SYNTHESIS, ASSEMBLY, AND SECRETION OF GAMMA GLOBULIN BY MOUSE MYELOMA CELLS

III. Assembly of the Three Subclasses of IgG*‡

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While a great deal is known about the refolding and assembly of purified proteins in vitro, there is much less information available on how complex proteins attain their final conformation within the cells of higher organisms (1). Immunoglobulins provide an excellent subject for such a study since: (*a*) they are multichain molecules which have been well characterized physically and chemically; (*b*) the constituent heavy (H)[1] and light (L) polypeptide chains of the fully assembled immunoglobulin G (IgG) molecule are covalently linked and IgG can therefore be easily separated from unassembled and partially assembled precursors; (*c*) the more than twofold difference in size between the H and L chains makes their separation and identification relatively simple; (*d*) mouse myeloma tumors provide homogeneous populations of cells producing large quantities of IgG so that its synthesis and assembly can be readily studied using radioactive precursors; (*e*) many different tumors, some of which are blocked in assembly, can be compared (2).

In the studies which follow, we have examined the synthesis, assembly, and secretion of the three major subclasses[2] of mouse IgG in 14 myeloma tumors and two cultured myeloma cell lines. The tumors and cell lines differed from each other in the details of synthesis, assembly, and secretion of IgG, although their average behavior resembled closely these processes in normal lymphoid cells. Both the covalent and noncovalent assembly of fully assembled immuno-

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[1] *Abbreviations used in this paper:* H, heavy chain; H_2, heavy chain dimer; H_2L_2, fully assembled immunoglobulin-G; HL, half molecule; L, light chain; L_2, light chain dimer; IgG, immunoglobulin G; SDS, sodium dodecyl sulfate.

[2] The nomenclature of the three major subclasses of mouse IgG used in the present study (IgG_{2a}, IgG_{2b} and IgG_1) are equivalent to the γG, γH, and γF subclasses, respectively, of Potter.

globulin (H_2L_2) occurred for the most part after release of the nascent H and L chains from the polyribosomes. The newly synthesized and released chains formed an intracellular pool from which the IgG molecule was assembled through a series of intermediates. In each myeloma, assembly occurred via one major and one or more minor pathways. The major pathway in the assembly of IgG_{2a} and IgG_1 differed from that of IgG_{2b}. The kinetics of assembly varied in the different myelomas and required between 10 and 30 min for completion.

Materials and Methods

Myeloma Tumors and Cell Lines.—The MPC-11 tumor was provided by Dr. John Fahey of the National Institutes of Health. The remaining tumors were induced by us except for the SQPC 11 tumor which was induced by Dr. Paul Anderson. The subclass of the paraproteins produced by each tumor was determined by double diffusion in agar. All of the tumors were of BALB/c origin except the MOPC 245 and MOPC 282 tumors, which were induced in mice of an introgressive backcross of the C57BL heavy chain gene cluster onto BALB/c (3). MOPC 245 arose in a third backcross mouse. The original host was heterozygous for C57BL and BALB/c heavy chain genes and the myeloma protein was shown by electrophoresis to be directed by the C57BL IgG_1 (F^s) heavy chain gene (3). The MOPC 282 tumor arose in a seventh backcross mouse that was homozygous for BALB/c genes. Both tumors have been successfully serially transferred in BALB/c mice. All of the tumors were maintained by subcutaneous passage in BALB/c mice.

The MPC-11 cell line was adapted to continuous culture in this laboratory and has been described in detail elsewhere (4, 5). The P_3 cell line was originally adapted to culture from the MOPC 21 tumor by Dr. Horibata at the Salk Institute, and has been described in detail by Schubert (6). This cell line was kindly provided by Dr. Corrado Baglioni of the Massachusetts Institute of Technology. The cell lines were maintained in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 20% horse serum and nonessential amino acids.

Immunization of Normal Mice.—BALB/c mice were immunized in the hind footpads with 1 mg of hemocyanin suspended in 0.1 ml of pertussis vaccine (Eli Lilly & Co., Indianapolis, Ind.) (7). This mixture was reinjected at 6 wk, and 1 wk later the popliteal lymph nodes were removed and examined as described below. Under these conditions, no IgM was synthesized at the time of analysis.

Incubation of Cells with Radioactive Precursors:—Single cell suspensions were prepared from tumor fragments and from popliteal lymph nodes as described previously (4, 5), and were suspended in Eagle's spinner medium (8) containing 1/20th the normal amounts of valine, threonine, and leucine. In the continuous labeling experiments, 15×10^6 cells in 3 ml were incubated at 37°C with 3 μCi each of L-valine-^{14}C, threonine-^{14}C, and leucine-^{14}C (uniformly labeled, specific activity > 0.15 Ci/mmole, New England Nuclear Corp., Boston, Mass.). 2 ml of the cell suspension was removed after 15 min, chilled, and cytoplasmic lysates were prepared as outlined below. Secreted material was prepared after a 3 hr incubation from the supernatant medium of the remaining 5×10^6 cells. In the pulse-chase experiments, 30×10^6 cells in 3 ml were incubated at 37°C with 20 μCi each of L-valine-^{14}C, threonine-^{14}C, and leucine-^{14}C. As has been described previously (5), after 2.5 min, the cells were chased by the addition of 3 ml of medium containing 20 times the normal amounts of valine, threonine, and leucine. 1 ml of cell suspension containing 5×10^6 cells was removed at varying intervals after the chase, the cells were chilled, and cytoplasmic lysates were prepared. There was never any increase in acid-precipitable radioactivity after the chase, indicating that it was effective.

