Immunoglobulin formation in B lymphoid cells

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Immunoglobulin (Ig) molecules are formed by thymus-independent lymphoid cells, the B cell series, at various stages of maturation from resting lymphocytes to mature plasma cells; the latter are the cells which produce very large amounts of Ig (50% of total protein synthesis by the cell), and most of this Ig is secreted into the circulation. Since we are still uncertain whether thymus-derived lymphocytes (T cells) are able to produce any immunoglobulin at all, my remarks will be confined to Ig synthesis in the B cell series.

The small B lymphocytes express genes for Ig molecules but produce only small amounts of these proteins, most of which are associated with the outer cell surface. Although Ig represents only a small proportion of the cell's total protein production, the surface Ig molecules are of course essential for the recognition of antigen. On stimulation by the appropriate antigen these cells can be triggered to divide and differentiate into mature plasma cells. Antigen alone is not a sufficient stimulus in most cases; the triggering events are also under the control of other cells which interact with the B cells, ie, T cells and macrophages. The aspects of Ig synthesis which will be discussed are concerned with (a) synthesis by fully mature differentiated cells; (b) synthesis by the small lymphocytes; (c) the induction of lymphocytes to divide and mature into plasma cells; and (d) defects in Ig synthesis in myeloma cells.

Ig synthesis in B lymphoid cells follows all the rules for protein synthesis found in other eukaryotic cells and no special mechanisms for translation or gene transcription have been discovered. Originally it was thought that some special translational events might contribute to the extensive heterogeneity of Ig molecules in the serum which contains 10^6 to 10^7 different Ig molecules. However, no such special mechanisms have been discovered and it is now quite clear that the diversity of Ig molecules reflects the heterogeneity of the cell population which synthesizes Ig. Many different clones arise in individuals, each clone and its progeny forming one type of Ig molecule. Clonal selection thus forms the

basis of a specific antibody response.

In lymphoid tissues there is the usual flow of information from DNA to messenger RNA (m-RNA) to protein. DNA contains the information for the amino-acid sequence of the heavy (H) and light (L) chains of Ig, but the problem we need to elucidate is whether each stem cell possesses a sufficient number of genes coding for the variable regions of H and L chains to account for the heterogeneous population of Ig molecules produced or whether there is only a small gene pool, antibody diversity being generated during the life of the animal by mutations. This problem is still being widely debated (eg, Tonegawa, Steinberg, Dube, and Bernardini, 1974; Barstad, Farnsworth, Weigert, Cohn, and Hood, 1974). In any event, at some stage in its development the precursor cell has to choose to express one variable region and one constant region gene for each chain from its gene pool, be it small or large.

Ig Synthesis by Plasma Cells

A good deal is known concerning the formation and assembly of Ig molecules in mature cells, mainly from *in-vitro* studies on cell suspensions of mouse or human plasma cells tumours and of spleen or lymph nodes from immunized animals. Such studies have relied on the addition of 'pulses' of radioactive amino acids followed by analyses of the Ig and its intermediates, both within the cells and in the medium (representing the secreted Ig), after specific precipitation with the appropriate antiserum. Recent work in this field has been extensively reviewed (eg, Bevan, Parkhouse, Williamson, and Askonas, 1972; Askonas, 1974) and therefore the general conclusions only will be summarized.

Ig molecules for export are formed by polyribosomes aligned on membranes of the endoplasmic reticulum (ER). Heavy and light chains are produced independently on differently sized polyribosomes which are of sufficient size for each chain to be formed as a single polypeptide unit. Since two genes code separately for the variable and constant regions of each chain the genes must be integrated

at the gene level or they must be transcribed into a single m-RNA molecule by some mechanism. This has been confirmed recently by the isolation of L and H m-RNA. These messenger RNAs consist of about 1200 and 1900 nucleotides respectively and are thus of sufficient size to code for the whole light chain and the whole heavy chain, and also contain some additional nucleotide sequences external to the code for variable and constant regions of L and H chains (eg, Stevens and Williamson, 1973; Tonegawa *et al*, 1974; Milstein, Brownlee, Cartwright, Jarvis, and Proudfoot, 1974b).

