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Some advantages and problems arising in the substitution of traditional antisera by monoclonal antibodies

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Abstract - Some of the advantages and limitations of the application of monoclonal antibodies to the preparation of diagnostic reagents are described. A discussion of crossreactions in reference to monoclonal antibodies is given special attention. Also the areas in which monoclonal antibodies can be expected to be of greater application are emphasized.

INTRODUCTION

Almost 10 years have elapsed since Köhler and Milstein (1) made the first fusions of mouse myeloma cells with normal mouse spleen cells and obtained clones of "immortalized" hybrid cells producing monoclonal antibody molecules.

The hybridization is a random process, but the normal mouse used as donnor of spleen cells can be preimmunized to a specific antigen and, more important, the clones of hybrid cells can be selected for the specificity of the antibody they produce, by a variety of very sensitive assays. Through this method it is now possible to obtain any desired amount of a single monoclonal antibody of precisely selected specificity. Compared to the classical method of obtaining antisera, by immunization of animals, this technic is far superior, at least theoretically, and has atracted the interest of immunologists and researchers in most biological fields. As a result, monoclonal antibodies are being successfully applied to the solution of a rapidly expanding spectre of scientific and practical problems (Ref. 1-8). The field of diagnostic reagents has been no exception (Ref. 9-13).

I would like to make some general comments on the production of monoclonal antibodies as raw material for the production of diagnostic reagents and kits, and to discuss some of the problems that arise from their application. There is a basic difference between this particular field and the obtention of monoclonal antibodies for research purposes. In the later case the ascitic fluid of one or, at most, a few mice is generally sufficient for an entire project. For the preparation of diagnostic reagents one must assure the production of large quantities of antibody of very high specificity and affinity during a prolonged period of time.

There is often the idea that, because monoclonal antibodies have been selected intentionally for their high specificity and affinity for a particular antigen, the problem of cross reactions has disapeared. The fact is that crossreactions are an inherent property of antibodies, and monoclonal antibodies are no exception. They may even present unexpected problems in this respect. It is important to have this in mind when discussing, in new applications, whether a monoclonal antibody, an heterogeneous antiserum or a mixture of monoclonal antibodies will constitute the best solution.

Another extended idea is that monoclonal antibodies have a lower cost of production compared to classical animal antisera. This is not necessarily true, it depends among other things on the quantity and quality of the antibodies required for a particular application. I will not comment any further this matter except to emphasize that when considering the production of monoclonal antibodies, it is important to distinguish between the cost of production proper (as ascitis fluid or by cell culture) and the cost of the obtention of the desired hybridomas (immunization, hybridization, selection, cloning). The obtention of the hybridomas involves an unpredictable amount of time and work that can add up to a very high cost. And then, once obtained it would be marvellous if all hybridomas could produce, indefinitely, any desired

amount of antibody. Unfortunately hybridomas are unstable and can be lost any time as antibody producers. So, whenever the sustained production of antibody is essential one has to keep intact the capacity to produce new suitable hybridomas, adding to the expense. Finally, when a blending of monoclonal antibodies is the desired goal there is a further burden of work and expense involved.

MONOCLONAL ANTIBODIES VERSUS CLASSICAL ANTISERA

The main advantages of monoclonal antibodies are:

a) They are a well defined substance produced by a selected clone of cells.

b) Can be obtained in an unlimited quantity with the same characteristics.

c) The selection of the clones permits the obtention of specific antibody, even when immunizing with a complex mixture of antigens. This is of great importante with cellular antigens, or generally, with antigens difficult to obtain in a purified state.

d) There is no need to carry absorbtions in order to get rid of unwanted specifities. This is important when dealing with very scarce or difficult to isolate crossantigens.

e) They facilitate the simultaneous use of two antibodies specific for two distinct determinants on the same antigenic molecule opening the way to two-site immunometric assays.

There are, of course, certain limitations to the use of monoclonal antibodies. Here are some of them.

a) Some immunologicals reactions (precipitation, complement fixation) may not be possible with antibody to a single non-repetitive determinant.

b) Some antigens of biological interest may involve a "cluster" of determinants with minor variations from case to case. The monoclonal antibody, seing only one narrow determinant, may define these antigens (or groups) in a way not corresponding to that generally accepted.

c) When the selected hybridoma is lost, it may be difficult to obtain a new hybridoma producing a monoclonal antibody with exactly the same narrow specificity.

