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Immunology

Induction of amplified synthesis and secretion of IgM by fusion of murine B lymphoma with myeloma cells

(B-cell differentiation/hybrid cells/cytoplasmic Ig/membrane IgM/Ig gene expression)

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ABSTRACT **Murine B lymphoma cultured cell lines bearing membrane IgM and lacking the ability to secrete measurable amounts of IgM were fused with drug-resistant cell lines derived from the IgG_{2b}-producing MPC-11 myeloma. Many of the hybrid clones synthesized and secreted large amounts of IgM, as judged by radial and double immunodiffusion in agar of culture supernatants and by polyacrylamide gel electrophoresis of biosynthetically labeled IgM. When fused with an IgG_{2b}-producing myeloma, many of the hybrids also produced IgG_{2b}, indicating that there was no suppression of the parental IgG synthesis in the hybrids. The amount of IgM or IgG_{2b} secreted by the hybrids was higher than that secreted by myeloma cell lines. The synthesis of the γ_{2b} heavy chain of the MPC-11 myeloma was not reexpressed by fusion of a γ_{2b} nonproducer myeloma cell variant with B lymphoma. No other classes of Ig heavy chains, besides the γ_{2b} and the μ chains, were found to be secreted by any of more than 100 B lymphoma–myeloma hybrids examined. The results of the present work suggest that myeloma cells may fuse not only with normal plasma cells but also with less-mature normal B lymphocytes and that this fusion "induces" the maturation of B lymphocytes into IgM-secreting cells.**

Stimulation of B lymphocytes by immunogens or by mitogens results in a series of maturational events leading to the formation of antibody-secreting cells (1). At the molecular level, these changes include the transition from the expression of minute quantities of IgM on the surface of B lymphocytes to the production and secretion of large quantities of IgM by the mature antibody-secreting cells. Evidence also exists that individual antigen-stimulated B cells or their progeny may express more than one class of Ig molecules of similar antigen-binding specificity (2–6). Therefore, in the same cell, one type of amino-terminal variable region is able to associate with more than one class of the heavy chains. The mechanisms responsible for these steps in antigen-induced B-cell maturation are not well understood. Attempts to elucidate the biochemical events occurring during the maturation of normal B lymphocytes are hindered by the heterogeneous nature of the population of B cells involved.

Recently, murine lymphomas that bear membrane IgM and having other properties of B lymphocytes have been characterized in several laboratories (7–10). These tumors are presumably arrested at various stages of differentiation and are considered to be of monoclonal origin. Successful induction of maturation in these tumors may provide a suitable experimental model system for study of the molecular events involved in B-cell maturation.

Attempts to induce the differentiation of B lymphomas by using various mitogens (11) and dimethyl sulfoxide (12) have previously resulted at most in partial maturation of these cells. Recently, some human B-lymphoid cell lines were induced by lipopolysaccharide and normal T cells to secrete low quantities of Ig (13).

In the present work, we have used an alternative approach to induce the maturation of membrane IgM-bearing murine B lymphomas. It was found that fusion of these nonsecreting lymphoma cells with IgG-producing myeloma cells resulted in the formation of hybrids of which many produced and secreted large quantities of IgM.

MATERIALS AND METHODS

Cells. B lymphoma tumors appear spontaneously in old BALB/c mice (>17 months). These tumors were collected and maintained for several years by Ruth Merwin (National Cancer Institute) and were found to have properties similar to those of B lymphocytes (R. Merwin, P. Campbell, and R. Asofsky, unpublished data). The three B lymphoma cell lines used in the present fusion experiments—L10A, K46, and X16—were recently adapted to grow in culture and continue to exhibit properties of B cells; the cells bear IgM, Ia, and Fc receptors and lack the Thy 1.2 antigen (unpublished data).

Two clones of the MPC-11 myeloma, kindly provided by Matthew Scharff (Albert Einstein College of Medicine) were used for fusion: clone 4T00.1 producing IgG_{2b} and clone 4T00.1L1 which produces only light chains. Both clones were resistant to 6-thioguanine and ouabain (14).

