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Immunology

Assembly and secretion of pentameric IgM in a fusion between a nonsecreting B cell lymphoma and an IgG-secreting plasmacytoma

(J chain/somatic cell hybridization/genetic complementation/B cell differentiation)

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ABSTRACT **A new immunoglobulin product has been obtained by hybridization of mouse cell lines arrested at different stages in B lymphocyte development. One line was shown to have the characteristics of an undifferentiated B cell that synthesizes monomeric IgM as a membrane receptor but does not express J chain. The second line was representative of a fully differentiated plasma cell synthesizing large amounts of IgG and J chain, but no IgM. Fusion of the two cell types yielded independent hybrid clones that secreted pentameric IgM, normally the first product of antigen-driven B cell differentiation. Analyses of the hybrid cells indicated that the IgM was expressed as a result of complementation between the synthetic capacities of the parental lines. The hybrid cells synthesized both monomeric IgM and J chain and assembled these components into a pentameric molecule with the expected stoichiometry of one J chain per five monomeric units. These findings provided further evidence that the induction of B cell differentiation includes a signal for *de novo* synthesis of the J chain. Moreover, the complementation achieved by this hybridization provides a system for identifying other intracellular events in B cell differentiation to IgM secretion.**

The process by which cells differentiate to express a limited number of specific gene products is one of the major unresolved problems in eukaryote biology. The B lymphocytes of the immune system provide a particularly suitable model for attacking this problem. First, the differentiation of B cells is not completed at birth, and the B cells that populate peripheral tissue in the adult are capable of further differentiation in response to the appropriate antigenic stimulus (1). Second, differentiation to antibody secretion can be achieved experimentally by exposing B cells to specific antigen or to nonspecific mitogenic inducers under controlled conditions *in vivo* or *in vitro* (1–3). Finally, cells have been transformed at various stages in the differentiative pathway and the resulting cell lines provide homogeneous populations of cells arrested at a given state (4–7).

Because of these advantages, considerable progress has been made in understanding the stimulatory events at the B cell surface and the subsequent appearance of secreted immunoglobulin products. Differentiation has been shown to be initiated by the interaction of macrophage-bound antigen with monomeric Ig surface receptors (8). The immunocompetent B cell responds to the antigen signal by assembling and secreting pentameric IgM (9). This process involves both the covalent assembly of monomeric IgM subunits and the incorporation of a third polypeptide, the J chain (10). Upon continued antigen stimulus a switchover in immunoglobulin production occurs, to monomeric IgG, monomeric IgE, or monomeric and polydisperse IgA. These later responses require not only an antigen signal but also the cooperation of helper T cells, which interact with B cells through cell surface components or through soluble factors (11).

Only limited progress has been made, however, in identifying the intracellular events in B cell differentiation. As in other eukaryotic systems, the investigations have been hampered by lack of adequate methods for selecting mutants defective in differentiation and for characterizing the genetic defect. Recent advances in somatic cell hybridization provide a means to overcome these difficulties (12). A number of studies have shown that the fusion of two closely related cells, each lacking a particular phenotype, can give hybrids in which the phenotype is expressed by complementation (13–15). The present fusion studies were undertaken with the aim of achieving secreted antibody by complementation and using such a system to analyze internal signal transmission in B cells. This paper reports the results obtained when a line of undifferentiated B lymphoma cells was fused with a line of fully differentiated plasma cells secreting IgG.

MATERIALS AND METHODS

Cell Lines. A thioguanine-resistant clone of the IgG2b-secreting BALB/c plasmacytoma, MPC 11 (45.6 TG1.7), was obtained from M. Scharff (Albert Einstein College of Medicine, Bronx, NY) (16). The ouabain-resistance marker (resistant to 1 mM ouabain) was introduced into this line at the Salk Institute. The (NZB × BALB/c)F_1 B lymphoma cell line, WEHI 231, was obtained from Noel Warner (University of New Mexico, Albuquerque, NM) (6). All cell lines and hybrids were grown in a culture medium of Dulbecco's modified Eagle's medium (DME) plus 10% fetal calf serum. WEHI 231 cells required the addition of 50 μM 2-mercaptoethanol to the medium for growth, but MPC 11 cells and hybrids obtained by fusion of MPC 11 and WEHI 231 cells lacked this requirement.

