in the serum. Thus it seems that there is no need for adult brain thiolase to adapt during fasting.

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References and Notes

1. T. Itoh and J. H. Quastel, *Biochem. J.* **116**, 641 (1970).
2. D. H. Williamson, M. W. Bates, H. A. Krebs, *ibid.* **108**, 353 (1968).
3. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
4. J. D. McGarry and D. W. Foster, *ibid.* **244**, 4251 (1969).
5. W. H. Goldwater and D. Stetten, Jr., *ibid.* **169**, 723 (1947); Z. Koren and E. Shafrir, *Proc. Soc. Exp. Biol. Med.* **116**, 411 (1954); O. W. Portman, R. E. Behrman, P. Soltys, *Amer. J. Physiol.* **216**, 143 (1969); H. J. Kayden, J. Dancis, W. L. Money, *Amer. J. Obst. Gynecol.* **104**, 564 (1969).
6. R. O. Scow, S. S. Chernick, B. B. Smith, *Proc. Soc. Exp. Biol. Med.* **98**, 833 (1958), R. O. Scow, S. S. Chernick, M. S. Brinley, *Amer. J. Physiol.* **206**, 796 (1964).
7. C. B. Klee and L. Sokoloff, *J. Biol. Chem.* **242**, 3880 (1967).
8. A. L. Smith, H. S. Satterthwaite, L. Sokoloff, *Science* **163**, 79 (1969).
9. ———, *Abstr. Mtg. Internat. Soc. Neurochem. Milan, 2nd* (September 1969), p. 371.
10. H. G. Britton, *Anal. Biochem.* **15**, 261 (1966).
11. A. Zlatkis, B. Zak, G. J. Boyle, *J. Lab. Clin. Med.* **41**, 486 (1953).
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Immunoglobulin Production by Human Lymphocytoid Lines and Clones: Absence of Genic Exclusion

Abstract. *Immunoglobulin production was studied in established lines of normal human lymphocytes. Three lines which produced both immunoglobulin G and immunoglobulin M were cloned. Among the 25 immunoglobulin-producing clones, 23 produced both classes of immunoglobulins. These findings suggest that the phenomenon of genic exclusion does not hold for immunoglobulin production in lymphocytoid cells in culture.*

The development of techniques for establishing human lymphocytes in long-term culture (*1*) and more recently for cloning these cells (*2*) has enabled us to test the widely held view that single cells are capable of producing only a single, heavy chain class of immunoglobulins (*3*). On the basis of immunodiffusion studies of the concentrated medium in which the lymphocytes grow, it appears that clonal sublines, established from parental lines that produce both immunoglobulin G (IgG) and immunoglobulin M (IgM) of the kappa type, actively synthesize both classes of immunoglobulins, of the same type specificity as the parental lines.

Peripheral blood lymphocytes from three hematologically normal persons were established with the use of lysates of previously established lymphocytoid lines, as described by Choi and Bloom (*1*). These three lines, designated UM-1, -5, and -21 (University of Michigan, first, fifth, and twenty-first lymphocyte lines, respectively), were established in the course of our cytological, biochemical, and immunological studies on lymphocytes in long-term culture. All had been established and subcultured for at least 3 months prior to the cloning experiments; and all were, on karyotype analysis, normal diploid cell lines. Occasional aneuploid and tetraploid cells were seen, but these never exceeded 15 percent of the examined cells of each line.

In order to test the hypothesis of genic exclusion for the immunoglobulin loci, a number of clonal sublines was established from each of the three parental lines. The cloning technique has been described in detail elsewhere (*2*), with approximately 50 percent of isolated, single cells, from established lymphocytoid lines, going on to form clones. Single cells from the lines were isolated in individual wells of plastic serotest plates (Falcon) and incubated for 4 to 8 weeks, with media changes and transfer to larger culture vessels, until clonal sublines were established. Eleven clones were established from the UM-1 line, eight from the UM-5 line, and ten from the UM-21 line.