Preparation of Cytoplasmic Lysates and Immunological Precipitation of the Immunoglobulin Molecules.—The labeled cells were pelleted by centrifugation at 1000 rpm for 5 min, and the pellets were resuspended in an isotonic buffer as previously described (4, 5). The detergent Nonidet P-40 (Shell Chemical Co., New York) was added to a final concentration of 0.5%, and iodoacetamide was added to a final concentration of 0.1 M to prevent disulfide interchange (4, 5). The nuclei and ribosomes were removed by sedimentation at 105,000 *g* for 30 min at 4°C. One-half of each cytoplasmic lysate was treated with 2% sodium dodecyl sulfate (SDS) for 1 min at 100°C. The remaining half was precipitated with an excess of rabbit antiserum to mouse immunoglobulin. All incubations were carried out for 16 hr at 4°C. The immune precipitates were washed and dissolved in 0.01 M sodium phosphate buffer (pH 7.0) containing 2% SDS by heating at 100°C for 1 min. Iodoacetamide was added to a final concentration of 0.1 M.

Preparation of Antiserum.—Antiserum reactive with both mouse γH and kappa L chains was prepared by immunizing rabbits with purified MPC-11 paraprotein and then boosting with completely S-carboxymethylated MPC-11 protein. Antiserum specific for γH chains was obtained by immunizing rabbits with purified Fc fragments. Antiserum reactive with kappa L chains but not with γH chains was prepared by immunizing rabbits with the purified IgA paraprotein produced by the Adj. PC 6A tumor (3). This antiserum reacts with L chains in combination with both γ and α heavy chains.

Acrylamide Gel Analysis of Labeled Proteins.—Cytoplasmic lysates, secreted material, and immunological precipitates were analyzed on SDS-containing acrylamide gels as described previously (4, 5). This technique separates immunoglobulin molecules on the basis of their size (9). The gels were fractionated mechanically as described by Maizel (10) and the samples were collected on planchettes and counted in a Nuclear-Chicago low background counter (Nuclear-Chicago Corporation, Des Plaines, Ill.).

RESULTS

Production of IgG.—The synthesis and secretion of the three major subclasses of mouse IgG has been examined using cells from 14 different tumors, two cell lines, and from the popliteal lymph nodes of immunized mice. The results with one tumor from each subclass are shown in Fig. 1. Cell suspensions were incubated with radioactive amino acids for 15 min and the labeled soluble cytoplasmic proteins were analyzed on SDS-containing acrylamide gels (top panels, Fig. 1). The cytoplasm of each of the three tumors contained a number of distinct radioactive peaks superimposed on a diffuse background of labeled cell protein. The labeled immunoglobulin molecules were identified by precipitating the cytoplasmic lysates with antiserum reactive with γH and kappa L chains and then analyzing the precipitates on SDS-containing acrylamide gels (middle panels, Fig. 1). The polypeptide composition of each of the immunoglobulin peaks was established by: (*a*) calculating their molecular weights (4, 9) based on their migration in SDS acrylamide gels (4, 9), and (*b*) by recovering the protein in each peak and determining the relative content of H and L chains after reduction and alkylation (4). Based on these determinations, a continuum of immunoglobulin intermediates was identified within the cells. The immunoglobulin molecules secreted into the culture fluid during a 3 hr incubation with radioactive precursors were also examined (bottom panels,

Fig. 1). Although the immunological precipitates of the secretions are not shown, all of the peaks could be precipitated with specific antiserum (4). A similar analysis was performed using the cells obtained from the popliteal lymph nodes of immunized BALB/c mice (Fig. 2).

Since SDS disrupts noncovalent bonds but has no effect on disulfide bonds, analysis in the presence of SDS does not necesarily reveal the true composition of all of the immunoglobulin molecules. Nevertheless, it can be concluded that

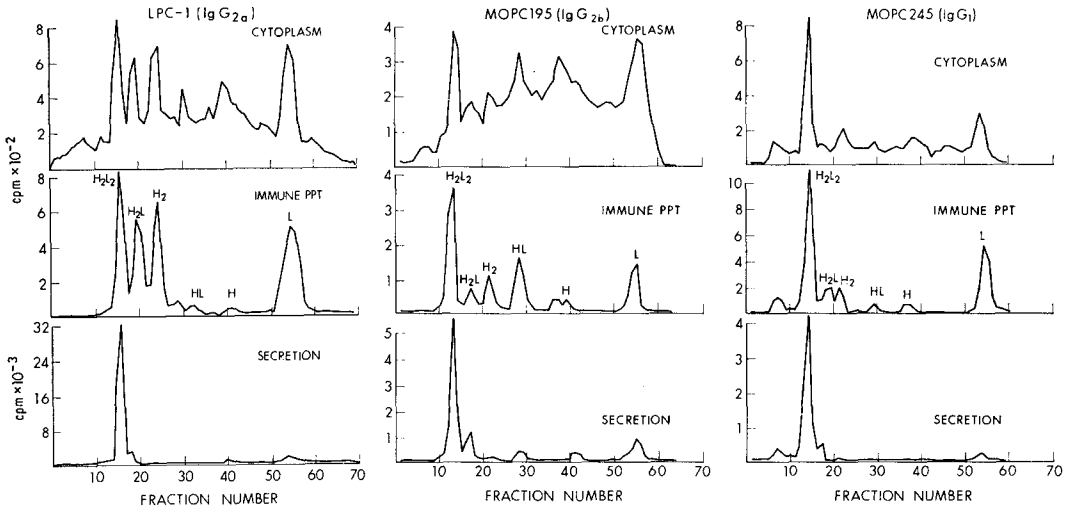

FIG. 1. Production of the three subclasses of IgG by mouse myeloma tumors. Cell suspensions were prepared from each of the three myeloma tumors shown. The cells were incubated with amino acids-^{14}C and labeled cytoplasm, immunologically precipitated cytoplasm, and secretion were prepared as described in the Materials and Methods. These samples were analyzed by electrophoresis on 5% 20-cm SDS-acrylamide gels for 16 hr at 70 v. The gels were fractionated into planchettes and the radioactivity in each of the planchettes was determined. The direction of migration on the above and on all subsequent electropherograms is from left to right.

