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Biosynthesis of Immunoglobulin A (IgA) and Immunoglobulin M (IgM)

CONTROL OF POLYMERIZATION BY J CHAIN

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Cell suspensions of mouse plasma-cell tumours secreting IgA (immunoglobulin A) and IgM (immunoglobulin M) were incubated with radioactive leucine for various periods of time. The secreted immunoglobulins were precipitated from the culture medium with specific rabbit antisera to determine the relative distribution of radioactivity among the different molecular species, and to estimate the fraction of total radioactivity in the J chain. For IgM-secreting cells there is a balanced synthesis of 7S subunits and J chains, and the secreted product is uniformly assembled to the pentamer. In cells secreting IgA, however, the results demonstrate that the pool of intracellular J chain is less than the intracellular IgA pool. The concentration of J chain is therefore limiting and is less than the requirement for complete polymerization. The major factor that determines whether an intracellular monomer is secreted as such or is polymerized with the addition of J chain is therefore the amount of intracellular J chain. When this is limiting, as it is in cells secreting IgA, then monomer will be secreted.

We have previously shown a requirement for J chain in the polymerization of IgA‡ and IgM (Della Corte & Parkhouse, 1973*b*). The assembly of polymeric forms from 7S (H_2L_2) subunits and J chain was catalysed by a disulphide-exchanging enzyme described by Fuchs *et al.* (1967). Whether the disulphide-exchanging enzyme functions *in vivo* is a matter of speculation. Certainly it is more likely to be involved in IgA polymerization than IgM, since polymerization of IgA *in vivo* never occurred, even at high protein concentrations, unless the enzyme was supplied. Evidence indicated that the polymerization process occurs close to the time of secretion (Parkhouse & Askonas, 1969; Bevan, 1971; Parkhouse, 1971*a*,*b*; Askonas & Parkhouse, 1971; Bargellesi *et al.*, 1972; Della Corte & Parkhouse, 1973*a*). It appears that the addition of carbohydrate does not play a controlling role in polymerization, as secreted IgA monomer and dimer are identical in carbohydrate composition (Della Corte & Parkhouse, 1973*a*), and as intracellular monomers of IgA and IgM, known to be deficient in galactose and fucose (Parkhouse & Melchers, 1971; Della Corte & Parkhouse, 1973*a*; Parkhouse, 1973) can be polymerized *in vitro* (Della Corte & Parkhouse, 1973*b*).

A fascinating question to ask is why secreted IgM is a uniform pentameric product, whereas IgA is secreted as a heterogeneous mixture of monomer and polymer. As the IgA-producing cell contains the biochemical machinery for polymerization, and as secreted (or intracellular) IgA monomer can be polymerized *in vitro* (Della Corte & Parkhouse, 1973*b*), we thought it possible that the amount of intracellular J chain might be a critical factor. From experiments described below, we conclude that synthesis of J chain in IgM-producing plasma cells is balanced with respect to μ chains. On the other hand, the amount of intracellular J chain is limiting in IgA-secreting plasma cells, and therefore probably explains the heterogeneity of the product.

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‡ Abbreviations: IgA, immunoglobulin A; IgM, immunoglobulin M.

Materials and Methods

Plasma cell tumours

These were kindly provided by Dr. M. Potter. Tumours TEPC 183 (IgM) and MOPC 315 (IgA) were maintained in Balb/C mice and 5647 (IgA) was kept in C_3H mice.

Labelling in vitro, isolation and analysis of secreted myeloma proteins

Cell suspensions were prepared and incubated with [4,5-^{3}H]leucine, and the secreted immunoglobulins were precipitated from the culture medium with class-specific rabbit antisera and added homologous carrier immunoglobulin as previously described (Parkhouse & Askonas, 1969). The radioactive antibody–antigen precipitates were electrophoresed in 4.25 % (w/v) sodium dodecyl sulphate–polyacryl-

amide gels (Summers, *et al.*, 1965), or reduced in urea, alkylated and electrophoresed in 5% (w/v) alkaline-urea–polyacrylamide gels (Reisfeld & Small, 1966). The methods are described in more detail in the preceding paper (Della Corte & Parkhouse, 1973*a*). Monomer/dimer IgA radioactivity ratios are given by the sodium dodecyl sulphate system, and the fraction of total radioactivity incorporated into the J chain is given by the alkaline-urea gels.

