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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 heterogeneous 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
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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 8-8 loops of about 100-120 residues each, and the one at the N-terminus is highly variable.](image)

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 \( \kappa \) 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 (Schreider 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.

<table>
<thead>
<tr>
<th>Light chains</th>
<th>V region domains</th>
<th>C region domains</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \kappa )</td>
<td>( V_{\kappa 1} )</td>
<td></td>
</tr>
<tr>
<td>( \lambda )</td>
<td>( V_{\lambda 1} )</td>
<td></td>
</tr>
<tr>
<td>Heavy chains</td>
<td>( V_{H1} )</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Light chains</th>
<th>V region domains</th>
<th>C region domains</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \kappa )</td>
<td>( V_{\kappa 2} )</td>
<td></td>
</tr>
<tr>
<td>( \lambda )</td>
<td>( V_{\lambda 2} )</td>
<td></td>
</tr>
<tr>
<td>Heavy chains</td>
<td>( V_{H2} )</td>
<td></td>
</tr>
</tbody>
</table>

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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.
antigen stimulation, have a very short life span and cannot be cultured in vitro. Codominant expression of immortality (from myelomas) and antibody production (from lymphoid cells) is achieved in somatic cell hybrids, when myeloma cells are fused with such normally occurring antibody-producing cells. The essential features of the derivation of hybrids secreting a specific antibody are shown in

![Diagram](image)

**Figure 4. Fixation of the specific antibody production of a transient spleen cell in a permanent tissue culture (t.c.) line. Anti-SRBC hybrids. (Taken from Milstein et al. (1980).)**

The myeloma parent confers to the hybrid the malignant phenotype and its ability to grow permanently in tissue culture. Being prepared as a mutant defective in the enzyme hypoxanthine guanine phosphoribosyl transferase, it cannot grow under conditions where incorporation of hypoxanthine is essential to cell growth (HAT medium; Szybalski et al. 1962; Littlefield 1964). The spleen parental cell provides the active enzyme and permits the hybrid cells to grow under these conditions. In addition it contributes with the rearranged V and C genes for both heavy and light chains which code for a specific antibody. In this way the transient property of antibody secretion can be fixed as a permanent property of an established cell line (figure 3).

The first permanent lines of cells secreting a predefined antibody were against sheep red blood cells and the hapten TNP (Köhler & Milstein 1975, 1976). Since
then many other hybrid myelomas have been derived secreting antibodies to small ligands (haptens), proteins, carbohydrates, cell surface components, glycolipids, viruses and enzymes. The experience accumulated tends to indicate that the method is general. The degree of difficulty in obtaining a specific hybrid myeloma seems to be correlated to the response of the immunized animal. When this response is very weak the search for the specific antibody-producing clone among the many hybrids secreting non-specific immunoglobulin may require special methods.

The derivation of hybrids between mouse myelomas and spleen cells from an immunized donor represented a departure from previous uses of cell fusion. Somatic cell hybrids had been used for two purposes (Ringertz & Savage 1976). For gene mapping, interspecific hybrids were made between cells of different species to correlate the segregation of chromosomes to the loss of species-specific properties (like the electrophoretic mobility of a given enzyme). For studies of gene expression, intraspecific hybrids obtained by fusion of two cell types from the same species were used. This permitted the study of the loss as well as complementation of a variety of cellular functions. In our experiments and for the immortalization of specific differentiated functions, hybrids were prepared of normal and transformed cells genetically and phenotypically as closely related as possible.

THE IMPORTANCE OF COMPATIBLE PHENOTYPES

There was a very welcome but unexpected feature in the derivation of specific antibody-secreting hybrids that we noticed very early on. As, in the spleens we were using, well below 1% of the cells secreted specific antibody, we would have been very pleased if 1% of the hybrids derived secreted the specific antibody. Of course, we were delighted but also a bit suspicious when the proportion turned out to be one order of magnitude better, around 10%. It soon became quite obvious that, together with immortalization, we had enrichment of our selected function. This apparent selectivity seems to have several components. One may be related to preferential survival of hybrids between actively dividing cells. Since the hybrids are prepared with immunized spleen cells, actively dividing cells are enriched with those triggered by antigenic stimulation.