In the differentiated cell the chains are released rectorially into the vesicles of the endoplasmic reticulum (Bevan *et al*, 1972), where they are assembled within one to two minutes of synthesis into the basic four-chain Ig molecule (L_2H_2) by disulphide bonding. A pool of light chains serves as an intermediate in assembly. This pool is small in normal plasma cells, turns over rapidly and is maintained within the cells. However, in plasma cell tumours the light chain pool varies in size, due to defects in the coordination of L and H chain synthesis and possibly in the alignment of polyribosomes on the ER membrane system. Ig is secreted via the smooth membranes while carbohydrate residues are added gradually during movement of the molecules through the rough and smooth membrane compartments of the cell.

The disulphide bonds in polymeric Ig are assembled more gradually. In mouse myeloma and normal spleen cells the intracellular precursor of IgM is the disulphide-linked, four-chain monomer (μ_2L_2) referred to as IgMs and approximately 7-8S in size. No significant amounts of polymer are present within the cell, so that polymerization into the pentameric 19S molecule occurs at the time of secretion and involves interchange of disulphide bonds, possibly mediated by an enzyme, and also the incorporation of one J chain per 19S pentamer (Parkhouse and della Corte, 1973; Parkhouse, 1974). In some patients with IgM macroglobulinaemia the cells do accumulate 19S IgM (Buxbaum, Zolla, Scharff, and Franklin, 1971) but there is no evidence that this occurs in normal cells. IgA also polymerizes into dimers and trimers in the same way from α_2L_2 monomers at the time of secretion. The fact that IgA is secreted mainly as a mixture of monomer, dimer and trimer is probably due to the formation of insufficient amounts of J chain to permit polymerization of all the IgA subunits (Parkhouse, 1974). The finding that IgM is present as 7S subunits within the cell has a bearing on the size of the surface Ig receptors of IgM class on lymphoid cells (see below).

Heavy and light chain synthesis in plasma cells

is normally regulated to provide a balanced synthesis of light and heavy chains to ensure that the major secretory products of the cell are fully assembled Ig molecules (Askonas, 1974). There is a small excess of light chain secretion observable in immune tissues which we found to be less than 5%; the results obtained by different investigators have been somewhat variable. It is, however, clear that control of H-chain synthesis is much more fragile than that of L-chains; cloning procedures of plasma cell tumour lines have shown that variant clones in which H-chain formation is partially or completely suppressed arise at a high frequency of 10^{-3} (Coffino, Lasteur, and Scharff, 1970). This suggests a regulatory variant rather than mutation of structural genes which appears to arise at a very low frequency in cultured myeloma cells (Milstein, Adetugbo, Cowan, and Secher, 1974a). Partial or complete suppression of H-chain formation is also a frequent observation *in vivo* in myeloma patients. The table summarizes some of the defects in Ig production frequently observed in human and mouse myeloma.

	Intracellular L Chain Pool	Production	Defect
Normal and immune spleen cells	Small	$L = H^1$	—
Myeloma cells	Variable Variable	$L > H$ Only L chain	Regulatory Regulatory (H chain suppression)
	Large —	L and H Variant H chains	No secretion Deletion or structural mutation
	Variable	L and H	Secretion of HL heavy chain deletions

Table Defects in Ig production by myeloma cells

¹L = light chain of Ig, H = heavy chain of Ig

Neoplastic plasma cells of mouse and man, both *in vivo* and in tissue cultures, frequently show disturbances in the synthesis and assembly of Ig molecules and their regulation.

Their susceptibility to these disturbances needs to be taken into consideration in the evaluation of such studies, and conclusions about normal control mechanisms in Ig synthesis therefore have to be based on studies of normal lymphoid cells as well.