Results

Three tumour-cell lines were selected for the experiments, one secreting IgM (TEPC 183 cells), and two secreting IgA (5647 and MOPC 315 cells). Cell suspensions were incubated with radioactive leucine for various periods of time, and the secreted radioactive immunoglobulins were precipitated with specific antisera for analysis in polyacrylamide gel. The molecular species present in the secretion were determined with the sodium dodecyl sulphate gel system; the fraction of radioactivity in the J chain was obtained with the alkaline-urea system by using reduced and alkylated samples.

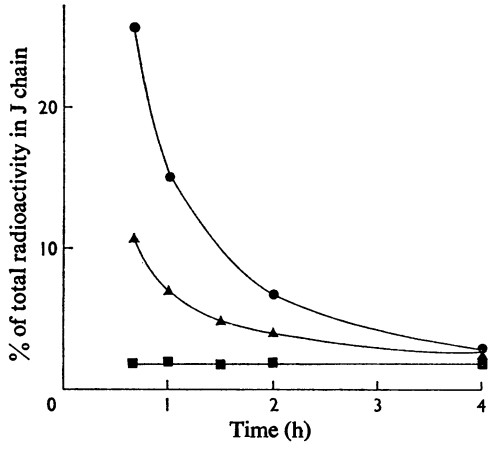

Fig. 1. *Amount of radioactivity in J chain as percentage of the total secreted radioactivity in secreted immunoglobulin*

Cultures incubated for 40 and 60min contained 100 μCi of [4,5-^{3}H]leucine/ml at 52 mCi/mmol. Cultures incubated for more than 1h contained 100μCi of L-[4,5-^{3}H]leucine/ml at 1mCi/mmol. To determine the amount of radioactivity in J chain, secreted radioactive immunoglobulin was precipitated from the culture medium with specific antibody, reduced, alkylated and electrophoresed in alkaline-urea–polyacrylamide gels as given in the Materials and Methods section. Cells: ●, MOPC 315; ▲, 5647; ■, TEPC 183.

The results (Fig. 1) clearly show that the fraction of radioactivity found in J chain from secreted IgM is constant (1.9% of the total) over the 4h time-period. On the other hand, in both IgA-secreting tumours studied the amount of radioactivity found in J chain was high at first and decreased with time, reaching values of 3.1% (MOPC 315) and 2.8% (5647) of the total radioactivity after 4h of labelling. At this time, however, a state of equilibrium labelling is apparently not established, since the values have not stabilized at a constant level.

Similar results were obtained when pulse–chase-type experiments were done. In Table 1 are presented the results of a continuous labelling and a pulse–chase experiment done with a suspension of MOPC 315 myeloma cells. Again the radioactivity found in J chains is high with short incubation periods and decreases as the time allowed for secretion is extended.

By using electrophoresis in sodium dodecyl sulphate–polyacrylamide gels the radioactive secreted products were defined as follows: TEPC 183 secreted 19S IgM (90%), 7S IgM (2%) and free light chains (8%); MOPC 315 secreted monomer (32.3%), dimer (51.3%) and trimer (16.4%) IgA; 5647 secreted monomer (15.0%), dimer (34.3%) and trimer (50.7%) IgA. In both IgA-producing cell lines the relative concentrations of secreted radioactive molecular forms remained constant over the period 1–4h.