Fusion Procedure. MPC 11 cells were grown in complete medium containing 10 μM thioguanine and 1 mM ouabain for 1 week before hybridization to select against revertants that might have accumulated in the population; 10^7 cells were then added to an equal number of WEHI 231 cells and the pelleted mixture was treated with 40% (wt/vol) polyethylene glycol (1500 daltons, BDH) in DME as described (17). After overnight culture the cells were distributed in Falcon 3040 microwell trays (2×10^5 cells per well) in a selection medium containing DME, 10% fetal calf serum, HAT ingredients (100 μM hypoxanthine/0.4 μM aminopterin/16 μM thymidine), and 1 mM ouabain. From 21 potential hybrid colonies in microwells two were selected, cloned, and analyzed in this study. The DNA contents of parental and hybrid cells were determined by the method of Crissman and Tobey (18). Chromosome numbers were estimated with BALB/c thymocytes (40 chromosomes) as the standard.

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Abbreviations: DME, Dulbecco's modified Eagle's medium; NaDodSO$_4$, sodium dodecyl sulfate.

Preparation of Antisera. The anti-immunoglobulin and anti-μ chain reagents used have been described (19). The antibody used for IgM radioimmunoassay was prepared against mouse μ chains isolated by mild reduction and alkylation of myeloma IgM and filtration through a Sephadex G-200 column equilibrated with 1 M acetic acid and 6 M urea. The IgM products of HPC 76 ($\kappa\mu$) and TEPC 183 ($\kappa\mu$) tumors were the gifts of Noel Warner and L. Herzenberg (Stanford University Medical School, Stanford, CA), respectively. Antisera were raised in rabbits by injection of the μ chains emulsified in complete Freund's adjuvant, and the IgG fraction of the antisera was isolated by ammonium sulfate precipitation and DEAE-cellulose chromatography. Anti-J chain antibody was prepared against mouse J chain isolated from MOPC 315 IgA ($\lambda\alpha$) by the method of Mosmann and Baumal (20) and further purified by preparative gel electrophoresis (21). The MOPC 315 plasmacytoma and some partially purified J chain were kindly provided by E. Simms (Washington University Medical School, St. Louis, MO). The antiserum was raised in a goat injected with J chain in complete Freund's adjuvant and the IgG fraction was obtained as described above.

Analysis of Immunoglobulin Products. Labeling of surface immunoglobulin with ^{125}I (New England Nuclear) was performed by using the procedure of Dennert and Raschke (19). Biosynthetic labeling was accomplished by culturing cells at densities of 1–1.5 $\times 10^6$ per ml for 4 hr in DME/10% fetal calf serum or in methionine-free RPMI medium/10% fetal calf serum containing 20–250 μCi of [^{35}S]methionine (Amersham or New England Nuclear) per ml (1 Ci = 3.7 $\times 10^{10}$ becquerels). Cells were lysed with 1% Nonidet P40 in phosphate-buffered saline containing either 1 mM phenylmethylsulfonyl fluoride (Sigma) and 1000 units of Trasylol (Sigma) per ml or 0.1 M iodoacetamide, and the remaining insoluble material was removed by high-speed centrifugation. Labeled IgG2b was isolated directly from the cell lysates and culture supernatants by absorption to formalin-fixed, heat-killed *Staphylococcus aureus* (22); labeled IgM and free light chain were removed by complexing with specific antisera and precipitation with goat anti-rabbit IgG antiserum or *S. aureus*. For analysis of immunoglobulin size, the washed precipitates were solubilized in 2% sodium dodecyl sulfate ($NaDodSO_4$) and 10 M urea and electrophoresed on 2% polyacrylamide/$NaDodSO_4$/agarose gels (23). The gels were frozen, sliced, and solubilized in NCS (Amersham), scintillation fluid was added, and radioactivity was measured in a Beckman scintillation counter, model LS100C. For determination of immunoglobulin chain content, the washed precipitates were dissolved by boiling in 2% $NaDodSO_4$/2% (vol/vol) mercaptoethanol and electrophoresed on polyacrylamide slab gels. The gels were dried and analyzed by radioautography (19). Alternatively, the washed precipitates were dissolved in 10 M urea/100 mM dithioerythritol and electrophoresed on alkaline urea/4.15% polyacrylamide gels (24), and the distribution of radiolabeled molecules was determined as described above. Tryptic peptide patterns of labeled immunoglobulin chains isolated from dried acrylamide gels were prepared according to Hutchinson *et al.* (25). Peptides containing [^{35}S]methionine were observed by fluorography using 2,5-diphenyloxazole dissolved in 2-methylnaphthalene as described by Bonner and Stedman (26).