Fusion Procedure. This was done by using polyethylene glycol (PEG-1000, Sigma) essentially according to Margulies *et al.* (14, 15). The cells were seeded into either 96-microwell or 24-well Linbro plates, and the hybrids were selected in growth medium containing 100 μM hypoxanthine, 1 μM aminopterin, 30 μM thymidine, and 1.5 mM ouabain (15).

Preparation of Monospecific Anti-Ig Sera. Antisera were raised in rabbits and goats against purified myeloma proteins of various Ig classes (16). The antisera were made Ig class-specific by absorption with other myeloma proteins as described (16).

Analysis of Secretion of Biosynthetically Labeled Ig. Hybrid and parent cells ($0.25–1 \times 10^6$ cells) were labeled with 5 μCi (1 Ci = 3.7×10^{10} becquerels) of ^{14}C-labeled L amino acid mixture (New England Nuclear, NEC-445) in 0.25–0.5 ml of Dulbecco's medium lacking amino acids and containing 1% dialyzed horse serum (GIBCO), 2 mM glutamine, 50 units of penicillin per ml, and 50 μg of streptomycin per ml. At the end of the incubation period (5 hr at 37°C) the cells were pelleted and aprotinin (Sigma, A-6012) and sodium dodecyl sulfate ($NaDodSO_4$) were added to the supernatants, to final concentrations of 200 units/ml and 0.5%, respectively. The supernatants were dialyzed at room temperature for 3 hr against 3–5 liters of 0.1% $NaDodSO_4$, lyophilized, and dissolved in 100 μl

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Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

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Table 1. Frequency of recoverable B lymphoma–myeloma hybrids

Fusion*		No. of clones recovered†	Mean no. of clones per well	No. of clones per 10^6 lymphoma cells
Lymphoma	Myeloma			
L10A	4T00.1L1	11	0.25	1.0
L10A	4T00.1	2	0.02	0.8
K46	4T00.1	3	0.03	0.6
X16	4T00.1L1	>48	>1.0	>9.0
X16	4T00.1	>72	>1.0	>13.0
L10A	—	0	<0.04	<0.2
K46	—	0	<0.02	<0.2
—	4T00.1L1	0	<0.04	<0.1

* The cells were fused by the polyethylene glycol method (15). Between 2.5 and 12 × 10^6 lymphoma cells were fused with a similar number of myeloma cells in the various experiments. Hybrids were selected in growth medium containing hypoxanthine, aminopterin, thymidine, and ouabain.

† Clones that grew to macroscopic size were scored as positive.

of 4% $NaDodSO_4$/10% (wt/vol) glycerol/0.1 M Tris·HCl, pH 6.8/0.02% bromophenol blue. The samples were then boiled for 1 min, and 20-μl portions were subjected to polyacrylamide gel electrophoresis on 3–20% gradient slab gels (17, 18). Electrophoresis of individual Ig chains were performed on 15% gels after the samples were boiled in the presence of 0.3 M 2-mercaptoethanol for 1 min. The gels were stained, dried, and placed in contact with XR-2 Kodak film for autoradiography. Labeled secreted Ig was precipitated by anti-Ig prior to electrophoresis as described (19).

Other Methods. Chromosomes were analyzed after treatment of the cells with Colcemid (0.04 μg/ml) for 20 hr according to the method of Dev *et al.* (20). Supernatants derived from growing clones were tested for the presence of various classes of Ig by double diffusion in agar (21) and radial immunodiffusion techniques (22). The presence of membrane and cytoplasmic Ig was examined by staining with affinity-purified fluorescein-labeled class-specific antibodies (23, 24).

RESULTS

Frequency of Recoverable Hybrid Clones. The clones that grew in the selective medium were composed of large cells, similar in size to the parent myeloma cells, in contrast to the smaller lymphoma cells.

Table 1 summarizes the results of several fusion experiments done with three B lymphomas and two myeloma cell lines. The frequency of recoverable clones varied between 1 and >10 hybrid clones per 10^6 input lymphoma cells, depending on the B lymphoma used for fusion. Low frequencies (up to 1 clone per 10^6 cells) were obtained with L10A and K46 lymphomas. With the X16 lymphoma multiple clones (3 to 10) were observed in all wells of the plates seeded after fusion. Thus, the frequency of recoverable clones obtained with the X16 lymphoma was at least 10-fold higher than that with the other B lymphomas tested.