Another component in the apparent selectivity is probably due to complementation. The myeloma parental line is an actively secreting cell and can provide to the hybrids the high production and secretion phenotype. This has been shown in fusions of myelomas and non-secreting lymphomas (Levy & Diley 1978; Laskov et al. 1978; Raschke 1980).

In a hyperimmune spleen, for each cell actively secreting antibody there are perhaps five more cells that synthesize but either do not secrete antibody or secrete it only in trace amounts. The fusion of either of them with the high-secretor myeloma may result in a high-secreting hybrid.

There seems to be another most interesting component of the selectivity related to phenotypes of the parental cells. If the same population of spleen cells, which
contain similar numbers of B and T cells, are fused with a myeloma or a T cell lymphoma, the results are quite different. When the fusion parent is the myeloma, the growing hybrids express the antibody-secreting phenotype of the parental spleen but do not seem to express the markers characteristic of T cells. On the other hand, when the fusion parent is a T cell lymphoma, the growing hybrids preferentially express the T cell characteristics (table 1).

**Table 1. Selective fixation of differentiated functions in established hybrids**

(Taken from Milstein et al. 1980.)

<table>
<thead>
<tr>
<th>cell phenotype</th>
<th>Ig secreted (%)</th>
<th>Thy-1 surface antigen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>parental and hybrid lines</td>
<td>parental</td>
<td>myeloma</td>
</tr>
<tr>
<td>X63 (myeloma)</td>
<td>&gt; 95</td>
<td>0</td>
</tr>
<tr>
<td>(X63 × spleen) hybrids</td>
<td>&gt; 90</td>
<td>ca. 65</td>
</tr>
<tr>
<td>spleen</td>
<td>0</td>
<td>ca. 5</td>
</tr>
<tr>
<td>(BW × spleen) hybrids</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BW (T lymphoma)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The selective effect of fusions with myeloma is more dramatically seen with rat myeloma cells than with mouse myelomas. This is illustrated by some recent experiments described in table 2. It shows that all or nearly all hybrids derived with the rat myeloma line express B cell characteristics of the parental spleen cell. It is not clear yet whether the higher apparent selectivity of the rat line when compared to the mouse lines is due to phenotypic compatibility, better stability, or a combination of both.

The practical message of these experiments is that 'for the recovery of a differentiated property of a given cell type in an established hybrid it may be best to use the parental partners cells of similar type' and that 'for the derivation of hybrids with specific T cell functions, thymomas are likely to be better parental partners' (Milstein et al. 1976). T cell hybrids prepared in this way constitute now a subject of their own.

The immortalization process therefore does not produce a random sample of all the cells from the spleen but it seems to be a random representation of the antibody-producing cells. For this reason it is considered a way of dissecting the immune response of the animal. But the precise meaning of such statements is not as clear as it sounds. One of the main reasons is that correlation of the antibody present in the serum, or even of the antibody-secreting cells, with the products of the isolated hybrids must take into consideration fast changes in the differentiated state of the cells in question. At present we do not have a definite picture of the relative survival advantages of hybrids derived from B cells at different states of differenti-
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The problem has considerable theoretical interest and could have practical implications. But this should not cast a serious shadow over the more general statement that roughly speaking the hybridization represents a random immortalization of the antibody-producing cells.

**Table 2. The expression of the spleen parental immunoglobulin is better in hybrids prepared with rat myelomas than in those prepared with mouse myelomas**

<table>
<thead>
<tr>
<th>Fusion</th>
<th>Parental Cells</th>
<th>Negative Hybrid Clones as a Percentage of Growing Clones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
</tr>
<tr>
<td>NN1</td>
<td>NSI/1 Ag.4.1 × B10.D2 spleen</td>
<td>18</td>
</tr>
<tr>
<td>NN2</td>
<td>NSI/1 Ag.4.1 × C3H spleen</td>
<td>35</td>
</tr>
<tr>
<td>NOA1</td>
<td>NSI/1 Ag.4.1/0 × BALB/c spleen</td>
<td>41</td>
</tr>
<tr>
<td>XOAI</td>
<td>X63 Ag 8.653 × BALB/c spleen</td>
<td>50</td>
</tr>
<tr>
<td>YS3/5</td>
<td>Y3 Ag.1.2.3 × DA spleen</td>
<td>0</td>
</tr>
<tr>
<td>YA5</td>
<td>Y3 Ag.1.2.3 × DA spleen</td>
<td>0</td>
</tr>
<tr>
<td>YA5</td>
<td>Y3 Ag.1.2.3 × DA spleen</td>
<td>0</td>
</tr>
<tr>
<td>YOL1</td>
<td>YB2/3.0 Ag × Lou spleen</td>
<td>10</td>
</tr>
<tr>
<td>NR5/6</td>
<td>NSI/1 Ag.4.1 × Lou spleen</td>
<td>50</td>
</tr>
<tr>
<td>YN5/6</td>
<td>Y3 Ag.1.2.3 × C3H spleen</td>
<td>3</td>
</tr>
</tbody>
</table>

