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Monoclonal antibodies from hybrid myelomas

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When the lymphoid cells from immunized animals are fused with myeloma cells adapted to grow permanently in culture, hybrid cells can be isolated that are capable of permanent growth in culture, or as transplantable myeloma tumours in animals, and that at the same time express the antibodies of the immunized donor. Such hybrid cells can be cloned and the antibody produced by each clone is monoclonal. By this procedure therefore it is possible to dissect the hetereogeneous immune response of an animal. The monoclonal antibodies can be permanently produced in unlimited quantities and the products are well defined chemical entities, unlike antibodies prepared in animals, which vary from animal to animal and even in different periods within a single animal. These properties have been of great importance in the use of antibodies as biochemical reagents in basic research in a variety of fields. They are also replacing conventional antibodies in standard laboratory practice.

It is with considerable trepidation that I am addressing you on the very happy occasion of this Royal Society Wellcome Foundation Lecture. It is not only the question of the responsibility of delivering this first lecture, but also a terror of failing you all.

Among you there are many who came, out of kindness, to be with me in this exciting moment. Some of you may have come with the hope of finding out what the fuss over monoclonal antibodies is all about. But at the other extreme there are those who are by now better informed about monoclonal antibodies from hybrid myelomas than myself. I really despair of my ability to cope with this situation.

I will feel sufficiently relieved if I can transmit to all of you the deep impression that living through the personal experience of these past years has left on me. Although the message has been repeated many times in the past, for some odd reason it needs to be repeated again and again. Even to someone like me, who was convinced before it all happened, such a clear example of the artificiality of the dissociation between so-called basic and applied research as I have experienced came somewhat as a shock. Yes, basic and applied research may appear to be well defined at times. How often have we heard someone saying: 'Oh, no! My research is of no practical use to anyone'? And then there is this shattering experience that

what seemed quite clearly basic, with no possible application, became very much applied. I do not plan to produce analogous examples of exactly the opposite, of which there are many.

It is not only that I was totally committed to basic immunology before the method for the derivation of monoclonal antibodies was developed, but also that the method itself evolved from one experiment, among others, performed to provide us with a more appropriate cell line with which we could continue our studies on the old problem of the nature and origin of antibody diversity.

I became involved in immunology in 1962, fascinated, as many others, by the diversity and specificity of antibodies. This was a problem that had been growing in theoretical interest since it was first recognized by Ehrlich at the beginning of the century. My involvement was prompted by the developments that were taking place at the time and which, in the words of R. R. Porter (1967), offered 'a feasible experimental approach to obtaining an answer to the question .... Does amino acid sequence alone control antibody specificity and, if so, how is it achieved?'.

The following period in basic immunology was as fruitful as in our wildest dreams. By 1970, our general ideas had settled down to a meaningful picture (Milstein & Pink 1970) which has not changed in its fundamentals although our understanding of the system has been revolutionized by the unfolding of its intricacies and complexities. Indeed, it was as a consequence of the advances of that period that I became convinced that to further our knowledge of the subject we needed a basic change in approach. So my priorities shifted from protein chemistry to nucleic acid chemistry and somatic cell genetics. In a short time I found myself and my collaborators trying to make mutants of myeloma cells in culture and at the same time fusing myeloma cells to alter the stability of their expression. The coexistence of those two aims and the need to evolve new ways to further them were the essential ingredients from which the research that I will describe to you developed.

Hybrid cell lines secreting predefined antibody

Antibodies are made up of light and heavy chains (as illustrated in figure 1), which are usually joined by disulphide bonds, each containing a variable and a constant region, usually referred to as the V region and the C region. Each V region is a folded polypeptide of about 100–120 amino acids and contains one intrachain disulphide bond. The C region contains between one and four similar pseudo-subunits in each chain. These define the class of the antibody molecule. The C regions are involved in effector functions, such as complement fixation and transport across membranes. Within a type or a subclass the C region is highly constant. On the other hand the V region is highly variable; with very few exceptions each antibody molecule has a different V region, even when the same antibody specificity is shared by more than one molecule. This dual role of recognition and effector functions, although expressed in a single polypeptide chain, is under

the control of independent genetic loci (figure 2). There are key elements in this genetic arrangement that make the antibody gene family a unique system: the final expression into protein requires a rearrangement of the genes and in addition there is insufficient coding DNA to account for the diversity of amino acid sequences to which the germ line genes can give rise.