Most of these difficulties can be overcome by blending several monoclonal antibodies. However, this may not be easy and, naturally, adds to the overall complication and expense of the process.

CROSSREACTIONS

Monoclonal antibodies are normal antibodies and there is no reason why they should not show crossreactions (Ref. 14). There are examples in the literature of unexpected and puzzling crossreactions with monoclonal antibodies (Ref. 15-17). This may happen through several mechanisms.

First of all a crossreaction may occur because two alternate antigenic determinants are structurally related. This is the easiest case to deal with and it should be possible to select an hybridoma that sees preferentially one of the alternate structures. Still, it might be difficult to select, at the same time, for high affinity.

The crossreaction can also be entirely accidental. The two antigens may share an area of reasonably similar spatial distribution of charge, polarity and hydrophobicity with the original molecule. This can happen, by chance, between entirely unrelated molecules. It can be compared with the fitting of the endorfins and the alkaloid extracted from opium to the same cell receptors. Of course, this case is more difficult to forecast and can be expected to occur more frequently with monoclonal antibodies. Being the size of the structure recognized by the monoclonal antibody smaller, more precise, than that recognized by a mixture of heterogeneous antibodies, the probability of finding this simpler determinant repeated, by chance, in other antigenic structures becomes greater.

A third possibility of crossreactions derives from the observation that an antibody molecule can bind to more than one epitope or "subepitope". And it

can do this not because the different epitopes are any similar but because the combining site is large enough to accommodate different epitopes through different areas (18). This may not be easily apparent in a specific antiserum containing a mixture of heterogeneous antibodies. But the use of monoclonal antibodies is likely to magnify the effect of this phenomenon.

The above considerations rise a very interesting question. Can, in some cases, an heterogeneous antiserum be "more specific" than monoclonal antibodies? I believe this is enterily possible. When an animal is immunized with an antigen, even if this is a very well defined chemical structure, responds with the production of a great variety of antibodies. They all recognize in different ways the same determinant, but they may have entirely unrelated possibilities of crossreaction with other antigenic determinants. In other words, all the different antibody molecules in the immunological response to an epitope have in common their capacity to combine (in different ways) with this epitope and, at the same time, each of them has a different "repertoire" of crossreactivity with other epitopes. When this antiserum is used in the laboratory with the "specific" antigen, all the heterogeneous molecules of antibody contribute to the positive reaction, combining with the antigen, whereas some of the possible crossreactions may be too disperse and weak to cause any detectable reaction with other antigens. If one single antibody, out of this vast variety, could be selected as a monoclonal, its crossreactivity may show up only in rare occasions, but then it will do so with full strength.

With the generalized use of monoclonal antibodies it is perhaps more important than ever to restate in modern terms the classical lock and key theory of the immune response. There is no such thing as one lock, one key, univocal correspondence. The immune system is like a factory of random keys with no reference whatsoever to the locks they will, eventually, fit. Every key fits a number of locks and every lock can be opened by a number of different keys. In an immunization it is the antigen that selects those antibodies that, by chance, fit, regardless of their fitting to other structures. And with monoclonal antibodies it is the experimenter that selects the antibodies that, by chance, fit, regardless of their fitting to other structures. But this is no warranty that it will not fit, by chance, to many other structures, except if they have been specifically negatively selected.

Obviously, a monoclonal antibody can be considered specific for the antigen for which it has been positively selected and to lack crossreactivity for each and every antigen for which it has been negatively selected. One should be very cautious, however, in inferring, even partial, molecular identity or common genetic origin of two molecules on the basis of a crossreaction with a monoclonal antibody. For the same reason, one has to accept the possibility of occasional unexpected strong crossreactions, to a greater extend than with classical antisera.

ANTIGENS FOR WHICH THE OBTENTION OF SPECIFIC ANTISERA BY THE CLASSICAL METHOD IS EXTREMELY DIFFICULT

They are, naturally, the best candidates for this new method. I would like to make reference to two broadly defined groups of antigens to which monoclonal antibodies have been extensively applied.

One corresponds to "families" of related molecules (hormones, enzimes, cell-transmitters), usually proteins, that share one or several common domains, or peptides, and show variations in part of the molecule. These substances often exist in minute quantities and this makes impossible or extremely difficult the correct absorbtion of heterogeneous antisera obtained by the classical immunization of animals. The following references represent an exemple (Ref. 19-21).