When the concentration of cells in the cultures of the parental and clonal lines reached 1×10^6 cells per milliliter, medium was obtained for immunoglobulin studies. Medium was obtained by decanting after centrifugation of the cells and medium at 1500 rev/min for 10 minutes. The cells were then returned to culture with fresh medium added. Since no effort was made to synchronize the cells, they were in various stages of interphase and mitosis at the time the medium was tested for immunoglobulin production (*4*). The used medium was tested for the presence of immunoglobulins, after a 40-fold concentration of it had been obtained by vacuum suction. Concentrates were stored at −4°C until tested.

Immunoglobulins were studied by a modification (*5*) of the double diffusion method of Ouchterlony (*6*), by using Oxoid Ionagar No. 2 (Colab), with a barbital buffer of *p*H 8.5. Equine antiserums (Kallestad) to human IgG (γ chain specific) and IgM (μ chain specific), and to kappa and lambda light chains, were placed in the center wells of the agar-covered slides, and run against the concentrated medium placed in the peripheral

wells. Slides were incubated at 37°C from 24 to 48 hours in a sealed box. With the emergence of precipitin bands, slides were stained with a 0.5 percent amido black solution, diluted in a 9 : 1 methanol : glacial acetic acid mixture. The precipitin bands were distinct, with no evidence of spurring; and no cross-reactivity was seen between the two heavy chain antiserums or between the two light chain antiserums.

Since the lymphocytes were cultured in the presence of 20 percent fetal calf serum, we first had to test for cross-reactivity between the calf serum and each of the antiserums used. No precipitin bands were seen when any of these antiserums were run against fetal calf serum alone, or against the concentrated mixture of tissue culture medium RPMI (Roswell Park Memorial Institute) 1640 (Grand Island Biological) with 20 percent fetal calf serum (Baltimore Biological Laboratories).

As seen in Table 1, each of the parental cell lines, UM-1, -5, and -21, produced detectable levels of both IgG and IgM, and each of the mediums was reactive with antiserum to kappa chain. No reactivity with antiserum to lambda chain was, however, seen. These immunoglobulins were repeatedly demonstrable in the parental lines.

Of the 29 clonal sublines, 25 were found to be immunoglobulin-producing. Twenty-three of the 25 immunoglobulin-producing clones had detectable levels of both IgG and IgM. Medium from all 25 clones reacted with antiserum to kappa chain.

These data suggest that the loci responsible for IgG and IgM synthesis in cultured human lymphocytes are operative simultaneously within the cells of a clone. Assuming that the immunoglobulin activity in the clone accurately reflects the genic activity of the cell of origin of the clone, we may conclude that genic exclusion does not hold for the immunoglobulin loci in established lymphocytoid cells. This conclusion is supported by the studies of Takahashi *et al.* (*7*) who demonstrated by immunofluorescence that single cells of established lymphocytoid lines produce both gamma and alpha heavy chains.

It is difficult to say at this time what, if any, influence the Epstein-Barr virus (EBV) may have on immunoglobulin production by established lymphocyte cell lines (*8*). While it is likely that virtually all well-established lines have EBV in a small percentage of cells (*9*), there is evidence that the proportion of EBV-infected cells declines with time, to under 0.2 percent of the total cell population once the line is established (*10*). Nonetheless, EB viral DNA is detectable after transformation has taken place (*11*), and this at least raises the possibility that the viral genome may affect the activity of the immunoglobulin loci. While our method of establishing these lines involves addition of lysates of previously established lymphoid lines, we are not as yet certain of the presence of EBV in the lysates, parental lines, or clones. In any event, even if there are EBV-positive cells in the parental lines, the number of positive cells in the clones should amount to less than 1 percent (*10*).

The fact that 23 of 25 clonal sublines produced the same classes of immunoglobulins caused us to ask whether our three parental lines might each be derived from a rare, perhaps mutant, cell in which these immunoglobulin loci were active. We obtained evidence recently that lymphocyte lines, established by our method, are, in fact, mixtures of at least two subpopulations, by study of the glucose-6-phosphate dehydrogenase (G6PD) isozymes in two established lymphocytoid lines (UM-26 and -27) from a female known to be heterozygous for X-linked G6PD. Electrophoresis of her erythrocyte G6PD (*12*) demonstrated both a fast (A) and a slow (B) moving band. Electrophoresis of lysates of her two established lymphocyte lines similarly revealed these two distinct bands, suggesting that these lines are a mixture of G6PD A- and B-producing cells.

