INDUCTION OF HUMAN IMMUNOGLOBULIN SYNTHESIS AND SECRETION IN SOMATIC CELL HYBRIDS OF MOUSE MYELOMA AND HUMAN B LYMPHOCYTES FROM PATIENTS WITH AGAMMAGLOBULINEMIA*

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Agammaglobulinemia is a heterogeneous antibody deficiency disease, usually associated with defective B lymphocytes (1, 2). Most patients with the common varied form of the disease have B lymphocytes which do not synthesize immunoglobulin either in vivo, or in vitro after mitogenic stimulation (3–6). Additionally, a few patients with the X-linked form of the disease also have B lymphocytes, which are blocked in differentiation (2, 7, 8).

Human immunoglobulin production has previously been observed in somatic cell hybrids between human lymphocytes and mouse myeloma cells (9). Hybrid cell colonies resulted only from fusions with human B lymphocytes (10). The three major classes of human immunoglobulin (IgM, IgA, and IgG) were produced by individual clones of hybrid cells (11).

In this study mouse myeloma cells were fused with B lymphocytes from three patients with agammaglobulinemia. Somatic cell hybrids were isolated and analyzed for Ig production.

Materials and Methods

Peripheral lymphocytes were obtained from three patients with a primary diagnosis of agammaglobulinemia. One patient (patient 4–8) had X-linked agammaglobulinemia (7, 8), while the other two patients (5–6 and 4–23) had common varied agammaglobulinemia (3, 6). All three patients had normal numbers of B lymphocytes in their peripheral blood as determined by EAC3 rosette formation and surface Ig (3, 7). Purified B lymphocytes (more than 90% EAC3 rosette forming) were tested for ability to synthesize immunoglobulin in vitro. Incubation with the lymphocyte mitogenic factor (LMF)[1] (from normal T cells) failed to stimulate immunoglobulin synthesis by B cells from each of the patients. T lymphocytes from these patients produced LMF when incubated with tetanus toxoid. These results indicate that, in these patients, there is a primary failure of immunoglobulin production intrinsic to the B lymphocytes. Long term B lymphoid cell lines from patients 4–8 and 4–23 did not synthesize immunoglobulin detectable by [^{14}C]leucine incorporation. A lymphoid cell line from patient 5–6 synthesized and secreted immunoglobulin. In all three patients, no evidence of increased suppressor cell activity was found. Their B lymphocytes failed to respond to in vitro mitogenic stimulation.

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[1] *Abbreviations used in this paper*: EBSS, Earle's balanced salt solution; FITC, fluorescein isothiocyanate; HAT, hypoxanthine, aminopterin, thymidme selective medium; LMF, lymphocyte mitogenic factor; MEM, minimal essential medium; SDS, sodium dodecyl sulfate.

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Participation in this study was approved by the institutional review committee, and consent was obtained.

Peripheral blood was anti-coagulated with preservative-free heparin. Mononuclear cells were separated by flotation on Ficoll-metrizoate (12). Because B lymphocytes uniquely form somatic cell hybrid colonies with mouse myeloma cell lines (10), no further fractionation of mononuclear cells was undertaken. The mononuclear cell preparation will be referred to as lymphocytes.

RPC 5.4, an IgG secreting mouse myeloma cell line resistant to 6 thioguanine (13), was obtained from the Cell Distribution Center, Salk Institute, San Diego, Calif.

All cultures were grown in Dulbecco's modified Eagle's minimal essential medium (MEM) supplemented with 15% heat inactivated fetal calf serum, 2 ml per 100 ml of medium of 200 mM L-glutamine and 0.4 ml per 100 ml medium of penicillin-streptomycin (10,000 U and 10,000 μg, respectively). HAT medium was composed of 1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin and 1.6×10^{-5} M thymidine as described by Littlefield (14). HAT was added as a times 100 concentrate to Dulbecco's medium as indicated.

Sendai virus was grown in embryonated chicken eggs as previously described (15) and inactivated by exposure to ultraviolet light (16).

