Developmental tumours

Do murine embryonal carcinoma cells express class I histocompatibility antigens?

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H-2 gene products are of central importance in immune recognition, and account for the strong transplantation resistance observed between mouse strains. Murine embryonal carcinoma (EC) cells are the tumourigenic stem cells of teratocarcinomas, and they elicit strong transplantation resistance in some allogeneic mouse strains (1) but do not express H-2 antigens. Since it is generally believed that all T cells recognize major histocompatibility complex (MHC) products, the failure to demonstrate murine cytolytic T cell lysis of EC cells under a variety of conditions is not surprising. This includes the use of lectin-dependent and cell-mediated cytotoxicity (LDCC) (2), a procedure capable of revealing the total cytolytic potential of stimulated murine T cell populations regardless of specificity. In contrast to these findings, the results presented here show that xenogeneic rat cytotoxic T cells can lyse EC cells in LDCC, and, further, that rat cytotoxic T cells generated by stimulation with mouse spleen cells in vitro can lyse murine embryonal carcinoma cells directly (3). These results prompted a re-evaluation of potential murine T cell lysis of EC cells. Murine cytotoxic T cells lytic for EC cells could be generated by immunisations and in vitro mixed lymphocyte cultures which are consistent with recognition of determinants coded by the Qa-Tla region. These results and other serological observations suggest that EC cells express MHC-class I related molecules probably specified by genes at the Qa-Tla region of chromosome 17.


Embryo-derived cell lines

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The multipotential stem cells from early mouse embryos may now be readily isolated and maintained in tissue culture. These cells will re-populate a mouse embryo when introduced into the cavity of a host blastocyst and give rise to chimaeric mice which show contributions from the cultured cells to many tissues including the germ line. This opens up a route from culture to the mouse gene pool. The results of genetic modifications of the cells in culture and their transfer to chimaeric mice will be presented.
Acute leukaemia in humans involves the transformation of single, stem cells or progenitor cells which are either multipotent in terms of lineage potentiality or lineage restricted. Transformed progenitor cells have selective growth advantage and frequently undergo an uncoupling of maturation and proliferation which results in an apparent maturation arrest. When leukaemic cells and normal bone marrow or thymus progenitor cells are compared using monoclonal antibodies to cell surface antigens, and immunoglobulin gene probes, it is clear that the leukaemic phenotype is (with the exception of chromosomal translocations) a conserved replica of the normal counterpart at the same development stage.

Leukaemic clones established as cell lines provide access to interesting gene products that may play a role in early haemopoietic differentiation. Several cell surface glycoproteins have been isolated and characterised from these cells, some chromosomally mapped and one, the transferring receptor, has been molecularly cloned. The corresponding molecules cannot be isolated from normal stem cells and progenitors but the expression of monoclonal antibody defined cell surface structures on these cells can be analysed using the Fluorescence Activated Cell Sorter and colony assays.

Embryonal carcinoma derived growth factor

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The purification, structural characterisation and biological properties of developmentally regulated PC13 embryonal carcinoma derived growth factor will be described.
Sequence heterogeneity at the *Drosophila* tumorgene lethal (2) giant larvae

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The *Drosophila* tumorgene l(2)gl has been localized by deletion mapping and Northern blot hybridization to DNA sequences at the tip of chromosome 2L. Two polyA RNAs of 4.3 and 5.7 kb length hybridize to DNA which cover two allelic breakpoints. Within or close to the coding DNA fragments other sequences are found, which hybridize to the transposon 297. Moreover, a large repetitive DNA region adjacent to the gene hybridize to several but not all chromosome tips, and probably comprise a simple sequence organization. Comparison of genomic DNA from several wildtype stocks revealed a considerable sequence heterogeneity in two particular stocks which is seen at the repeat region and in the fragment containing the transposon sequences. The structural features and possible implications on the high frequencies of mutations found at this locus are discussed.

Factors that affect the growth of teratocarcinoma and embryonic stem cells

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In an earlier study (Martin, 1981) it was demonstrated that cells of the inner cell mass (ICM) of a mouse blastocyst could give rise to pluripotent embryonic stem cell (ESC) lines when they were cultured in medium that had previously been conditioned by teratocarcinoma stem cells (embryonal carcinoma cells, EC cells). It was suggested that the EC-conditioned medium (EC-CM) might contain a growth factor, produced by the EC cells, which stimulated the growth of ICM cells. It was further hypothesized that this growth factor might have autostimulatory activity, inducing the EC cells that produce it to proliferate. Our studies of EC-CM indicate that it does indeed contain factors that stimulate the proliferation of several cell types, including the EC cells that produce it. Purification of the growth factors in EC-CM and their characteristics will be described.

In the course of further studying the phenomenon of ICM cell growth *in vitro* we have determined that EC-CM is not required for the establishment of ESC lines. However, we have observed that there are differences among strains of mice in the frequency with which their ICMs give rise to ESC lines. Whereas the ICMs of some strains give rise to ESC lines at relatively low frequency (5–10 %), ICMs of other strains do so at relatively high frequency (35–45 %). We have taken advantage of this observation to increase the frequency of ESC lines isolated from embryos carrying various mutations. In particular, we have been interested in obtaining cells homozygous for mutations in the mouse t-complex. We have studied three such mutations, t\textsuperscript{w5}, t\textsuperscript{i} and t\textsuperscript{wl8}, each of which is a recessive lethal mutation that causes death of homozygous embryos early in the post-implantation period. Our results indicate that whereas it is possible to obtain an ESC line homozygous for t\textsuperscript{w5}, it has not been possible to obtain an ESC line homozygous for t\textsuperscript{i}. Studies of the t\textsuperscript{wl8} mutation are still in progress.