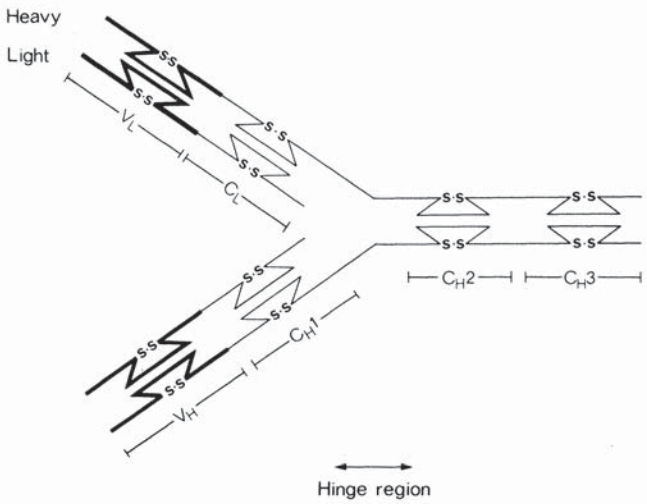

FIGURE 1. The IgG molecule: light and heavy chains are joined by S–S bridges, which have not been drawn as they vary in classes and subclasses of antibodies. They are made up of S–S loops of about 100–120 residues each, and the one at the N-terminus is highly variable.

The DNA rearrangements occur somatically at some stage during differentiation of the stem cells into antibody secreting cells. These changes commit the relevant cells to the production of a single antibody structure. But, since the genetic changes are independent for each cell, the antibody molecule secreted by each cell is different (figure 3). The antibody response is the result of the proliferation of some of these cells triggered by the antigenic stimulus.

Many of the important advances in our present understanding of this system have come from studies of myelomas and related lympho-proliferative disorders. Myelomas are tumours of antibody-secreting cells that arise spontaneously in animals, but that can be induced in mice by injections of mineral oil. They do not arise as the result of a specific antigenic stimulation, but they produce and secrete an immunoglobulin, myeloma protein, with no defined antibody function.

Myeloma tumours in experimental animals can be transplanted and adapted to grow in tissue culture. On the contrary the naturally occurring antibody-producing cells, which proliferate in the spleen and other lymphoid organs as a result of

FIGURE 2. A schematic representation of the genes coding for antibodies. The three chains are probably in different chromosomes. In the mouse the κ and heavy chains are probably on chromosomes 6 and 12, respectively (Hengartner *et al.* 1978) and the heavy chains on chromosome 14 in man (Croce *et al.* 1980) and in rat (Schröder *et al.* 1980). The V regions are coded by V fragments and J fragments of DNA occurring many thousands of bases apart. The number and detailed arrangements of genes in each case vary in different species. For the expression of an antibody, individual V, J and C fragments are associated combinatorially within a horizontal array.

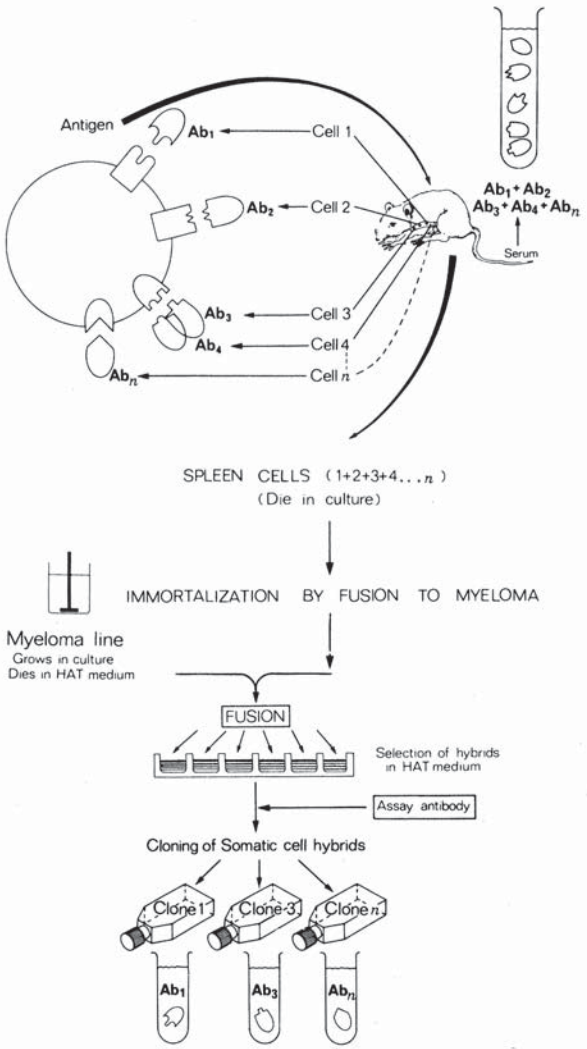

FIGURE 3. When an animal is injected with an immunogen the animal responds by producing an enormous diversity of antibody structures directed against different antigens, different determinants of a single antigen, and even different antibody structures directed against the same determinant. Once these are produced they are released into the circulation and it is next to impossible to separate all the individual components present in the serum. But each antibody is made by individual cells. The immortalization of specific antibody-producing cells by somatic cell fusion followed by cloning of the appropriate hybrid derivative allows permanent production of each of the antibodies in separate culture vessels. The cells can be injected into animals to develop myeloma-like tumours. The serum of the tumour-bearing animals contains large amounts of monoclonal antibody.

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