RPC 5.4 mouse myeloma cells and human peripheral blood lymphocytes were fused with the aid of Sendai virus (10, 11). 5–10 million mouse myeloma cells were fused with $10\text{–}30 \times 10^6$ human lymphocytes by the addition of 1,000–3,000 hemagglutinating units of inactivated Sendai virus in medium without serum at pH 7.8–8.0. After overnight culture in medium with serum, the culture was centrifuged and the cells were resuspended in medium with HAT. Selection was continued for 4 days, after which the cells were collected and cloned in medium without hypoxanthine, aminopterin, thymidine selective medium (HAT). Hybrid cultures were cloned in 96 well microplates (Linbro Chemical Co., New Haven, Conn.) as described by Margulies et al. (17). Experiments with RPC 5.4 mouse myeloma cells showed that after 3 days in HAT selective medium there were no mouse parental cells capable of forming colonies and after 4 days there were no mouse parental cells that excluded trypan blue dye.

Fusion experiments were performed on at least two different occasions with each patient's lymphocytes. Parental myeloma cells and hybrid colonies were tested for mycoplasma by growth on agar mycoplasma plates (Grand Island Biological Co., Grand Island, N. Y.) at frequent intervals during these experiments and were found to be without contamination.

The chromosome constitution of each clone was determined. Cells were incubated for 24 h with 4 μg/ml of 33258 Hoechst (American Hoechst, Somerville, N. J.) (18). They were centrifuged and resuspended in medium without the dye. Metaphase chromosome spreads were then prepared as previously described (11, 19, 20), including a Giemsa banding technique (21). Pretreatment with 33258 Hoechst resulted in the elongation of the mouse parental chromosomal centromeres except for chromosomes 1 and 7, permitting rapid discrimination of human and mouse chromosomes. The species identity of the human chromosomes was confirmed by the Giemsa banding pattern.

Hybrid cells were examined for EAC3 rosette formation (22). Sheep erythrocytes were coated with IgM anti-sheep hemolysin and then reacted with the first four components of complement, to prepare EAC3 (EAC1423). 0.1 ml of a cell suspension containing $1\text{-}2 \times 10^6$ cells was mixed with 0.1 ml of EAC3 suspension, centrifuged at 150 *g* for 5 min, and incubated at 37°C for 1–3 h. Percent EAC3 rosette formation was determined by counting at least 200 cells in a hemocytometer.

Rabbit antiserum to human heavy chains and to mouse Ig were purchased from Behring Diagnostics, Inc., Woodbury, N.Y. They were rendered species specific by cross species absorption with myeloma proteins coupled to Sepharose 4B (23). For fluorescent antibody procedures, the IgG fraction of each antiserum was isolated by column chromatography on DEAE cellulose (0.015 M phosphate buffer, pH 6.8). They were conjugated to fluorescein isothiocyanate (FITC) (BioQuest, BBL & Falcon Products, Div. of Becton, Dickinson & Co., Cockeysville, Md.) as previously described (24). Before use, fluorescent antisera were centrifuged at 100,000 *g* for 1 h to remove aggregated material (25). For immune precipitations, monospecific antiserum to rabbit IgG was raised in a goat.

Hybrid cells ($1\text{-}2 \times 10^6$ per ml, >90% viable by trypan blue dye exclusion) were washed three times with Earle's balanced salt solution (EBSS) with heat inactivated fetal calf serum, resuspended in 50 μl of EBSS and mixed with 50 μl of appropriately diluted antiserum. They

were incubated for 30 min at 4°C, washed three times with cold EBSS, resuspended in glycerol:EBSS at 1:1, and placed on microscope slides. Slides were examined with a Zeiss fluorescence microscope with an HBO 200 W mercury lamp, a BG 12 primary filter, and a K 510 secondary filter (Carl Zeiss, Inc., New York).

