# IMMUNOLOGY PRACTICAL CLASSES

## GENERAL NOTES

These notes amplify the practical class notes and are not intended to replace a textbook of immunology.

The aim of these practicals is to help you understand some of the properties of antibody-antigen interactions, to look at some of the properties of the cells responsible for making antibody and to illustrate a few of the applications of the reaction of antibody with antigen.

## 1. ANTIGEN-ANTIBODY REACTIONS

The binding of antigen to antibody is very similar to the binding of a substrate to the active centre of an enzyme except with antibodies no chemical alteration of the antigen takes place. As all antibodies have at least two combining sites, antibodies will in most cases agglutinate particle antigens, for example, red cells and bacteria. With soluble antigens such as proteins and carbohydrates, provided there are several sites on each molecule of antigen where antibodies can simultaneously attach, then large three-dimensional lattices of antibody and antigen may form and precipitate from solution.

Complete precipitation will only occur at an optimal ratio of antibody and antigen. This ratio defines the volume of undiluted antibody which is equivalent with one volume of undiluted antigen of known concentration. At this ratio (equivalency of optimal proportions) there is neither antibody nor antigen left in solution after removing the precipitate.

The optimal ratio is determined by carrying out an optimal proportions titration. This can be done by titrating the antigen and adding a constant amount of antibody or vice versa. In the presence of excess antibody or excess antigen, small complexes can form and remain in solution.

**A) GEL DIFFUSION PRECIPITATION.** Antigen and antibody diffuse towards each other from wells in a semi-solid medium (agar). The speed with which molecules diffuse is determined largely by size. Different antigens and their specific antibodies will also start at different concentrations. A line of precipitation forms where the antigen and antibody meet in optimal proportions. In a system with several antigens and corresponding antibodies, each antigen and its corresponding antibody will usually form a line at a distinct position because it will have different optimal proportion requirements, starting concentration and/or diffusion rate. If two different antigen preparations diffusing from separate wells are tested with one antiserum any antigens common to both preparations will give lines which merge. Lines due to different antigens will cross, and lines due to antigens with some common determinants will merge but show spurs. (see poster).

**B) IMMUNOELECTROPHORESIS.** The antigen is submitted to electrophoresis in a semi-solid medium (agar). This causes separation of components according to electrophoretic mobility. An antiserum is then placed in a trough cut parallel to the electrophoretic migration. The antigen fractions and the antibodies diffuse through the agar and lines of precipitation form where they meet at optimal proportions (see poster). Immunoglobulins can be separated electrophoretically in the same way and different classes of antibody identified by placing antigen in the trough.

**C) AGGLUTINATION.** A variety of particles can be agglutinated by antibody. The agglutination of red blood cells (haemagglutination) will be studied in the practicals. Bacterial agglutination is a method for identifying bacteria using specific antisera and for the diagnosis of a causative agent during and following disease. A rising titre indicates recent antigenic stimulus.

Particles such as bentonite and latex can be coated with antigen for the detection of antibody by agglutination (in these cases it is sometimes called aggregation or flocculation). Many methods have been developed for attaching antigens to red cells so that antibodies to soluble antigen can be demonstrated by haemagglutination. Similarly red cells can be coated with antibody and used to detect soluble antigens by haemagglutination.

## 2. COMPLEMENT

Complement is the name given to a system of plasma proteins which circulate in an inactive form as proenzymes awaiting the action of the predecessor in the sequence to convert them to the active form. The conversion frequently involves a proteolytic step. In these practicals complement will be used to lyse antibody-coated red cells. This reaction forms the basis of the plaque assay and of the complement fixation assay.

Guinea-pig serum is the most commonly used source of complement as it has a high titre of haemolytic activity. The lysis of red cells can be seen by the release of haemoglobin. As complement is an enzyme cascade, the components are used up or fixed during its activation. The complement cascade can be triggered by antibody-antigen complexes either in solution or as an antibody attached to cell membrane. If complement is added to a solution containing antibody-antigen complexes, the complement cascade will be activated and the complement used up. This can be shown by then adding antibody-coated red cells which fail to be lysed. This forms the basis of the complement fixation test which is performed by first mixing the serum under investigation with antigen in the presence of a defined amount of complement. The second part of the test involves adding antibody-coated red cells as an indicator system for residual complement. The antibody-coated red cells are referred to as EA (erythrocyte-antibody). The test is positive when the complement has been consumed and the antibody-coated red cells remain unlysed. The test is negative when sufficient complement remains to lyse antibody-coated red cells. In the complement fixation test it is important to use a defined amount of complement. The more complement that is used the less sensitive the test will be; it is therefore first necessary to titrate the activity of the complement.