The results were obtained three to four weeks after fusion, by analysis of the immunoglobulin secreted by all successfully growing hybrids followed by a statistical calculation of the number of growing clones. (Taken from Milstein et al. (1980).)

**The importance of compatible genotypes**

Early fusions were performed between cells of the same inbred strains. We soon found that using cells from different mouse strains did not alter the results. We then established that fusions between mouse myeloma cells and rat spleen cells (mouse × rat) were equally successful (Galfre et al. 1977). However, other combinations were more difficult. Efforts have been made by us and by others to immortalize the antibody production of rabbit and human cells by means of myeloma cells of mouse or rat origin. Although hybrids can be derived that express the antibody of the donor, the expression is quickly lost. It has been a common experience of ours and of several other laboratories that the stabilization of the expression of interspecific hybrids is possible but rather difficult. So the mouse × rat hybrids seem to represent the exception rather than the rule.

An interesting point concerning the stability of expression of rat antibodies in
the mouse × rat hybrids is that it happens in spite of the preferential loss of rat chromosomes. Table 3 summarizes the karyotype analysis of 23 hybrid clones, which shows that losses of chromosomes were mostly due to rat chromosomes. Furthermore, the small mouse chromosome losses were fairly random, particularly among trisomies. On the other hand the losses of rat chromosome were not random and some of the chromosomes (i.e. 1, 2, 4, 5, 13, X) were present in all cells. We have concluded that the expression of the rat antibodies is stable because the immunoglobulin genes themselves are located on stable chromosomes (Schröder et al. 1980). Other controlling elements must be also on stable rat chromosomes or are not species-specific.

TABLE 3. KARYOTYPES OF ESTABLISHED MOUSE MYELOMA – RAT
SLEEN CELL HYBRID CLONES
(Data taken from Schröder et al. (1980).)

<table>
<thead>
<tr>
<th>Chromosomes</th>
<th>Total</th>
<th>Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>X63 mouse myeloma</td>
<td>63–64</td>
<td>—</td>
</tr>
<tr>
<td>Normal rat</td>
<td>42</td>
<td>—</td>
</tr>
<tr>
<td>23 hybrid clones</td>
<td>74–89</td>
<td>17–31</td>
</tr>
<tr>
<td>Mouse chromosomes</td>
<td>55–60</td>
<td>3–8</td>
</tr>
<tr>
<td>Rat chromosomes</td>
<td>(13) 15–26 (28)</td>
<td>14–27</td>
</tr>
</tbody>
</table>

The mouse × human hybrids also show non-random losses of chromosomes, and chromosome 14, which is thought to include the H chain gene locus, is preferentially retained (Croce et al. 1980). Therefore, the preferential loss of human chromosome does not seem to be the reason for the preferential loss of the expression of the human immunoglobulins, at least as far as the heavy chains are concerned.

The disappointing results with interspecific hybrids have reinforced the idea that for the production of human (or most other species’) monoclonal antibodies one needs to use appropriate parental myeloma lines derived from the same species. The derivation of such lines is tedious and takes a long time, and for human myelomas it has proved difficult. The human myelomas do not grow easily in tissue culture. We, and quite a number of other laboratories, have been searching for a suitable line. But they are very slow growers and do not give viable hybrids. A promising exception may be a line recently derived (N. Kaplan, personal communication). But a more general solution to the problem would be desirable. We still need to define more clearly the reasons for the phylogenetic restrictions and see how these can be circumvented. In collaboration with G. Galfré we have been experimenting with double fusions. Mouse × human hybrids are first prepared and from them hybrids expressing human immunoglobulins are selected. These are then tested to see if they can be used as myeloma parents to fuse with human
lymphocytes. The hope is to select a mouse–human line suitable for the derivation of \((\text{mouse–human}) \times \text{human}\) hybrids which will express human antibodies in a more stable manner.