each of the three tumors depicted in Fig. 1 differs from the others in the relative amounts of immunoglobulin and cell protein produced, and in the relative amounts of partially assembled or unassembled molecules present intracellularly and secreted. The characteristics of each of the tumors examined are summarized in Table I. There was considerable individual variation and each tumor appeared to have distinctive patterns of immunoglobulin production which was reproducible. All of the tumors produced large amounts of immunoglobulins and contained significant amounts of the various partially assembled or unassembled molecules. In addition, all of the tumors secreted some incompletely assembled molecules. These findings could not be ascribed to

cellular heterogeneity since a number of spleen clones of the MPC-11 and MOPC 173 tumors were identical with their parent tumors in their patterns of IgG production (4, 11). The presence of the partially assembled molecules seen in Fig. 1 and Table I was not because of abnormal metabolic conditions

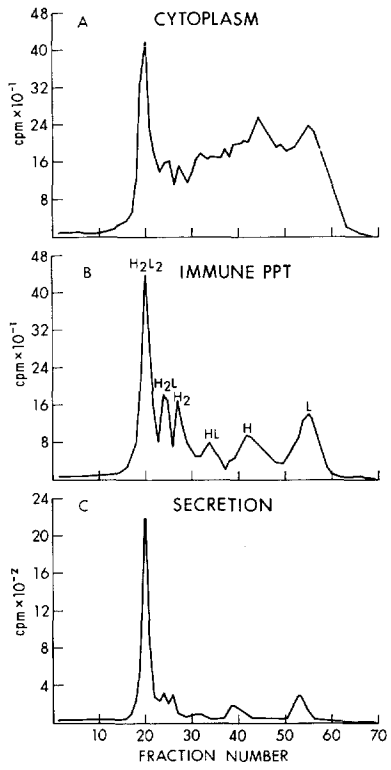

FIG. 2. Production of IgG by lymphoid cells of hyperimmunized mice. An isolated cel suspension was prepared from the popliteal lymph nodes of hyperimmunized mice. The cells were incubated with amino acids-^{14}C and labeled cytoplasm, immunologically precipitated cytoplasm, and secretion were prepared as described in the Materials and Methods. All samples were analyzed by electrophoresis on SDS-acrylamide gels.

during short-term in vitro incubation of the cells since similar results were obtained when MPC-11 (12), LPC 1, and MOPC 31C, 494, 21A, 173, were labeled in vivo (see below).

The IgG_{2b}-producing tumors differed from tumors of the other two subclasses in that they all contained more half molecule (HL) intracellularly and, as a group, appeared to secrete more free L chains (Table I). However, the production of excess L chains cannot be reliably evaluated by examining se-

TABLE I

Characteristics of IgG Production by Mouse Myeloma Tumors, Cultured Myeloma Cell Lines, and Normal Lymphoid Cells

	% Immunoglobulin	Intracellular immunoglobulin						Secreted immunoglobulin					
		H_2L_2	H_2L	H_2	HL	H+L_2	L	H_2L_2	H_2L	H_2	HL	H+L_2	L
Immune lymph node	30	45	10	10	5	10	20	75	9	4	0	5	7
IgG myelomas (average)	29	41	13	11	8	4	23	76	7	2	4	3	8
I. IgG_{2a}													
Adj. PC-5	29	32	24	22	3	3	16	91	8	1	0	0	0
MOPC 494	36	38	21	10	4	6	21	82	7	2	2	2	5
MOPC 173	15	35	17	8	4	2	32	90	10	0	0	0	0
LPC 1	32	27	22	21	2	1	27	80	8	2	2	4	4
II. IgG_{2b}													
MOPC 282	21	54	6	4	14	2	20	80	3	1	0	4	12
MOPC 195	21	41	7	10	20	6	16	57	17	1	8	1	16
TEPC 129	38	20	7	19	16	4	34	50	8	1	4	8	31
MPC 11 tumor	30	25	3	4	21	13	34	36	2	2	28	4	28
MPC 11 culture	25	46	10	9	7	13	15	80	2	0	4	6	8
SQPC 11	40	50	12	6	15	5	12	70	14	3	6	2	5
MOPC 141	28	33	16	4	16	9	22	70	14	4	8	3	1
III. IgG_1													
MOPC 245	43	49	11	8	2	3	27	84	7	2	0	1	6
MOPC 160	23	53	9	2	0	2	34	71	4	3	1	7	12
MOPC 31C	31	37	20	25	2	2	14	91	4	2	0	1	2
MOPC 21A tumor	30	49	7	19	2	4	19	92	6	2	0	0	0
MOPC 21A culture	24	61	10	6	3	2	18	96	0	3	0	3	1

Cell suspensions were prepared from each of the mouse myeloma tumors listed and from the popliteal lymph nodes of hyperimmunized mice. These cells and the cultured myeloma cell lines were incubated with amino acids-^{14}C and labeled cytoplasm, immunologically precipitated cytoplasm, and secretion were prepared as described in the Materials and Methods. The per cent immunoglobulin was estimated by planimetry from the gels of the cytoplasmic lysates. The area under the immunoglobulin peaks was measured and expressed as a percentage of the area of the total labeled cell protein. The values so obtained were in close agreement with those derived by specifically precipitating an aliquot of cytoplasm lysate and expressing the trichloroacetic acid (TCA)-precipitable counts in the immunological precipitate as a percentage of the total cytoplasmic TCA-precipitable counts. The radioactivity in all the peaks on the gels of the immunologically precipitated cytoplasm and secretion was calculated and the amount of intracellular and secreted immunoglobulin molecules in each peak was expressed as the per cent of total. The identity of the various peaks was established on the basis of their molecular weights and relative content of H and L chains as outlined in the Results. It was difficult to separate the H and L_2 peaks on the gels, so that the amount of immunoglobulin in these peaks is shown together.

creted material, since some tumors such as MOPC 21 (6) and MOPC 173 appear to degrade the excess L chains which they produce. Finally, it was observed that the average pattern of immunoglobulin production of the 14 tumors and two cultured lines was similar to that of the heterogeneous lymphoid population in the lymph nodes of immunized mice (Table I).