Similar frequencies of hybrid clones were obtained when B lymphomas were fused to either the IgG_{2b} myeloma (clone 4T00.1) or to the κ chain-producing myeloma variant (clone 4T00.1L1). Hybrids were not detected in control experiments in which each of the parental cell lines was fused to itself.

Ig Secretion by Hybrids. Supernatants from clones grown to confluence were tested for the presence of Ig by double diffusion in agar. According to the Poisson distribution, it was assumed that single clones were present in wells when <30% of the wells in a Linbro plate contained growing cells. Fig. 1 shows an example of analysis made on the supernatants of four different hybrid clones, derived from fusion of L10A lymphoma with 4T00.1L1 myeloma and from fusion of X16 with 4T00.1 myeloma. In contrast to the parent lymphoma cell lines which did not secrete detectable amounts of Ig, all four clones

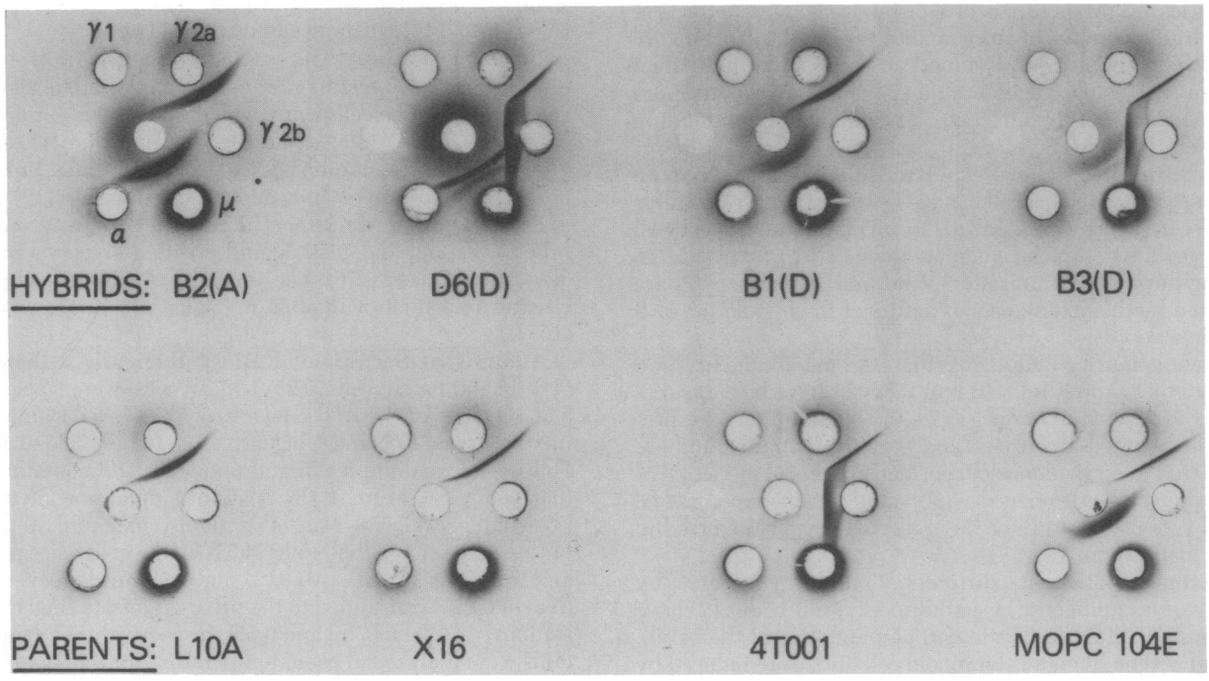

FIG. 1. Ouchterlony analysis of Ig secretion by four hybrid clones. Cultured medium from cloned hybrid cells was concentrated 2–4 times and applied to the central wells. Class-specific antisera were placed in the outer wells as shown for clone B2(A). The parent lymphoma cell lines L10A and X16 did not secrete detectable amounts of Ig. 4T00.1 myeloma secreted IgG_{2b}. IgM-secreting MOPC 104E myeloma was included as a control. The precipitation line between the anti-γ_{2a} and anti-γ_{2b} antisera is due to cross absorption of these antisera with purified myeloma proteins.