Radioimmunoassays. For measurements of cellular IgM content, monomeric IgM was prepared from MOPC 104E ($\lambda\mu$) and TEPC 183 pentameric IgM by reduction with 3 mM dithioerythritol and alkylation with iodoacetamide. The monomeric MOPC 104E preparation was radioiodinated (27) and used as the indicator protein, whereas the monomeric TEPC 183 preparation served as the reference standard. Because 104E IgM contains λ chains, this procedure eliminated the possibility that anti-light chain activity in the anti-μ antiserum might contribute to the measurements. For assays of secreted IgM, purified MOPC 104E pentamer was used as both the indicator and the reference protein, and for J chain assays highly purified MOPC 315 J chain served both roles. The assays were carried out by diluting cell lysates or supernatants in phosphate-buffered saline containing 0.45% Nonidet P40 and 1% bovine serum albumin and determining their inhibitory capacity. The details of the procedure are given by Brown and Koshland (28).

Table 1. Properties of the parental cell lines, WEHI 231 and MPC 11, and the hybrid clones, M × W 231.1a and M × W 231.2

Property	WEHI 231	MPC 11	Hybrids
Immunoglobulin			
Internal	IgM ($\mu_2\kappa_2$)	IgG ($\gamma 2b_2\kappa'_2$)	IgM ($\mu_2\kappa'_2$) IgG ($\gamma 2b_2\kappa'_2$)
Surface	IgM ($\mu_2\kappa_2$)	0	0
Secreted	0	IgG ($\gamma 2b_2\kappa'_2$) κ' light chain	IgM ($\mu_2\kappa'_2)_5$ IgG ($\gamma 2b_2\kappa'_2$) κ' light chain
J chain			
Internal	0	Free	Free
Secreted	0	0	IgM-bound
Cell diameter,* μm	11.6 ± 1.0	16.8 ± 3.1	20.0 ± 2.8
Chromosomes	45	75	155 (231.1a) 127 (231.2)

* Mean ± SD.

RESULTS

Characterization of Parental Lines. The properties of the parental cell lines used in these investigations are summarized in Table 1. The WEHI 231 B lymphoma cells have many characteristics in common with antigen-sensitive B cells. Their membranes contain monomeric IgM receptors, which are readily demonstrated by cell surface radioiodination (Fig. 1A).

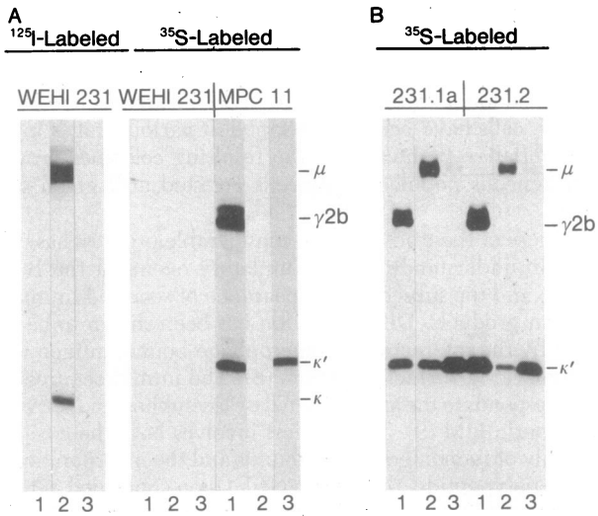

FIG. 1. $NaDodSO_4$/polyacrylamide gel electrophoresis of the immunoglobulin chains produced by the parental (*A*) and hybrid (*B*) cell lines. Lysates from ^{125}I-surface-labeled cells or supernatant fluids from [^{35}S]methionine-labeled cell cultures were treated first with *S. aureus* to precipitate IgG2b (track 1), second with anti-μ chain IgG antibody and *S. aureus* to precipitate IgM (track 2), and finally with anti-Ig serum and *S. aureus* to assay for any remaining free light chain (track 3). The precipitates were dissolved in 2% $NaDodSO_4$/2% mercaptoethanol and electrophoresed on slab gels.

