

Technical methods

Latex agglutination method for IgA deficiency used for large scale screening of blood donor sera

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Washed red blood cell suspensions and various plasma fractions contain substantial amounts of IgA, so that transfusion of blood or blood products in IgA deficient patients with anti-IgA antibodies is potentially hazardous because of possible anaphylactic reactions to IgA.¹ IgA deficient blood donors could be a source of non-hazardous blood and blood products for such patients. Various studies have estimated the prevalence of IgA deficient subjects in blood donor populations from 1 in 500 to 1 in 1000.^{2,3} The identification of such IgA deficient blood donors would, therefore, entail extensive screening of donor sera IgA values. Commonly used serum IgA quantitation techniques, including immunodiffusion, laser nephelometry, and immunoassay can be unwieldy or expensive for such large scale screening. Agglutination techniques such as haemagglutination⁴ offer an inexpensive and feasible alternative screening method. We established an indirect latex agglutination test for preliminary screening of donor sera to eliminate sera with normal IgA values and identify sera potentially low in IgA for more accurate measurements.

Material and methods

PREPARATION OF IgA COATED LATEX

Fractions of IgA myeloma sera enriched with immunoglobulin were prepared by ammonium sulphate precipitation (serum: saturated ammonium sulphate solution, 1:1). Precipitates were recovered by centrifugation and dialysed overnight against 0.15M saline: their volumes were adjusted to twice the original serum volume with saline.

A suspension of 40% latex (Intex-191, International Rubber, Southampton) was diluted 1/100 in glycine-saline buffer (0.1M, pH 8.2, containing 0.2% w/v sodium azide). Latex suspension, IgA-myeloma

Ig fraction, and glycine-saline buffer were mixed in the ratio 5:5:3, respectively, and incubated for 20 minutes at 60°C. The coated latex was recovered by centrifugation (6000g for one hour), resuspended in phosphate buffered saline (Dulbecco 'A', Oxoid) containing 1% w/v bovine serum albumin and 0.2% w/v sodium azide, and passed several times through a 21 gauge needle to break up granules.

INDIRECT LATEX AGGLUTINATION TEST

Test sera (35 μ l) and anti-IgA serum (5 μ l) (sheep antihuman IgA, SAPU, Law Hospital, Carlisle) were mixed in wells of a U-well vinyl microtitre plate (Flow Laboratories) for 30 minutes at room temperature. Aliquots (20 μ l) of the mixtures were transferred to wells of Dispo trays (96 CV, Flow Laboratories), mixed with IgA coated latex suspension (20 μ l) and shaken gently on an orbital mixer for five minutes. Clearly visible flocculation was detected for IgA deficient sera where the residual sheep anti-IgA agglutinated the coated latex, while no flocculation was found with normal serum IgA, which neutralised the anti-IgA activity on preincubation.

Serum IgA concentrations were accurately determined by laser nephelometry.⁵

Results and Discussion

Initial attempts to establish a direct latex agglutination test for serum IgA with anti-IgA coated latex were impractical, but an alternative "indirect" test, giving agglutination of IgA coated latex in the presence of added anti-IgA in the absence of serum IgA, was eventually established. Using a panel of 200 sera of known IgA content ranging from 0.2 to 13.0 g/l (determined by laser nephelometry) it was possible to adjust the amount of added sheep antihuman IgA in the test to give a required cut off for serum IgA values above which IgA coated latex agglutination did not occur due to neutralisation of added anti-IgA by test serum IgA. In the above method this cut off was set at 0.5 g/l and was checked against a small panel of 12 sera of differing IgA content each day. Adjustments or changes in cut off could be made by varying the anti-IgA to test serum ratio, or by varying the final mixing and incubation time with IgA coated latex (three to eight minutes) for changed sensitivity near to cut off.

Using the indirect latex agglutination test, 10 045 blood donor sera were screened, and 239 (2.4%) were positive for agglutination. Of these 239, 18 were found to be IgA deficient by laser nephelometry (IgA <0.5 g/l), representing 1:503 (0.18%) of the original 10 045

donors screened, similar to the proportions of IgA deficient blood donors reported in other British studies.^{2,4} None of the 239 sera agglutinated uncoated latex, but 228 agglutinated IgA coated latex directly in the absence of added anti-IgA (including seven of the 18 confirmed IgA deficient sera). With ethical committee approval, follow up serum samples were obtained from the 18 IgA deficient blood donors six months after the initial samples had been taken: 14 of these still showed IgA deficiency (IgA < 0.5 g/l) by laser nephelometry while four had returned to normal serum IgA values. One IgA deficient donor had mild but clinically important recurrent infections (sinusitis, otitis media), two had mild allergies, and two had possible autoimmune disorders. No other important clinical abnormalities were elicited at interview.

These results show that the manual indirect latex agglutination test can provide a simple and satisfactory preliminary screen for IgA deficient blood donors, reducing the requirement for quantitative IgA testing by more sophisticated and expensive methods. It is more rapid than the indirect passive haemagglutination test described by Hunt *et al*⁴ which uses a similar preincubation step; it is free of interference from any anti-red cell antibodies; and IgA coated latex has been found to be stable for at least two months, unlike IgA coated red cells which must be prepared about every week. The relatively simple ammonium-sulphate precipitation for enrichment of IgA myeloma immunoglobulin for coating renders coated latex highly sensitive to agglutination by anti-IgA and enables accurate adjustment of cut off to desired values to be made. With this degree of sensitivity the test does not distinguish between IgA deficient donors and donors with autologous anti-IgA. The latter group would tend not to be distinguishable from donors with anti-red cell antibodies in passive haemagglutination techniques. The importance of finding donors with normal serum IgA values and anti-IgA antibodies is not clear and may merit further studies.

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PLP fixation for combined routine histology and immunocytochemistry of liver biopsies

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The development of monoclonal antibodies has facilitated both histopathological diagnosis and research by allowing the presence of various cytoplasmic and cell surface antigens to be shown on lymphoid and other cells.^{1,2}

Showing the presence in liver biopsy specimens of surface markers for lymphocytes, accessory immune cells, and epithelial cells by using monoclonal antibodies has largely been limited to frozen tissue³⁻⁸; reliable and reproducible results have not been obtained in paraffin embedded tissue. This creates several problems. Frozen sections are inferior in morphological detail to paraffin sections and are less easily stored. Dividing the biopsy specimen into two for frozen and paraffin sectioning increases the possibility of sampling error, and frozen sectioning introduces a risk of infection for laboratory staff, especially when material from patients with viral hepatitis is handled.⁹

Recently Collings *et al*¹⁰ reported the successful use of several monoclonal antibodies using paraffin embedding after fixation of tissues in periodate-lysine-paraformaldehyde (PLP).¹¹ In this study we applied this technique to liver biopsy specimens to assess its suitability for routine diagnostic purposes.

Material and methods

The study was carried out in two parts. The first part was designed principally to test "routine" staining methods after PLP fixation and paraffin embedding;

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