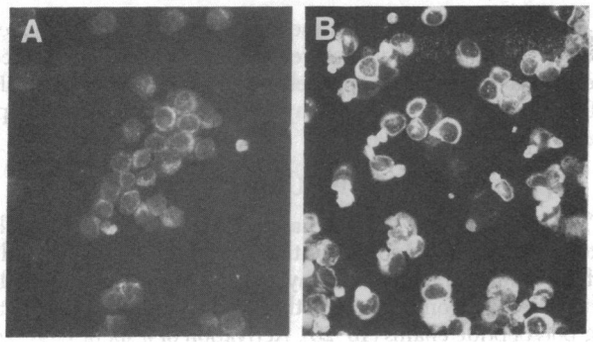

FIG. 2. Cytoplasmic IgM in hybrid cells. Cytocentrifuged preparations of cells were fixed and then stained with fluorescein-labeled affinity-purified anti-μ serum (24). (*A*) L10A lymphoma cells. (*B*) B2(A) hybrid cells (L10A–4T00.1L1). (×160.)

secreted IgM and two secreted both IgM and IgG_{2b}. No other Ig class (IgG_1, IgG_{2a}, or IgA) was detected on repeated examinations of the supernatant fluids derived from more than 100 wells containing growing hybrid cells.

Table 2 summarizes the analysis of the secreted Ig derived from individual clones, as defined above, and shows that (*i*) significant numbers of clones secreted IgM; (*ii*) when the parent myeloma secreted IgG_{2b}, many of the hybrids secreted both IgG_{2b} and IgM, and (*iii*) secretion of the γ_{2b} heavy chain was not detected when the parent myeloma was a variant that had lost the ability to produce γ_{2b}.

Chromosomal Analysis of Hybrid Clones. The hybrid nature of the growing clones was confirmed by chromosomal analysis done on cells from individual clones or on cells derived from wells containing multiple clones. The mean number of chromosomes (±SE) in L10A and X16 lymphomas was 68 ± 3 and 70 ± 4, respectively. The mean number of chromosomes in the 4T00.1 and 4T00.1L1 myeloma clones was 60 ± 3 and 64 ± 2. On the other hand, the mean number of chromosomes in the hybrid clones exceeded that of the parental cell lines and ranged from 82 ± 2 to 123 ± 3. Clearly, there was a considerable loss of chromosomes in the hybrids because they contained less than the sum of chromosomes of their parental cell lines.

Cytoplasmic and Membrane Ig in Hybrid Clones. Many of the hybrid clones revealed cytoplasmic IgM by immunofluorescence. Fig. 2 shows the cytoplasmic staining obtained with a hybrid clone compared to the "membrane" staining pattern obtained with the parent L10A lymphoma. The pattern of cytoplasmic staining revealed in the hybrids was indistinguishable from that obtained with myelomas (not shown). Hybrids that secreted both IgM and IgG_{2b} also contained both of these Ig classes in the cytoplasm. Immunofluorescence of viable cells revealed surface IgM on the B lymphoma cells and no Ig on the surface of the myeloma cells. Initially, the hybrid cells showed variable amounts of fluorescence. However, no staining was revealed in subsequent experiments. It was not clear whether the membrane staining initially observed was due to surface IgM. Membrane staining was not found when anti-γ_{2b} antiserum was used, even when the hybrids produced and secreted both IgG2b and IgM.