**Monoclonal Antibodies as Specific Reagents**

The potential of the hybrid myeloma technique stems from the fact that the antibody-producing cells can be cloned, that the individual clones can be maintained indefinitely and that they are capable of large-scale production of monoclonal antibody. These, therefore, are well defined chemical reagents reproduced at will. This contrasts with the conventional antisera, which represent an ill defined mixture, a mixture that can never be reproduced once the original supply is exhausted. For this reason monoclonal antibodies are likely to substitute conventional antibodies as standard reagents in laboratory practice.

An interesting example is provided by the well established ABO grouping reagents used for blood transfusions. The reagents used at present are obtained from human serum, preferably of hyperimmunized human volunteers. In countries like the United Kingdom, where this procedure has not been adopted, the reagents tend to be of considerably lower quality. The preparation of ABO typing reagents in the U.K. uses 1200 l of human serum annually from 6000 blood donations. Each donation must be carefully scrutinized for the presence of unwanted antibody specificities that are likely to be expressed together with the anti-A or anti-B specific reactions. From among a variety of hybrid myeloma clones we have chosen one (MH2/6D4) that displays the necessary qualities as a provider of a standard anti-A reagent (Vosk et al. 1981). The McAb was used with 1421 samples in manual tests, including 169 cord samples. They all gave satisfactory reaction and no false reactions were detected. In automatic tests, using the Auto Grouper 16C machine, 1911 random samples including many A1B and A2B gave satisfactory results. The reagent was prepared as the spent medium of stationary phase MH2/6D4 cells. We have concluded that mass culture production of this monoclonal anti-A provides a cost-effective model for the use of monoclonal antibodies as a new generation of better-standardized potent reagents for routine blood typing. The reagent is at present being tested in the U.K. on a national scale.

The preparation of monoclonal antibodies does not require pure antigens and this is the most powerful aspect of the hybrid myeloma method. It therefore represents a new strategy for the detection, characterization and purification of unknown antigens or minor components of a mixture. Purification of the permanent hybrid cell line capable of producing unlimited amounts of specific antibody is achieved by cloning the somatic cell hybrids (figure 3). The specific antibody can then be attached to Sepharose or other solid supports and used as immunoadsorbants to purify the corresponding antigen by affinity chromatography. A strin-
C. Milstein

gent test of this scheme has been the purification of human leucocyte interferon (Secher & Burke 1980). When this project was started, no pure interferon was available. Preparations enriched with interferon were used to immunize mice. Spleen cells from such animals were fused to myeloma cells and the hybrids tested for the ability of the spent culture medium to neutralize interferon. The biological assay is rather lengthy and not very suitable for screenings of this type, and it was

**Table 4. Purification of crude interferon† (IF) by Sepharose immunoabsorbant chromatography with a monoclonal antibody**

(Data taken from Secher & Burke (1980).)

<table>
<thead>
<tr>
<th>applied to column</th>
<th>eluted</th>
</tr>
</thead>
<tbody>
<tr>
<td>volume/ml</td>
<td>100</td>
</tr>
<tr>
<td>IF titre/(unit/ml)</td>
<td>7.2 x 10⁴</td>
</tr>
<tr>
<td>total activity/unit</td>
<td>7.2 x 10⁵</td>
</tr>
<tr>
<td>total protein/mg</td>
<td>220</td>
</tr>
<tr>
<td>specific activity/(unit/mg)</td>
<td>3.3 x 10⁶</td>
</tr>
<tr>
<td>purification factor</td>
<td>100</td>
</tr>
<tr>
<td>yield (%)</td>
<td>97</td>
</tr>
</tbody>
</table>

† Culture medium from stimulated Namalva cellsafter removal of material that precipitated at pH 2.

**Figure 5.** Monoclonal antibody cascades provide a means for the dissection of all antigens of a complex mixture. (Taken from Milstein & Lennox (1980).)
supplemented with very simple assays to detect the secretion of immunoglobulin regardless of antibody activity. Eventually a hybrid clone was isolated and large amounts of antibody were prepared and used to make immunoadsorbent columns. With these columns it was possible to purify interferon in excellent yields. The purification achieved in a single passage of crude material through the columns was 5000-fold (table 4). Furthermore, the same antibody is being successfully used in a radioimmunoassay of interferon, which will be invaluable in the control of interferon production and purification as well as for clinical studies.

