資 料

Coombs 博士来日記念講演

一昨年 3 月、クームス教授は日本学術振興会 (JSPS) の招聘により来日されましたが、その折に「クームス教授を歓迎する会」(発起人代表 森 亘先生、世話人代表 福岡良男先生)主催により札幌、東京、京都、福岡の 4 カ所で「Coombs Reaction」と題した来日記念講演会が開催されました。クームス反応は輸血領域において最も重要な検査の一つであり、それは医学、医療の場に多くの益をもたらしました。先生の御好意によりその来日記念講演の内容を掲載致しました。なおクームス教授は現在、英国ケンブリッジ大学の病理学教室の教授で免疫学的部門を担当されております。

The Anti-Globulin Reaction its History, Development and Applications

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The subject of my lecture, as perhaps you can imagine, was not my choice—I am only doing what I was commanded to do! The historical side of the antiglobulin reaction (but see later) goes back to 1945. I was, at that time, working on the Conglutination Reaction, a variant of the Complement Fixation Test.

Over coffee in the laboratory, there was a discussion with Dr. Race and Dr. Mourant (both working in Cambridge at that time) on the nature of so-called “incomplete” Rh antibodies and on the need for a satisfactory test for the detection of these antibodies. Returning from London one night shortly afterwards in a dirty old dark train, I was delighting myself in reading some early papers by Ehrlich and there were illustrations such as I can show on the accompanying slide. Next day I got up early and hurried to the laboratory and attempted to detect Rh antibody (Ehrlich’s amboceptor) with anti-amboceptor, i.e., antiglobulin. By the afternoon it was clear that the idea would work. The test³⁰⁾, as then performed, was subsequently called the “indirect antiglobulin test or reaction”—a test to detect “incomplete” Rh antibody in serum.

After quite a while (certainly not immediately) we suddenly thought that the antiglobulin test might be used diagnostically on red cells of infants with haemolytic disease of the newborn¹⁾ to test whether the red cells were sensitized with antibody *in vivo* (direct antiglobulin reaction). Interestingly, out of the first fifteen suspected cases tested, two gave us a certain amount of concern. One, which seemed at first to be a false-positive, as there was no Rh antibody in the mother’s serum, was in fact not false at all and turned out to be the first finding of the Kell System. In another case of severe haemolytic jaundice, the antiglobulin test on the infant’s red cells was negative. Was this a false-negative? No, it turned out to be a case of congenital syphilis.

Soon, my own niece (brother’s daughter), by name “Coombs”, was a case of haemolytic disease of the newborn—and was antiglobulin positive! At this time we also suggested that the direct antiglobulin test might also be positive in acquired haemolytic anaemia.

Excursion into animal diseases: The antiglobulin reaction then took us on an excursion into animal diseases. The mule industry (the mule is the off-spring of a donkey father and horse mother) in France was suffering serious losses of newborn foals (newborn mules) with haemolytic jaundice. This was thought to be caused by protozoal infestation. But Caroli and Bessis of Paris thought that the haemolytic jaundice might be comparable with Rh disease. They invited me to France and together we were able to show, with the antiglobulin reaction, that it was similar to Rh disease. Back in England, we showed the comparable disease in thoroughbred horses in Newmarket—the centre of horse racing in Great Britain and very close to Cambridge. In the horse, natural sensitization occurs as in man. Later again, haemolytic disease was found to occur in newborn piglets—the red cells of the jaundiced piglets giving a positive direct antiglobulin

test. The disease in piglets occurred only after the Ministry of Agriculture introduced a vaccine for swine fever which is a very serious epidemic disease of pigs. The vaccine (almost impossible to believe now) consisted of the whole blood of other pigs containing the virus which had been inactivated with crystal violet.

Experimental investigations in the laboratory: In the 1950's there was still no recognition of the different classes (now called isotypes) of immunoglobulin; there was one type of antiglobulin serum or reagent. Inhibition of an antiglobulin reaction was used by Wiener in the U.S.A.²⁾ and by Grubb in Sweden³⁾ as a sensitive assay for γ -globulin— measuring down to 0.1 μ g. The antiglobulin reaction was used to enhance not only red cell agglutination but also bacterial agglutination, especially of recent isolates⁴⁾. The antiglobulin procedure made it possible to reveal antibody (now known to be IgG) to *Brucella abortus* in human cases of chronic brucellosis⁵⁾. Chronic cases of brucellosis have always been difficult to diagnose.

