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Haematological Oncology

Multiple Myeloma: The Biology of Malignant Plasma Cells

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SUMMARY. **Multiple myeloma remains a difficult disorder to treat and cures are virtually unknown. Most modalities of treatment have been tried on an empirical basis, and a greater understanding of the nature of myeloma progenitors may lead to more specific therapies. In the past few years interest in the biology of myeloma plasma cells has increased and the current state of knowledge is summarised in this review.**

Myeloma clonogenic, or colony, assays have been attempted by many groups. Despite this, no direct equivalent is available of the CFU-GM assay for granulocyte-macrophage progenitors in normal marrow. No published methods have been exported widely to other laboratories.

Recently, myeloma plasma cells were found to express a wide range of adhesion molecules permitting cell to cell and cell to stroma interactions. This finding may explain the difficulty of myeloma colony assays, since adhesive clumping must be prevented.

The observation that interleukin (IL)-6 can stimulate myeloma plasma cells led to further work with other cytokines such as IL-3 and GM-CSF. The precise role of IL-6 in the usual case of bone marrow myeloma remains unclear however.

Multiple myeloma has in the past been a relatively little studied disorder. It is primarily a disease of the elderly that has proved relatively difficult to treat, and leads often to a prolonged period of ill health associated with gradual deterioration, bone pain, repeated infection and death. A few research groups prior to 1983 had an interest in this condition and some useful work had been done, but it was not until the use of high dose melphalan in younger patients by the late Tim MacElwain and his colleagues at the Royal Marsden Hospital,[1] that interest in this condition began to increase exponentially. The possibility that myeloma might be amenable to aggressive treatment and cure, together with increasing knowledge of normal human haemopoiesis, provided the right environment for further research. Clinical studies have increased in number but the investigation of the biology of the causative cells, malignant plasma cells and their precursors, has also increased greatly. The areas of particular interest include:

1. In vitro culture of myeloma bone marrow
2. Identification of progenitors and growth factor requirements
3. Antigen expression by myeloma plasma cells with a particular emphasis on adhesion molecules.

This review sets out to discuss critically the current status with regard to each of these areas and to suggest future developments. The subsequent article considers other aspects of plasma cell biology and discusses the treatment of multiple myeloma.

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Culture of Myeloma Bone Marrow In Vitro

The culture in vitro of myeloma plasma cells might be considered as the holy grail of myeloma research.

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Enormous efforts have been expended by a large number of groups in an attempt at producing a reliable clonogenic assay of myeloma plasma cells in bone marrow. Such a method would be useful for both characterising the nature of the progenitor cells and also for monitoring active disease and also the effectiveness of bone marrow purging.

The characteristics of clonogenic assays of malignant cells were reviewed by Selby et al in 1983.[2] These may be summarised as colonies grown being proportional to numbers of cells seeded on to the plate and that adhesion or clumping phenomena should be excluded. Also, with current knowledge of haemopoietic growth factors, further refinement of the growth requirements of such clonogenic cells should be possible. For example, movements away from the use of conditioned media, accessory cells and serum toward defined serum free media. For ultimate utility, as for assays of normal human haemopoietic progenitors, methodology should be readily exportable to other centres competent in cell culture techniques.

In the middle 1970s Hamburger and Salmon, working in Tucson, Arizona, developed a system of clonogenic assay of myeloma plasma cells. This utilised a fairly complex tissue culture system involving mineral oil conditioned T-cell medium from mice.[4] A review of the literature suggests that this technique has not been exported to other groups and in Tucson tended to be used mainly for drug resistance studies. In the early 1980s Izaguire and colleagues, working in Toronto,[4] developed a more simple method using T-cell conditioned medium that appeared to be fairly straightforward. This produced interesting data at that time but it is now believed unlikely that the colonies identified by this method were clonogenic myeloma progenitor derived (Bergsagel, personal communication).

