Plasma Cell Tumour-formation and Antigen-Binding Myeloma Proteins in Mice

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Plasmacytoma can be induced in the inbred BALB/c mouse by the injection of mineral oil or implants of solid plastics. This induction depends on three factors: firstly, an abnormal peritoneal granulomatous tissue; secondly, genetically determined factors in the strain BALB/c genotype; and, thirdly, the presence of a gastrointestinal and respiratory microbial flora. The incidence of plasmacytomas following three injections of mineral oils or purified isoparaffins ranges from 40 to 60%. The tumours develop in the peritoneal oil granuloma and often remain localized in the peritoneal connective tissues.

In several series of myeloma proteins in this strain of mice the IgA class predominates; 60-66% of all tumours that produce a detectable heavy chain class myeloma protein produce an IgA class protein. This finding strongly implies that a portion of the total immunocyte population is more prone to neoplastic transformation. Because IgA myeloma proteins in BALB/c mice are usually polymeric molecules, these proteins can be tested for their ability to bind polyvalent antigens by precipitin reactions. Using this method myeloma proteins with antigen-binding activity have been found to several different antigens. The chemical determinants have been identified for many and include: nitrophenyl compounds, $\alpha 1 \rightarrow 3$ dextrose (nigerotriose), methyl α-D-galactoside, methyl β-D-galactoside (probably more specifically to $\beta 1 \rightarrow 6$ linked galactose), N-acetyl glucosamine in $\beta 1 \rightarrow 3$ linkages, N-acetyl D-mannosamine, phosphoryl choline, choline, and a trypsin-sensitive determinant common to antigenic substances produced by salmonella.

Other myeloma proteins have been found to precipitate with antigens of bacterial origin. The chemical determinants for these have not yet been established. The actual incidence of active M-components in a series of tumours ranges from 5 to 10% when test antigens containing the determinants mentioned above are used. Many of the antigens can be shown to be produced by organisms in the gastrointestinal and respiratory microbial flora. The chemical determinants, however, to which the myeloma proteins are directed may be found in some cases on autogenous macromolecules. Nevertheless, bacterial antigens containing these determinants are probably highly immunogenic and the precursors of the tumour cells were probably responding to these antigens before neoplastic transformation. The finding of several plasmacytomas of independent origin that produce myeloma proteins which identify the same antigen or chemical antigenic determinant supports this suggestion.

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Studies on a Biclonal Gammopathy

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The partial amino-acid sequences of the variable regions (V-regions) of 40 human immunoglobulin heavy chains were examined by automatic sequencing methods. At least three distinct classes of the heavy chain sequences exist; members of any V_H class may be found in association with different heavy chain constant regions for (example, with constant regions of μ, α_1, α_2, γ_1, γ_2, γ_3, and γ_4 chains).

In addition, the proteins of a patient (Til) who was producing two serum paraproteins were studied; one protein was an IgG_2-K and the other an IgM-K immunoglobulin. The light chains of the two proteins through the hypervariable region in position 30-35 were identical. A hypervariable region (HYP-R) was noted in the sequence (30-35) of the heavy chains of 8 V_{HIII} proteins homologous to the HYP-R in K chains. The Til IgG and IgM contained common idiotypes as evidenced by fluorescein and rhodamine staining with anti-idiotype serum; 60% of plasma cells stained with anti-IgG_C, 40% with anti-IgM_C, and 100% with anti-Til idiotype. These findings of reciprocal sharing of any V_H with any constant region are stronger evidence for the existence of (at least) two separate genetic systems coding for a single polypeptide chain than any evidence to date. Complete sequence of the two proteins is in progress.

Most probably the genetic systems contributing to the synthesis of a single Ig chain must be close to each other on the chromosomes. The actual number of genes coding for members of a V-region class is uncertain, but analysis of our sequence data suggests that it is probably not less than ten. Our findings also suggest that the IgM and IgG antibodies to a single determinant in the immune response of a single animal have identical variable regions, and may be derived from a single precursor cell—presumably, one which has had no previous exposure to antigen. We postulate that the first exposure to antigen of such a cell stimulates production of first IgM and then IgC within the same cell, followed rapidly by division into separate IgG and IgG-producing cell lines sharing three of the four genes involved in producing each molecule. Hence, if one looks at the right time after antigenic stimulation both IgG and IgM antibodies of the same specificity should be present within the same cell.

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Proliferation and Differentiation in Human Lymphoid and Plasma Cell Lines

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The use of lymphoid cultures in which synchrony of division was achieved has established that immunoglobulin synthesis occurs only during late G1 and early S phases of the cell cycle. Clearly, expression of immunoglobulin genes is a carefully controlled cellular process. Plasma cells differ from lymphoid cells largely in synthesizing greater quantities of immunoglobulin and in employing a greater proportion of the available protein synthetic machinery in the formation of specific immunoglobulin. Current studies indicate several mechanisms by which this aspect of maturation of lymphoid and plasma cell may occur.