In 1950 an interesting observation came to our attention. The Paul-Bunnell antibody in the serum of patients with glandular fever agglutinates sheep red cells; but does not agglutinate bovine red cells, despite the fact that the antibody is readily absorbed by these bovine red cells. Bovine red cells treated with the Paul-Bunnell antibody haemolyse when complement is added, but do not agglutinate even when subsequently washed and exposed to an antiglobulin serum. Here was a real challenge to find the explanation. During a Christmas holiday I was looking at the candles on the branches of a Christmas tree and this conjured up, in my mind, the possible explanation, i.e., that the antigens (seen as candles) could be so situated at some distance from the outer slip-plane of the bovine red cells (Christmas tree) and that more than one layer of antiglobulin could be needed to build up the bridging lattice. This indeed was found to be the case. Preliminary enzyme-treatment of the red cells to remove the extending muco-peptide chains (peripheral branches of the Christmas tree) also rendered the bovine red cells directly agglutinable by antibody^{6,7,8)}. A similar explanation can be given for the relative inagglutinability of recent isolated capsulated bacteria. An instructive illustration of this was reported by Cappellini⁹⁾ in Italy, focussing on the Vi lipopolysaccharide of *Salm. typhi*.

In the early 1950's we made attempts using the antiglobulin reaction to measure the almost mythical (at that time) "reaginic" antibody. With allergens such as egg albumin and horse dander, linked in a very special way to carrier red cells (a special way to avoid subsequent non-specific absorption of γ -globulin) we attempted to measure adsorbed "reaginic" antibody with our (crude) antiglobulin reagents. This was the so-called "Red Cell-Linked Antigen-Antiglobulin Reaction"¹⁰⁾. However, success had to await future developments with class or isotype-specific discriminating antiglobulin reagents.

Fluorescent-labelled antiglobulin: In 1954 Weller and Coons¹¹⁾ incorporated the principle of the antiglobulin reaction to enhance immunofluorescent staining and at the same time to afford a universal reagent for such tests. They developed the so-called "Indirect Immunofluorescent Technique" by labelling with fluorescein the antiglobulin reagent instead of the primary antibody reacting with the cells under study. This was an important development, with other labels such as radio-isotopes and enzymes being subsequently introduced into the antiglobulin molecule— so-called labelled antiglobulin reagents.

Different classes and subclasses of immunoglobulin: The scope and analytical usefulness of the antiglobulin principle was greatly extended in the 1960's with the discovery of the different classes (isotypes) of human immunoglobulin, i.e., IgG, M, A, D and E; also the subclasses and even allotypic forms.

The very careful researches of Ishizaka in the U.S.A and of Johansson in Sweden showed that the "reaginic" antibody (underlying Type 1 hypersensitivity) was IgE and was present in only very small amounts in normal serum (100 ng/ml). The antiglobulin principle, but now using class-specific anti-IgE, made it possible to measure this "reaginic" antibody *in vitro* either using radiolabelled antiglobulin as in the RAST (Radio Allergo Sorbent Test)¹²⁾ or even without any label in the antiglobulin, in the Red Cell-Linked Antigen Antiglobulin Reaction (RCLAAR)¹³⁾.

Also, of course there could now be discriminating antiglobulin tests for IgG, IgA, IgM or IgD. It is interesting that the non-agglutinating antibodies to *Brucella abortus* in chronic brucellosis were shown to be IgG (as opposed to the agglutinating IgM antibodies) as indeed were the "incomplete" Rh antibodies.

Also in the Jerne placquing methods¹⁴⁾ it is the IgG antibodies that are enhanced with antiglobulin.

Mixed Antiglobulin Reaction: A rather special further development—namely the “Mixed Antiglobulin Reaction” brought together two distinct principles, that of mixed agglutination and that of the antiglobulin reaction. Mixed agglutination, if I may remind you, is the agglutination together of two distinct cell types, which only occurs if the two cell types have a common antigen. The reaction allowed the demonstration of antigens on any type of tissue cell (not suitable for ordinary agglutination) as long as the same antigen(s) was found naturally, or could artificially be put on, a second (indicator) cell, such as the red cell. Mixed agglutination was harnessed to show the presence of the ABO blood group antigens on skin epidermal cells¹⁵⁾ and as a means of checking the species of origin of cultured tissue cells¹⁶⁾.