When it is possible to measure accurately the amount of released haemoglobin it is usual to use a 50% lysis at the end point. For the purpose of these practicals, the

end point will be taken as the highest dilution of serum that will give 100% lysis of the antibody-coated red cells. Because the amount of complement used is arbitrary, it is important to define the conditions of the test when giving fixation titres. Complement is inactivated by a variety of substances other than antibody-antigen complexes. This is referred to as 'anti-complementary activity' and the complement fixation test must include the appropriate controls to test for the anti-complementary activity of the test sera and test antigen.

Finally, the serum from the patient is first heated at 56° C for thirty minutes to inactivate the complement in the serum. The complement in the test serum would otherwise interfere with the complement fixation test.

## 3. LYMPHOCYTES

Antibody is made by B lymphocytes. The Jerne plaque assay allows you to detect single cells making antibody. This assay depends upon the ability of complement to lyse antibody-coated red cells. Consequently if a lymphocyte secretes antibody that is specific for the red cell surface, red cells close to the lymphocyte will lyse in the presence of complement and give a clear plaque visible in the hazy background of unlysed cells. In these practical you will use lymphocytes from an animal injected with sheep red cells and enumerate the number of cells making antibody specific for sheep red cells. Only plasma cells which are derived from B lymphocytes, secrete sufficient antibody to detect in this type of assay.

## 4. BLOOD GROUPING

Blood grouping is the detection of molecules on the surface of red cells which show antigenic differences amongst different individuals of the same species. In man, the two most commonly measured blood group systems are the ABO system and the Rhesus system.

**i) THE ABO SYSTEM**. The detection of ABO blood groups on red cells is usually done by using an agglutination reaction. Specific antibody to the A or to the B antigens, anti-A and anti-B are mixed with a suspension of red cells. If agglutination occurs then the red cells express the antigen. Anti-A can be obtained from the serum of an individual who is group B and anti-B from a group A individual. Such Anti-A and anti-B are sometimes referred to 'natural' antibody and have arisen as a result of exposure to the A and B antigens present on many microorganisms. As an individual is tolerant to his own molecules, Anti-A or anti-B are only present in the serum of an individual when the antigen with which they react is absent from their own red cells. Today, monoclonal antibodies are used instead of human sera as ABO typing reagents and these are what you will use in Class 8.

**II) THE RHESUS SYSTEM**. Many of the antibodies to Rhesus antigens e.g., anti-Rh D, are non-agglutinating and are sometimes referred to as 'incomplete' antibody. It is important to realise that such antibodies are not incomplete in any physical sense and are normal bivalent molecules which fail to bring about agglutination. The reaction of these antibodies with antigen on the surface of the red cells can be detected by using an antiglobulin to bring about agglutination. This is known as the Coombs test. An antiglobulin antiserum is prepared by injecting an

animal with a preparation of purified heterologous immunoglobulin (e.g. rabbit anti-human immunoglobulin).

## 5. BLOOD TRANSFUSION

Blood transfusion is an essential part of medical practice. Although it can truly be regarded as life-saving, it can also lead to the death of patients. It is therefore important that stringent measures be taken to ensure the suitability and compatibility of the blood to be given to the patient.

**A) FACTORS ASSOCIATED WITH THE DONOR:** It is important that the donor, for his/her own sake, should not be anaemic. It is equally important that (s)he should not be suffering from any transmissible disease, currently all blood donations in the UK are tested for 5 infections by specific tests (HBV,HCV,HIV,HTLV,Treponema).

**B) FACTORS CONCERNED WITH STORAGE:** It must be realised that red cells are metabolically active and have a finite life span. In order to prolong their life storage, it is necessary:

i. to prevent bacteria from entering the blood during collection,
ii. to collect the cells into a solution containing some form of nutrient, usually glucose,
iii. to store the blood at a low temperature (4°- 6°C) in order to reduce metabolic activity.

Under these conditions blood can be stored for up to three weeks before being given to a recipient. Blood stored for a period longer than this can be used but the number of effete cells in the stored bottle rises rapidly with time.

**C) FACTORS ASSOCIATED WITH TRANSFUSION:** Following the collection of blood from a donor the ABO group and the Rh (D) group will have been determined and the presence of abnormal antibodies in the serum of the donor will have been excluded. At some stage the bottle/bag will be selected for transfusion into a recipient on the basis of the blood group. It is insufficient, however, to select blood on this basis alone and it is necessary to perform further tests.

These tests involve testing the donor's red cells against the recipient serum by a variety of techniques designed to detect antibodies which may be active over a wide variety of temperatures. It must be realised that the presence of a very weak antibody in a recipient may be sufficient to cause a transfusion reaction, and the test system must therefore detect very weak antibodies. For this reason all blood grouping tests are not carried out on a tile, but use tubes or plastic wells that allow an incubation period of cells and serum of between one or two hours.

The tests used may involve either the addition of protein or the use of antiglobulin serum to enhance the reactions of any antibody that should be present. And, in addition, most of these reactions are examined with a microscope in order that even small agglutinates of cells which may be present are not overlooked.