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METHODS FOR THE DETECTION OF AUTO-ANTIBODIES

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Although the concept of auto-immunisation is by no means new in the history of medicine (as early as 1900, Metalnikoff had shown that guinea pigs, when injected with homologous spermatozoa formed antibodies capable of reacting even with their own spermatozoa), nevertheless it is only in recent years that extensive research has been carried out in this field.

The methods used to reveal the presence of auto-antibodies consist in the main of precipitation, agglutination, complement fixation reactions and immunofluorescence.

PRECIPITATION REACTIONS

The precipitin reactions were those first used by Roitt et al. in detecting antithyroglobulin antibodies. Although they are not very sensitive, they continue to be used because they are very easy to conduct.

It is possible to perform this reaction by bringing the antigen and the serum containing the antibodies into contact in small test tubes and studying the precipitation which occurs at the liquid-liquid interphase. However, best results are obtained when the antigen-antibody contact occurs in an agar gel. In this case, there is an easily perceptible precipitate.

In performing these gel reactions, it is possible to use either the Oudin method in which the reaction takes place in test tubes or the Ouchterlony method, in which the reaction occurs

in agar plates and the antigen and antibody are placed in separate wells in the agar itself. The last method is used more frequently because it is simpler and also because of the excellent results obtained it is also possible to compare the antigenic relationship between two different proteins.

As we have already pointed out, these methods are not very sensitive. In fact, the presence of antibodies is not, in itself, always sufficient to give a positive result either because their concentration is too low or because they form, with the antigen, a soluble complex which does not yield a line of precipitation.

The precipitation reactions may be employed to give a quantitative analysis of the serum antibody content by combining constant quantities of the serum with increasing quantities of antigen in a liquid medium and studying the point of maximum precipitation.

Precipitating antibodies have been shown in Hashimoto's disease, ulcerative colitis, Sjögren's syndrome and chronic pancreatitis.

AGGLUTINATION REACTIONS

Among the agglutination reactions the method most commonly used is that developed by Boyden. This consists in using the antigen to coat red cells which have been previously treated with weak solutions of tannic acid after which the cells acquire the property of absorbing into their own surface the proteins with which they come in contact. These cells become agglutinated by the antibodies which are specific for the antigen adhering to their surface.

This test is very sensitive and even though it does not parallel precisely the true antibody content of the serum, it provides a very useful indication which can be sufficient for clinical purposes. If a more accurate indication of the true antibody content is required, the serum titration must be carried out in the presence of a small but constant amount of inhibiting antigen.

At first, it proved difficult to apply this type of reaction for routine investigations, as it was impossible to preserve the sensitized cells. This difficulty has been overcome by treating the cells with a solution of formalin either before or after sensitization or by freeze drying the cells which have already sensitized. In this way, the preparation can be kept active for several months if stored in the cold.

In addition to treatment with tannic acid, it is possible to absorb the antigens on cells by treating the latter with solutions of some aldehydes; it would seem that the best results are obtained with pyruvic aldehyde. This method yields more or less the same results as those obtained from treatment with tannic acid.

Antigens can be combined with cells using bisdiazotised benzidine. However, this method has not been used on a wide scale as it is difficult to apply in practice: the cells must be sensitized immediately prior to use as they cannot be preserved. Moreover, the quantity of antigens which must be used is highly critical and even very slight variations might considerably affect the results.

Relatively purified preparations of antigens must be used for coating the cells; with crude preparations it is more difficult to get unequivocal results.

Different particles such as bentonite and latex have also been sensitized with antigens. However, these methods offer no particular advantage as compared with the Boyden method. On the contrary, they are generally found to be less sensitive and the results more difficult to read.

These agglutination reactions have been used in the study of rheumatoid arthritis, Hashimoto's disease, ulcerative colitis and post commisurotomy syndrome.

COPRECIPITATION REACTIONS

Another method which has been used to estimate antibody content of serum involves coprecipitation reactions with radioactive antigens and anti-gammaglobulin serum. Antigens and

antibody are left to react together and the soluble complex is precipitated with an anti-gamma-globulin serum. The amount of radio-activity in the precipitate gives an indication of the antibody content in the serum under investigation.

This method makes it easy to follow changes in the antibody level in serial samples from a subject treated in various ways during the course of the disease.

This method has been applied for the detection of thyroglobulin and anti-intrinsic factors antibodies in Dr. *Roitt's* laboratory.

COMPLEMENT FIXATION

This reaction forms the basis of the classical Wasserman reaction, and it depends on the ability of the antigen-antibody complex to fix a group of constituents present in the normal plasma and normally referred to as complement.

As this method is widely used it will not be necessary for us to go further into it. However, it has been proved to be a most valuable method for the detection of auto-antibodies.

Complement fixing antibodies may also be capable of destroying the target cells in tissue culture.

Complement fixing antibodies have been found in cases of auto-immune thyroiditis, pernicious anaemia and Addison's disease. Non-organ-specific complement fixing antibodies are frequently found in connective tissue disease particularly systemic lupus erythematosus, Sjögren's syndrome and also in some liver diseases.

IMMUNOFLUORESCENCE

The immunofluorescence method was developed a few years ago by *Coons & Kaplan* and is now available to all laboratories thanks to the production on a commercial basis of fluorescein isothiocyanate and of sera which have already been conjugated with fluorescent substances. The main difficulty lies, in fact, in the preparation of the fluorescent substances.

The method is particularly useful and easy to apply in revealing the presence of many auto-antibodies. It consists in treating with the serum under study a histological frozen section of fresh organ. If antibodies to the substances composing the tissue are present, these become fixed to the antigen. Subsequently, an anti-human gammaglobulin conjugated with fluorescent substances, normally fluorescein or Rhodamine B 200, is added. These anti-gammaglobulins react with the antibodies, themselves gammaglobulins, which have become fixed to the section. Thus if they are examined under the fluorescent microscope they will reveal fluorescent areas if the serum in question contains antibodies to the substances present in the tissue.

For this reaction, either fresh or fixed specimens can be used. Fixation is essential when antibodies to soluble antigens are sought; otherwise, these would be removed from the tissue during the various washings through which the specimens pass.

This method is particularly useful for the detection of the anti-nuclear antibodies which are characteristic of systemic lupus erythematosus and which probably imply a break down of immunological tolerance.

As we have seen, many different methods are available for the demonstration of auto-antibodies. As a guide to selection of the most apposite method in each case we would add that, generally speaking, the precipitation and agglutination reactions are more suitable for revealing antibodies to soluble antigens; the efforts made to absorb particulate antigens on cells or particles have so far not yielded very satisfactory results. On the other hand, complement fixation is recommended when it is thought that the antigens are of the particulate type. As regards immunofluorescence, this can be used in all circumstances remembering, however, that when soluble antigens occur it is frequently necessary to fix the specimen first in such a way as to ensure that the antigen is not removed during the washing of the specimen in question. It should also be remembered that although this method is very useful it is neverthe-

less qualitative and not quantitative, so that it is frequently advisable to employ it in conjunction with other methods which will also give a quantitative indication of the antibody content.

In conclusion, we may state that the various methods frequently complement one another and that it is therefore undesirable to opt for one rather than another. However, we feel that in many cases preference should be given to those methods which also give some indication of the quantities of the antibodies present, remembering in particular that auto-antibodies to some constituent of the body may be present in clinically healthy subjects. However, in these cases a positive reaction will be obtained only at very low dilutions of the serum in contrast to the titre usually found in the auto-immune diseases.

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