Pathways and Kinetics of Covalent Assembly of IgG_{2a}.—H and L chains could

be assembled into H_2L_2 through a number of distinct pathways (Table II). In order to determine the sequence of formation of the interchain disulfide bonds in each of four IgG_{2a}-producing tumors, pulse-chase experiments were carried out. The intracellular immunoglobulin molecules present at various times after the chase were examined by electrophoresis of immunologic precipitates. The

TABLE II

Possible Pathways of Assembly of the IgG Molecule

1	2	3	4	5
$H + H \rightarrow H_2$	$H + L \rightarrow HL$	$H + L \rightarrow HL$	$H + L \rightarrow HL$	$L + L \rightarrow L_2$
$H_2 + L \rightarrow H_2L$	$HL + HL \rightarrow H_2L_2$	$HL + H \rightarrow H_2L$	$HL + L \rightarrow HL_2$	$L_2 + H \rightarrow HL_2$
$H_2L + L \rightarrow H_2L_2$		$H_2L + L \rightarrow H_2L_2$	$HL_2 + H \rightarrow H_2L_2$	$HL_2 + H \rightarrow H_2L_2$

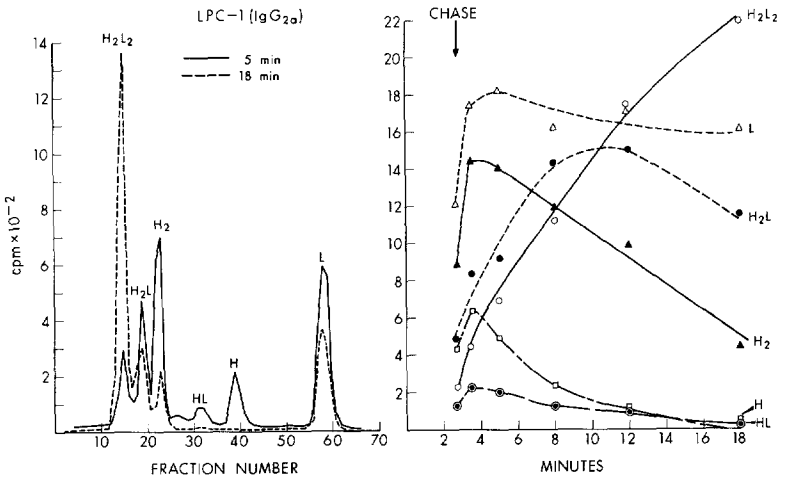

FIG. 3. Pathways and kinetics of covalent assembly of IgG_{2a} in the LPC 1 tumor. A cell suspension was prepared from the LPC 1 tumor. The cells were incubated with amino acids-^{14}C for 2.5 min, chased by adding a 200-fold excess of unlabeled amino acids, and cytoplasmic lysates were prepared and immunologically precipitated as described in the Materials and Methods. Gel electropherograms are shown on the left for the samples obtained 5 and 18 min after addition of label. On the right, the radioactivity in each peak on the gel electropherograms was tabulated for all of the samples obtained.

results with the LPC 1 tumor are presented in Fig. 3. The gel electropherograms at an early and late time after the chase are shown in the left-hand panel, while the results of all the time points are summarized in the right-hand panel. In these experiments, the ribosomes had been removed so that only the assembly of released polypeptide chains was examined. However, since previous studies have shown that all of the nascent peptides are released from the polyribosomes within 2 min after the chase (4, 13), it can reasonably be assumed

TABLE III

Kinetics of Formation of IgG by Mouse Myeloma Tumors, Cultured Cell Lines, and Normal Lymphoid Cells

	Intracellular	
	7 min	18 min
Immune lymph node	29	71
IgG myelomas (average)	49	78
I. IgG_{2a}		
Adj. PC-5	33	56
MOPC 494	50	80
MOPC 173	52	74
LPC 1	20	40
II. IgG_{2b}		
MOPC 282	63	85
MOPC 195	58	100
TEPC 129	50	80
MPC 11 tumor	72	100
MPC 11 culture	70	83
SQPC 11	60	83
MOPC 141	47	91
III. IgG_1		
MOPC 245	51	81
MOPC 160	51	86
MOPC 31C	11	39
MOPC 21A tumor	48	80
MOPC 21A culture	52	82

Pulse-chase experiments were performed in short-term culture with normal lymph node cells and the myeloma tumors and cultured cell lines, as described in the Materials and Methods. The radioactivity in all the immunoglobulin peaks on the gel electropherograms obtained at 7 and 18 min after the addition of label was calculated. The intracellular H_2L_2 present at 7 and 18 min is expressed as the per cent of total radioactivity, corrected by a factor obtained from Table I, showing the percentage of H_2L_2 which was ultimately secreted at 3 hr.

that nascent chains play no role in immunoglobulin assembly which occurs later than 4.5 min. As in previous studies with the MPC-11 tumor (5), experiments have shown that no immunoglobulin molecules were lost in the nuclear pellet, that the amount of antiserum used precipitated all of the immunoglobulin

molecules, and that similar results are obtained without immunological precipitation.

In the LPC 1 tumor, the major intermediates in the assembly of H_2L_2 were heavy chain dimer (H_2) and H_2L (Fig. 3). Immediately after the chase, there were large amounts of H_2 present. As this molecule decreased in amount there was a progressive increase and then decrease in H_2L, followed in time by an increase in H_2L_2. This suggested that pathway 1 (Table II) was the major pathway of assembly of IgG_{2a} in this tumor. Since there was a small amount of HL present which was assembled into H_2L_2, pathway 2 and/or 3 was also