To examine immunoglobulin synthesis and secretion, cells were washed with EBSS and resuspended at 4×10^6 cells per ml in Eagle's MEM with 1/10th the normal concentration of leucine supplemented with 10% dialyzed fetal calf serum, 1 ml of 200 mM L-glutamine per 100 ml medium, and 2 μCi. [^{14}C]leucine per ml medium (New England Nuclear Corp., Boston, Mass.). The cells were incubated for 12–16 hours, after which the medium was collected, the cells were washed with EBSS, resuspended in isotonic buffer (0.01 M Tris, 0.15 M NaCl, 0.0015 M $MgCl_2 \cdot 6H_2O$ and 1 N HCl to neutral pH), and lysed by the addition of Nonidet P-40 (Bethesda Research Laboratories, Rockville, Md.) to 0.6%. After 30 min, the cell lysate was centrifuged at 2,000 *g* for 20 min to remove nuclei and large cell debris.

Samples of the cell medium and of the cell lysate were examined for Ig by the addition of specific antiserum in excess at 4°C followed 1 h later by the addition of goat antiserum to rabbit IgG at equivalence. The mixture was incubated at 4°C for 24–48 h, washed three times with cold EBSS, and resuspended in 2% sodium dodecyl sulfate (SDS), 1% 2-mercaptoethanol.

Immune precipitates were heated to 100°C for 2 min, and electrophoresed in 7.5% acrylamide slab gels with SDS-phosphate buffer (26). The slab gels were dried in a Hoefer gel drying apparatus (Hoefer Scientific Instruments, San Francisco, Calif.) and autoradiographed with Kodak RPR 54 X-ray film (Eastman Kodak Co., Rochester, N.Y.) to detect de novo immunoglobulin synthesis and secretion.

Results

Sendai virus-mediated fusion of RPC 5.4 mouse myeloma cells and human peripheral blood lymphocytes from patients diagnosed to have agammaglobulinemia resulted in proliferating clones of hybrid cells 4–6 wk after fusion. Five clones of hybrid cells derived from patient 4-8 were examined for immunologic properties; six clones of hybrid cells examined were derived from lymphocytes of patient 5-6 and of patient 4-23.

To establish the hybrid nature of the clones, metaphase chromosome spreads were prepared. Clones were pretreated for 24 h with 33258 Hoechst and chromosome spreads were stained for Giemsa banding. The modal numbers of total chromosomes and of human chromosomes were determined for each hybrid clone (Table I). RPC 5.4 mouse parental myeloma cells had a modal number of 59 chromosomes, all of which were acrocentric. All but one of the hybrid clones from the three patients had modal numbers of chromosomes which were close to the modal number of RPC 5.4 (58 to 68). One clone, 5.4 5-6 4-1, had a modal number of 110 chromosomes, not quite double the modal number of the mouse parental cells. In agreement with previous studies of mouse-human hybrid cells, only a few human chromosomes were retained in the hybrid cells. The modal numbers of human chromosomes present in hybrid clones were either 2 or 3, with ranges from 0 to 5 chromosomes (Table I).

Hybrid cells derived from fusion of lymphocytes from patients 4-8 and 5-6 were examined for the B-cell characteristic of EAC3 rosette formation. RPC 5.4 mouse parental myeloma cells did not form EAC3 rosettes. Lymphocytes from patients 4-8 and 5-6 included 17 and 19% EAC3 rosette-forming B cells, respectively. None of the 5.4 4-8 or 5.4 5-6 hybrid cell clones formed EAC3 rosettes. These results are contrasted with 53% rosette-forming cells from the Epstein-Barr virus transformed lymphoid cell line, LAZ 135, derived from the B lymphocytes of patient 5-6.