Table 1. Immunoglobulin production by three lymphocytoid cell lines (UM-1, -5, and -21) and their clones. The three lines were established from hematologically normal persons, and were cloned within 6 months of establishment. Equine antiserums (Kallestad) to γ-chain specific human IgG, μ-chain specific IgM, and kappa and lambda chains were tested by the double diffusion method.*

Cell line	Heavy chain		Light chain	
	IgG(γ)	IgM(μ)	K(κ)	L(λ)
UM-1	+	+	+	−
Clones 1 to 11	+	+	+	−
UM-5	+	+	+	−
Clones 1 to 8	+	+	+	−
UM-21	+	+	+	−
Clones 1, 6	+	−	+	−
Clones 2, 4	−	−	+	−
Clones 3, 5, 8, 10	+	+	+	−
Clones 7, 9	−	−	−	−

* The converse of this experiment was also done, that is, concentrated medium from each of the parental cell lines and clones were placed in the center wells and run against the chain-specific antiserums in the peripheral wells. In all instances, a continuous precipitin band with no spurs was formed, linking the antiserums to the gamma and kappa chains and the antiserums to the mu and kappa chains. This suggests that the heavy and light chains are incorporated into the immunoglobulin molecule, and not free in the medium.

It is clear from the evidence presented here that the ability of established lymphocytoid cells to produce multiple classes of immunoglobulins distinguishes such cells from both normal lymphocytes (*13*) and myeloma cells (*14*) in vivo. The cultured lymphocyte may be in a less highly differentiated state than either of the other two cell types; or, alternatively, a specific, as yet undefined, mechanism of derepression of the immunoglobulin loci may exist in vitro.

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References and Notes

1. G. E. Moore, E. Ito, K. Urid, A. A. Sandberg, *Cancer* **19**, 713 (1966); S. W. Broder, P. R. Glade, K. Hirschhorn, *Blood* **35**, 539 (1970); K. W. Choi and A. D. Bloom, *Science* **170**, 89 (1970).
2. K. W. Choi and A. D. Bloom, *Nature* **227**, 171 (1970).
3. B. Pernis, G. Chiappino, A. S. Kelus, P. G. H. Gell, *J. Exp. Med.* **122**, 853 (1965).
4. D. Buell and J. L. Fahey, *Science* **164**, 1524 (1969).
5. G. J. Ridgway, G. W. Klontz, C. Matsumoto, *Bull. Int. North Pac. Fish. Comm.* **8**, 1 (1962).
6. O. Ouchterlony, *Acta Pathol. Microbiol. Scand.* **32**, 231 (1953).
7. M. Takahashi, N. Takagi, Y. Yagi, G. E. Moore, D. Pressman, *J. Immunol.* **102**, 1388 (1969).
8. G. Martin, B. R. Schwartz, M. A. Derr, *Lancet* **1970-II**, 772 (1970).
9. P. Gerber, J. Whang-Peng, J. H. Monroe, *Proc. Nat. Acad. Sci. U.S.* **63**, 740 (1968).
10. D. P. Stevens, L. F. Barker, H. E. Hopps, H. M. Meyer, *J. Immunol.* **105**, 1040 (1970).
11. H. Zur Hausen, H. Schulte-Holthausen, G. Klein, W. Henle, G. Henle, P. Clifford, L. Santesson, *Nature* **228**, 1056 (1970).
12. R. S. Sparkes, M. C. Baluda, D. E. Townsend, *J. Lab. Clin. Med.* **73**, 531 (1969).
13. D. Gitlin and T. Sasaki, *Science* **164**, 1532 (1969).
14. D. Feinstein and E. C. Franklin, *Nature* **212**, 1496 (1966).
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