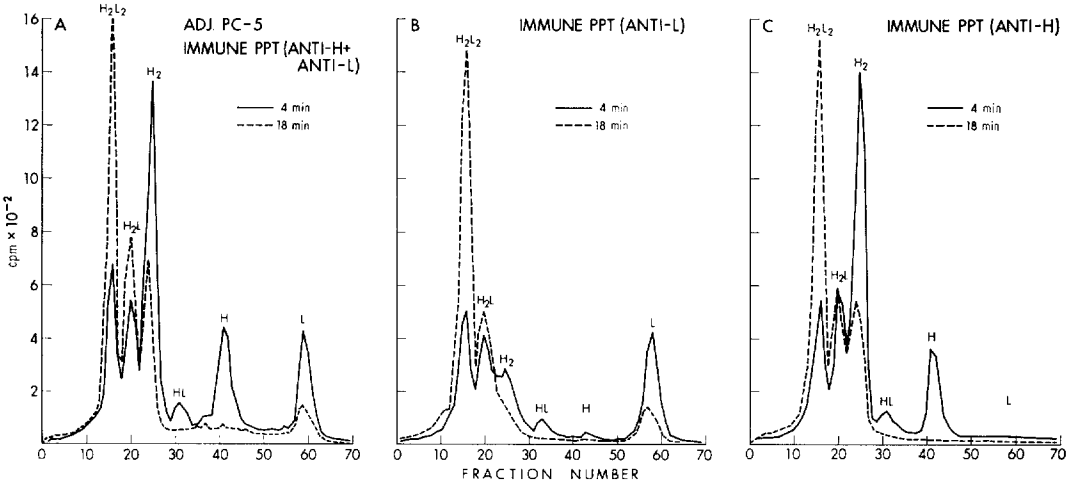

FIG. 4. Covalent and noncovalent assembly of IgG_{2a} in the Adj. PC-5 tumor. A pulse-chase experiment was performed on the Adj. PC-5 tumor as described in the Materials and Methods and in Fig. 3. One-third of the cytoplasmic lysates obtained at 4 and 18 min after the addition of label was precipitated with (*a*) antiserum reactive with mouse γH and kappa L chains (Fig. 4 *A*); (*b*) antiserum reactive with mouse kappa L chains (Fig. 4 *B*); (*c*) antiserum specific for mouse γH chains (Fig. 4 *C*).

operative, although quantitatively minor. In this and subsequent experiments with other tumors, 90% or more of the radioactivity present in the various intermediates at 5 min could be accounted for in H_2L_2 and the intermediates remaining at 18 min. Of the H and L chains which were ultimately assembled into H_2L_2 in LPC 1, 20% were assembled by 7 min and 40% by 18 min (Table III).

Three additional IgG_{2a}-producing tumors were analyzed in the same way as the LPC 1 tumor. Although they differed somewhat from one another in the details of the assembly process, all utilized H_2 and H_2L (pathway 1) as their major pathway of assembly, and all contained small amounts of HL which were also assembled into H_2L_2.

Noncovalent Assembly of IgG_{2a}. —The sequence of formation of the interchain disulfide bonds need not necessarily reflect the noncovalent associations of the polypeptide chains. For example, the free H chains seen on SDS acrylamide gels could have been associated noncovalently with L chains and H_2 could have noncovalently linked L chains attached to it. In order to study noncovalent assembly, pulse-chase experiments were carried out and the cytoplasmic lysates were precipitated with: (*a*) antiserum reactive with both γH and kappa L chains; (*b*) antiserum reactive with kappa L chains but not with γH chains; and (*c*) antiserum specific for H chains. When the Adj. PC-5 tumor was examined at 4 and 18 min after labeling, the results with antibody against H and L

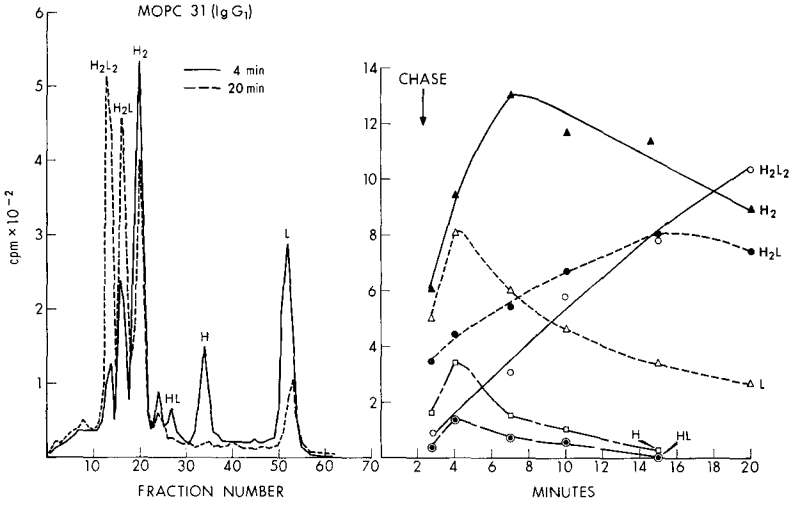

FIG. 5. Pathways and kinetics of covalent assembly of IgG_1 in the MOPC 31C tumor. A pulse-chase experiment was performed on the MOPC 31C tumor, as described in the Materials and Methods and in Fig. 3.

chains (Fig. 4 *A*) were generally similar to those presented for the LPC 1 tumor (Fig. 3). When the portions of the same lysates were precipitated using antibody against L chains (Fig. 4 *B*), all of the L chain–containing molecules were precipitated but only negligible amounts of H and H_2 were identified. When antibody specific for H chains was used (Fig. 4 *C*), all of the H_2 and H were precipitated but no free L chains were detected. These results indicate that most of the H and H_2 did not have noncovalently linked L chains associated with them, and strongly suggest that pathway 1 was the major pathway of noncovalent as well as of covalent assembly of IgG_{2a}.

Assembly of IgG_1. —The kinetics and pathways of covalent assembly of IgG_1 were examined in four tumors and one cultured cell line. Two of these experiments are shown in Figs. 5 and 6. MOPC 31 assembled IgG_1 relatively slowly,

while MOPC 245 assembled it much more rapidly (Table III). As with IgG_{2a}, H_2 and H_2L (pathway 1) were the major precursors of H_2L_2, while HL was a minor precursor. The noncovalent assembly of some of these IgG_1 tumors was examined as described for the LPC 1 tumor with similar results.

