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Desert locusts settle for the night. See pages 484, 486.
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Continuous cultures of fused cells secreting antibody of predefined specificity

THE manufacture of predefined specific antibodies by means of permanent tissue culture cell lines is of general interest. There are at present a considerable number of permanent cultures of myeloma cells[1,2] and screening procedures have been used to reveal antibody activity in some of them. This, however, is not a satisfactory source of monoclonal antibodies of predefined specificity. We describe here the derivation of a number of tissue culture cell lines which secrete anti-sheep red blood cell (SRBC) antibodies. The cell lines are made by fusion of a mouse myeloma and mouse spleen cells from an immunised donor. To understand the expression and interactions of the Ig chains from the parental lines, fusion experiments between two known mouse myeloma lines were carried out.

Each immunoglobulin chain results from the integrated expression of one of several *V* and *C* genes coding respectively for its variable and constant sections. Each cell expresses only one of the two possible alleles (allelic exclusion; reviewed in ref. 3). When two antibody-producing cells are fused, the products of both parental lines are expressed[4,5], and although the light and heavy chains of both parental lines are randomly joined, no evidence of scrambling of *V* and *C* sections is observed[4]. These results, obtained in an heterologous system involving cells of rat and mouse origin, have now been confirmed by fusing two myeloma cells of the same mouse strain, and provide the background for the derivation and understanding of antibody-secreting hybrid lines in which one of the parental cells is an antibody-producing spleen cell.

Two myeloma cell lines of BALB/c origin were used. P1Bul is resistant to 5-bromo-2′-deoxyuridine[4], does not grow in selective medium (HAT, ref. 6) and secretes a myeloma protein, Adj PC5, which is an IgG2A (κ), (ref. 1). Synthesis is not balanced and free light chains are also secreted. The second cell line, P3-X63Ag8, prepared from P3 cells[2], is resistant to 20 μg ml^{-1} 8-azaguanine and does not grow in HAT medium. The protein secreted (MOPC 21) is an IgG1 (κ) which has been fully sequenced[7,8]. Equal numbers of cells from each parental line were fused using inactivated Sendai virus[9] and samples contining 2×10^5 cells were grown in selective medium in separate dishes. Four out of ten dishes showed growth in selective medium and these were taken as independent hybrid lines, probably derived from single fusion events. The karyotype of the hybrid cells after 5 months in culture was just under the sum of the two parental lines (Table 1). Figure 1 shows the isoelectric focusing[10] (IEF) pattern of the secreted products of different lines. The hybrid cells (samples *c–h* in Fig. 1) give a much more complex pattern than either parent (*a* and *b*) or a mixture of the parental lines (*m*). The important feature of the new pattern is the presence of extra bands (Fig. 1, arrows). These new bands, however, do not seem to be the result of differences in primary structure; this is indicated by the IEF pattern of the products after reduction to separate the heavy and light chains (Fig. 1*B*). The IEF pattern of chains of the hybrid clones (Fig. 1*B*, *g*) is equivalent to the sum of the IEF pattern (*a* and *b*) of chains of the parental clones with no evidence of extra products. We conclude that, as previously shown with interspecies hybrids[4,5], new Ig molecules are produced as a result of mixed association between heavy and light chains from the two parents. This process is intracellular as a mixed cell population does not give rise to such hybrid molecules (compare *m* and *g*, Fig. 1*A*). The individual cells must therefore be able to express both isotypes. This result shows that in hybrid cells the expression of one isotype and idiotype does not exclude the expression of another: both heavy chain isotypes (γ1 and γ2a) and both V_H and both V_L regions (idiotypes) are expressed. There are no allotypic markers for the C_κ region to provide direct proof for the expression of both parental C_κ regions. But this is indicated by the phenotypic link between the *V* and *C* regions.

Figure 1*A* shows that clones derived from different hybridisation experiments and from subclones of one line are indistinguishable. This has also been observed in other experiments (data not shown). Variants were, however, found in a survey of 100 subclones. The difference is often associated with changes