On the same principle the Mixed Antiglobulin Reaction allowed the detection of antibody (as immunoglobulin) on tissue cells, when red cells coated with immunoglobulin were added. The reaction was intended as a means of measuring tissue—or organ-specific antibodies and hopefully in those days of detecting tumour-specific antibodies. These reactions and their bases were reviewed by Coombs and Franks¹⁷⁾. It is a special pleasure today to remember the researches I did with Mori-San in 1968. We were attempting to show the organ of origin of metastatic ascitic tumour cells in cancer patients¹⁸⁾. However, such serological discrimination requires the specificity of monoclonal antibodies as are available today! Mori-San also showed that the Mixed Anti-Globulin Reaction was well suited for detecting “tumour-specific” antibodies in C3H mice immunized with killed BP8 tumour cells¹⁹⁾.

The Mixed Antiglobulin Reaction as the Mixed Antiglobulin *Rosetting* Reaction (MARR) was one of the very early methods for showing SmIg on B lymphocytes²⁰⁾ giving beautiful rosettes around B cells, but with no rosettes around T cells. The MARR has been greatly improved in the last decade by evolution to the DARR (Direct Antiglobulin [or Antibody] Rosetting Reaction) which is much more efficient and effective. These reactions have been, I think, cornerstones in the development of the so-called “Rosetting Reactions”.

Rosetting Reactions: The essential characteristic of all rosetting reactions is the final formation of rosettes (a rosette means literally a little rose). I must not digress into the intricacies of rosetting reactions except to say that essentially there are four main categories of these reactions²¹⁾. Firstly, (i) rosetting reactions to reveal antigen-specific receptors on tissue cells (e.g., innate receptors on B lymphocytes) using antigen on the indicator red cells or to reveal passively acquired cytophilic antibody (e.g., reaginic antibody on basophils). Secondly, (ii) those rosetting reactions to reveal cell surface antigenic determinants on tissue cells (e.g., MARR, DARR or an indirect antiglobulin form of the reaction, IARR—Indirect Antiglobulin Rosetting Reaction). Thirdly, (iii) there are rosetting reactions to reveal and measure receptors on tissue cells for γ Fc or complement—these receptors are very important in conveying signals for cellular activities. Finally, (iv) there are so-called “special affinity” rosetting reactions (e.g., sheep red cells rosetting around human T lymphocytes and affording a marker for these cells).

These rosetting reactions now form a minor technology of their own with both analytical and preparative capabilities.

An antiglobulin step in immunoassays: An increasing number of well established radio-immunoassays or enzyme-immunoassays have now introduced an antiglobulin step into the scheme of the assay, changing, for instance, radioimmunoassays into non-competitive immuno-radiometric assays (a particular example of this is the RAST) and there are considerable advantages. Another label—fluorescein was first introduced into antiglobulin reagents by Weller and Coons in 1954 as already mentioned.

While discussing the rosetting reactions above, I mentioned yet another label for antiglobulin reagents—namely the red cell itself. Red cells may be linked to antiglobulin molecules (or *vice versa*!) by chromic chloride and as such used in the DARR mentioned above.

Assays based on red cell-labelled antibodies: In the last 10 years or so, I have been specially concerned in developing a whole system of immunoassay based on red cell-labelled antibodies. In many of these assays (but by no means all!) the labelled antibody has been antiglobulin, which has again added a new dimension to the antiglobulin reaction.

In these assays the antibody reagent (antiglobulin or otherwise) is electrovalently linked to red cells by chronic chloride. Preliminary treatment of the red cell with enzyme is essential (in many cases) to achieve the ultimate sensitivity of 1.0—0.2 ng/ml. Of course the use of monoclonal antibodies has revolutionized the whole picture. Essentially the assays are performed by diluting out the sample (or analyte) for assay in microtitre plates. The end/point is read by the sedimentation pattern of agglutinated red cells (in rosetting tests of course, by rosette formation).