Since all of the foregoing experiments were carried out with tumor cells in short-term culture, it was important to determine whether similar pathways and kinetics of assembly existed in vivo. Mice bearing myeloma tumors were injected intravenously with radioactive amino acids and 5 min later they received an injection of unlabeled amino acids. Tumors were removed at

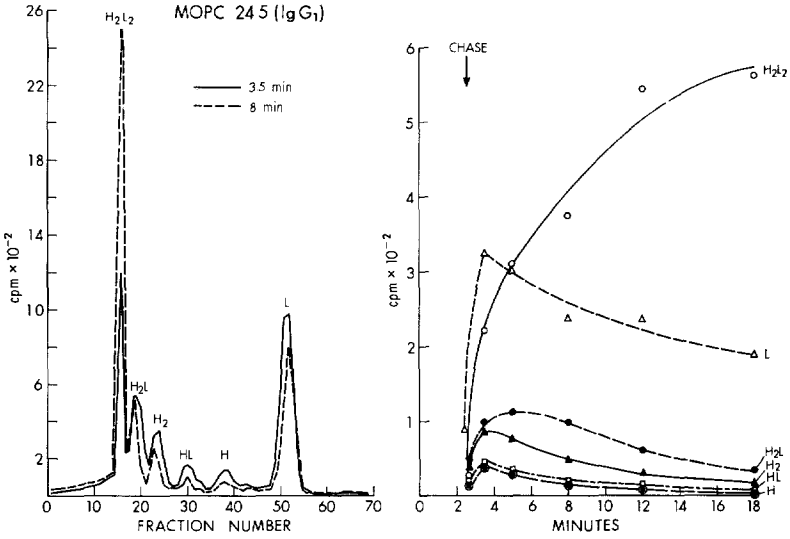

FIG. 6. Pathways and kinetics of covalent assembly of IgG_1 in the MOPC 245 tumor. A pulse-chase experiment was performed on the MOPC 245 tumor as described in the Materials and Methods and in Fig. 3.

various times after the chase and cytoplasmic lysates were analyzed. In these experiments it was difficult to achieve an identical degree of labeling in all mice and to be certain of the effectiveness or time of onset of the chase. Nevertheless the in vivo results (Fig. 7) were quite comparable to those found in vitro (Fig. 5).

Assembly of IgG_{2b}. —As discussed above, the initial characterization of the various tumors (Table I) indicated that, after 15 min of incubation with label, the cytoplasm of the six IgG_{2b}-producing tumors and cultured cell lines contained larger amounts of HL than did any of the tumors of the IgG_{2a} or IgG_1 subclasses. When pulse-chase experiments were performed with IgG_{2b} tumors, they were found to differ from the other two subclasses in that HL was a major precursor of H_2L_2. Two examples are shown in Figs. 8 and 9. Since, in all

IgG_{2b}-producing tumors, H_2 and H_2L were also significant intermediates, it was not possible to be certain whether pathway 2, 3, or both (Table II) was operative. The MPC-11 tumor differed from the other IgG_{2b}-producing tumors in that it was unable to form a covalent bond between the HL precursors. The details of its assembly and mechanism of the block have been described previously (5). However, it is interesting to note that, in the MPC-11 tumor, significant amounts of H_2L_2 were assembled via pathway 1.

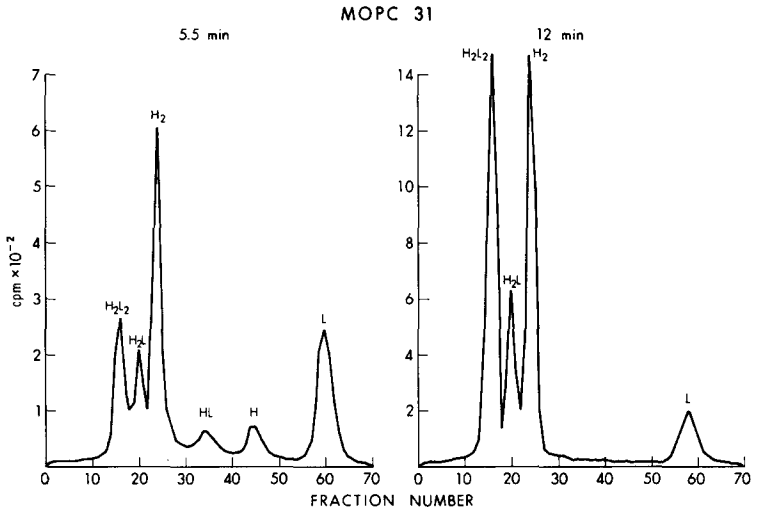

FIG. 7. Pathways and kinetics of covalent assembly in vivo of IgG_1 in the MOPC 31C tumor. Mice bearing the MOPC 31C tumor received an intravenous injection of 30 μCi of valine-^{14}C, threonine-^{14}C, and leucine-^{14}C. 5 min later, they were given an intravenous injection of 0.25 ml of a 200-fold concentrated solution of these same amino acids and the tumors were removed at 5.5 and 12 min after the administration of label. The tumors were immediately chilled and cytoplasmic lysates were prepared, immunologically precipitated, and analyzed on SDS-acrylamide gels as described in the Materials and Methods. The different scales for the gel electropherograms shown resulted from the difficulty in obtaining equal labeling in the two mice used for this experiment, as noted in the text.

Assembly in Lymph Node Cells.—BALB/c mice were immunized using conditions that would lead to predominantly IgG antibody formation. Popliteal lymph nodes were removed and pulse-chase experiments were carried out on cell suspensions using the same techniques as with the myeloma tumors (Fig. 10). H_2 and H_2L were the major precursors of H_2L_2, but HL was also chased into H_2L_2. The relative amounts of the various subclasses vary enormously depending on a variety of conditions, but IgG_1 and IgG_{2a} are usually present in serum in greater amounts than IgG_{2b} (14).

