Technical Monograph No. 2

The *ABO* Blood Group System and *ABO* Subgroups

History

Blood has always held a fascination for man and it was Landois[1] in 1875 who mixed lamb red cells with serum from a dog and incubated the mixture at 37°C. The red cells lysed within two minutes. This discovery prompted Landsteiner[2] in 1900 to experiment with human blood. His Nobel prize-winning discovery of the *ABO* system was the foundation stone of modern blood transfusion science. But Landsteiner's nomenclature was not clear and understanding it led to uncertainty. Twenty years after Landsteiner's discovery, in Britain blood groups were still being referred[3] to as groups I, II, III and IV because of this uncertainty. These groups corresponded to *AB*, *A*, *B* and *O* using the Moss[4] nomenclature. Alexander Fleming, famous for his work in Microbiology was one of the authors of the above referenced paper[3]. It was published in 1919 after the First World War and refers to blood groups I to IV. Fleming and Porteous were reporting that they had successfully given blood transfusions to 100 injured soldiers. It was in the 1920's that the forerunner of the World Health Organisation added clarity to the confusion of nomenclature and approved the name of the present ABO system.

The early part of the 20th century was a time of scientific advancement and the "War to end Wars" promoted rapid developments in blood transfusion. It was generally believed particularly in the early 1940s that people whose blood group was *O* could donate blood for use by anyone. During this period, hundreds of thousands of bottles of group *O* blood were transfused as a remedy for war injuries, the majority with satisfactory results. As a consequence it became generally accepted that blood transfusion was a therapy with minimal risk. This attitude prevailed until the 1960's when the discovery of the 'Australia antigen' (HBsAg) in the serum of an Australian aborigine, had a profound affect on our attitude towards blood transfusions and viral infections. This has been followed by the AIDS alert and the current suspicions concerning the transmission of vCJD (variant Creutzfeldt Jakob Disease).

Antigenic Determinants

The most frequently occurring antigens on the red cell surface are the antigens of the *ABO* system. These antigens are synthesised during foetal development by the sequential addition of sugar residues to a common precursor substance. *A* and *B* red cell antigens are distinguished by the nature of their immunodominant terminal monosaccharides on the red cell surface. These sugars are *N*-acetylgalactosamine in group *A* individuals and galactose in group *B*. The absence of these sugars is denoted by *O* and the presence of both by *AB*.

The immune system protects the body so that when a foreign antigen is introduced, an individual reacts to that antigen by producing an antibody, which is specific and reacts with that antigen in an observable way. This is often portrayed as a lock and key mechanism. In the *ABO* system anti-A reacts (agglutinates) specifically with *A* substance on the red cell surface and anti-B agglutinates *B* red cells. A group *A* person has anti-B in their serum and a group *B* person has anti-A. Group *O* individuals have both anti-A and anti-B in their serum and a group *AB* person has neither anti-A nor anti-B. Anti-A and Anti-B that are demonstrated in this reverse grouping process are called isoagglutinins. *In vitro* agglutination by these antibodies is optimal with cells suspended in saline at about 5°C and the strength of the reaction decreases as the temperature of testing is raised. For convenience, *ABO* testing is performed at room temperature (18-24°C). At lower temperatures non-specific cold agglutinins interfere with reverse grouping and at temperatures above 30°C, the activity of the reagents and the isoagglutinins is lessened. This factor is particularly important in hot climates.

The pH and conductivity of the diluents also affect the potency of reaction, as does bacterial contamination. The potency of a reagent is its titre i.e. the amount by which it can be diluted to give a "one plus" reaction. From the pattern of results obtained with any individual's red cells and serum their blood group can be determined. The presence or absence of isoagglutinins confirms the interpretation of the *ABO* blood group and these phenomena are unique to the *ABO* system. The exceptions to this reverse grouping check are individuals with hypo- or a gammaglobulinemia, some elderly individuals and infants too young to produce their own antibodies (usually less than 6 months).

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If antibodies are detected in cord serum, it may be assumed that they have been passively transferred from the maternal circulation. Any discrepancy between the red cell group and the reverse group demands that further testing should be done to establish the true group of an individual. It is thought that the development of anti-A and anti-B isoagglutinins occurs during childhood as a natural consequence of exposure to the analogous antigen. The occurrence of these naturally occurring IgM antibodies indicates how widely distributed in nature these antigens are. The titre of these antibodies reaches its maximum between the ages of 5 and 10 years and then starts to fade in old age. The consequences of a major *ABO* incompatible transfusion i.e. *A* blood to *O* or *B* patient, *B* to *O* or *A* and *AB* to *A*, *B* or *O*, usually results in serious organ damage and/or death. If transfusions were to be given without regard to the *ABO* groups, about one-third of the population of the U.K. would be incompatible. It is essential therefore that testing for the *ABO* system be thorough and accurate.

Blood groups are inherited and the gene locus for the *ABO* system is situated on chromosome 9. The inheritance of *ABO* groups follows Mendelian principles, the presence of *A* or *B* being dominant over their absence (*O*). To determine heterozygosity (*AO* or *BO*) from homozygosity (*AA* or *BB*) of the groups *A* and *B* requires a detailed family study.

Blood groups have formed the basis of many studies and the genes encoding 20 of the 23 major blood group systems have been cloned and sequenced[5]. This now allows the determination at the molecular level of many blood group antigens and phenotypes. Great progress is being made in the area of the molecular mechanisms that generate all the diverse blood group antigens. One of the many potential uses for molecular genotyping is to confirm weak A and B antigenic determinants (see earlier).