Surface immunoglobulin was examined by immunofluorescence with FITC-con-

TABLE I
Chromosome Constitution of Hybrid Clones

Clone	Modal chromosome number (range)	Modal human chromosome number (range)
5.4 4-8		
1-1	64 (61-68)	3 (1-4)
1-3	65 (62-66)	2 (1-3)
2-3	65 (63-66)	2 (1-3)
3-2	59 (57-60)	2 (1-3)
3-3	63 (60-64)	3 (2-4)
5.4 5-6		
1-2	65 (62-68)	2 (1-3)
1-3	64 (62-67)	2 (1-3)
1-4	63 (58-64)	2 (1-4)
3-2	63 (60-64)	2 (0-5)
3-3	64 (61-65)	2 (1-3)
4-1	110 (100-119)	3 (0-5)
5.4 4-23		
1-1	58 (55-60)	2 (0-3)
2-2	60 (58-62)	2 (1-3)
2-4	59 (52-60)	2 (1-2)
3-1	58 (55-62)	2 (1-4)
3-2	59 (59-61)	2 (0-3)
4-2	62 (61-65)	2 (1-4)
RPC 5.4	59 (56-62)	0

jugated antisera to mouse Ig and to human γ, α, or μ-heavy chains. The percentage of cells reactive with each antiserum was determined. Immunoglobulin production was determined by SDS-gel electrophoresis of [^{14}C]leucine labeled cytoplasmic and secreted proteins isolated by indirect immune precipitation with antiserum directed against mouse Ig or human heavy chains. The immunoglobulin bearing and producing properties of hybrid clones will be presented according to the patient from whom the human parental lymphocytes were obtained.

The classes of human and mouse immunoglobulin heavy chain molecules produced by the hybrid clones can be determined by migration in SDS-acrylamide gels. A representative group of autoradiographs demonstrate the range of variation of classes of immunoglobulin molecules produced. Some clones produced only mouse immunoglobulin (Tables II-IV); γ and L chain molecules which comigrated with RPC 5.4 IgG myeloma protein (Fig. 1-RPC 5.4 and Fig. 2-clone 5.4 5-6 3-2). Other clones synthesized mouse and human Ig molecules, but secreted Ig molecules of only one species. Clone 5.4 4-23 4-2 had cytoplasmic human γ, μ, and L-chain molecule and mouse γ- and L-chain molecules (Fig. 3). Only the mouse γ- and L-chain molecules were secreted. Other clones synthesized and secreted both human and mouse immunoglobulin molecules (Fig. 4, clone 5.4 4-8 3-2). In each of these samples, the primary class of mouse immunoglobulin produced was IgG, which comigrated with RPC 5.4 myeloma protein. When human immunoglobulin was produced it included molecules of the γ, μ, and α-heavy chain classes, as well as L-chains.

RPC 5.4 mouse parental myeloma cells produced mouse IgG (Fig. 1). 93–95% of the live cells fluoresced with antiserum to mouse Ig. They did not have receptors for

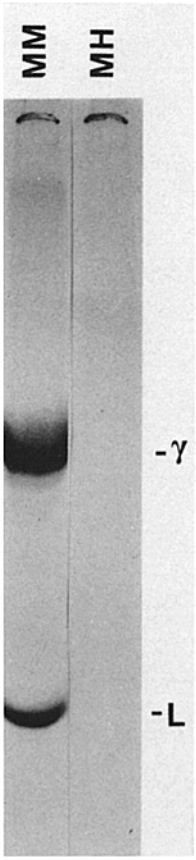

FIG. 1. RPC 5.4 IgG. 10^8 RPC 5.4 parental mouse myeloma cells were incubated for 14 h in medium with 1/10th normal concentration of leucine supplemented with 2 μCi [^{14}C]leucine per ml of medium. The supernatant (medium) fraction was collected after centrifuging the cells into a pellet and divided into two aliquots. One portion was incubated with rabbit antiserum to mouse Ig, the other with rabbit antiserum to human heavy chains. Goat antiserum to rabbit IgG was added at equivalence to form a precipitate. The precipitates were washed, redissolved, reduced, and electrophoresed in a 7.5% SDS-acrylamide slab gel. The gel was fixed, dried, and autoradiographed. The autoradiograph is shown. MM, medium precipitated with antiserum to mouse Ig; MH, medium precipitated with antiserum to human heavy chains.

heat aggregated rabbit Ig. Cultures incorporated [^{14}C]leucine into cytoplasmic and secreted mouse immunoglobulin which migrated as γ- and L-chains in SDS gels. 9 of 10 subclones secreted the myeloma protein.