The monoclonal antibody strategy for the purification of biological products is being taken one step further as a means of isolating all the components of biological extracts. In the monoclonal antibody cascade purification (figure 5) an unknown mixture is used to immunize an animal and a random set of monoclonal antibodies is prepared. Immunoadsorbant columns are then prepared and used to remove the corresponding antigens from the original mixture. The remaining simplified mixture is then used to immunize new animals and derive new hybrid myelomas. These, in turn, are used to prepare new immunoadsorbant columns to simplify further the original mixture before repeating a new cycle. In this way the minor antigenic elements of the mixture are slowly enriched and eventually monoclonal antibodies to them can also be prepared. So hybrid myelomas provide the analytical tool for the characterization of all the individual components and at the same time are the permanent source for the preparation of immunoadsorbant columns and successive purification of all the elements of the mixture.

MONOCLONAL ANTIBODIES AND CELL SURFACE ANTIGENS

The specificity of the cell surface is a subject of increasing interest in terms of functional properties like transport phenomena and cell–cell interactions. But, regardless of their functional properties, cell surface antigenic determinants constitute a simple means of defining cell types and cell lineages. Since the early successes with the preparation of monoclonal antibodies to define hitherto unknown differentiation antigens (Williams et al. 1977; Springer et al. 1978), the application of the method is being extended by many laboratories. This is an area in which fast and rather dramatic progress is being made. Of particular interest to clinical immunologists are monoclonal antibodies defining subsets of lymphoid cells in humans (McMichael et al. 1979; Reinherz et al. 1979). Monoclonal antibodies to cell surface antigens are valuable not only in defining the functional differences of cell subpopulations (White et al. 1978; Reinherz et al. 1980) but also as a means to classify leukaemias and to define the anomalous expression of antigenic determinants in malignant cells (Bradstock et al. 1980a b). There are monoclonal antibodies, like YD1/23, that react well with certain types of leukaemic cells but seem not to react with bone marrow precursors (Janossy et al. 1980). Such reagents can be tested for their capacity to remove leukaemic cells from the bone marrow of patients. This could increase the effectiveness of autologous marrow transplants.
The nature of antigen-antibody interactions has been one of the fascinating subjects of classical immunology. The vast majority of these studies were performed with mixtures of molecular species. The use of monoclonal antibodies requires a fresh approach to the interpretation of serological reactions. For instance, the lattice theory (Marrack 1938) predicts that immunoprecipitation reactions should not be observed with monoclonal antibodies. Precipitation requires the formation of three-dimensional lattices and as monoclonal antibodies bind to a single determinant no such lattices can be formed unless the same determinant is repeated in the molecule, as in polymeric structures. Figure 6 illustrates that individual monoclonal antibodies fail to precipitate human IgG but mixtures of them do not. This may be the first formal proof, if one is needed, of the lattice theory. On the other hand, we have observed that three monoclonal antibodies directed against different sites of human C3 complement failed to give precipitates (Lachmann et al. 1980). Non-precipitating antibodies are an old serological puzzle and monoclonal antibodies may be the way to solve it.

Complement-mediated cytotoxicity is affected not only by the class of antibodies but also by their local distribution on the cell surface. Local concentrations can be increased by multiple antibodies recognizing neighbouring determinants of the same antigen. The importance of such synergistic effects is illustrated in figure 7 by monoclonal antibodies that recognize two independent sites of the same rat histocompatibility antigens. These were called P and S sites. Individual monoclonal antibodies to either site were poorly cytotoxic or not cytotoxic but the blend was strongly cytotoxic (Howard et al. 1979).

In a more general way, the interactions between antigen and antibodies have been always clouded by the heterogeneity problem. This was true when measuring thermodynamic and kinetic parameters as well as following serological reactions. The studies of antigen–antibody interactions by crystallography and other modern physical methods need no longer depend on a few myeloma proteins for which a more or less reasonable binding to randomly tested ligands has been detected. It will be most instructive to be able to define the multiplicity of antibody structures that are capable of interacting with a single antigenic determinant. And here I am referring to the general problem of molecular recognition and the diversity of
protein–ligand interactions. The use of hybrid myelomas to define the complete repertoire of antibodies to single antigens (Reth et al. 1977) is now expanding very rapidly. Although its major motivation has been the problem of genetic diversity and idiotypic regulation, the purely structural aspect of the diversity is of no less interest.