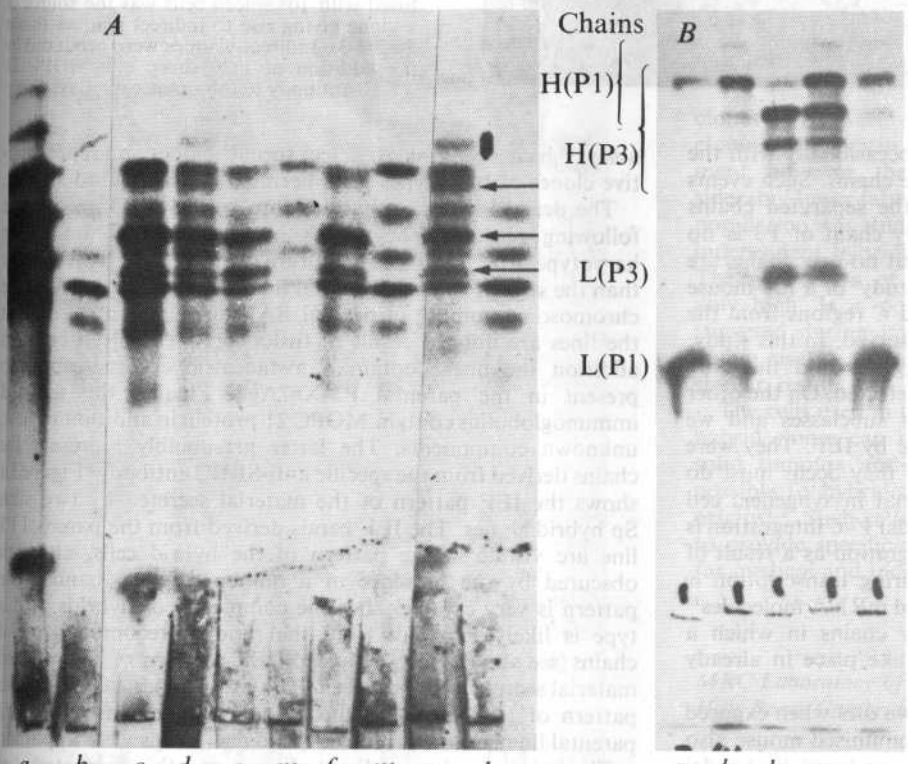

Fig. 1 Autoradiograph of labelled components secreted by the parental and hybrid cell lines analysed by IEF before (*A*) and after reduction (*B*). Cells were incubated in the presence of ^{14}C-lysine[14] and the supernatant applied on polyacrylamide slabs. *A*, *p*H range 6.0 (bottom) to 8.0 (top) in 4 M urea. *B*, *p*H range 5.0 (bottom) to 9.0 (top) in 6 M urea; the supernatant was incubated for 20 min at 37 °C in the presence of 8 M urea, 1.5 M mercaptoethanol and 0.1 M potassium phosphate *p*H 8.0 before being applied to the right slab. Supernatants from parental cell lines in: *a*, P1Bu1; *b*, P3-X67Ag8; and *m*, mixture of equal number of P1Bul and P3-X67Ag8 cells. Supernatants from two independently derived hybrid lines are shown: *c–f*, four subclones from Hy-3; *g* and *h*, two subclones from Hy-B. Fusion was carried out[4,9] using 10^6 cells of each parental line and 4,000 haemagglutination units inactivated Sendai virus (Searle). Cells were divided into ten equal samples and grown separately in selective medium (HAT medium, ref. 6). Medium was changed every 3 d. Successful hybrid lines were obtained in four of the cultures, and all gave similar IEF patterns. Hy-B and Hy-3 were further cloned in soft agar[14]. L, Light; H, heavy.