Hybrid clones derived from patient 4-8 continued to produce mouse Ig (Table II). Fewer hybrid cells fluoresced with FITC-antiserum to mouse Ig than the parental myeloma cells (31–90% of hybrid cells). All five of the clones contained some cells reactive with antiserum to human heavy chains. Three of these had only a few cells bearing human Ig. The two remaining clones had moderate numbers of cells bearing human surface Ig.

All five of the hybrid clones synthesized and secreted mouse parental Ig. Four of the five clones incorporated [^{14}C]leucine into cytoplasmic protein identified as human Ig. Three of these clones secreted the human Ig; one clone synthesized but did not

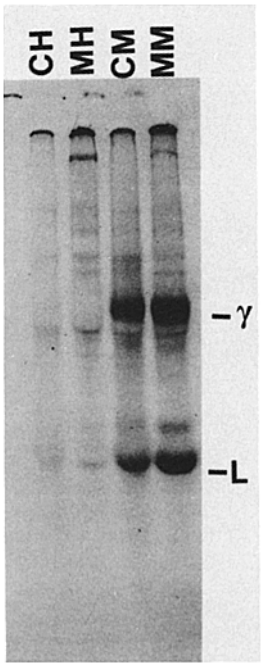

FIG. 2. Autoradiograph of SDS-acrylamide gel electrophoresis of proteins from clone 5.4 5-6 3-2. Cells were incubated with [^{14}C]leucine for 12–14 h. Medium (supernatant) fraction was collected after centrifugation. The pelleted cells were resuspended in isotonic Tris buffer and were lysed by the addition of Nonidet P-40 to 0.6%. Large cell debris was removed by centrifugation at 2,000 *g* for 20 min. Portions of the cytoplasmic (cell) fraction and the supernatant (medium) fraction were immunologically precipitated with antiserum to mouse Ig or to human heavy chains in excess, followed by goat antiserum to rabbit IgG at equivalence. The immune precipitates were redissolved, reduced, and electrophoresed in 7.5% SDS-acrylamide slab gels. The gels were fixed, dried, and autoradiographed. CH, cell fraction, precipitated with antiserum to human heavy chains; MH, medium fraction, precipitated with antiserum to human heavy chains; CM, cell fraction, precipitated with antiserum to mouse Ig; MM, medium fraction, precipitated with antiserum to mouse Ig.

secrete human Ig. Clone 5.4 4-8 1-1 produced mouse Ig but did not produce human Ig.

Six clones derived from B lymphocytes of patient 5-6 were examined. Five of the six clones reacted with FITC-antiserum to mouse Ig (Table III). One clone, 5.4 5-6 3-3, had only a few cells (5%) which reacted with FITC-antiserum to mouse Ig. All six of the clones had more than 70% of the cells reactive with FITC-antiserum to at least one class of human Ig heavy chain. Most of the clones were reactive with FITC-antiserum to more than one class of human heavy chain. More than 90% of the cells from 5.4 5-6 3-2 reacted with each of the FITC-antisera to mouse Ig and to the three classes of human Ig heavy chains.

All six of the clones synthesized and secreted radioactively labeled mouse Ig (Table IV). Five of the six clones incorporated [^{14}C]leucine into cytoplasmic material identified as human Ig. Three of these clones secreted radioactively labeled human Ig. Two clones synthesized human Ig which was not secreted. One clone did not synthesize or secrete human Ig.

