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Cloning Cells of the Immune System

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The purpose of this introductory note is to explain why the immunological papers in this collection concentrate on cloning. There are three good reasons for choosing cloning as an appropriate subject at the present time. One is that cloning provides a valuable means of acquiring information about the working of the immune system. Another is that several of the technical problems which prevented satisfactory cloning have just been solved, so that rapid progress can and is being made. The third is that application of the new procedures is providing insight into leukaemia.

The value of cloning follows from the way in which the immune system is arranged as a loose population of cells which traffic from place to place interacting through transient contacts and soluble factors. In consequence cells are differentiated from one another not by their anatomical position and connections but by their genetic and epigenetic makeup. There is a strong contrast here between the nervous and immune systems, the two most complex and highly integrated systems of the body which otherwise share many features in common. No doubt clones from the nervous system can provide interesting information about such topics as receptor function and metabolic control, but they cannot be expected to tell us directly how neurones work. This is not true of lymphocytes and to a lesser extent, of antigen-presenting cells: here we can expect clones to express all major functions.

The major technical problem has been to find ways of keeping cells alive and multiplying outside the body. The first step forward was to maintain clones of B cells under antigenic stimulation in irradiated mice (Askonas and Williamson 1972). For B cells the in vitro problem has now largely been solved by the hybridoma technique (Köhler and Milstein 1976). Monoclonal antibodies produced by this technique turn out to be immensely powerful tools in biochemistry, cell biology, and medicine. Their application is well exemplified by Beverley's study of stem cell surface markers described in this volume. The hybridoma revolution is sweeping all before it, leaving only little room for alternatives such as the immortalization of human immunoglobulin-secreting cells by Epstein-Barr virus infection (Steinitz et al. 1977).

For T cells, hybridomas have thus for proved less successful. Our own experience has been that immunoregulatory activity can be maintained in this way for a while, but tends to decline in an unpredictable and uncontrollable way (Kontiainen et al. 1978). Other laboratories find the same decline. On the other hand, T cells are proving highly amenable to less drastic cloning procedures.

One such procedure is to maintain them on T cell growth factor (TCGF). Another is to restimulate cultures with antigen at intervals. Both of these procedures are discussed and evaluated in detail at the International Congress of Immunology this year, and the latter is well exemplified by Hengartner's study described here.

Our approach (Czitrom et al. 1980) has been to generate allospecific helper T cells by stimulation in vitro. Our previous work had shown that the adoptive secondary response in mice could be successfully adapted for the study of helper T cells directed at cell surface antigens. We generate helper T cells by alloantigen-induced proliferation in vitro directed at I^k (A.TH anti-A.TL) and test for their ability to help in vivo primed B cells directed at D^b (A.TH anti-B10) in an adoptive

secondary response with 2000-R irradiated boosting antigen – a cell carrying both the I^k and the D^b antigens [B10.A(2R)]. Helper T cells did increase the anti-D^b response, as judged by Cr^{51} cytotoxicity titrations 9 days after cell transfer. The in vitro generated specific helper T cells in primary and repeatedly stimulated mixed lymphocyte cultures were more effective in helping these B cell responses than equivalent helper T cells induced by in vivo priming.

Similar results have been obtained with helper T cells boosted in vitro and directed at H-minor antigens (CBA anti-B10.Br) in helping in vivo primed B cells directed at Thy.1 (CBA anti-AKR). Thus, we are still at an early stage in our attempt to generate clones. The point of our approach is that it utilizes a powerful and important group of antigens, the murine alloantigens, at the expense of having to use a rather cumbersome assay for function.

How far will these approaches take us with leukaemia? The use of TCGF for growing leukaemic and normal lymphocytes in vitro are just beginning to be explored and will be made easier by the purification of the agent as here described by Gallo. TCGF is itself both a candidate agent and a target for therapy in immunological diseases, including leukaemia. Lymphocytes can be generated in vitro with the capacity to kill MHC-identical human leukaemic cells (Sondel et al. 1976). There are still many questions about these cells, such as their relationship with natural killer (NK) cells. These can surely best be answered by cloning.

On the B cell side, the main application of monoclonal antibodies to leukaemia thus far has been in (1) the identification of markers on lymphocyte subsets and their use in defining leukaemic phenotypes, topics discussed here by Greaves and (2) the characterization of transformation proteins such as $ppSRC^{60}$ (for references see Mitchison and Kinlen 1980).

Some fascinating questions are beginning to arise in ontogeny as one attempts to relate the stages of lymphocyte development to events affecting immunoglobulin genes. At what stage, for instance, do V_H and V_L genes move to their "differentiated" position close to J and C genes? If, as seems likely in the mouse at least, V_H genes are expressed (as idiotypes) earlier than V_L genes, why does the intervening interval (the pre-B cell) last so long? Could it be that movement of V_H is a difficult and dangerous process for the cell, as the evidence of mistaken movements on the unexpressed chromosome suggests; if so, may not the rapid proliferation of pre-B cells represent a mechanism for expanding a premium cell before it has to undergo the equally costly business of moving a V_L gene? Such speculations may at least begin to explain why so many ALLs are of pre-B types (this discussion of pre-B cells draws on M. Cooper's unpublished data and is derived from discussion with him).

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