In general each cell produces a single molecular species of immunoglobulin although there are some exceptions to this, as a proportion of circulating lymphocytes carry both IgD and IgM on their surface (Rowe, Hug, Forni, and Pernis, 1973). For example, one patient with Waldenström's macroglobulinaemia was found to have B lymphocytes in

peripheral blood with both membrane-bound IgM and IgD, and both classes of surface receptors showed reactivity with IgG. After capping the receptors with aggregated IgG as antigen, both IgD and IgM reappeared on the lymphocyte surface suggesting that both of these molecules were being formed by the same cell (Pernis, Brouet, and Seligmann, 1974). Lymphoma cells in tissue culture often express more than one heavy or light chain, but these cells are malignant and it is not clear whether they suffer from faulty regulatory mechanisms or whether cells at an early stage of development have the ability to express more than one of the constant region genes simultaneously. There is, however, no doubt that at a later stage of normal differentiation only genes for a single species of Ig molecule are expressed, and that the immunoglobulin product of these cells remains the same throughout the life of a clone. This has become clear from our studies with antibody-forming cell clones in mice, which we selected and then propagated by sequential transfer of spleen cells in syngeneic mice (Askonas and Williamson, 1972). We found that the immunoglobulin product of these clones is remarkably stable. We have passaged such clones into as many as 500 mice and through up to eight transfers with an estimated 60-80 successive cell divisions. Unfortunately we cannot passage these clones for even longer periods because these normal cells lose their proliferative capacity after about 60 to 80 cell divisions, unlike the myeloma cells which divide indefinitely (Williamson and Askonas, 1972). The Ig products of mouse plasmacytomas also remain unchanged when passaged over many years in syngeneic mice. In a myeloma patient the structure of the L or H chains of the myeloma protein produced also remains constant during the life of the patient, which may be several years. Although variants of mouse myeloma cells in cloned tissue culture cells have been detected (Morrison, Bauml, Birsthein, Knehl, Preud'homme, Frank, Jasek, and Scharff, 1974; Milstein *et al* 1974), structural variants of the Ig (deletions of part of the heavy chain gene or amino acid replacements) arise at very low frequency (10^{-6} cell generation) unless mutagens are used.

Ig Production in Small B Lymphocytes

Small lymphocytes form very small amounts of Ig, most of which is associated with the plasma membrane. The fact that Ig represents less than 1% of total cellular protein synthesis has been a handicap in the study of Ig formation by these cells as compared with plasma cells. However, recent techniques, including that of separation of the small lymphocytes

from larger secretory cells (Miller and Phillips, 1969) have assisted these studies. By iodination of plasma membrane proteins, surface IgM molecules on B cells have been identified as the μ_2L_2 7S monomeric subunits, IgMs (Vitetta and Uhr, 1973; Marchalonis and Cone, 1973), whereas mature cells secrete the IgM in its 19S pentamer form (Parkhouse, 1974). Estimates of turnover time of the IgMs surface receptors are variable. Following iodination, fairly rapid shedding of IgMs from B cells has been observed with a half life of several hours (Marchalonis and Cone, 1973; Vitetta and Uhr, 1973); on the other hand, after labelling such receptors biosynthetically with radioactive amino acids, the half life is 20-48 hours (Melchers and Andersson, 1973; Andersson, Lafleur, and Melchers, 1974). Similarly, a very slow release of surface IgMs, 10% in eight hours, was reported following iodination of Daudi lymphoma cells in tissue culture (Grundke-Iqbal and Uhr, 1974). These differences might be attributed to subpopulations of B lymphocytes with various rates of Ig receptor turnover or possibly to the deleterious effect of iodination on cell membrane structure leading to cell death; hence, at present, work with biosynthetically labelled receptors probably gives the more reliable estimates.

According to Andersson, Lafleur, and Melchers, (1974) about 90% of the Ig in small lymphocytes is associated with the surface membrane. These cells do not secrete Ig, but slow shedding of the receptor molecules may occur thus accounting for the very slow turnover of these molecules (half life of 24 to 48 hours). This contrasts with the pattern of Ig synthesis in the mature secreting cell, which forms about 100 times more immunoglobulin than the immature B lymphocyte. The density of Ig receptors on the surface is low and most of the Ig formed is secreted from the cell. Up to 50% of the cell's total protein synthesis is represented by Ig and there is a very rapid secretion of the intracellular Ig with a half life of about two hours. Ig secretion by many myeloma cells is much less efficient than secretion by the normal plasma cell; intracellular Ig tends to turn over more slowly in myeloma cells with a half life of about four to six hours.