In 1984, Ludwig et al[5] reported a system of growing myeloma progenitors using a plasma clot method. This appears not to have been utilised by other groups and little further information is available. Adhesion phenomena were not prevented by this method. From 1984 through to 1989 our own group studied culture systems for short term clonogenic growth of myeloma cells. Using a modification of Izaguire's technique we felt that this was producing reliable data but on more rigorous phenotyping of cells obtained from these colonies we considered that we were observing growth of macrophage-like cells combined possibly with some clumping phenomena of plasma cells.

Rhodes et al (1990)[6] used a culture system using adherent layers of cells as feeders with some success in about 55% of cases. Further data on this method is awaited with interest but it does appear quite arduous and the workers were exhaustive in their exclusion of adhesion phenomena.

Millar et al in 1988[7] reported an apparently simple method for growing myeloma progenitors using a system that produced two principle types of colonies mainly small lymphoid cells and larger plasmacytic cells. This and subsequent work using this method has concentrated on drug resistance studies to assist clinicians in determining therapy. It does not appear to have been used by other groups. Despite efforts to avoid adhesion phenomena, it remains possible that these contribute to the colonies in this method because of the very thin agar (0.28%) required in the method. Durie et al (1991)[8] has shown recently how motile plasma cells can be. Also, more detailed phenotyping of colonies using clustered antibodies would improve the strength of these data.

Long-term culture of myeloma bone marrow has been carried out for many years by Durie in Arizona and also by our own group. Observations have tended to be rather empirical, the main value of this technique being the production of myeloma plasma cell lines from extra-medullary plasma cell proliferations (e.g. pleural fluid or ascites).[9,10] They are nevertheless very useful in providing tools for the investigation of the biology of myeloma, but in themselves long-term culture systems appear not to have made a significant contribution to date. Klein's group in Montpellier have used a variation on this technique in which the cytokines GM-CSF and interleukin-6 (IL-6) were found to generate a much greater proportion of cell lines including some from myeloma bone marrow.[11] However, these studies have not produced a reliable clonogenic assay equivalent to, for example, CFU-GM for growing out granulocyte-macrophage precursors.[12]

A very substantial number of laboratory years has so far been spent trying to achieve a highly reproducible clonogenic assay of human myeloma plasma cells. To date none of the methods fulfils entirely the criteria laid down at the outset of this section[1] or those aspects that have become important since. Long-term bone marrow culture of myeloma marrow has its uses in the development of plasma cell lines but little fundamental information about the biology of myeloma or the nature of the progenitor cells has yet emerged from all these studies. Further attempts to develop methods for short term myeloma progenitor cell assays should wait until more information is available about the nature of the clonogenic cell in myeloma, and that cell culture studies should concentrate on longer term cultures supported by bone marrow stroma.

The Nature of the Myeloma Progenitor Cell and its Growth Factor Requirements

The lack of a true progenitor cell or clonogenic assay that has become widely accepted for studying the biology of myeloma progenitors has been discussed above. In normal B-cell ontogeny the full repertoire of the human immunoglobulin gene pool is made available in germinal centres by the production of usually short lived clones which then die through a

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process of apoptosis if they do not have antigen presented. Surviving clones will mature and give rise eventually to plasma cells. It is assumed that at some stage these cells migrate from lymph nodes to bone marrow where plasma cells develop. The possibility that such cells in myeloma may be produced in lymph node and then circulate in the blood prior to homing to bone marrow and other secondary lymphoid organs has been a subject of interest for some years. The search for peripheral blood lymphoid cells that are part of the myeloma clone has continued since Kubagawa et al[13] found idiotype positive lymphoid cells in the blood. This paper has been much quoted but it is possible that at this distance in time from 1979 the methodology used was not sufficiently rigorous to exclude idiotypic antibody having been acquired passively by non idiotype producing cells. Other studies on peripheral blood lymphoid cells in myeloma include those by Pilarski et al[14] and more recently techniques such as the polymerase chain reaction (PCR) and in situ hybridisation have been used to try to determine whether or not myeloma clone B-cells are present in the blood. Clofent et al[15] used PCR and were unable to detect the presence of circulating lymphoid cells of the same immunoglobulin rearrangement as the myeloma itself, but other groups have managed to do so.[16] The difficulty in interpreting these data is that those studies which have found clonal B-cells in blood may in fact be including some circulating plasma cells which will cause PCR positivity whereas those which have not found the presence of re-arranged genes may be excluding the relevant cell population. Studies to investigate the development of pre-plasma cells have been few although Caligaris-Cappio in 1985[17] suggested the possibility that the lymph node phase of B-cell ontogeny was not necessary in myeloma. Although an attractive hypothesis further evidence for this has not been forthcoming.