DISCUSSION

The assembly of the three major subclasses of mouse IgG has been studied in 14 myeloma tumors and two cultured cell lines and has been compared to the assembly of IgG by popliteal lymph node cells of hyperimmunized mice. In earlier studies, we (13, 15) and others (6, 16) have presented evidence that some of the initial covalent and noncovalent association of H and L chains occurs while the H chains are still associated with the polyribosomes. However,

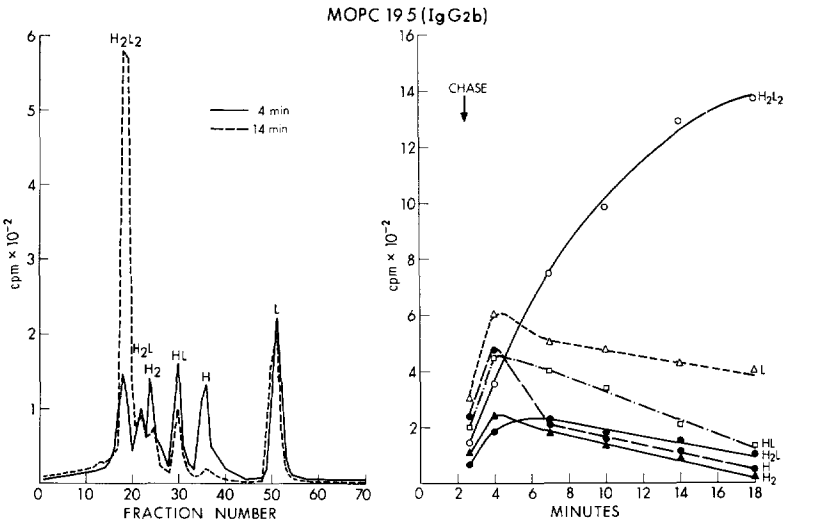

FIG. 8. Pathways and kinetics of covalent assembly of IgG_{2b} in the MOPC 195 tumor. A pulse-chase experiment was performed on the MOPC 195 tumor as described in the Materials and Methods and in Fig. 3.

the kinetics of the assembly process described above indicate that most of the covalent and noncovalent assembly must occur after release of H and L chains from the ribosomes. Studies on the MPC-11 (5) and MOPC 173 (11) tumors have shown that newly released chains continued to assemble even when the remaining nascent chains are frozen on the polyribosomes after treatment with cycloheximide. In contrast, the studies of Schubert (6) suggested that all of the interchain disulfide bonds were formed in the P_3 cell line while the newly synthesized chains were still associated with the polyribosomes. In our own studies with the P_3 cultured cell line and with the MOPC 21A tumor from which it was derived, we found significant amounts of H, L, HL, H_2, and H_2L in the cytoplasm and these intermediates subsequently were assembled into H_2L_2 (17). The fact that Schubert did not find intermediates off the polyribosomes

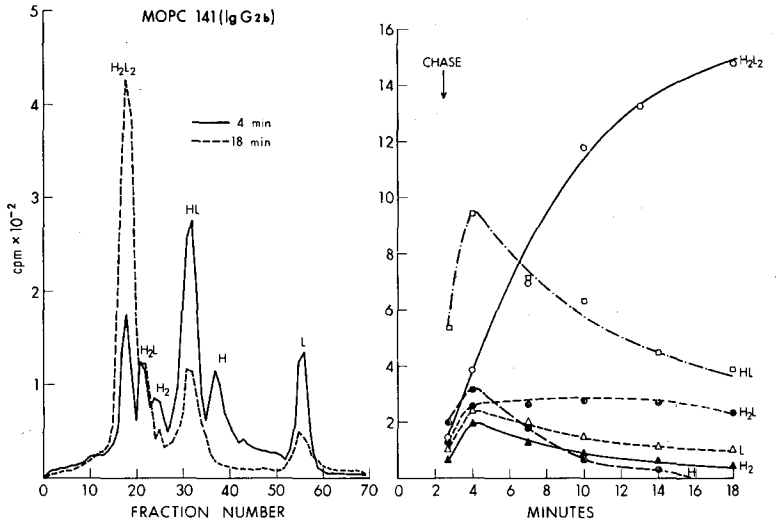

FIG. 9. Pathways and kinetics of covalent assembly of IgG_{2b} in the MOPC 141 tumor. A pulse-chase experiment was performed on the MOPC 141 tumor, as described in the Materials and Methods and in Fig. 3.

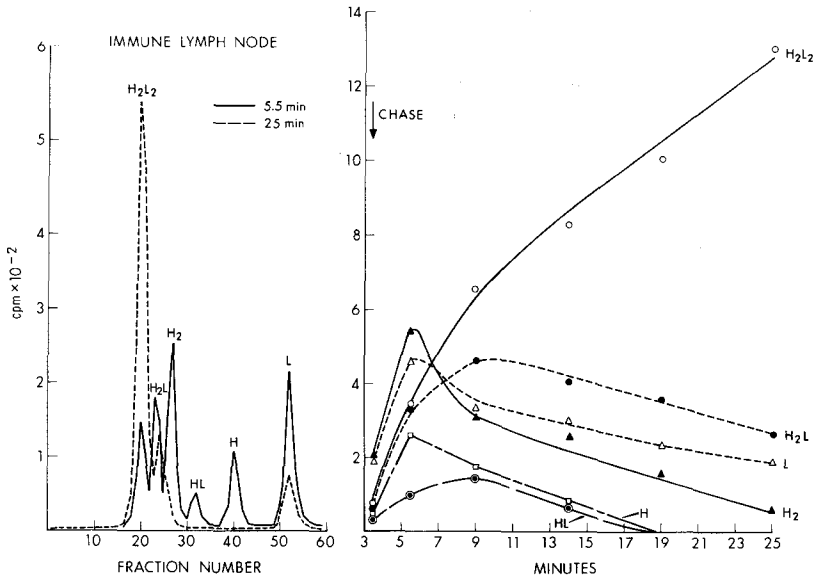

FIG. 10. Pathways and kinetics of covalent assembly of IgG by lymphoid cells of hyperimmunized mice. A pulse-chase experiment was performed on the popliteal lymph node cells of hyperimmunized mice as described in the Materials and Methods and in Fig. 3.

may have been because he did not add iodoacetamide to the reaction mixture during immunological precipitation so that self assembly could have occurred. With the exception of the MPC-11 tumor (13), we have not examined the polyribosomes of the remaining tumors for the presence of assembled nascent chains.