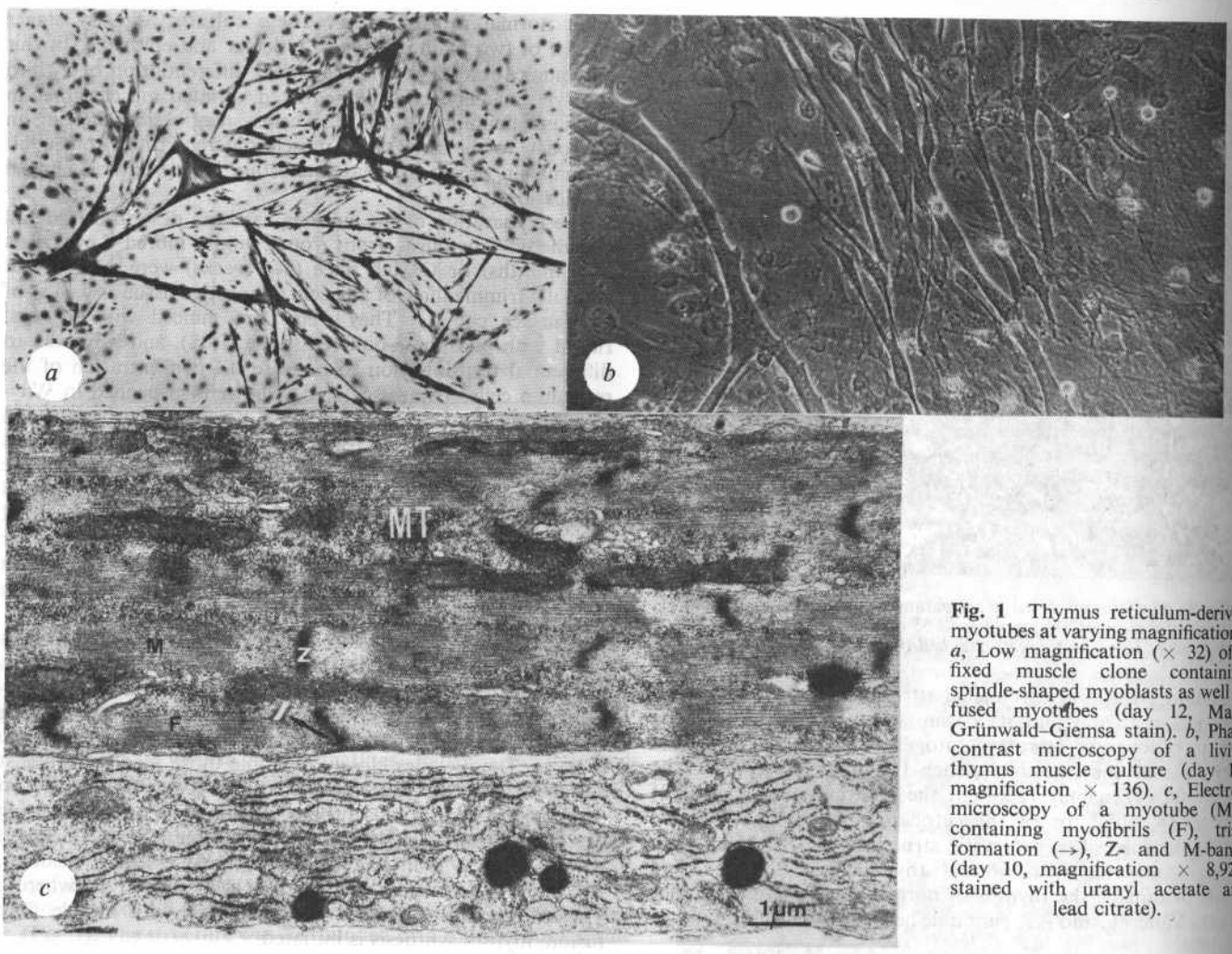

Fig. 1 Thymus reticulum-derived myotubes at varying magnifications. *a*, Low magnification (× 32) of a fixed muscle clone containing spindle-shaped myoblasts as well as fused myotubes (day 12, May-Grünwald–Giemsa stain). *b*, Phase contrast microscopy of a living thymus muscle culture (day 10, magnification × 136). *c*, Electron microscopy of a myotube (MT) containing myofibrils (F), triad formation (→), Z- and M-bands (day 10, magnification × 8,925, stained with uranyl acetate and lead citrate).

malian organs[14] might argue against the possibility that thymus muscle clones are derived from pre-existing committed precursor cells.

Thus, we are left with the third possibility, namely, differentiation of thymus muscle clones from pluripotential stem cells. Several arguments seem to favour this possibility. It has been shown that, apart from muscle, tissues as different as osteocytes[15] and chondrocytes (D. Yaffe, personal communication) can differentiate from thymus cells in particular conditions *in vitro*. In our cultures, the relatively long latency period of 8 d preceding muscle cell fusion could be due to differentiation processes of originally pluripotential stem cells. This is well in line with the strikingly similar behaviour of mouse teratoma cells. Undifferentiated OTT 6050 embryoid body cells differentiate into muscle tubes when they are cultured in very similar conditions to our thymus cells[16].

The relationship of thymic cells and neoplastic pluripotential stem cells may be more than coincidental. It should be noted that the anterior mediastinal space shows the highest rate of teratomas, next to the gonads, and that these teratomas are thought to originate from thymic tissue[17]. It is tempting to speculate that mediastinal teratomas arise from neoplastic transformation of pluripotential stem cells in the thymus. Such stem cells, accumulated in this organ, may have a physiological role in the regeneration of the body's tissues. Experimental studies of congenital aplasias, for example in nude mice, should yield further information.

A final, very obvious implication could concern the correlation of thymic lesions and muscle autoimmune diseases like myasthenia gravis in particular. As we demonstrated before, thymocytes can be *in vitro* sensitised against autochthonous thymus reticulum cells[3]. It seems possible, therefore, that, following a defect of control, thymus lymphocytes could be autosensitised against a thymus component bearing muscle antigens, and that such autosensitised lymphocytes cause the pathological lesions leading to myasthenia. Whether or not this is the case, our culture system provides the means to approach this problem experimentally.

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