The various reactions or assays in the system are shown diagrammatically in the Figure. Reverse Passive Haemagglutination (RPH) is the simplest yet possibly the most important reaction. The principle is not new, but recent technicalities have greatly increased the sensitivity (to less than 1 ng/ml). The only requirement is for the analyte to have multiple epitopes to enable a bridging lattice to be built up. RPH has the great advantage of being an homogeneous reaction. RPH has been found, for example, to be a very satisfactory way of measuring serum IgE²²⁾ and human chorionic gonadotropin luteinizing hormone in urine²³⁾. It is of special value in detecting microbial antigens in body fluids, secretions and excretions and is thus of great potential value in the early and rapid diagnosis of infective diseases (e.g., rota virus infection²⁴⁾ RS virus infection²⁵⁾ and chlamydial infection²⁶⁾.

DARR (see the Figure) with either linked antiglobulins or monoclonal antibodies marker to antigens can be very helpful in recognizing and characterizing lymphocyte sub-populations²⁷⁾ or neo-antigens in transfected tissue cells. The IARR can be used as an *indirect* antiglobulin test to measure sensitizing antibodies reacting with such cells. MRPAH (mixed reverse passive antiglobulin haemagglutination—see also the Figure) measures bacterial antibodies, both titre and isotype in the one test²⁸⁾. The “s” in MRSPA H indicates “solid-phase”. The reaction, using red cell-labelled antiglobulin reagents, measures antibody to antigens simply adsorbed on a solid-phase such as the well of a plastic microtitre plate. The test is performed in a similar manner to an ELISA assay but is tow steps shorter. Antigen or allergen-specific IgE antibodies can be measured as also antibodies to alcohol-soluble gliadin in the sera of coeliac patients²⁹⁾. MR and MRSPA H are entirely antiglobulin reactions, whereas RPH and DARR can be antiglobulin, but much

Fig. 1. Scheme of assays using red cell-labelled antibodies.

else besides.

Comment on assays based on red cell-labelled antibodies: This system of assays might simply be a set of tools or playthings for laboratory workers were it not for the following considerations. It requires no radio-isotopes or γ -counting—neither photometry as in ELISA. The assays surprisingly are extremely sensitive, especially now that monoclonal antibodies are increasingly available. The assays are extremely simple to perform, involving only stepwise dilutions of the sample or analyte. And possibly most important—the antibody-coupled red cells can now be stabilized and freeze-dried ensuring a long shelf-life. Ampoules of freeze-dried antibody-coupled red cells can be sent anywhere in the world.

The implications are that very simple and sensitive assay and diagnostic tests became available almost as “side-room” techniques. The assay “package” consists really of a special reagent only, rather than a whole “kit”. The assays can be carried out in very unsophisticated laboratories and, for instance, an IgE assay on serum could easily be performed in the middle of the Sahara Desert or even on top on Mount Fuji! Personally, I would like these assays to be taken up in countries of the so-called Third World.

Concluding remarks: in 1945 antiglobulin tests began with free antiglobulin in solution to test for antibodies (Rh antibodies) reacting with red cells, now we have antiglobulin electrovalently coupled to red cells to probe for immunoglobulin, either free in solution or combining with tissue cells—a complete change-about! I should say that over the years I have made repeated attempts to get away from the red cell; but as you can see, I have not been very successful.

Epilogue: I have tried to give you a bird’s eye view of research on the antiglobulin reaction since 1945. But in 1945 I (together with my colleagues, Dr. Race and Dr. Mourant) made one of the commonest errors that scientists often make. We referred to the reaction as a “new test”³⁰⁾. After we had published this and submitted a more detailed subsequent paper, Dr. Mourant unearthed two papers written in 1908^{31,32)} by a certain Carlo Moreschi. With both red cells and bacteria, Moreschi reported an enhancement of agglutinating activity with that we would now recognise as antiglobulin sera (crude as they were!).

In a lecture in Rome in 1954³³⁾ I acknowledged this and paid tribute to Carlo Moreschi. Six months later when this lecture was published in Italian, I received a most delightful letter of a Dr. Ruggieri of Milan (a nephew of Carlo Moreschi) saying that it was the first time that such homage had been paid to the memory of his uncle who had died in 1921. Apparently, Professor Moreschi, then director of a medical clinic, died of smallpox contracted from a patient.

So you see, the history of the antiglobulin reaction goes back much earlier than 1945—as of course does much in our science of Immunology!

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