![Diagram](image)

**Figure 6.** Double-diffusion analysis with monoclonal antibodies. (Top) The centre well contains human serum (diluted 1:3). The outer wells contain ascites fluid from mice bearing the following hybrid myeloma clones secreting anti-human IgG antibodies: 1, NH2/17 (anti-γ); 2, NH3/130 (anti-γ); 3, NH3/41 (anti-κ); 4, NH3/75 (anti-γ). Notice that a single McAb does not give a precipitin line. However, between two neighbouring wells containing different McAb (1 and 2) a precipitation zone occurs where the two McAb mix. (Bottom) The centre well contains NH3/41 (anti-κ on the left), NH3/130 (anti-γ on the right) and a mixture of the two in the centre. The outer wells contain myeloma proteins of the following subclasses and types: a, IgG1 (κ); b, IgG2 (γ); c, IgG3 (κ) (probably sometimes aggregated); d, IgG4 (γ); e, IgG4 (κ); f, Bence-Jones protein (κ). The fact that IgG3 (κ) is precipitated by NH3/41 alone is interpreted as due to multivalency of the antigen due to aggregation. Note the shortening of the precipitin line in e and a due to competition by the fast diffusing Bence-Jones protein in f. (Taken from Milstein et al. (1980).)

**Concluding Remarks**

The use of monoclonal antibodies has spread into many areas of biological research and clinical medicine. They are slowly beginning to replace conventional antisera in standard kits for radioimmunoassays and many commercial companies are beginning to market them. More importantly, they will allow widespread use
of diagnostic reagents that, until now, were not available or were considered highly specialized. Their impact in areas like virology, parasitology and bacteriology is beginning to be felt. Great hopes are placed on their application to organ transplantation, and world standardization of tissue typing is but one of its aspects. In basic research, their use is even more widespread and their reported uses extend

![Figure 7](image)

**Figure 7.** The combined effect of two monoclonal antibodies is demonstrated by the lysis of rat red blood cells. The two monoclonal antibodies recognize two independent sites (labelled P and S) of the same histocompatibility antigen. The red cells have been made radioactive with $^{51}$Cr and the cell lysis is measured by the release of the radioactivity to the extracellular medium following addition of antibody and complement. Each antibody separately does not affect the cells but the two together completely disrupt them. (Taken from Howard et al. (1975).)

into embryology and pharmacology and to hormone and neuron receptors. The possibility of their use in direct therapy is being seriously explored in several directions. The most obvious is passive immunization: injection of antibody into patients. Treatment with antibody, for instance in prevention of Rh disease, may be much better done with monoclonal antibodies. Because of their specificity, monoclonal antibodies are also being considered as candidates for tumour therapy. I have already mentioned their possible use in transplants of bone marrow cells, either to free them from tumour cells or to remove those cells that cause graft versus host reactions.

Another exciting possibility is to use monoclonal antibodies as carriers of toxic substances for specific treatment of tumours: a variation on the old ‘magic bullet’ theme of P. Ehrlich. Preliminary experiments are at present being performed and there are many problems to be solved. For instance, one must ensure that under in vivo conditions the antibodies will ‘home’ preferentially in the tumour. This may not always be the case. In one model experiment, performed in collaboration with H. M. Warenius, N. Blee hen and G. Galfrè, we have observed preferential homing in the liver probably due to antigen–antibody complexes arising from
circulating antigen due to breakdown of the tumour. But this may vary from tumour to tumour. It is possible that Fab fragments will be better targeting agents than intact antibody.

What next?

I have said at the beginning of my lecture that the preparation of monoclonal antibodies by the hybrid myeloma method depended on the state of immunization of the animal. There are many tricks that we can use to enrich and select for hybrids producing a specific antibody with particular properties. But there are no miracles; if the animal does not make it there is no hope for immortalizing it.

