Assembly of Immunoglobulin G: the Role of the Light-Chain Pool

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Studies on mouse plasmacytomas have shown that IgG† is assembled from its constituent heavy chains and light chains, which are synthesized on separate polyribosomes (Shapiro *et al.*, 1966a; Askonas & Williamson, 1966a). The cells of these tumours contain a pool of free light chains but very little heavy chain (Askonas & Williamson, 1966a; Scharff *et al.*, 1967; Schubert, 1968). Light-chain pools have also been found in normal lymph-node and spleen cells of immunized mice and rabbits (Shapiro *et al.*, 1966b; Askonas & Williamson, 1967) and therefore are not a peculiarity of neoplastic cells. Kinetic experiments, using a plasmacytoma that does not secrete light chain, suggest that the pool is involved in the assembly of IgG (Askonas & Williamson, 1966b; Williamson & Askonas, 1968). Additional evidence for this is presented here obtained by using plasma-cell tumour Adj PC5, which secretes both IgG and an excess of free light chains.

The experiments were designed to distinguish between three possibilities. (a) The accumulated light chains are present in mutant cells that do not synthesize heavy chains (Scheme 1, model a). Schubert *et al.* (1968) have shown that such mutants can arise. (b) The pool is a by-product of protein synthesis, resulting from overproduction of light chains, and is not used for IgG assembly (Scheme 1, model b). (c) After release from the polyribosome, light chains enter the pool, which may be randomly mixed or time-ordered. The pool is then drawn on for IgG assembly (Scheme 1, model c). In each of these alternatives assembly may take place either on the polyribosome, heavy-chain release being controlled by light chains (Schubert, 1968; Askonas *et al.*, 1969; Lisowska-Bernstein *et al.*, 1971), or released heavy chains and light chains may associate in solution to form IgG.

Materials and methods

Plasmacytoma Adj PC5. This tumour, which secretes IgG_{2a} and a κ Bence-Jones protein, was kindly provided by Dr. M. Potter, National Cancer Institute, Bethesda, Md., U.S.A. It was maintained by serial subcutaneous transplantation in Balb/c mice.

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† Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M (macroglobulin).

Scheme 1. Alternative pathways for light chains in cells synthesizing IgG

H, heavy chain; L, light chain; $\boxed{L}$, light-chain pool; $\boxed{P}$, polyribosomes; $\boxed{A}$, assembly process.

Labelling *in vitro*. Cell suspensions were prepared from 2–3-week-old tumours by the method of Jacob & Bhargava (1962), incubated in Eagle's medium without valine (Vogt & Dulbecco, 1963) for 30 min at 3×10^7 – 6×10^7 cells/ml, at 37°C, and then in fresh Eagle's medium with L-[G-³H]valine (500 μ Ci/ml; 29 Ci/mmol) or L-[U-¹⁴C]valine (10 μ Ci/ml; 270 mCi/mmol). Both radioactive amino acids were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Samples were removed at various times and chilled rapidly to 0°C. The cells were washed twice in Hanks salt solution containing 10 mM-DL-valine and quickly frozen in an ethanol–solid CO₂ bath.

Purification of intracellular and secreted Adj PC5 IgG: chain separation. The purification of ³H-labelled intracellular IgG and ¹⁴C-labelled secreted IgG, separation of heavy chains and light chains and measurement of the ³H/¹⁴C ratios of the chains have been fully described by Stott & Munro (1972).

Results and discussion

IgG was purified from Adj PC5 plasma cells incubated *in vitro* with [³H]valine for 5–40 min, and also from the medium of cells incubated with [U-¹⁴C]valine for 4 h, after addition of Adj PC5 serum as a source of carrier IgG. More than 98% of the total

Table 1. *Relative specific radioactivity of light chains and heavy chains from the intracellular IgG of Adj PC5 plasma cells incubated for various times with [^3H]valine*

For experimental details see the text. Results are given $\pm 2\sigma$, calculated from the total number of disintegrations measured in each sample.

Time (min)	$^3\text{H}/^{14}\text{C} (\text{L})/[^3\text{H}/^{14}\text{C} (\text{H})]$
5	0.255 ± 0.005
10	0.255 ± 0.005
20	0.425 ± 0.005
40	0.588 ± 0.003
240	0.925 ± 0.035

radioactivity in each IgG preparation was specifically precipitable with anti-IgG after correction for non-specific precipitation. ^3H -labelled intracellular IgG was combined with ^{14}C -labelled secreted IgG, and the heavy chains and light chains were prepared by reduction and gel filtration on Sephadex G-100 in 1 M-propionic acid. The purity of the separated chains was tested by disc polyacrylamide-gel electrophoresis (Summers *et al.*, 1965; Choules & Zimm, 1965); cross-contamination was less than 3%.

By mixing ^3H -labelled intracellular IgG and ^{14}C -labelled secreted IgG, differences in amino acid composition and chain length, and errors due to differential recoveries of heavy chains and light chains, were eliminated. Consequently, comparison of the $^3\text{H}/^{14}\text{C}$ ratio of light chains [$^3\text{H}/^{14}\text{C} (\text{L})$] and heavy chains [$^3\text{H}/^{14}\text{C} (\text{H})$] from the same IgG preparation gives a measure of the specific radioactivity of light chains relative to that of heavy chains. Since the heavy-chain pool is either zero or very small (Williamson & Askonas, 1968), changes in the ratio [$^3\text{H}/^{14}\text{C} (\text{L})$]/[$^3\text{H}/^{14}\text{C} (\text{H})$] indicate changes in the specific radioactivity of the light chains of IgG. Table 1 shows that the ratio rises from 0.25:1 after 5 and 10 min labelling, approaching 1:1 after 4 h. This indicates that at short labelling times the newly synthesized light chains are diluted by a pool of unlabelled light chains before assembly into IgG. After longer labelling times the ratio approaches 1:1 because the pool becomes saturated with radioactive light chains. These results are in agreement with model (c) (Scheme 1), whereas if

model (a) or (b) were correct the specific radioactivity of the light chains of IgG would be constant and the ratio [$^3\text{H}/^{14}\text{C} (\text{L})$]/[$^3\text{H}/^{14}\text{C} (\text{H})$] would be 1:1 for all labelling times. These results for cells secreting excess of light chains are in agreement with the results obtained by Cioli & Baglioni (1967), and by Williamson & Askonas (1968) for plasma cells secreting IgG only. Similar conclusions were also reached by Parkhouse (1971) for a mouse plasmacytoma that secretes IgM and an excess of light chains. Thus model (c) appears to be applicable to the synthesis of both IgG and IgM.

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