Work with mouse myeloma had shown that B-cell stimulating factor 2 (BSF-2) could stimulate the growth of plasmacytomas in mice.[18] BSF-2 is now known as IL-6 and Kawano et al in 1988 provided evidence[19] to suggest that IL-6 was produced in excessive amounts in myeloma bone marrow, by the plasma cells themselves. The possibility that the IL-6 was actually produced by monocytic cells contaminating the plasma cell preparation cannot be excluded as being responsible for this observation but subsequent data have shown that there is an unequivocal effect of IL-6 in plasma cell neoplasms.[20–22] However, despite much work the precise role of IL-6 in human myeloma remains unclear although a few patients with very aggressive disease have shown regression of their tumours when treated with anti-IL-6 antibodies.[23]

Following these observations, Bergui et al studied the growth factor requirements of circulating mononuclear cells in myeloma and were able to show stimulation, in particular by the combination of IL-3 and IL-6.[24] This caused cells to develop from peripheral blood mononuclears that expressed the same antibody isotype as the plasma cell neoplasm and end stage plasma cells were seen. However the precise nature of these progenitors and the subpopulation of the mononuclear cells responsible was not investigated further. This group investigated the role of peripheral blood mononuclear cells further by seeding these on to autologous stromal layers from myeloma bone marrow and again showed that colonies of myeloma plasma cells bearing the same isotype were produced.[25] Clearly these data are of fundamental importance and further work needs to be done to try to identify the cells responsible for the plasma cell proliferations within the mononuclear cell fraction of peripheral blood.

These interesting data on the stimulation of plasma cell proliferation by combinations of growth factors led Klein's group[11] to expose marrow in long-term culture to various combinations of growth factors. As mentioned in the previous section, they found that it was possible to generate cell lines using GM-CSF and IL-6. This finding, although of course of great interest, is also rather alarming in that it implies that granulocyte or granulocyte macrophage colony stimulating factors may have an effect on myeloma plasma cells and this may make problematic the use of these cytokines as support for patients receiving high dose chemotherapy for myeloma.

In the absence of data on the nature of myeloma progenitor cells in fresh bone marrow or peripheral blood, the use of cell lines derived from patients with myeloma has acquired a particular importance. In the mid 1980s there were only a very few such cell lines but there has been a significant increase in their availability since. It is of particular importance to ensure that lines obtained from patients with myeloma are not EBV transformed lymphoblastoid lines since although these may have their own intrinsic interest they do not represent in our view the myeloma clone. Such lymphoblastoid cell lines are relatively easy to produce from myeloma marrow and should not be considered to be human plasma cell lines.

Research on plasma cell lines is now extensive. Nilsson developed the first true myeloma cell line, U266,[26] and more recently has used cell lines to propose that the role of IL-6 is as a paracrine growth factor.[21] This is consistent with Klein's work[27] but there is evidence for a degree of autocrine IL-6 activity in U266.[28,29] The advantage of working with myeloma plasma cell lines in such studies is that one is sure of a pure population of cells with which to work.

Antigen Expression by Myeloma Plasma Cells

As a consequence of a number of studies of myeloma plasma cell phenotype it has emerged that myeloma plasma cells express a wide range of surface antigens

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some of which are responsible for cell adhesion, lymphocyte homing and cell localisation. There is no specific plasma cell antigen that is present both on every plasma cell and in all cases of myeloma. PCA-1 is generally specific to plasma cells[30] but in our hands only about 50% of cases express it. The majority of plasma cells also express CD38[31] although this is also expressed on activated T-lymphocytes.[32] The HB8 epitope of CD24 is also expressed commonly[33] and up to 10% of myeloma cases express the CALLA antigen as recognised by CD10 antibodies.[34]