Of six clones of hybrid cells resulting from the fusion of RPC 5.4 mouse myeloma

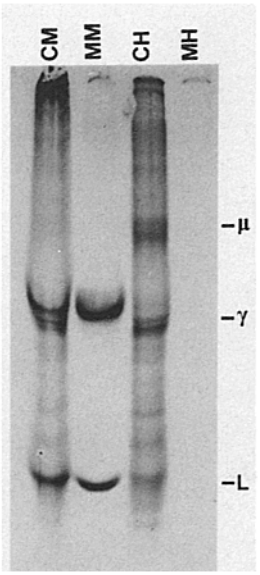

FIG. 3. Autoradiograph of SDS-gel electrophoresis of precipitated immunoglobulins from clone 5.4 4-23 4-2. Conditions and labels are as in legend to Fig. 2. CH, cell fraction, human heavy chains; MH, medium fraction, human heavy chains; CM, cell fraction, mouse Ig; MM, medium fraction, mouse Ig

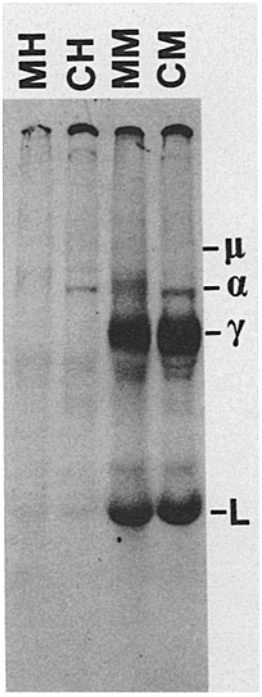

FIG. 4. Autoradiograph of precipitated immunoglobulins from clone 5.4 4-8 3-2 electrophoresed in SDS acrylamide slab gel. For details, see legend to Fig. 2. CH, cell fraction, human heavy chains; MH, medium fraction, human heavy chains; CM, cell fraction, mouse Ig; MM, medium fraction, mouse Ig

TABLE II
Characteristics of Clones of Patient 4–8 Hybrid Cells

Clone	% Cells reactive with FITC-antiserum to:				Human Ig production detected by SDS-acrylamide gel electrophoresis	
	Mouse Ig	Human heavy chains			Cytoplasmic	Secreted
		γ	α	μ		
RPC 5.4	93	0	0	0	–	–
5.4 4-8						
1-1	43	6	2	8	–	–
1-3	66	12	3	7	+	+
2-3	60	38	3	30	+	+
3-2	31	3	5	9	+	–
3-3	90	41	43	65	+	+

TABLE III
Characteristics of Clones of Patient 5-6 Hybrid Cells

Clone	% Cells reactive with FITC-antiserum to:				Human Ig production detected by SDS-acrylamide gel electrophoresis	
	Mouse Ig	Human heavy chains			Cytoplasmic	Secreted
		γ	α	μ		
RPC 5.4	93	0	0	0	–	–
5.4 5-6						
1-2	100	35	70	40	+	+
1-3	95	95	10	90	+	+
1-4	95	83	2	5	+	–
3-2	90	90	90	90	–	–
3-3	5	60	80	80	+	+
4-1	60	35	75	15	–	–

cells and lymphocytes from patient 4-23, five had more than 50% of the cells reactive with FITC-antiserum to mouse Ig (Table IV). The remaining clone, 5.4 4-23 2-4 had only a few cells reactive with FITC-antiserum to mouse Ig. Only two of the six clones had significant numbers of cells reactive with FITC-antiserum to human Ig heavy chains. Clone 5.4 4-23 3-2 had 63% cells reactive with anti μ-chain serum; clone 5.4 4-23 4-2 had 43% cells reactive with anti-α-chain serum. The other clones had a maximum of 32% cells reactive with any single antiserum to human heavy chains. In contrast to hybrids from normal individuals (8, 10), each clone had one predominant class of human Ig heavy chain. For example, clone 5.4 4-23 3-1 had 26% cells reactive with anti-human γ-chain serum, and no cells reactive with anti-μ or α-chain serum.