Developmental tumours

Isolation of the different clusters of embryoid bodies: ultrastructural analysis and capacity for differentiation in vivo

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By means of intraperitoneal passages in mice of strain 129/Sv, of an experimental teratoma Stevens (1959) obtained an ascitic form of tumour, which, because of its similarity to mice embryos of 3 to 6 days development, are called Embryoid Bodies (EB). Morphologically, two types of EB have been described: simple, which are formed by endoderm cells which surround a cluster of embryonary carcinoma (EC), and cystic ones, which have an endoderm layer which simultaneously surrounds EC cells and liquid cavities (Martin, 1978). However, the term EB as Gaillard (1976) points out, is more etymological than real, since the cluster of EB which exist in an ascitis is morphologically extremely heterogeneous.

Our work consists in the first place of isolating, by means of discontinuous Ficoll gradients, the different morphological types of EB existent in an ascitis in a mouse of 129/Sv strain, obtaining in this way four clusters of EB which are analysed ultrastructurally, and in the second place we studied the capacity for differentiation of each one of these EB clusters, when they are subcutaneously injected in 129/Sv mice.

The analysis of the tumours obtained in this way, indicates that each morphological type of EB can be found in different phases of cellular determination, since each one of these develops very concrete capacities for differentiation.


Recombinant retroviruses, a tool to express genes in multipotential embryonic cells

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Moloney Murine Leukemia Virus (M-MuLV) can efficiently infect and integrate into totipotent cells. However, the integrated copies of the virus are inactive and become methylated. An approach to express genes carried by retroviruses stems from our observation that the SV40 enhancer activates adjacent promoters both in differentiated and multipotential cells (1). Considering this fact we constructed retroviral vectors containing various genes under the independent transcriptional control of either the SV40 early promoter or a composite SVtk promoter (1). This transcriptional unit was placed after the first intron of a cloned copy of the M-MuLV proviruses whose gag-pol and env genes were deleted. When these vectors are introduced into a virus producing cell, the recombinant retroviruses are produced. These viruses infect and stably express the new genes in both differentiated and multipotential cells. Therefore these recombinant retroviruses overcome the block(s) in the expression of retroviruses in multipotential cells (2).

Furthermore, we have recently shown that transcription of the antiparallel strand of a gene (producing nonsense RNA) inhibits expression of that gene (3). We will show how recombinant retrovirus vectors can be used to express nonsense RNA in order to study the function of specific genes.

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Embryonary carcinoma cells obtained by immunosurgery, from the external layer or teratocarcinoma embryoid bodies OTT-6050

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Embryoid bodies (EB) are structures derived from an experimental teratocarcinoma. Morphologically, they are formed by pluripotent cells, called Embryonary Carcinoma (EC), and which are surrounded by a layer of endoderm cells.

The interesting point about the EB is that its EC cells remain indifferented for many years, keeping their full potency when they injected subcutaneously (Pierce and Dixons, 1959a) or are introduced in the interior of a blastocyst, and contribute in a totally normal way to the development of all the tissues, including the germinative cells (Papaioannou, 1981), which demonstrates their fully potent characteristics.

In our laboratory and basing our findings on the technique of immunosurgery (Solter et al. 1975), we have used a method which allows us to eliminate selectively the external cellular layer of the EB. In order to do this, we have isolated, using discontinuous Ficoll gradients, a cluster of EB made up exclusively of endoderm cells, which injected in a rabbit gives an antiserum which, in the presence of the complement and embryoid bodies eliminates the external layer.

This work presents the first results obtained using the technique of immunosurgery.


Reversibility of malignancy: molecular control of clonal growth and differentiation in haematopoiesis and normalisation of leukemic cells

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An understanding of the mechanism that controls growth and differentiation in normal cells would seem to be an essential requirement to elucidate the origin and reversibility of malignancy. For this approach I have mainly used normal and leukemic haematopoietic cells, and in most studies have used myeloid cells as a model system. Our development of systems for the in vitro cloning and clonal differentiation of normal haematopoietic cells made it possible to study the controls that regulate growth (multiplication) and differentiation of these normal cells and the changes in these controls in leukemia. Experiments with normal haematopoietic precursors have shown that normal cells require different proteins to induce growth and differentiation. Differentiation-inducing protein, but not growth-inducing protein, is a DNA-binding protein. We have also shown that in normal myeloid precursors, growth-inducing protein induces both growth and production of differentiation-inducing protein, so this ensures the coupling between growth and differentiation that occurs in normal developmental. The origin of malignancy involves uncoupling of growth and differentiation. This can occur by changes from inducible to constitutive expression of specific genes which results in asynchrony in the co-ordination required for the normal developmental programme. Normal myeloid precursors require an external source of growth-inducing protein for growth, and we have identified different types of leukemic cells. Some no longer require, and others constitutively produce their own growth-inducing protein. But addition of the normal differentiation-inducing protein to these malignant cells still induces their normal differentiation in vitro and in vivo, and the mature cells are then no longer malignant. Genetic changes which produce blocks in the ability to be induced to differentiate by the normal inducer occur in the evolution of leukemia. But even these cells can be induced to differentiate by other compounds that induce the differentiation programme by other pathways. In some tumors, such as sarcomas, reversal from malignant to a non-malignant phenotype can be due to karyotypic changes that suppress malignancy. But in myeloid leukemia the stopping of growth in mature cells by induction of differentiation by-passes the genetic changes that produce the malignant phenotype. These conclusions can also be applied to other types of cells.


Developmental tumours
Growth factors and embryos
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