Some diseases can cause problems by influencing the blood group. In acute leukaemia the A and B antigens can become weakened. In carcinomatous tissue these antigens may even be lost. The acquisition of a B antigen has also been noted in some cases of cancer. Special care is needed when blood grouping all of these case types since mis-grouping can be fatal. Clinical detail is very important.

Subgroups

It was von Dungern and Hirszfeld[6] in 1911 who discovered that group *A* individuals can be divided into sub-groups A_1 and A_2. In Europeans about 80% of group *A* individuals belong to sub-group A_1, almost all the rest being A_2. Individuals of group A_2 have fewer *A* antigen sites on their red cells (approximately 250,000 per cell) when compared to A_1 individuals (approximately 1,000,000). This has led to the view that the difference is purely quantitative. To a large extent the minor blood groups (sub-groups of *A* and the sub-groups of *B*) are of academic interest to the biomedical scientist. Their importance is only realised for donations and when organ transplants are being considered. Approximately 2% of group A_2's and 25% of A_2B's have a naturally occurring anti-A_1. Careful microscopic examination of the reverse group (patients serum versus known pooled *A* cells and known pooled *B* cells) reveals a "mixed-field" appearance when an anti A_1 antibody reacts with the pooled A cells. The A_1 cells within the pooled *A* cells are agglutinated specifically but not the A_2 cells. This anti-A_1 reacts at 20°C and not normally at 37°C and is therefore not clinically significant. It can cause confusion in grouping when *A* cells agglutinate with a group *A* individual's serum due to the presence of an anti-A_1 antibody. The ideal anti-A_1 used to type sub-groups of *A* is a lectin, produced from *Dolichos biflorus* seeds. The extract from the seeds when appropriately diluted is specific for A_1 substance. Although lectins are essentially plant extracts they have been discovered in many organisms from bacteria to mammals.

Many other weaker forms of *A* have been described and their characterisation depends on a number of serum and secretor factors and molecular analysis (see earlier). About one in 1000 of group *A* individuals belong to subgroup A_3. The subgroups A_X, A_M, A_{el} and A_{end} give varying reactions with anti A,B typing sera and they may or may not have a naturally occurring anti-A_1 present. The serum of selected group O individuals is used to prepare this additional reagent, anti-A,B which has the ability to detect subgroups of A and B antigens as well as confirm the results of tests with anti-A and anti-B. The occurrence of these subgroups is very rare.

There are no subgroups of *B* that are analogous to group A_2 but some types of *B* cells have been described that react weakly or not at all with anti-B.

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Blood Group Frequencies

The frequency of blood group *A* is quite high (25-55%) in Europe, especially in Scandinavia and parts of central Europe. High group *A* frequency is also found in the Aborigines of South Australia (up to 45%) and in certain American Indian tribes where the frequency reaches 35%.

The frequency of A_2 in Lapland reaches 37% but elsewhere in Europe it does not exceed 1%. Group A_2 is found mainly in Europe, the Near East, and Africa, but is either very rare or absent from indigenous populations throughout the rest of the world.

High frequencies of *B* are found in Central Asia especially in the Indian Sub-Continent (20-30%). In Europe group *B* frequency diminishes from about 15% in the east to less than 0.5% in the Netherlands, France, Spain and Portugal. Group *B* is almost absent from American Indians and most Australian Aborigines and was probably totally absent prior to the arrival of Europeans.

Populations with a relatively high frequency of blood group *O* (greater than 70%) are found in North and South America, and in parts of Africa and Australia. The European and Asian populations are more diverse and blood group *O* does not reach these proportions. Some Indians of South and Central America are virtually all group *O*, and probably were entirely so before the European invasion.

Monoclonal antibodies

Monoclonal antibodies have had a major positive impact on blood transfusion. The potential to harvest unlimited amounts of high quality antiserum that does not show batch to batch variation is a major advancement in Transfusion Medicine. A line of plasma cells derived from a single lymphocyte can make each type of antibody molecule. The progeny of a single cell is called a clone and the identical molecules produced by such a clone constitute a monoclonal antibody. Murine monoclonal antibodies specific for various human antigen components have proved to be very satisfactory for grouping purposes. The BIOTEC [7] range of ABO antisera manufactured from murine monoclonal antibodies are suitable for use by slide and tube methods. The range extends from the *ABO* system, Rhesus anti D and anti-Human Globulin products to Bovine Albumin.

References

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7. BIOTEC Laboratories Ltd., 32 Anson Road, Martlesham Heath, Ipswich, IP5 3RG. Telephone +44 (0) 1473 612158.

BIOTEC ABO Reagents

Listed below are ABO reagents available from BIOTEC Laboratories Ltd. Please contact your local BIOTEC distributor for further details.

Cat. No.	Description	Size	Cat. No.	Description	Size
1/008i	Anti A	10 ml	1/028i	Anti A,B	10 ml
1/008	Anti A	10 x 10 ml	1/028	Anti A,B	10 x 10 ml
1/008-1L	Anti A	1000 ml	1/028-1L	Anti A,B	1000 ml
1/018i	Anti B	10 ml	1/128	Anti A1 (Lectin)	2 ml
1/018	Anti B	10 x 10 ml	1/132	Anti A1 (Lectin)	5 ml
1/018-1L	Anti B	1000 ml	1/132d	Anti A1 (Lectin)	10 ml

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