Myeloma plasma cells express a variety of adhesion molecules including members of the integrin and immunoglobulin superfamilies.[35,36] ICAM-1, a member of the immunoglobulin superfamily, is positive in the majority of normal and malignant plasma cells. LFA-1 (CD11a and the beta chain CD18), its ligand, appears to be present on plasma cells in some studies but not others. The specific anti-LFA-1 antibody used appears to be critical since Ahsmann et al, using a new unclustered antibody to LFA-1, F8.8, has found a strong correlation between LFA-1 positivity and disease activity.[37] N-CAM (CD56) and LFA-3 (CD58) are commonly expressed in malignant plasma cells. We showed that LFA-3 is expressed only rarely on normal plasma cells but it appears to be strongly expressed in myeloma.[38] As the ligand for LFA-3 is CD2 found on T-lymphocytes this suggests the possibility of interactions with T-cells which may provide a mechanism for the promotion of cytokine production necessary for myeloma growth. Van Camp and Durie observed that the LE19 antibody for N-CAM was positive in the majority of myeloma plasma cells.[39] We have confirmed this finding[38] and found also that normal plasma cells are usually negative for N-CAM. It would appear that more aggressive myeloma and plasma cell leukaemia cells also tend to lack expression of N-CAM and there is some association between increasing malignancy (e.g. plasma cell leukaemia) and lack of CD56 expression. N-CAM mediates homotypic cell adhesion and like ICAM-1 and LFA-1 may play a role in the clumping phenomenon observed in myeloma cell line growths.[40] The expression of CD56 may also allow interaction between plasma cells and natural killer cells. CD44 is widely expressed by normal and malignant plasma cells and in addition to playing a role in lymphocyte homing it also has a function in linking cell surface molecule interactions with the cyto-skeleton.[36,41] More recent work has shown that myeloma cell lines express some of the very late antigens (VLA), in particular VLA-1 and VLA-4 as well as some VLA-5 and 6.[42,43] These integrins have a role, in particular, in binding cells to extra cellular matrix proteins such as collagen or fibronectin. The frequent expression of these adhesion molecules by plasma cells suggests great potential for interaction with marrow stroma and other regulatory systems such as T-cell interaction.

Conclusions

Myeloma is now being studied much more, and the insights into the characteristics of plasma cells are suggesting new experiments. We still do not understand how the malignancy begins, and the role of earlier stages in B-cell ontogeny and the nature of the myeloma clonogenic cell remain obscure. Why is myeloma so much a disease of bone, rather than of other sites where plasma cells are found such as tonsil and lamina propria of gut? What is the importance of circulating cells in the disease? Are myeloma precursors stimulated by GM-CSF? The answers may be of critical importance as we move into an era of ever more aggressive therapy and the use of peripheral blood derived haemopoietic progenitors mobilised by cytokines to support such therapy. Clearly, if clonogenic myeloma cells circulate in blood, then peripheral cells may be an inappropriate source of support. If therapies that will benefit the majority of patients with myeloma, which has a median age of 70 years at death, are to be developed these are likely to follow a much improved understanding of the biology of the cells involved, and such therapy is likely to be targeted at the origin of the tumour, and not its expression, the plasma cell.

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References

1. McElwain T J, Powles R L. High-dose intravenous melphalan for plasma cell leukaemia and myeloma. Lancet 1983; 2: 822–824.
2. Selby P, Buick R, Tannock I. A critical appraisal of the 'Human tumor stem-cell assay'. N Engl J Med 1983; 308: 129–134.
3. Hamburger A, Salmon S E. Primary bioassay of human myeloma stem cells. J Clin Invest 1977; 60: 846–854.
4. Izaguire C A, Minden M D, Howatson ?, McCulloch E A. Colony formation by normal and malignant human B-lymphocytes. Br J Cancer 1980; ?: 430–437.
5. Ludwig H, Fritz E, Peest D. A plasma clot culture system for growing and antiproliferative drug sensitivity testing of myeloma stem cells. Leuk Res 1984; 8: 702–711.
6. Rhodes E G H, Colleen O, Flynn M P. A serum-free culture method for myeloma progenitors in vitro: Proliferative and immunophenotypic characteristics. Exp Hematol 1990; 18: 79–83.
7. Millar B C, Bell J B G, Lakhani A, Selby P J, McElwain ?. A simple method for culturing myeloma cells from human bone marrow aspirates and peripheral blood in vitro. Br J Haematol 1988; 69: 197–203.
8. Durie B G M, Vela E E, Richardson-Andrews R, Wolff S, Knight I, Shephard J. Evaluation of the functional role of adhesion molecules in myeloma using immunophenotyping combined with time lapse video analysis. Blood 1991; 78: 127a.
9. Durie B G M, Vela E, Baum V et al. Establishment of two new myeloma cell lines from bilateral pleural effusions. Evidence for sequential in vivo clonal change. Blood 1985; 66: 548–555.