Studies from a number of laboratories have suggested that the newly synthesized H and L chains are released from the polyribosomes into the rough endoplasmic reticulum and then are transported to the outside of the cell via the Golgi apparatus, smooth endoplasmic reticulum, and perhaps some sort of secretory vacuole (19–22). The postribosomal assembly which we have described presumably occurs during the course of intracellular transport. Vassalli et al. (23) have shown that an intact endoplasmic reticulum is necessary for assembly in a cell-free immunoglobulin synthesizing system.

The myeloma tumors which we have examined differed from one other in a number of ways, including the rate at which they assembled their H and L chains into the completed H_2L_2 molecule (Table III). At 7 min, there was a distinct difference between the average kinetics of the H_2L_2 formation by the tumors and cultured cell lines on the one hand, and the immune lymph node cells on the other (Table III). However, almost all of the tumors and cultured lines were relatively efficient in that they ultimately assembled most of the intermediates into H_2L_2 (Table I, secreted immunoglobulin). There were exceptions, however, such as the MOPC 195 and MPC-11 tumors, which secreted relatively large amounts of H_2L and HL, respectively. Some excess free L chains were synthesized and secreted by most of the tumors, cultured lines, and immune lymph node cells.

Studies on some of the IgG_{2a} and IgG_1 tumors suggested that the noncovalent association of the H and L polypeptide chains occurred over a period of many minutes and that the interchain disulfide bonds were formed at about the same time as the noncovalent bonds. We have no information on the kinetics of folding of the individual chains or the time of formation of the intramolecular disulfide bonds. Studies on the refolding of a number of different enzymes in vitro (24, 25) have shown that there is an initial rapid (30–60 sec) folding of the polypeptide chain followed by a series of slow relatively minor rearrangements leading to the final biologically active enzyme. Studies on the association of purified rabbit H and L chains in vitro have suggested that the native configuration of the whole molecule can be achieved more readily if the individual chains are allowed to refold before association with each other (26, 27). The present experiments show that there is ample time for folding of the individual chains to occur before final assembly.

Perhaps the most interesting observations made in the present study relate to the pathways of assembly of the IgG molecule. Each tumor assembled IgG through one major and one or more minor pathways. As noted above neither cellular heterogeneity nor abnormal metabolic conditions could account

for these findings. Two, and perhaps three, of the theoretical pathways (Table II) were observed. Pathways 4 and 5 (Table II), involving light chain dimer (L_2) as an intermediate, do not appear to occur since: (*a*) L_2 is not present in large amounts in any of the IgG tumors; and (*b*) when L_2 was present, as in MPC-11 (4), it was an end product since it did not chase into H_2L_2.

The tumors producing IgG_{2a} and IgG_1 utilized H_2 and H_2L (pathway 1) for both covalent and noncovalent assembly of the immunoglobulin molecule (Fig. 4). The tumors producing IgG_{2b} utilized HL preferentially (pathway 2 or 3) as the major precursor in the covalent assembly of H_2L_2 (Figs. 8 and 9). In a previous study with the MPC-11 tumor, it was demonstrated that two HL were associated by noncovalent interaction, although there was a block in the formation of the inter–H chain disulfide bonds (5). Therefore, at least in this IgG_{2b}-producing tumor, noncovalent assembly also proceeded via pathways 2 and/or 3.

Askonas and Williamson have shown that in an additional IgG_{2a}-producing tumor, 5563, H_2 and H_2L were also the major precursors of H_2L_2 (28). Sutherland et al. (29) have also shown that the major precursors of the IgG_{2a} protein produced by the Adj. PC-5 tumor were H, H_2, and H_2L. Hanaoka reported that HL was a major precursor of H_2L_2 in a cell line derived from MOPC 31B (30). Our own studies with the MOPC 31C tumor (Fig. 5) indicated that H_2 and H_2L but not HL were the major precursors of H_2L_2. This discrepancy could reflect the difference in the cells previously noted (2). The importance of HL as a precursor of IgG_{2b} presumably reflects some property of the H chain. This subgroup specificity in pathways of assembly is not correlated with the location of the inter H-L disulfide bond which appears to be similar for IgG_{2a} and IgG_{2b} and different of IgG_1 (31), or with the structural differences related to the ability to fix C′, since both IgG_{2a} and IgG_{2b} fix complement while IgG_1 does not (32).

Although assembly of the immunoglobulin molecule has not been studied as extensively in other species, HL has been shown to be a precursor in one human IgG myeloma (33), in a number of IgM macroglobulinemias (34), and in normal rabbit IgG (29). Continuous human lymphoid cell lines secreting human IgG use H_2 and H_2L as major intermediates (35). Additional studies using human cells will be necessary to determine if the subgroups of IgG assemble through different pathways.

Finally, both H_2 and H_2L have been shown to be precursors of H_2L_2 in hyperimmunized lymphoid cells of BALB/c mice, indicating that the pathways observed in the tumors are also used in assembly of normal mouse IgG.

SUMMARY

The synthesis, assembly, and secretion of the three major subclasses of mouse IgG has been examined in 14 myeloma tumors and two cultured cell lines as well as in the cells from the popliteal lymph nodes of immunized mice.

The total amount of IgG synthesized was between 15 and 43% of the cytoplasmic proteins made during a 15 min period. H_2 and H_2L were the major precursors of IgG_{2a} and IgG_1 but, in all of the tumors, HL was also an intermediate. In contrast, HL was a major precursor of IgG_{2b}. Most of the noncovalent and covalent assembly of IgG occurred after release of the newly synthesized H and L chains from the polyribosomes and assembly was not completed until 10 min or more after the synthesis of the polypeptide chains.

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