The six clones synthesized and secreted mouse Ig. All six of the clones incorporated [^{14}C]leucine into cytoplasmic protein identified as human Ig. Only two of these clones

TABLE IV

Characteristics of Clones of Patient 4-23 Hybrid Cells

Clone	% Cells reactive with FITC-antiserum to				Human Ig production detected by SDS-acrylamide gel electrophoresis	
	Mouse Ig	Human heavy chains			Cytoplasmic	Secreted
		γ	α	μ		
RPC 5.4	93	0	0	0	–	–
5.4 4.23						
1-1	93	4	1	10	+	+
2-2	54	14	3	29	+	+
2-4	7	0	32	1	+	–
3-1	81	26	0	0	+	–
3-2	85	4	27	63	+	–
4-2	53	10	43	26	+	–

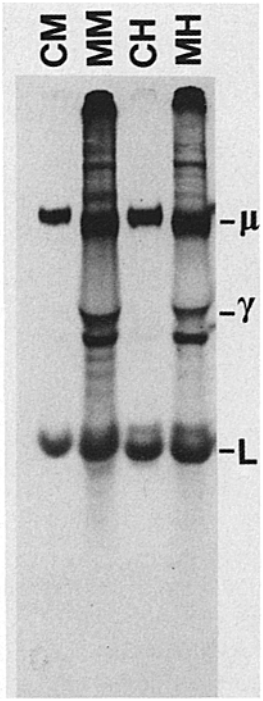

FIG. 5. Autoradiograph of immunoglobulins precipitated from clone 5.4 4-23 1-1 and electrophoresed in SDS-acrylamide slab gel. Conditions were as described in legend to Fig. 2. CH, cell fraction, human heavy chains; MH, medium fraction, human heavy chains; CM, cell fraction, mouse Ig; MM, medium fraction, mouse Ig.

secreted radioactively labeled human Ig. The other four hybrid clones did not secrete human Ig.

An apparent mouse immunoglobulin molecule other than, or in addition to the RPC 5.4 parental myeloma protein was found on an SDS-acrylamide gel autoradi-

ograph. Clone 5.4 4-23 1-1 had cytoplasmic μ, γ, and L chains which precipitated with antiserum to human heavy chains and to mouse Ig (Fig. 5). Only μ- and L-chains were secreted. We cannot exclude the possibility that the molecules which precipitated with antiserum to mouse Ig might represent interspecies hybrid molecules composed of human μ-chains combined with mouse L chains.

Discussion

Human peripheral blood lymphocytes from three patients with agammaglobulinemia were fused with RPC 5.4 mouse myeloma cells. Chromosome analysis confirmed the hybrid nature of each clone, indicating that most of the mouse parental chromosomes were retained by the hybrids whereas few of the human parental chromosomes persisted. Like their myeloma parent, no clone expressed the B-cell associated surface receptor for the third component of complement. Most clones from each of the three patients synthesized and secreted mouse Ig. In addition, some clones from each of the three patients synthesized and secreted human Ig. Most of the clones had mouse Ig on their cell surface, while clones from only one of the patients (5-6) had significant numbers of cells with surface human Ig.

Long-term B lymphoid cell lines derived from each of the three patients by in vitro infection with Epstein-Barr virus have been studied (6, 8). Cell lines from patients 4-8 (LAZ 166) and 4-23 (LAZ 153) were primarily surface IgD bearing with a small population of surface IgM bearing cells. Neither cell line secreted immunoglobulin nor was synthesis of cytoplasmic immunoglobulin detectable by SDS-acrylamide gel electrophoresis. The cell line derived from patient 5-6 (LAZ 135) had cells with surface IgG, A, M, and D and synthesized and secreted immunoglobulin.

Some somatic cell hybrid clones from all three patients synthesized and secreted human immunoglobulin. This demonstrates the presence of structural genes coding for immunoglobulin in the genome of each of these agammaglobulinemic individuals. It further demonstrates that these genes direct the synthesis of immunoglobulin only under the influence of the mouse parental myeloma cell genome. Examination of the classes of human immunoglobulin demonstrated the production of human μ, γ, and α-heavy chains by one or more clones.

Current evidence indicates that IgG and IgA producing cells develop from IgM bearing cells (27). B cells from all three patients do not progress beyond the stage of surface IgM and IgD bearing lymphocytes. The presence of surface IgD suggests development of the patients' B cells beyond the stage at which individual cells' isotypes are specified (27, 28). Whether isotype specification has occurred cannot be determined with certainty. Fusion with mouse myeloma cells results in the production of the three major classes of human immunoglobulin. This suggests that these surface IgD and IgM bearing B lymphocytes may differentiate into IgM, IgG, or IgA producing cells.