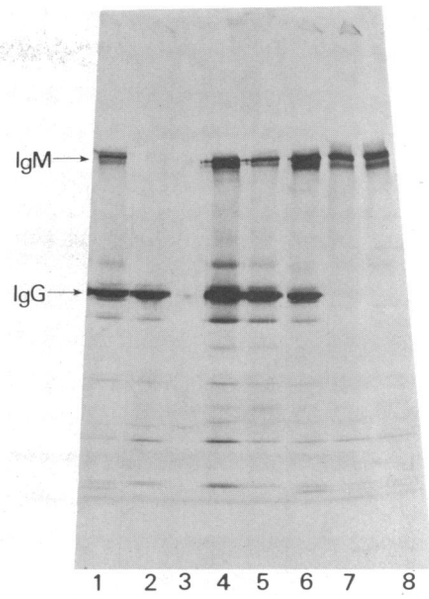

FIG. 3. $NaDodSO_4$/polyacrylamide gel electrophoresis of nonreduced Ig secreted by the hybrids. ^{14}C-Labeled amino acids were incorporated into secreted proteins of the parental and hybrid cells. The secreted proteins were treated with $NaDodSO_4$ and electrophoresed without reduction on 3–20% gradient gels with a 3% spacer gel. Purified IgM (TEPC 183) and IgG_1 (MOPC 300) were used as markers (arrows). Lanes: 1, clone D6(D), hybrid of L10A and 4T00.1L1; 2, parent myeloma 4T00.1; 3, parent lymphoma L10A. Lanes 4 and 5, clones A6 and G8, hybrids of K46 and 4T00.1; 6, clone B2(3), hybrid of X16 and 4T00.1L1; 7 and 8, clones C5(8) and C6(8), hybrids of X16 and 4T00.1L1.

B Lymphoma–Myeloma Hybrids Secrete Primarily Pentameric IgM. Gel electrophoresis of biosynthetically labeled and secreted IgM is shown in Figs. 3 and 4. Hybrid cells secreted relatively large quantities of IgM which was present mainly in its pentameric form and appeared as a double band (Fig. 3). Upon reduction of the disulfide bonds, both μ and light chains were evident (Fig. 4). The μ chains were similar in size to those of MOPC 104E and TEPC 183 IgM markers which were electrophoresed in parallel. Clones derived from fusion of lymphomas with the IgG_{2b}-producing myeloma secreted both IgG_{2b} and IgM. Virtually no secretion of IgM by the parental lymphoma L10A (Figs. 3 and 4) or X16 and K46 (not shown) was detected. The γ_{2b} chain of the MPC-11 myeloma parent cells revealed a double band that was also obvious in the hybrids (Fig. 4). Secretion of IgM by the hybrids was confirmed by gel electrophoresis of the specific immunoprecipitates obtained from the secreted material (not shown). Upon prolonged exposures of the autoradiographs, the specific immunoprecipitated secreted material of the X16 and the L10A lymphomas revealed a light chain band having a faster mobility than that of the MPC-11 myeloma. In the hybrids, the two parental light chain bands were evident, indicating that the light chains of both parents were expressed.

Kinetics and Amounts of Ig Secretion by Hybrids. The

Table 2. Immunoglobulin polypeptide chains secreted by B lymphoma–myeloma hybrids

Fusion		Ig secreted	No. of clones	No. of clones secreting per total clones		
Lymphoma	Myeloma	by myeloma	analyzed*	μ	γ_{2b}	$\mu + \gamma_{2b}$
L10A	4T00.1L1	κ	9	3/9	0/9	0/9
X16	4T00.1	γ_{2b}, κ	6	3/6	5/6	2/6
K46	4T00.1	γ_{2b}, κ	3	3/3	3/3	3/3

* Hybrid cells that grew in less than 30% of the wells were considered to be clones.

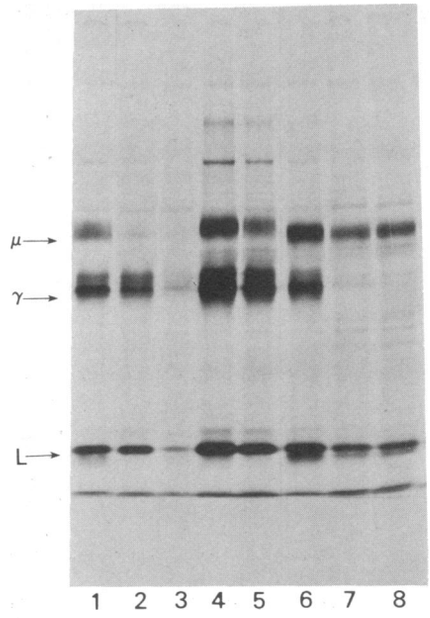

FIG. 4. $NaDodSO_4$/polyacrylamide gel electrophoresis of reduced Ig secreted by the hybrids. Samples and markers were similar to those shown in Fig. 3; electrophoresis was on 15% polyacrylamide slab gels with a 5% spacer gel.