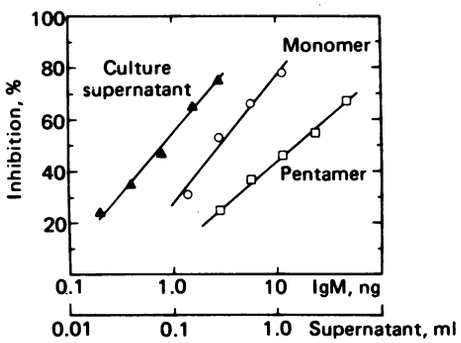

FIG. 2. Radioimmunoassay of IgM content of WEHI 231 culture fluid. WEHI 231 cells were cultured for 27 hr in 5 ml of DME/10% fetal calf serum at a density of 2.4×10^6 cells per ml. Aliquots of the supernatant fluids were tested for their ability to inhibit the binding of ^{125}I-labeled monomer IgM to anti-μ chain antibody. ▲, WEHI 231 supernatant fluid; ○, monomer IgM standard; □, pentamer IgM standard.

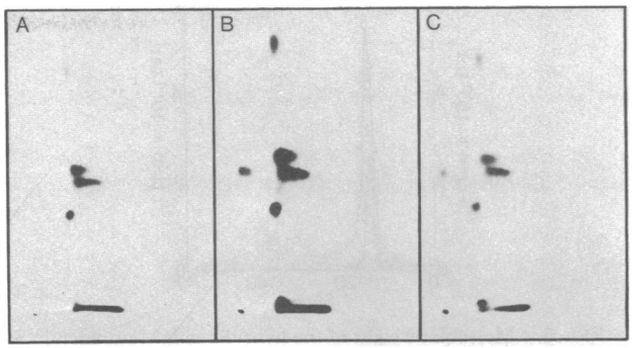

FIG. 4. [^{35}S]Methionine-containing tryptic peptides from the nonsecreted μ chain of WEHI 231 (*A*) and the secreted μ chains of the hybrids M × W 231.1a (*B*) and M × W 231.2 (*C*). [^{35}S]Methionine-labeled μ chains were eluted from polyacrylamide slab gels, digested with trypsin, and analyzed by electrophoresis (27 min at 1 kV, 50 mA, with cathode to the right) followed by chromatography on cellulose thin-layer chromatography plates.

The cells do not secrete detectable immunoglobulin as measured by slab gel electrophoresis and radioautography of cell supernatants after 4-hr labeling with [^{35}S]methionine (Fig. 1*A*). Although some extracellular IgM could be detected by radioimmunoassay of 27-hr supernatants from high-density cultures (Fig. 2), the amounts present were very small, equivalent to an output of 1800 molecules/hr per cell, and the slope of the inhibition curve indicated the IgM was monomeric. On the basis of these data, it would appear that the extracellular IgM consisted of receptor molecules released into the external medium. This conclusion was supported by the finding that the WEHI 231 cells do not synthesize the J chain, which is known to be required for pentamer IgM assembly (10). Analyses of cell lysates (Fig. 3*B*) showed that each cell contains less than 100 molecules of J chain.

The MPC 11 line, on the other hand, displays the characteristics of a fully differentiated plasma cell secreting IgG antibody. The cells produce an IgG protein of the γ2b subclass (Fig. 1*A*), and no IgM could be detected within the limits of sensitivity of the radioimmunoassay—i.e., 100 molecules per cell (Fig. 3*A*). The MPC 11 line does, however, contain appreciable quantities of J chain (Fig. 3*B*), implying a previous commitment to synthesis of pentameric IgM (29). The average level of 4×10^5 molecules per cell must reflect the net balance between the amount of J chain synthesized and the amount internally degraded because only traces of J chain were detected in the extracellular medium. The amounts found could be accounted for by the lysis of one in 500 cells per hour.

