SHORT ARTICLES

THE DETECTION AND AVOIDANCE OF FALSE-POSITIVE REACTIONS IN TESTS FOR RUBELLA-SPECIFIC IgM

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A CONFIDENT laboratory diagnosis of rubella is often dependent on the detection of specific IgM (Pattison and Dane, 1975). This is usually measured, as rubella haemagglutination-inhibiting (HI) antibody, in the IgM fraction of the serum, separated from the IgG by sucrose density-gradient centrifugation (Best, Banatvala and Watson, 1969) or gel filtration (Pattison and Mace, 1975). Recently, however, there have been reports of occasional unreliability of the rubella HI test due to chick erythrocyte factors (Saeed and Murray, 1975), inadequate removal of non-specific inhibitors (Haukenes and Blom, 1975) or bacterial contamination of sera (Bruce-White and Tinnion, 1975). We describe here our own findings, during the past 6 years, of false-positive results obtained in tests for rubella-specific IgM.

MATERIALS AND METHODS

Rubella HI antibody tests were carried out with four units of haemagglutinin, overnight incubation of virus-serum mixtures and pigeon erythrocytes (Pattison and Mace, 1973). Serum immunoglobulins were fractionated by gel filtration with Sephadex G-200 as previously described (Pattison and Mace, 1975). Before gel filtration, serum was treated with an equal volume of a mixture of 0.2M-MnCl₂ and heparin (1000 units per ml) for 20 min. at 4°C to precipitate β -lipoprotein non-specific inhibitors; the precipitate was removed by centrifugation at 3000 r.p.m. for 10 min. to leave a clear supernate. Sensitivity to 2-mercaptoethanol (2-ME) was tested by the method of Caul, Smyth and Clarke (1974).

RESULTS

Stored sera. During our earlier investigations of the gel filtration technique (Pattison and Mace, 1975), we examined three sera, collected from pregnant women, for rubella-specific IgM antibodies, both before and after storage at -20°C. Before storage, two of the sera (each with a rubella HI-antibody titre of 2560) had no detectable rubella HI activity in the IgM fraction; after storage for 7 months, both showed low levels of activity in the IgM fraction (titres of 20 and 40, respectively). This activity was resistant to 2-ME, and was, therefore, more likely to be due to the presence of non-specific inhibitors than to specific antibody. The third serum was examined only after it had been stored for 2 months; it had a rubella HI-antibody titre of 2560 and rubella HI activity was demonstrable in the IgM fraction to a titre of 60; it was again resistant to 2-ME. The patient from whom this serum was collected subsequently gave birth to a normal child. At that time, a blood specimen from the mother and cord blood had rubella HI-antibody titres of 2560 and 1280, respectively, but neither had any detectable rubella-specific IgM; at 6 months of age the baby's rubella HI-antibody titre was only 40 and there was no evidence of congenital rubella.

Other sera stored at -20°C demonstrated more obvious retention of non-specific

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inhibitors after MnCl_2 -heparin pretreatment, the low-speed centrifugation failing to clarify the serum. On gel filtration, the lipaemic material in the cloudy supernate eluted with the IgM fraction and exhibited rubella HI activity, resistant to 2-ME. Such false-positive reactions were obtained with 10 of 12 sera that had been stored for 12–24 months at -20°C , and with two sera that had been stored for only 4–5 weeks; false-positive reactions were not obtained with sera that had been stored for shorter periods than this.

Contaminated sera. Four sera were contaminated with bacteria. None of the patients had any clinical evidence of rubella but had been referred for IgM antibody tests because they had high serum rubella HI-antibody titres (640 or greater). All four sera showed rubella HI activity in the IgM fraction, resistant to 2-ME. Two of the sera were cultured and both yielded pure growth of an organism that was identified, by the Central Public Health Laboratory, Colindale, as either *Flavobacterium* or *Pseudomonas*.