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10. Hamilton M S, Ball J, Bromidge E, Lowe J, Franklin I M. Characterization of a new IgGo myeloma plasma cell line (EJM): a further tool in the investigation of the biology of multiple myeloma. Br J Haematol 1990; 75: 378–384.
11. Zhang X G, Bataille R, Jourdan M et al. GM-CSF synergizes with interleukin-6 in supporting the proliferation of human myeloma cells. Blood 1990; 76: 2599–2605.
12. Pike B L, Robinson W A. Human Bone Marrow colony growth in Agar-gel. J Cell Physiol 1970; 76: 77–84.
13. Kubagawa H, Vogler L B, Capra J D, Lawton A, Cooper ?. Studies of clonal origin of multiple myeloma. J Exp Med 1979; 150: 792–807.
14. Pilarski L M, Mant M, Ruether B A. Pre-B cells in peripheral blood of multiple myeloma patients. Blood 1985; 66: 416–422.
15. Clofent G, Klein B, Commes T, Ghanem N, Lefranc M P, Bataille R. No detectable malignant B cells in the peripheral blood of patients with multiple myeloma. Br J Haematol 1989; 171: 357–361.
16. van Riet I, Heirman C, Lacor P, de Waele M, Thielemans ?, van Camp B. Detection of monoclonal B lymphocytes in bone marrow and peripheral blood of multiple myeloma patients by immunoglobulin gene rearrangement studies. Br J Haematol 1989; 73: 289–295.
17. Caligaris-Cappio F, Bergui L, Tesio L et al. Identification of malignant plasma cell precursors in the bone marrow of multiple myeloma. J Clin Invest 1985; 76: 1243–1251.
18. Van Damme J, Opdenakker G, Simpson R J et al. Identification of the human 26-kD protein, interferon B2 (IFN-B2), as a B cell hybidoma/plasmacytoma growth factor induced by interleukin 1 and tumour necrosis factor. J Exp Med 1987; 165: 914–919.
19. Kawano M, Hirano T, Matsuda T et al. Autocrine generation and requirement of BSF-2/IL6 for human multiple myelomas. Nature 1988; 332: 83–85.
20. Zhang X G, Klein B, Bataille R. Interleukin-6 is a potent myeloma-cell growth factor in patients. Blood 1989; 74: 11–13.
21. Nilsson K, Jernberg H, Petterson M. IL-6 as a growth factor for human multiple myeloma cells-a short overview. In: Melchers F, Potter M, eds Current topics of microbiology and immunology. Berlin: Springer Verlag, 1990; 166: 3–12.
22. Tanabe O, Kawno ?, Tanaka H et al. BSF-2/IL-6 does not augment Ig secretion but stimulates proliferation in myeloma cells. Am J Hematol 1989; 31: 258–262.
23. Klein B, Wijdenes J, Zhang X-G et al. Murine anti-interleukin-6 monoclonal antibody therapy for a patient with plasma cell leukemia. Blood 1991; 78: 1198–1204.
24. Bergui L, Schena M, Gaidano G, Riva M, Caligaris-Cappio F. Interleukin 3 and interleukin 6 synergistically promote the proliferation and differentiation of malignant plasma cell precursors in multiple myeloma. J Exp Med 1989; 170: 613–618.
25. Caligaris-Cappio F, Bergui L, Gregoretti M G et al. Role of bone marrow stromal cells in the growth of human multiple myeloma. Blood 1991; 77: 2688–2693.
26. Nilsson K, Bennich H, Johansson S G O, Ponten J. Established immunoglobulin producing myeloma (IgE) and lymphoblastoid (IgG) cell lines from an IgE myeloma patient. Clin Exp Immunol 1970; 7: 477–489.
27. Klein B, Zhang X G, Jourdan M et al. Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6. Blood 1989; 73: 517–526.