Both parental lines also exhibit abnormal features. The cells were shown to be larger than their normal counterparts and to contain extra DNA (Table 1). The WEHI 231 nucleus has the equivalent of 45 chromosomes compared to the normal mouse complement of 40, and the MPC 11 nucleus is essentially tetraploid. The WEHI 231 line also lacks membrane IgD and Ia antigens commonly found on B cells, because precipitation of surface-labeled cell extracts with anti-δ and anti-H-2^d (C57BL/6 anti-BALB/c) reagents, which precipitate IgD and Ia molecules from labeled BALB/c spleen cell extracts (17), failed to bring down these molecules (data not shown). In addition, immunoglobulin synthesis in both cell lines appears to be less regulated compared with their normal cell counterparts. The WEHI 231 cells were found to have an unusually high content of receptor IgM, averaging 5×10^5 molecules per cell (Fig. 3*A*). Even when this value was corrected for the larger size of the WEHI 231 cell, it represented a receptor density at least 3-fold higher than that of normal mouse B cells (30). The MPC 11 cells were found to synthesize and secrete a large excess of light chain. After sequential treatment of MPC 11 culture supernatants with anti-IgG and anti-IgM reagents, free light chain could be recovered in amounts comparable to the Ig-bound light chain that was secreted (Fig. 1*A*).

Characterization of MPC 11–WEHI 231 Hybrids Secreting IgM. When the products of WEHI 231 and MPC 11 cell fusion were examined, the two clones tested were found to secrete not only the IgG2b protein and the light chain of the MPC 11 parent but also pentameric IgM, a product characteristic of a cell intermediate in the differentiative pathway between an antigen-sensitive B cell and an IgG-secreting plasma cell. The properties of these hybrids, M × W 231.1a and M × W 231.2, are summarized in Table 1. Analyses of the secreted IgM indicated it was assembled from the WEHI 231 μ chain and the MPC 11 light chain. In maps of the [^{35}S]methionine-

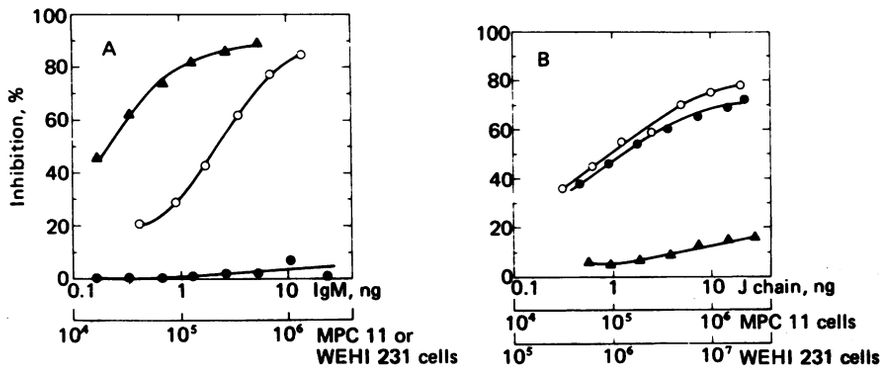

FIG. 3. Radioimmunoassays of IgM (*A*) and J chain (*B*) contents of parental cell lines. ●, MPC 11 lysates; ▲, WEHI 231 lysates; ○, mon IgM or J chain standard.

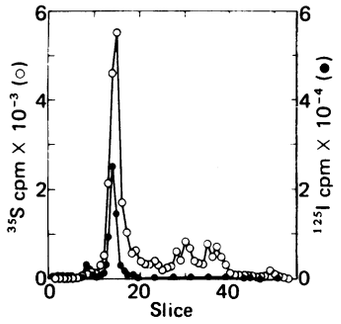

FIG. 5. Molecular weight of the hybridoma IgM product. [^{35}S]-Methionine-labeled supernatant fluid was treated with rabbit anti-μ chain IgG antibody and goat anti-rabbit IgG antiserum. The precipitate was dissolved in 10 M urea/2% NaDodSO$_4$ and electrophoresed on 2% polyacrylamide/NaDodSO$_4$/agarose gels. ○, Anti-μ chain precipitate of [^{35}S]methionine-labeled M × W 231.2 supernatant fluid; ●, ^{125}I-labeled pentamer IgM control.