amount of Ig secreted by the cloned hybrid cells was measured by radial immunodiffusion in agar. The validity of comparing the amounts of IgM secreted determined this way depends on the physical form of the secreted IgM—i.e., whether it is secreted in its monomeric (8 S) or pentameric (19 S) form. Because analysis of biosynthetically labeled IgM showed that most of the IgM is secreted as 19S in both hybrids and in an IgM producing myelomas (see above), it was possible to determine (*i*) the absolute amount of IgM secreted by the hybrids and (*ii*) to compare this amount to that secreted by an IgM-secreting myeloma. Fig. 5 shows the kinetics of secretion of IgM and IgG by three hybrid clones and the corresponding parental cells. The amount of IgM secreted by the hybrids exceeded the amount secreted by the IgM-producing MOPC 104E. The L10A lymphoma and the MPC-11 myeloma did not secrete measurable quantities of IgM. One of the clones that secreted both IgM and IgG secreted the latter in amounts exceeding that of an MPC-11 IgG_{2b}-producing myeloma cell line.

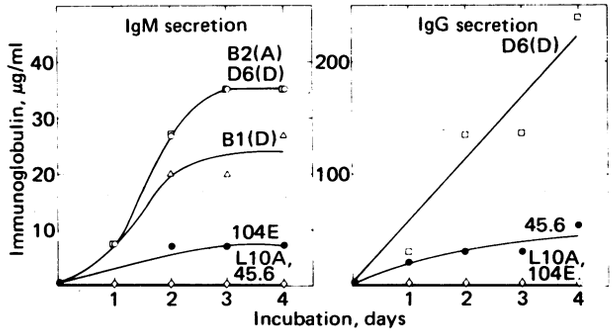

FIG. 5. Kinetics and amounts of Ig secretion by parents and hybrid cell lines. The cells were washed twice in the growth medium, seeded at a concentration of 0.8×10^6 cells per ml in plastic dishes (3.0 mm diameter), and incubated for 4 days. Samples (0.25 ml) were withdrawn and analyzed for IgM and IgG content by radial immunodiffusion. Purified IgM (TEPC 183) and IgG (MOPC 300) myeloma proteins were used as standards. L10A, B lymphoma; 45.6, a cloned cell line of MPC-11 myeloma; clones B1(D) and D6(D), hybrids of X16 and 4T00.1; clone B2(A), hybrid of L10A and 4T00.1L1; MOPC 104E, IgM-secreting myeloma cell line.

From the initial slopes of the kinetic curves, it can be calculated that the hybrid cells secreted about 20 pg of IgM per cell per day and 40 pg of IgG per cell per day. The values for MOPC 104E myeloma and MPC-11 myeloma were <10 and 15 pg per cell per day, respectively.

DISCUSSION

The technique of cell fusion has recently been used as a tool to study the regulation of Ig synthesis in lymphoid cells. Studies of myeloma–myeloma cell hybrids have demonstrated that these hybrids continue to synthesize the parental types of the Ig polypeptide chains (15, 25). Activation of a silent Ig gene or modification of the expression of an already activated Ig gene was never observed in these hybrids. In addition myeloma–myeloma hybrids did not reexpress the heavy chain of a nonproducer myeloma variant (15, 26).

Similar to the results obtained with myeloma–myeloma hybrids, the fusion of a B lymphoma with myeloma cells, as described in the present work, resulted in no alteration in the expression of the Ig genes contributed by the parent myeloma. Thus, the hybrids continue to express the γ_{2b} heavy chain produced by the myeloma and they fail to synthesize the γ_{2b} chain of the nonproducer variant. On the other hand, the hybrids exhibited apparent changes in the expression of the IgM gene products seemingly contributed by the parent lymphoma cells. Whereas the parent lymphoma cells synthesize only minute quantities of IgM which is displayed on the cell surface and was not secreted, the B lymphoma–myeloma hybrids synthesize considerably larger quantities of IgM which are also secreted into the extracellular medium. These findings can be interpreted according to the following possibilities.