The selection of hybridomas making an antibody specific for the desired determinant can be carried out using very sensitive assays that require extremely small amounts of antigens carrying either the desired or the unwanted crossreacting determinants. Once the correct hybridoma has been selected, there is no further need of those substances for the production of large amounts of highly specific antibody. In these cases the monoclonal antibodies are far superior and, in some cases, they may be the only alternative.

There is another large group of antigens for which monoclonal antibodies have represented a real break through. These are the numerous molecules present on cell membranes (receptors, differentiation molecules, groups...) of great biological interest, often present in very low concentration and/or difficult to extract and purifie in sufficient quantity for the immunization of animals, much less for the adsorbtion of the antisera obtained. Mice can be immunized with whole cells bearing the antigen of interest and, after fusion of the spleen cells with myeloma cells, the hybridomas so obtained can be selected by sensitive methods (cytotoxicity, immunoassais) using appropiate cells as substrate. This obviates completely, not only the need to isolate the antigen, but also its previous identification. Monoclonal antibodies may actually be a decisive factor in the discovery of some of these molecules. The following references are a few exemples (Ref. 22-27).

In this field, the application of the monoclonal technology has opened a vast horizon of possibilities and has been the basis of an accelerated progress in the knowledge of these important cell structures, that was not possible a few years ago. Many of these monoclonal antibodies are used for research purposes only, but an increasing number are being applied to various analytical methods as cell typing and cell counting and sorting with flow cytofluorimeters.

ANTIGENS FOR WHICH SPECIFIC AND POTENT ANTISERA ARE READILY AVAILABLE

There are several reasons for not attempting the substitution of these antisera by monoclonal antibodies (Ref. 14). Cost is certainly one factor, but the substitution of these sera rises more interesting problems.

There is the problem of specificity and cross reactions. For some antigens it may be very difficult to match the specificity of classical sera. Of course, this could be solved by blending several monoclonal antibodies, but this adds up to the complication and expense of the project.

Another factor may, in some cases, delay the application of monoclonal antibodies. Many sera are used for tests involving precipitation, or some sort of lattice formation, or the activation of complement. This is best achieved by a mixture of antibodies to the same antigen molecule. A blending of monoclonal antibodies could be again the solution but this may not always be practical.

Nevertheless, the need for reagents of constant characteristics may slowly open the way to monoclonal antibodies in this area too.

IMMUNOASSAYS

Monoclonal antibodies offer definite advantatges for the design of immunoassays. First of all the homogeneous nature of the antibody facilitates the calibration of the dose-response curves and the "purity" of the antibody reagent eliminates many of the problems inherent to these assays.

But what makes probably the greatest contribution of monoclonal antibodies in this area is the possibility of selecting two antibodies specific for two different determinants of the same molecule. This permits to take full advantatge of the immunometric methods and will, no doubt, be a field of increasing applications (Ref. 28-30).

Another interesting observation is that two antibodies to the same antigen can cooperate resulting and enhanced binding and a greater sensitivity of the reaction (Ref. 31).

AFFINITY CHROMATOGRAPHY

Affinity chromatography may be a powerful tool for the analysis and separation of certain substances, and combined with the use of monoclonal antibodies has aquired a new dimension (Ref. 32-36).

Its greatest potential is probably the obtention of pure substances starting with complex mixtures. What is interesting for this particular application

is that in view of obtaining a better yield of the substance and of using milder methods of elution it may be advisable to select monoclonal antibodies of low affinity.

CONCLUDING REMARKS

Monoclonal antibodies are a very potent tool in all biological fields. In their application to the preparation of diagnostic reagents a few facts of special interest have been emphasized.

Monoclonal antibodies have become indispnesable whenever there is some impediment to the obtention of specific antisera by classical methods. This is specially the case of families of very scarce homologous antigens and with cell-membrane determinants.

Monoclonal antibodies find in immunoassays a very promissing field of application.

Monoclonal antibodies may eventually substitute readily avalable classical antisera. In some occasions this may require blending several antibodies and it may not always be possible to match the behaviour of some antisera.

Crossreactions are to be expected. They are part of the system, but usually can be taken into account at the time of selecting the appropriate hybridomas.

There is no doubt that monoclonal antibodies will substitute classical antisera in an increasingly large number of applications. We canvision a future with "cell factories" producing large amounts of many monoclonal antibodies. However this will require a sustained effort and will certainly not be easy nor cheap.

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