Most of the somatic cell hybrid clones produced immunoglobulin which was immunologically identified as of mouse parental origin composed of γ- and L-chains that could be identified as the RPC 5.4 parental myeloma protein. Some clones produced IgM which precipitated with antiserum to mouse Ig. Because this antiserum reacts with light chains, we could not be sure that this IgM was not composed of human μ-chains with mouse L-chains. Intra- and interspecies hybrid molecules similar to this have been previously reported (11, 17, 29, 32). The antiserum to human Ig was specific for the γ, α, and μ-heavy chains, which permitted positive identification of human μ-chain production in these clones.

The hybrid cells resulting from the fusion of mouse myeloma cells with human B lymphocytes morphologically resemble plasma cells. The absence of receptors for the third component of complement on the clones also suggests this stage of differentiation. This finding is expected from the predominance of the mouse parental genome and from the synthesis and secretion of immunoglobulin.

Hybrid clones derived from patient 5-6 had large numbers of cells with human and mouse surface Ig. This result is in agreement with studies of such hybrids using B lymphocytes from normal individuals (11, and J. Schwaber, unpublished results). Clones derived from patients 4-8 and 4-23, however, had fewer cells with human surface Ig. This difference in the expression of surface Ig molecules is in accord with the behavior of lymphoid cell lines derived from these patients. Lymphoid cell lines from patients 4-8 and 4-23 had restricted expression of surface Ig classes, with IgD as the primary surface Ig molecule, whereas the lymphoid cell line derived from patient 5-6 expressed IgG, A, M, and D as surface molecules. These findings suggest that there are different defects in differentiation of the B lymphocytes from these patients.

The induction of a gene product by somatic cell hybridization has been reported only a few times. Double fusion of rat hepatoma cells with each other and then with mouse fibroblasts resulted in the induction of mouse albumin production (30). Fusion of mouse hepatoma cells with human leukocytes resulted in the induction of human albumin production (31). Attempts to induce immunoglobulin production (17, 32–36) have failed. However, fusion of two parental cells capable of producing immunoglobulin has permitted continued immunoglobulin production (11, 17, 32, 34).

Fusion of mouse myeloma cells with human lymphocytes resulted in the induction of human immunoglobulin production. A previous study has shown that these hybrid cells result exclusively from the fusion of human B lymphocytes (10). The B cells from these agammaglobulinemic patients are abnormal forms, incapable of differentiation to immunoglobulin secretion. The production of human immunoglobulin by the hybrids represents induction of a hitherto unexpressed gene product i.e., γ- and α-heavy chain. The parental human B cells were incapable of producing immunoglobulin for export before fusion, or of even expressing surface IgG or IgA. We presume that this results from a molecular defect which is complemented by the mouse parental genome.

Summary

Somatic cell hybrid clones were isolated from the fusion of RPC 5.4 mouse myeloma cells and B lymphocytes from three patients with agammaglobulinemia. One patient had X-linked agammaglobulinemia; the remaining two patients had common varied agammaglobulinemia. All three patients had B lymphocytes which fail to secrete immunoglobulin. The hybrid nature of the clones was established by examination of metaphase chromosome spreads. Most of the clones from all three patients expressed surface immunoglobulin of mouse and human parental origin. Clones from two of the patients had fewer cells with surface Ig than hybrids from normal persons, while clones from the third patient had large numbers of surface Ig fluorescent cells. Most of the clones from all three patients synthesized and secreted human and mouse immunoglobulin. As determined by sodium dodecyl sulfate acrylamide gel electrophoresis of radioactively labeled proteins, clones from each of the patients produced human γ, α, and μ-heavy chains. These studies demonstrate the presence of functional

structural genes coding for human immunoglobulin heavy chains in B lymphocytes of patients with agammaglobulinemia. Further, they represent induction in the somatic cell hybrids of a gene product not expressed in the parental B lymphocytes.

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