containing tryptic peptides, the WEHI 231 and hybrid μ chains gave similar patterns (Fig. 4). At least seven of the expected eight methionine-containing peptides (31) were resolved in the secreted μ chain preparations and one less in the cellular μ chain preparation. This difference could have reflected the oligosaccharide content of the peptides because cellular and secreted μ chains have been shown to differ in the amounts of sialic acid, galactose, and fucose present (32). In one-dimensional slab gel electrophoresis the light chain of the hybrid IgM exhibited the slower mobility characteristic of the MPC 11 κ' chain (Fig. 1*B*). Thus, each hybrid appeared to express the heavy chain phenotype of both parents, and failed to express the light chain phenotype of the WEHI 231 parent. None of the resulting immunoglobulin products was incorporated into the cell membrane. Analysis of ^{125}I-surface-labeled cells showed that the hybrid clones, similar to the MPC 11 parent, contained only traces of membrane immunoglobulin (data not shown).

The secreted IgM was identified as a pentamer from measurements of its size and polypeptide composition. When IgM was immunoprecipitated from supernatants of [^{35}S]methionine-labeled hybrid cells and the precipitate was analyzed directly on 2.0% polyacrylamide/NaDodSO$_4$/agarose gels, the radioactive label appeared in a single major peak that comigrated with a pentamer control (Fig. 5). Moreover, when the precipitate was reduced and electrophoresed on alkaline urea/4.15% polyacrylamide gels, J chain, as well as μ and light chains, was recovered (Fig. 6). The yield of each chain was determined from these [^{35}S]methionine measurements and the known number of methionine residues per chain. The results

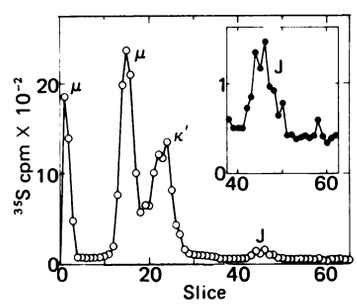

FIG. 6. Polypeptide composition of the hybridoma IgM product.]Methionine-labeled supernatant fluid of M × W 231.1a was ted with rabbit anti-μ chain IgG antibody and goat anti-rabbit antiserum. The precipitate was dissolved in 10 M urea/100 mM oerythritol and electrophoresed on 4.15% alkaline urea/polyac- ide gels. Under these conditions some of the μ chain remained zated (peak 1). Note 10-fold expanded vertical scale on *Inset*.

Table 2. Polypeptide composition of hybridoma IgM product

Chain (gel slice)	Total ^{35}S cpm	Total pmol recovered Met	Total pmol recovered Chain*	Chains per pentamer†
μ (1–18)	12,091	166.4	20.8	10
κ' (19–28)	7,126	98.0	24.5	11.8
J (42–50)	440	6.06	2.03	0.98

* Calculated from the [^{35}S]methionine specific activity of 3.3 μCi/μmol and values of 8 (31), 4 (33), and 3 (unpublished observation) for the methionine contents of mouse μ, κ', and J chains, respectively.

† Calculated by normalizing to a value of 10 for the number of μ chains per pentamer.

(Table 2) showed that the chain composition clearly matched that expected for pentameric IgM—i.e., 1 J chain for every 10 μ chains (10). The slightly high yield of light chain was probably due to the coprecipitation of a small amount of the free light chain present in the supernatant.

The two hybrid clones differed, however, in their output of pentameric IgM. As the radioimmunoassay data in Table 3 show, the M × W 231.1a cells were found to secrete 5 times as much pentameric IgM as the M × W 231.2 cells, 3.2×10^5 versus 6.6×10^4 molecules/hour per cell. This result could not be attributed to a secretion defect because a comparable difference was observed in the intracellular contents of the two hybrids. Analyses of the cell lysates (Table 3) showed that the M × W 231.1a cells contained 4- to 5-fold more monomeric IgM and J chain than the M × W 231.2 cells. Moreover, the analyses indicated that the hybrid IgM assembly was normal. In each case the ratio of intracellular monomer to J chain and the ratio of intracellular components to the secreted product were similar to those obtained previously for normal IgM-secreting B cells. In view of these data, it would appear that the pentameric IgM output was determined by the amounts of μ and J chain synthesized and thus some parameter regulating the translation or transcription of μ and J chain was differentially active in the two clones.