Conversion of Small Lymphocytes into Ig-secreting Cells

It is not fully understood how a small lymphocyte matures into a plasma cell with high levels of antibody secretion. In a preliminary attempt to analyse this problem we have recently tried to relate the kinetics of DNA synthesis, of the transcription of Ig genes and of Ig synthesis, using a model system in

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which mouse spleen cells are triggered with lipopolysaccharide (LPS) from *E coli* to divide and mature into IgM-secreting cells (Stevens, Askonas, and Welstead, 1975). In this system, short-term culture for four days does not induce IgG production but a major proportion of the B lymphocytes is stimulated to divide and mature so that by the second day 40-50% of the cells have been activated into blast cells (Greaves and Janossy, 1972; Andersson, Sjöberg, and Möller, 1972). It would, of course, be preferable to carry out this type of study on B memory cells stimulated with antigen but, despite the multiclonal response to a single antigen, only a very small proportion of the cells (about 0.05 to 0.2%) is stimulated by a given antigen so that too few cells take part in the reaction for satisfactory biochemical analysis. After LPS administration H chain m-RNA could be isolated in multiple samples using the technique of Stevens and Williamson (1973) who found that m-RNA for H chain (but not for L chain) interacts with Ig molecules. This interaction is neither species-specific nor Ig class-specific.

Mouse spleen cells in tissue culture stimulated with LPS show a 10- to 30-fold stimulation of DNA synthesis on the first or second day, as measured by the incorporation of ^3H -thymidine, but peak levels of IgM synthesis and secretion occur on days 3 and 4, lagging at least 24 hours behind the peak of DNA synthesis. The transcription of m-RNA for H-chain parallels the DNA synthesis pattern, and is reduced by days 3 and 4 when Ig production is at its peak (Stevens *et al*, 1975). The results suggested a translation block on the freshly formed H chain m-RNA until maturation of the cell had occurred. There was no apparent block on the transport of H m-RNA from the nucleus during early stages of activation. Examination by electron microscopy (Shohat, Janossy, and Dourmashkin, 1973) confirms this pattern; 16-28 hours after the addition of LPS many B cells have become lymphoblasts with cytoplasmic polyribosomes, but development of the endoplasmic reticulum commences only after 28 hours and plasmablasts appear on day 3. Translation of the Ig message thus appears to be blocked until the cells are forming the endoplasmic reticulum to which polyribosomes can attach so that synthesis for export from the cell can commence.

Summary

A considerable amount is known about Ig biosynthesis by mature plasma cells, which form large amounts of Ig for secretion from the cell. A brief summary is given of the formation of light (L) and

heavy (H) chains by polyribosomes aligned on the endoplasmic reticulum and the rapid assembly of the chains into 7S molecules (H_2L_2) by disulphide bonding. There is a time-ordered secretion from the cell of 7S Ig molecules; the polymeric forms of Ig, ie, IgM and IgA, are formed from monomers by disulphide bond interchange and J chain incorporation at the time of secretion.

Myeloma cells from mouse and man have proved very useful in this type of study but such malignant cells show many defects in regulatory mechanisms; therefore, no conclusions can be drawn about normal control mechanisms without analysis of lymphoid tissues from normal or immunized animals.

The pattern of Ig synthesis by the mature cell contrasts with that by small B lymphocytes which form 1/50 to 1/100 the amount of Ig produced by mature cells. Most of the small lymphocyte Ig is associated with the cell surface, and in IgM-producing cells the surface receptors are 7S monomer subunits of IgM. Such receptors turn over slowly (24-48 hours); they may be gradually shed from the cell surface but the small lymphocyte does not actively secrete Ig.

Antigen- and cell-cell interactions stimulate small B lymphocytes to divide and mature into Ig-secreting cells. Little is known about the associated intracellular events, but preliminary data on lipopolysaccharide-stimulated mouse spleen cells indicate that transcription of m-RNA for H-chain mirrors the kinetics of DNA synthesis. A translational block then occurs during cell maturation and there is a lag of at least 24 hours before Ig production rises sharply and reaches peak levels.

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