Heat-inactivated sera. Five other sera, referred to us because of high rubella-antibody titres, were also found to possess rubella HI activity in the IgM fraction, resistant to 2-ME. These sera were not contaminated with bacteria. The results were suspect, however, not only because of the resistance to 2-ME, but also because none of the patients from whom the sera had been obtained gave a history of recent, rubella-like illness (in our experience, sub-clinical rubella is rare in adult, fair-skinned women). All these sera had been sent to us already inactivated at 56°C for 20 min. We normally process only unheated sera and it was therefore decided to investigate the effect of heating on our IgM test. Four sera with rubella HI-antibody titres of 2560, 1280, 640 and 320 were examined. None showed rubella HI activity in the IgM fraction before heating; after heating, the three sera with the highest rubella HI-antibody titres (2560, 1280 and 640) had developed HI titres of 40, 60 and 20, respectively, in the IgM fraction. It is likely, therefore, that heating alters some of the serum IgG which then elutes from the Sephadex column with the IgM. Presumably only a small proportion of the total IgG is changed since only the sera with very high antibody titres (640 and greater) had rubella HI activity in the IgM fractions.

DISCUSSION

Non-specific inhibitors of rubella virus haemagglutination (HA) belong predominantly to the β or low-density lipoprotein (LDL) class, but all serum lipoproteins possess some inhibitory activity (Blom and Haukenes, 1974). Pretreatment of serum with MnCl_2 -heparin mixture effectively precipitates LDL but leaves behind most pre- β or very low-density lipoproteins and all the α or high-density lipoproteins (Haukenes and Blom, 1975). Although we do not favour pretreatment with MnCl_2 -heparin as an alternative to kaolin for routine rubella HI-antibody tests, we do use it before gel filtration because of the minimal dilution of serum involved. Provided that the serum is tested fresh, the further dilution due to gel filtration makes the small quantity of non-specific inhibitor left behind by the MnCl_2 -heparin treatment undetectable (Pattison and Dane, 1975; Pattison and Mace, 1975). However, storage at -20°C can alter the electrophoretic mobility and composition of serum lipoproteins (G. L. Mills, 1975, personal communication), and the false-positive IgM results obtained with stored sera are, therefore, probably due to the failure of MnCl_2 -heparin adequately to remove lipoproteins altered by storage.

Our results with contaminated sera are similar to those described by Bruce-White and Tinnion (1975) who showed that contamination of serum with an organism closely related to *Pseudomonas fluorescens* led to the appearance of non-specific inhibitors of rubella HA that could not be removed by MnCl_2 -heparin pretreatment.

Heating of serum has been used extensively as a method of aggregating IgG; up to 30% of normal IgG is aggregated by heating at 63°C for 30 min. (Augener and Grey, 1970), and 56°C for 30 min. also causes some aggregation (Christian, 1960). Our findings indicate that, at 56°C , 3–5% of rubella-specific IgG in serum aggregates, and then fractionates with IgM on gel filtration through Sephadex G-200.

Serum specimens sent to routine virus laboratories are likely to be inactivated at 56°C for complement-fixation tests, or kept at -20°C . Our investigations demonstrate that both

procedures should be avoided for sera that are to be tested for rubella-specific IgM. This applies whether the serum IgM fraction is to be separated by gel filtration or sucrose density-gradient centrifugation; aggregated IgG is deposited with IgM in a density gradient (Christian, 1960) and it is likely that storage-altered lipoproteins will, like the high density inhibitor of Al-Nakib, Best and Banatvala (1974), behave in a similar fashion. It is recommended that IgM fractions showing rubella HI activity at low titre should be re-examined after treatment with 2-ME, to exclude false-positive results due not only to the various causes described above but, perhaps, also to others as yet undefined.

SUMMARY

Serum specimens tested for rubella-specific IgM by the haemagglutination-inhibition technique may give false-positive results due to (a) storage at -20°C , (b) bacterial contamination, or (c) inactivation at 56°C . These false-positive reactions can be distinguished from rubella-specific IgM activity by their resistance to 2-ME.

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