(*i*) The myeloma parent cell provides the necessary machinery for the synthesis and secretion of large quantities of IgM. Accordingly, the B lymphoma cells may contain a large pool of IgM mRNA molecules that are translated at a very slow rate. In the hybrids, these specific mRNA molecules are efficiently translated to form the IgM polypeptide chains, using the rough endoplasmic reticulum contributed by the myeloma cells. Similarly, the Golgi apparatus of the parent myeloma may enable the secretion of the IgM produced.

(*ii*) The lymphoma cells may be truly induced to differentiate and form mature IgM-secreting cells. Induction may be caused by the presence of activator, competition for a limited quantity of a repressor, or the loss of chromosomes carrying a repressor gene from the hybrids (27). This possibility seems unlikely in view of the observed high frequency of IgM secretion among the hybrids. The simplest explanation is that induction is caused by an activator contributed by the myeloma cell. This activator is not specific for Ig class because induction of enhanced synthesis of IgM occurs by fusion with IgG-producing myeloma.

(*iii*) The explanation may be a combination of the above possibilities in which, for example, amplified synthesis of IgM is induced and IgM secretion is taking place by the Golgi apparatus provided by myeloma cells.

The possibility that the genome of the myeloma cell was induced to form IgM is unlikely because IgM synthesis was not induced in myeloma–myeloma (15, 25), T cell–myeloma (28), as macrophage–myeloma (29) cell hybrids. Further studies are needed to decide among these alternative possibilities. The "induction" of IgM secretion in B lymphoma–myeloma hybrids seems to be due to cell fusion because control experiments in which B lymphoma cells were fused to themselves did not result in the formation of viable hybrids. Also, repeated attempts to induce the lymphoma cells with the aid of lipopolysaccharide and dextran sulfate mitogens as well as dimethyl sulfoxide did

not result in IgM secretion as measured by a reverse plaque assay (30) (R. Laskov and Y. Rosenberg, unpublished results). It is also unlikely that the hybrids were derived from a small subpopulation of B lymphoma cells that already secreted IgM because no reverse plaques were detected in repeated examinations of the various B lymphoma cell lines (unpublished results).

Results somewhat similar to ours were recently obtained when murine myeloma cells were fused with lymphocytes derived from human patients with chronic lymphatic leukemias or other lymphoid neoplasias (31). In that case, the lymphocytes were not derived from cultured cell lines and might have contained a small percentage of normal Ig-secreting cells. Nevertheless, the hybrids seemed to be derived from the monoclonal leukemic cell population because the secreted IgM had the same type of light chains as that present on the surface of the parent leukemic cells (31).

B lymphocytes differentiate to form not only IgM-secreting but also IgG-secreting cells (32). It is not clear why the B lymphoma–myeloma hybrids were not also induced to secrete IgG. One possibility is that different precursors exist for IgM- and IgG-secreting cells and that the particular B-cell lines used for fusion in the present work represent the malignant cell counterparts of the precursors of IgM-secreting cells. Alternatively, an additional stimulus may be required to induce the maturation to an IgG-secreting cell (33), and this stimulus was lacking under the experimental conditions used.

In the fusion experiments originally done by Kohler and Milstein (34), myeloma cells were fused to immunized spleen cells, and hybrids forming specific antibodies were obtained. Based on the results of the myeloma–myeloma fusion experiments (15, 25) it was assumed that the antibody-secreting hybrids were due to fusion of myeloma cells with mature antibody-secreting cells ("like–like" fusion) (34). The results of the present study suggest, however, that myeloma cells may also fuse and induce antibody formation in a less-mature normal B lymphocyte.

Hybrids between B lymphoma and myeloma cells may prove to be a useful tool to answer the question whether the secreted IgM is structurally identical to the membrane IgM and also whether the somatic diversification of the antibody-combining sites is taking place in IgM-bearing nonsecreting B cells.