28. Jernberg H, Petterson M, Kishimoto T, Nilsson K. Heterogeneity in response to interleukin 6 (IL-6), expression of IL-6 and IL-6 receptor mRNA in a panel of established human multiple myeloma cell lines. Leukemia 1991; 5: 255–265.
29. Schwab G, Siegall C B, Aarden L A, Meckers L M, Nordan R P. Characterization of an interleukin-6-mediated autocrine growth loop in the human multiple myeloma cell line, U266. Blood 1991; 77: 587–593.
30. Anderson K C, Park E K, Bates M P et al. Antigens on human plasma cells identified by monoclonal antibodies. J Immunol 1983; 130: 1132–1138.
31. Ruiz-Arguelles G J, Katzman J A, Greipp P R, Gonchoroff N J, Garton J P, Kyle R A. Multiple myeloma: circulating lymphocytes that express plasma cell antigens. Blood 1984; 64: 352–356.
32. Reinherz E L, Kung P C, Goldstein G, Levey R H, Schlossman S F. Discrete stages of human intrathymic differentiation: Analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc Natl Acad Sci USA 1989; 77: 1588–1592.
33. Jackson N, Ling N R, Ball J, Bromidge E, Nathan P, Franklin I M. An analysis of myeloma plasma cell phenotype using antibodies defined at the IIIrd international workshop on human leucocyte differentiation antigens. Clin Exp Immunol 1988; 72: 351–356.
34. Durie B G M, Grogan T M. CALLA-Positive myeloma: An aggressive subtype with poor survival. Blood 1985; 66: 229–232.
35. van Riet I, de Waele M, Lacor P, Schots R, van Camp B. Expression of cytoadhesion molecules (CD56, CD54, CD18 and CD29) by myeloma plasma cells. Br J Haematol 1991; 79: 421–427.
36. Hamilton M S, Ball J, Bromidge E, Franklin I M. Surface antigen expression of human neoplastic plasma cells includes molecules associated with lymphocyte recirculation and adhesion. Br J Haematol 1991; 78: 60–65.
37. Ahsmann E J M, Lokhorst H M, Dekker A W, Bloem A C. Lymphocyte function-associated antigen-1 expression on plasma cells correlates with tumour growth in multiple myeloma. Blood 1992; 79: 2068–207.
38. Barker H F, Hamilton M S, Ball J, Drew M, Franklin I M. Expression of adhesion molecules LFA-3 and N-CAM on normal and malignant plasma cells. Br J Haematol 1992; 81: 331–335.
39. Van Camp B, Durie B G M, Spier C et al. Plasma cells in multiple myeloma express a natural killer cell-associated antigen: CD56 (NKH-1; Leu-19). Blood 1990; 76: 377–382.
40. Kawano M M, Huang N, Tanaka H et al. Homotypic cell aggregations of human myeloma cells with ICAM-1 and LFA-1 molecules. Brit J Haem 1991; 79: 583–588.
41. Coombe D R, Rider C C. Lymphocyte homing receptors cloned—a role for anionic polysacharides in lymphocyte adhesion. Immunol Today 1989; 10: 289–291.
42. van Riet I, Waele M, Schots R, Lacor P, van Camp B. The expression of cytoadhesion molecules in normal and malignant plasma cells. In: Radl J, van Camp B, eds. Monoclonal gammapathies III-clinical significant and basic mechanisms. Netherlands: Eurage, 1991: 75–78.
43. Uchiyama H, Barut B A, Chanhan D, Cannistra S, Anderson K C. Characterization of adhesion molecules on human myeloma cell lines. Blood 1991; 78: Suppl. 1, 120a.

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