So all that we seem to have acquired is the potential ability to select from an animal any of the antibodies of his repertoire. It is somewhat like selecting individual dishes out of a very elaborate menu: antibodies 'à la carte'. But surely our 'immunological gourmandizing' cannot be satisfied by the menu that the animals are offering us, astonishingly comprehensive and varied as it may be. A gastronomer worth his salt cannot be satisfied by selecting what somebody else has cooked. He wants to experiment with new ingredients, new combinations. His dream is to invent new dishes and not only to taste what others are doing. I am sure that our next step will be to move from the dining table, where we order and consume our antibodies 'à la carte', to the kitchen, where we shall attempt to mess them up!

In other words, the way that I imagine the next stage is to move away from the animals. Bearing in mind the recent developments in DNA cloning and directed mutagenesis, it is perhaps not too premature to start thinking along these lines.

Basically one would start by isolating the genes corresponding to a given antibody and to modify separately the heavy and light chain DNA sequences corresponding to the complementarity-determining regions. Modifications of the type of which I am thinking (predefined mutants) are probably best done by biochemical means and chemical synthesis. For instance, if suitable restriction sites are available, one could remove from the DNA coding for immunoglobulin small DNA fragments containing the stretch that one wants to modify. A replacement stretch will have to be chemically synthesized to include the intended changes. The tailor-made stretch will then have to be stitched back to the original DNA to derive the final predefined antibody mutant DNA.

The modifications introduced will have to be translated into protein. The mutant antibody will then have to be compared to the original antibody. This will tell us something about how the mutations affect the antibody activity. From here one might be able to design modifications to increase or decrease the association constant for a given antigen and modify the fine specificity (i.e. the relative reactivities towards cross-reactive antigens) in a predictable way. Here I should return to the problem of the diversity of molecular recognition and the development of our ability to predict modifications to suit our aims. The effect of modifications on the ligand stability is but one of the subsidiary aspects to look forward
It is pertinent here to refer to a recently isolated monoclonal antibody that increases the hydrolysis of the ester bond of a hapten (Kohen et al. 1980). Is this one way towards enzymes 'à la carte'?

Of course, there are very serious problems to be solved before such a scheme is carried out. The preliminary steps are, however, being taken. The derivation of antibody-producing cell lines, from which the appropriately rearranged genes can be prepared and used for bacterial cloning has been the subject of this lecture. From them mRNA for heavy and light chains or the corresponding genomic DNA fragments can be isolated. The way to proceed from here is clouded by uncertainties and multiple possibilities. Somehow the DNA fragments will have to go into cells capable of transcribing and translating the information with adequate efficiency. This is necessary to test the activity of the antibodies at first and later to make enough of them for practical applications. Keeping this in mind, should we try to put and translate the genes into bacteria or should we try to put them into animal cells; for instance, back into a myeloma-type of cell? If we prefer bacteria, we have to face the possibility that bacteria may not be able to handle properly the separated heavy and light chains so that correct assembly becomes possible. We know that myeloma cells are capable of correct assembly and secretion, but transformation of animal cells with naked DNA is still rather inefficient and not so well established. And then should we use genomic DNA or cDNA made from mRNA? cDNA is simpler to use, but animal cells may not like it very much. Fragments of genomic DNA containing both V and C regions do not seem a viable proposition because V and C regions are too far apart in the genome. If this is the approach that we prefer, we will have to learn how to stitch together genomic fragments containing V and C regions without destroying the ability of the final product to be properly transcribed and translated. Since we do not yet know the role of intervening sequences, there is always a danger that we will run into unexpected complications. Perhaps this danger is not too great, because we have recently learned that there are certain deletions that remove very large segments of DNA. These are located precisely where we need to remove DNA, between the V and C regions, and they do not impair the synthetic ability of the cell (Dunnick et al. 1980).

In this lecture I have made no attempt to review the literature and hence I apologize to the authors of many exciting papers that were not referred to. I have, however, tried to quote those papers that have been important to the development of my own ideas. It is my pleasure to acknowledge many lively discussions on 'What next?' with my colleagues at the Laboratory of Molecular Biology.

But in addition I would like to specifically acknowledge my immediate collaborators and colleagues who, in one way or another, were critical to the development of the hybrid myeloma method: R. G. H. Cotton, D. S. Secher, G. Köhler,
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G. Galfrè, T. Pearson, J. C. Howard, A. R. Williams and my technical assistants S. H. Howe, J. M. Jarvis and B. W. Wright. Without them I am certain that I would not have been here today.

REFERENCES


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