Table 1. *Amount of radioactivity in J chain as percentage of the total radioactivity in secreted IgA from MOPC 315 cells incubated with* [4,5-^{3}H]L-*leucine*

For the continuous labelling experiment cultures were incubated with L-[4,5-^{3}H]leucine (100μCi/ml at 1 Ci/mmol) and harvested after 1, 2 and 4h. For the pulse–chase experiment cultures were incubated with L-[4,5-^{3}H]leucine (100 μCi/ml at 52 Ci/mmol) for 15 min, chased by the addition of L-leucine (0.75mg) to each culture of 1ml, and then harvested at 1, 2 and 4h. Secreted immunoglobulin was precipitated from the culture medium with specific antibody, reduced and alkylated and electrophoresed in alkaline-urea–polyacrylamide gels as given in the Materials and Methods section.

Incubation period (h)	Radioactivity in J chain (% of total secreted radioactive immunoglobulin)	
	Continuous labelling	Pulse–chase
1	9.1	5.9
2	5.0	3.6
4	3.7	2.6

Discussion

The molecular heterogeneity of secreted IgA contrasts with the uniform degree of polymerization characteristic of IgM and, as demonstrated in the present paper, can be explained by the intracellular content of J chain.

In the experiments that we have described there was a striking difference between cells secreting IgM and IgA. When cells of the former category were incubated with [^{3}H]leucine, radioactive IgM secreted at all times contained a constant fraction of radioactivity in the J chain. With secreted IgA, on the other hand, the proportion of radioactivity in J chain was relatively high in immunoglobulin collected after short periods of culture, but decreased as the time of incubation was prolonged.

For IgM-secreting cells, therefore, there is a balanced synthesis of 7S subunits and J chains. In cells secreting IgA, however, the results demonstrate that the pool of intracellular J chain is less than the intracellular 7S IgA pool.

The balanced synthesis of immunoglobulin subunits in IgM-secreting cells thus determines the fact that the major secreted molecule is the pentamer. As previously suggested (Parkhouse, 1972), the occurrence of 7S IgM in sera from a variety of pathological conditions may simply reflect a deficiency in J chain synthesis. The absence of polymeric forms of IgM other than the pentamer must indicate that this is the most thermodynamically stable assembly form.

In IgA-secreting cells the size of the intracellular J chain pool is low, relative to the amount of intracellular monomer IgA. The concentration of J chain is therefore limiting and is less than the requirement for complete polymerization. We have shown that isolated IgA monomer, secreted or intracellular, can be polymerized provided that a source of J chain is supplied (Della Corte & Parkhouse, 1973*b*). The major factor which determines whether an intracellular monomer molecule is secreted as such or is polymerized with the addition of J chain is therefore the amount of intracellular J chain. When this is limiting, then monomer will be secreted.

The variable degree of polymerization found among different clones of IgA-secreting cells may be explained if there is a preferred conformation for each clonal product. In some cases the preferred assembly form will be dimer (e.g. MOPC 315 cells), in others trimer (e.g. 5647 cells), whereas in others perhaps a variety of forms are equally permissible. At present it is difficult to understand how a given degree of assembly can be selected, since we must assume that the only structural variable between different IgA molecules resides in the varlable-region sequences; these are remote from that part of the molecule involved in inter-7S-subunit assembly.

Finally, it is instructive to present some calculations based on the data and the following leucine analyses: 501 mol of leucine/mol of IgM, based on an average of the values reported for human IgM (Chaplin *et al.*, 1965; Suzuki & Deutsch, 1967); 13 mol of leucine/mol of human J chain, mol.wt. 24000 (Morrison & Koshland, 1972); 8 mol of leucine/mol of human J chain, mol.wt. 15600 (Schrohenloher, 1973). To calculate the predicted radioactivity fraction of the molecule found in J chains we have assumed 1 mol of J chain/mol of polymer. No calculation has been made for the IgA experiments because the amount of radioactivity found in the J chain had not reached a constant value.

For IgM the calculated values were 1.6% and 2.6% when the two analyses for human J chain were used. Thus the experimental finding of 1.9% would suggest that the molecular weight of 15600 (Schrohenloher *et al.*, 1973) is closer to the fact than the value of 24000 (Morrison & Koshland, 1972). Furthermore, the molecular weight of mouse J chain (16800; R. M. E. Parkhouse, unpublished work) is in reasonable agreement with the lower value of 15600 for human J chain.

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