DISCUSSION

The hybridization studies described here show that a new immunoglobulin product can be obtained by the fusion of mouse cell lines representing different stages in the life history of a B lymphocyte. One parental line had the properties of an antigen-sensitive B cell, which synthesizes monomeric IgM only for deposition in the plasma membrane. The second parental line had the properties of a plasma cell, which no longer synthesizes IgM but is terminally committed to IgG secretion. Fusion of these lines yielded two hybrid clones that continued to secrete IgG, but that also secreted pentameric IgM, a product characteristic of an intermediate cell in the differentiative pathway. Induction of IgM secretion has also been reported for

Table 3. Cellular levels and secretion rates on J chain and IgM in M × W 231.1a and M × W 231.2

Molecule	M × W 231.1a	M × W 231.2
Cellular	Molecules/cell	
IgM monomer	1.5×10^6	2.9×10^5
Free J chain	1.0×10^6	3.7×10^5
Secreted	Molecules/hr per cell	
IgM pentamer	3.2×10^5	6.6×10^4
IgM-bound J chain*	3.2×10^5	6.6×10^4

* Calculated from the known stoichiometry of one J chain per pentamer of IgM.

the interspecies hybrids produced by fusing human B leukemia cells with mouse myeloma cells secreting IgG (34).

The most reasonable explanation of these findings is a direct complementation between the synthetic capacities of the parental cells. It seemed likely that if the plasmacytoma had a previous history of IgM secretion, it could continue to express gene products required for pentameric IgM assembly, and thus fusion with an undifferentiated B lymphoma synthesizing monomeric IgM would yield a pentameric product. Evidence to support this mechanism was obtained by the J chain radioimmunoassays of the parental and hybrid cells. The analyses showed that the WEHI 231 B lymphoma did not express J chain within the limits of detection of the method, 100 molecules per cell. The MPC 11 plasmacytoma synthesized but did not secrete J chain, whereas the hybrid clones synthesized J chain and exported it as a covalently linked component in the secreted pentamer molecule. These results indicated that the capacity of the MPC 11 genome to synthesize J chain was one of the factors that promoted the assembly of the WEHI 231 μ chain into pentamer IgM. It is possible, however, that the complementation involved a more complex mechanism. Thus, the MPC 11 genome could have provided a component that activated J chain synthesis by the WEHI 231 genome. Although this possibility seems less likely, it cannot be rigorously excluded until markers are available to discriminate between the J chain products of the parental lines.

Whatever the origin of the hybridoma J chain, the analyses clearly indicate that J chain synthesis was essential for the fusion-induced secretion of pentameric IgM. Similar results have been obtained in studies of mitogen stimulation of normal B cells. J chain has been shown to be absent in the unstimulated B lymphocyte; after exposure to mitogen, however, J chain synthesis could be detected and pentameric IgM assembly and secretion ensued (35, 36). The hybridization data, therefore, provide additional evidence that induction of B cell differentiation includes a signal for *de novo* synthesis of J chain.

Other, as yet unidentified, synthetic capacities of the MPC 11 genome may also be required for the fusion-induced secretion of IgM. Because the cell lines used in this study represent pure populations of cells arrested at various differentiative stages, the system provides a powerful tool for identifying such critical gene products. By extending the comparison of the parental and hybrid cells, it should be possible to determine whether components other than the J chain are synthesized by the MPC 11 parent and the M × W hybrids but are not expressed by the WEHI 231 parent. The absence of any such component from non-IgM-secreting M × W fused cells would then strongly support its participation in the complementation by the MPC 11 genome and, by analogy, its role in normal B cell differentiation. Likely candidates would include gene products required to effect intracellular IgM polymerization, such as an enzyme that specifically catalyzes disulfide bond formation between monomeric IgM subunits and J chain (36). Moreover, by use of this system it might be possible to identify the gene products responsible for activating the synthesis of J chain and any other intermediate required for IgM secretion.