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1. Williamson, A. R. (1975) *Transplant. Rev.* **23,** 258–265.
2. Nossal, G. J. V., Szenberg, A., Ada, G. L. & Austin, C. M. (1964) *J. Exp. Med.* **119,** 485–502.
3. Pernis, B., Forni, L. & Amante, L. (1971) *Ann. N. Y. Acad. Sci.* **190,** 420–429.
4. Nossal, G. J. V., Warner, N. L. & Lewis, H. (1971) *Cell. Immunol.* **2,** 41–53.
5. Gearhart, P. J., Sigal, N. H. & Klinman, N. R. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1707–1711.
6. Wabl, M. R., Forni, L. & Loor, F. (1978) *Science* **199,** 1078–1080.
7. Warner, N. L., Harris, A. W. & Gutman, G. A. (1975) in *Membrane Receptors on Lymphocytes,* eds. Seligman, M., Preudhomme, J. L. & Kourilsky, F. M. (Elsevier, New York) p. 203.
8. Premkumar, E., Potter, M., Singer, P. A. & Sklar, M. D. (1975) *Cell* **6,** 149–159.
9. Bergman, Y. & Haimovich, J. (1977) *Eur. J. Immunol.* **7,** 413–417.
10. Slavin, S. & Strober, S. (1978) *Nature (London)* **272,** 624–626.
11. Maino, V. C., Kurnick, J. T., Kubo, R. T. & Grey, H. M. (1977) *J. Immunol.* **118,** 742–748.
12. Weimann, B. J. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41,** 163–164.
13. Kishimoto, T., Hirano, T., Kuritani, T., Yamamura, Y., Ralph, P. & Good, R. A. (1978) *Nature (London)* **271,** 756–758.
14. Margulies, D. H., Kuehl, W. M. & Scharff, M. D. (1976) *Cell* **8,** 405–415.
15. Margulies, D. H., Cieplinsky, W., Dharmgrongartama, B., Gefter, M. L., Morrison, S. L., Kelly, T. & Scharff, M. D. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41,** 781–791.
16. Morse, H. C., III, Pumphrey, J. G., Potter, M. & Asofsky, R. (1976) *J. Immunol.* **117,** 541–547.
17. Maizel, J. V. (1971) in *Methods in Virology,* ed. Maramorosch, K. & Koprowski, H. (Academic, New York), Vol. 5, pp. 179–246.
18. Flemming, H. & Haselkorn, R. (1974) *Cell* **3,** 159–170.
19. Laskov, R. & Scharff, M. D. (1970) *J. Exp. Med.* **131,** 515–541.
20. Dev, V. G., Miller, D. A., Allderdice, P. W. & Miller, O. J. (1972) *Exp. Cell. Res.* **73,** 259–262.
21. Ouchterlony, O. (1949) *Acta Pathol. Microbiol. Scand.* **26,** 507–515.
22. Mancini, G., Carbonara, A. O. & Heremans, J. F. (1965) *Immumochemistry* **2,** 235–254.
23. Pernis, B., Forni, L. & Amante, L. (1970) *J. Exp. Med.* **132,** 1001–1018.
24. Morse, H. C., III, Neiders, M. E., Lieberman, R., Lawton, A. R., III & Asofsky, R. (1977) *J. Immunol.* **118,** 1682–1689.
25. Milstein, C., Adetugbo, R., Cowan, N. J., Kohler, G., Secher, D. S. & Wilde, C. D. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41,** 793–803.
26. Kohler, G., Howe, S. C. & Milstein, C. (1976) *Eur. J. Immunol.* **6,** 292–295.
27. Davis, F. M. & Adelberg, E. A. (1973) *Bacteriol. Rev.* **37,** 197–214.
28. Mohit, B. & Fan, K. (1971) *Science* **171,** 75–77.
29. Yelton, D. E., Diamond, B. A., Kwan, S.-P. & Scharff, M. D. (1978) *Curr. Top. Microbiol. Immunol.* **81,** 1–7.
30. Molinaro, G. A. & Dray, S. (1974) *Nature (London)* **248,** 515–517.
31. Levy, R. & Dilley, J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2411–2415.
32. Zauderer, M. & Askonas, B. A. (1976) *Nature (London)* **260,** 611–613.
33. Bretscher, P. A. & Cohn, M. (1970) *Science* **169,** 1042–1049.
34. Kohler, G. & Milstein, C. (1976) *Eur. J. Immunol.* **6,** 511–519.
35. Laskov, R., Kim, K. J. & Asofsky, R. (1978) *Curr. Top. Microbiol. Immunol.* **81,** 173–175.