Proliferation of lymphoid and plasma cell cultures in vitro has many similarities to proliferation in vivo. Several phases of growth are evident, including an initial lag phase, followed by logarithmic proliferation, until growth slows and a stationary phase is achieved. Similar observations have been reported for tumour cells in vivo. Cell kinetic studies indicate that cells in the stationary phase are continuing through the cell cycle but at a much reduced rate. The phases G2 and S are prolonged, but

the major prolongation is in the G1 phase of the cell cycle. When nutritional limitations on growth are overcome evidence is obtained that crowding and contact inhibition as well as soluble metabolic factors may contribute to the limitations on proliferation.

The lymphoid and plasma cells in culture provide an interesting, though not completely analagous, model for studies of cell proliferation in vivo. The stationary culture shift to prolonged G1 phase, like a comparable event in vivo, should make cells resistant to chemotherapeutic agents that act primarily on DNA synthesis (S phase events). Mechanisms for affecting G1 cells or bringing arrested cells out of G1 are beginning to be explored.

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Immunoglobulin Synthesis and Tumour Cell Number and the Natural History of Multiple Myeloma

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Our recent studies of the synthesis, secretion, and metabolism of monoclonal immunoglobulins suggest that such observations may explain some of the features of multiple myeloma and related disorders. Essential for these studies was the development of radioimmunoassays capable of measuring nanogramme amounts of immunoglobulins produced by a few cells obtained freshly from the patient's bone marrow. Serial studies showed that for a given patient the average secretion rate of myeloma protein per myeloma cell may remain fixed for at least several years during the clinical course (both before and during chemotherapy).

So far over 40 patients with monoclonal IgG, IgA, IgM, and K or L secretion have been studied. Cellular immunoglobulin secretion rates were found to range from 12,500 to 87,500 molecules per minute (5 to 35 pg per day per plasma cell; average 12). If the measured or calculated total body synthesis rate for the patient's monoclonal protein (M-component) is divided by the average cellular rate, the resultant gives the total number of myeloma cells in the body. Our initial measurements of IgG myeloma showed that patients who can be clinically diagnosed as having myeloma have from 0·3 to somewhat over $3{\cdot}0\times10^{12}$ myeloma cells (about 3 kg) in the body. Similar quantities of tumour were present in monoclonal malignancies of other immunoglobulin types. Minor M-components generally cannot be detected by electrophoresis unless the tumour mass is at least 40 g.

These studies also indicated that even patients with "benign monoclonal gammopathies" usually have over 100 g of the progeny of a single plasma cell clone in the body. In IgG myeloma the degree of skeletal involvement correlated significantly with the total number of tumour cells in the body. Bence Jones proteinuria, hypercalcemia, and short survival were also much more frequent in patients with over $2{\cdot}0\times10^{12}$ tumour cells. The variation in serum concentration of the M-component in different patients with similar numbers of myeloma cells is a result of the variation in the secretory rate of the myeloma clones in these patients. During the clinical course little more than four doublings of tumour cell number occur from the time of "early" diagnosis until death with far advanced bone disease. The relation of the number of tumour cells to the natural history of disease and to the effects of treatment can be assessed by relating the total number of tumour cells in patients with early and advanced disease with their average life expectancy. Similar estimations can also be made by measuring the rate of change of the total body synthetic rate of M-component in patients who are untreated, those who are treated, and those who relapse during treatment.

If clonal doubling occurred at a constant exponential rate from its initiation one would calculate that the tumour had been present for over 16 years before clinical diagnosis. Though the concept of a simple exponential growth mode is attractive—and superficially might seem likely from evaluation of electrophoretic patterns—this mode of growth is rarely observed in tumour in animals, including various plasmacytomas, for in these growth slows increasingly as the tumour enlarges. This phenomenon can be described with a Gompertzian growth equation, which contains terms not only for the growth rate at any given time (A), which is a function of the number of tumour cells, but also a constant (a) that sets the degree of slowing of tumour growth.

Growth of Myeloma in Man

To define the actual mode of growth of myeloma in man we have done a detailed study using frequent electrophoretic, plasma volume, and tumour-cell number determinations. Together with Dr. Peter Sullivan we have recently designed very precise computer programmes which can indicate whether growth of myeloma is exponential or Gompertzian. These new techniques have shown already that in man myeloma does indeed grow in Gompertzian fashion and that the exponential models grossly overestimate the duration of the disease. Though a tremendous "iceberg" of tumour cell proliferation occurs asymptomatically before diagnosis, this growth appears to occur in under five years in the average patient. Possibly "benign monoclonal gammopathies" also grow rapidly at first, with retardation in the growth rate as the clone enlarges.

These studies have also been useful for analysing the effects of treatment of myelomatosis. Patients who have good responses to treatment generally have reductions of about two logs in the number of myeloma cells in the body. Interestingly, the mode of regression of the tumour during treatment with melphalan, alone or combined with prednisone, may also be described with the Gompertz equation. Using this equation we have predicted successfully the eventual plateau level of tumour in the body from a series of measurements made during the initial six to eight weeks of treatment, even though the plateau might not be reached for more than six to eight months. Such useful predictive techniques seem likely to bring about further improvement in the treatment of multiple myeloma.

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Natural History of Monoclonal Immunoglobulins

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In this contribution we discuss the fate of monoclonal immunoglobulin spikes detected in the absence of overt myeloma or Waldenström's macroglobulinaemia. Monoclonal immunoglobulins may occur transiently in the serum, disappearing without treatment. In recent years we have seen nine such