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1. Greaves, M. F., Owen, J. J. T. & Raff, M. C. (1973) *T & B Lymphocytes: Origins, Properties and Roles in Immune Responses* (Am. Elsevier, New York).
2. Greaves, M. F. & Janossy, G. (1972) *Transplant. Rev.* **11**, 87–130.
3. Andersson, J., Sjöberg, O. & Möller, G. (1972) *Transplant. Rev.* **11**, 131–177.
4. Potter, M. & Fahey, J. L. (1960) *J. Natl. Cancer Inst.* **24**, 1153–1165.
5. Andersson, J., Bauxbaum, J., Citronbaum, D. S., Forni, L., Melchers, F., Pernis, B. & Stott, D. (1974). *J. Exp. Med.* **140**, 742–763.
6. Warner, N. L., Harris, A. W. & Gutman, G. A. (1975) in *Membrane Receptors of Lymphocytes*, eds. Seligmann, M., Preud'homme, J. L. & Kourilsky, F. M. (Am. Elsevier, New York), pp. 203–216.
7. Bergman, Y., Haimovitch, J. & Melcher, F. (1977) *Eur. J. Immunol.* **8**, 574–579.
8. Mosier, D. E. (1967) *Science* **158**, 1573–1575.
9. Melchers, F. & Andersson, J. (1974) *Eur. J. Immunol.* **4**, 181–188.
10. Koshland, M. E. (1975) *Adv. Immunol.* **10**, 41–69.
11. Katz, D. H. (1977) *Lymphocyte Differentiation, Recognition, and Regulation* (Academic, New York).
12. Davidson, R. L. (1974) *Annu. Rev. Genet.* **8**, 195–218.
13. Sekiguchi, T. & Sekiguchi, F. (1973) *Exp. Cell Res.* **77**, 391–403.
14. Hyman, R. & Stallings, V. (1974) *J. Natl. Cancer Inst.* **52**, 429–436.
15. Köhler, G., Howe, S. C. & Milstein, C. (1976) *Eur. J. Immunol.* **6**, 292–295.
16. Margulies, D. H., Kuehl, W. M. & Scharff, M. D. (1976) *Cell* **8**, 405–415.
17. Raschke, W. C. (1978) *Cur. Top. Microbiol. Immunol.* **81**, 70–76.
18. Crissman, H. A. & Tobey, R. A. (1974) *Science* **184**, 1297–1298.
19. Dennert, G. & Raschke, W. C. (1977) *Eur. J. Immunol.* **7**, 352–359.
20. Mosmann, T. & Baumal, R. (1975) *J. Immunol.* **115**, 955–962.
21. Wilde, C. E., III & Koshland, M. E. (1973) *Biochemistry* **12**, 3218–3224.
22. Kessler, S. W. (1975) *J. Immunol.* **115**, 1617–1624.
23. Dingman, C. W. & Peacock, A. C. (1968) *Biochemistry* **7**, 659–668.
24. Reisfeld, R. A. & Small, P. A. (1966) *Science* **152**, 1253–1255.
25. Hutchinson, M. A., Hunter, T. & Eckhart, W. (1978) *Cell* **15**, 65–77.
26. Bonner, W. M. & Stedman, J. D. (1978) *Anal. Biochem.* **89**, 247–256.
27. Marchalonis, J. J. (1969) *Biochem. J.* **113**, 299–305.
28. Brown, J. C. & Koshland, M. E. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5682–5686.
29. Kaji, H. & Parkhouse, R. M. E. (1974) *Nature (London)* **249**, 45–47.
30. Grey, H. M., Colón, S., Campbell, P. & Rabellino, E. (1972) *J. Immunol.* **109**, 776–783.
31. Kehry, M., Sibley, C., Fuhrman, J., Schilling, J. & Hood, L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2932–2936.
32. Melchers, F. (1972) *Biochemistry* **11**, 2204–2208.
33. Smith, G. P. (1978) *Biochem. J.* **171**, 337–347.
34. Levy, R. & Dilley, J. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2411–2415.
35. Mather, E. L. & Koshland, M. E. (1977) in *Immune System: Genetics and Regulation*, eds. Sercarz, E. E., Herzenberg, L. A. & Fox, C. F. (Academic, New York), Vol. 6, pp. 727–733.
36. Roth, R. A., Mather, E. L. & Koshland, M. E. (1978) in *Cells of Immunoglobulin Synthesis*, eds. Vogel, H. & Pernis, B. (Academic, New York), in press.