Technical methods

Staining of the A antigen on human erythrocytes

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This report describes an immunoperoxidase technique which can be used to demonstrate the A antigen (of the ABO system) on human red cells.

Materials and Methods

RED CELLS

Red cells were washed thoroughly in normal saline, suspended in a bovine serum albumin solution (5-10% in normal saline), and smeared on clean glass slides. The smears were air dried and immersed in methanol containing 0.3% hydrogen peroxide for 30 minutes. This step serves both to fix the red cells and to block endogenous peroxidase activity.

BUFFER

Tris-HCl buffer, pH 7.6, 0.5 molar, was used throughout.

ANTISERA

The anti-A reagent was provided by group O serum in routine use as an ABO grouping reagent. Rabbit antiserum to human IgG, swine antiserum to rabbit serum protein, and soluble immune complexes of rabbit anti-peroxidase and peroxidase (PAP reagent—prepared according to the method of Sternberger *et al*, 1970) were obtained from Dakopatts, Copenhagen (UK distributors: Mercia Diagnostics, Sandown Road, Watford, Herts). All antisera were diluted in buffer for use.

IMMUNOPEROXIDASE STAINING OF RED CELLS

After fixation the red cell smears were air dried and exposed to each of the four antisera in turn for 30 minutes at room temperature. Following each application the slides were washed with Tris saline (buffer diluted 1 in 10 in normal saline).

The resulting antibody 'sandwich' is illustrated (fig 1).

After application of the fourth reagent (PAP) the localization upon red cells of the antibody bound

Fig 1 Diagram illustrating the immunoperoxidase 'sandwich' for staining the A antigen.

peroxidase was demonstrated by the addition of a suitable substrate (diaminobenzidine reaction¹) producing a stable brown reaction product visible by light microscopy. Slides were further washed in tap water, dehydrated through the alcohols to xylol, and mounted in DPX.

Results

Figure 2 shows the result of staining a 50:50 mixture

Fig 2 A 50:50 mixture of group O and group A red cells stained for the A antigen. $\times 2000$

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¹A solution of 0.6 mg/ml of diaminobenzidine tetrahydrochloride (3, 4, 3', 4'-tetra-amino-biphenyl hydrochloride—soluble form—BDH Chemicals, Poole, England) in Tris buffer with 0.01% hydrogen peroxide—for 15 minutes).

Fig 3 Red cells from a patient with myelomonocytic leukaemia stained for the A antigen. $\times 2000$

of group A and group O cells by this method. It can be seen that the two cell populations are clearly distinguishable. Preparations of group A cells alone stained uniformly positive, while all cells in group O smears were negative.

Figure 3 shows the staining reaction of red cells from a myelomonocytic leukaemic patient who had given a 'mixed field' reaction with anti-A serum on routine grouping. A minority of strongly staining cells can be seen against a background of weakly or negatively staining cells. This reaction was thought to represent an example of leukaemia-associated 'loss' of the A antigen (Race and Sanger, 1968) although the possibility that the patient was group A₃ could not be excluded.

Discussion

The demonstration of the A antigen on smears of human red cells by an immunoperoxidase technique has not to our knowledge been previously reported. The procedure opens several avenues for further investigation.

The study of the cellular distribution of the antigen in patients giving 'mixed field' agglutination reaction has already been touched upon. A₂, A₃, and weaker subgroups of A, as well as chimeric states, can be investigated in this way. It would also be feasible to follow the survival of group O cells in a group A recipient by this procedure, in cases where isotopic labelling was contraindicated.

Substitution of alternative antisera for the first layer of the 'sandwich' would permit the examination of other red cell antigens, such as B, I, i, etc. Those antigens to which rabbit antisera are available, for example, M, N, LW, can be investigated using a triple rather than a quadruple layer system.

Finally, by substituting reagents such as anti-IgM, anti- κ , and anti- λ for anti-IgG in the second stage of the reaction, the light and heavy chain class of the first antiserum can be studied. This is of potential value in investigating monoclonal antibodies such as anti-I.

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