Recruitment and plasmapheresis of donors to provide human antitetanus immunoglobulin

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SYNOPSIS A method is described whereby about 100 litres of plasma containing 10-50 IU/ml tetanus antitoxin was obtained, from which were prepared 5000 × 250 IU doses of human antitetanus immunoglobulin. Of 40 blood donors who received a booster injection of tetanus vaccine BP, 33 were plasmapheresed each week over a 10-12 week period starting three weeks after the injection. Twenty-two of these donors provided 90% of the total plasma, the antitoxin content of which averaged 23·6 IU/ml over the 10-12 week period.

The adverse reactions of patients to horse tetanus antitoxin and the possible medicolegal repercussions for any doctor using this therapy are now well known. Consequently, human antitetanus immunoglobulin (ATIG) is now gaining widespread acceptance. It has been used solely for at least 10 years at the Johns Hopkins Hospital, Baltimore (Shirkey, 1965) and has been available for a similar time in Canada (Perey, 1966), Australia, Switzerland, and Sweden. In the United Kingdom, ATIG is usually restricted to casualties whose previous tetanus immunity is in doubt, and/or in whom complete debridement of the wound may not be advisable, and/or in whom there is demonstrable evidence of hypersensitivity to heterologous protein. Its use is combined with an injection (0·5 ml-20 Lf) of adsorbed tetanus vaccine BP (TT ads). The main advantage is that ATIG is free of the anaphylactic reactions resulting from horse antitoxin. The 250 unit dose of ATIG reliably gives a protective antitetanus level (over 0·01 unit/ml) for about four weeks. This product should also be effective in clinical situations where antibiotic therapy would be unsuitable, e.g. severe wounds, those seen late after injury or those with a secondary infection, and in the treatment of cases of established tetanus where it could be used intravenously combining (a) rapidity of effect, (b) safety, and (c) maintenance of effective blood levels.

In Scotland, the five Regional Transfusion Centres are normally self-supporting in the supply of blood and blood products. Commercial blood products are rarely used as such items are supplied by the Protein Fractionation Centre at Edinburgh. The need to find a suitable source of high-titre plasma for the production of ATIG stimulated a search among various donor groups.

The following were approached:
1 Service personnel (1965-70).
3 Random casualties (not blood donors) receiving a standard course of three injections of TT ads were contacted and asked to volunteer to have their blood level assayed two to four weeks after the third injection, i.e. about seven months after the first injection. In these three groups blood levels were assayed by mouse tests.

3 In April 1974, 200 donors were contacted at random regarding a positive history of the three-injection standard course. Forty-five replied and 40 volunteered to receive a booster injection of 0·5 ml tetanus vaccine BP (TT). Starting three weeks later, 33 were plasmapheresed weekly for 10 weeks (double donation of 420 ml blood per donor), yielding an average of 500 to 550 ml plasma per weekly session.

Methods

Until 1972 tetanus antitoxin titres were estimated in vivo by the mouse neutralization test (Glenny and Stevens, 1938) by courtesy of P. Knight and D. Gall (Burroughs Wellcome & Co). In 1972, we modified the Counter current immunoelectrophoresis (CIE) method of Milne and Barr (1971) used for testing HBsAg and anti-HBs to measure ATIG. This proved to be less satisfactory than a simple Ouchterlony or Mancini (radial immunodiffusion) assay. The
results obtained by these two methods and by neutralization tests in mice were compared using two standards—(1) the International Standard antitoxin serum (Statens Serum Institut, Copenhagen), and (2) a solution of antitetanus immunoglobulin solution containing 50 IU, prepared by the Protein Fractionation Centre, Edinburgh (Director, J. Watt). The latter solution was used at 12.5, 25.0, and 50.0 IU/ml respectively.

Reagents

(a) Tetanus Vaccine BP (Tet/Vac/Ft) containing 20 Lf in 0.5 ml (Wellcome)
(b) Michaelis buffer, pH 8.2, as used with HBsAg/CIE method
(c) Agarose (BDH)
(d) 0.01% sodium azide as a preservative
(e) Immunodiffusion plates (Hyland).

0.9 agarose/100 ml buffer was dissolved by steaming in an autoclave at 5 lb for 15 minutes. The toxoid at 3 Lf/ml and the sodium azide at 0.01% were added after the agarose had been allowed to cool to approximately 60°C. The 3 Lf/ml concentration permitted the measurement of sera containing between 10 and 50 IU/ml. After being well mixed, 2.5 ml were pipetted into each Hyland plate and allowed to solidify on a level surface. The plates were stored at 4°C in sealed plastic bags until used. A template and cutting needle were used to cut 3.0 mm holes. The ratio of antibody to antigen is fairly critical, and, to maintain this, a micropipette was used to fill each hole. After the three standards (12.5, 25, 50 IU/ml) and the sera had been added, the plate was placed in a sealed bag and left overnight at room temperature. Increasing the temperature to 37°C did not appear to influence the results. Positive reactions could be seen after one hour, and a preliminary reading and estimate could be made at four hours. Final readings were made after leaving the plate overnight if no further change in diameters had occurred. The diameters were measured with the aid of a magnifier over a reading box using a millimetre graduated plastic card which was black except for the guide-lines and graduations which were transparent. The square of the diameter of our standard was plotted on graph paper and compared with the test results (fig 1).

Results

Comparison of the results of assays by mouse tests and those obtained by immunodiffusion showed a correlation within ±5% in 46 donors. This correlation was sufficiently accurate to select donors having levels over 10 IU/ml for plasmapheresis.

Between 1965 and 1970 our intermittent contacts at blood donor sessions involving Service personnel produced only 2-4% who had had a TT injection (either booster or third injection) within the previous four weeks. At that time the clinical demand for ATIG in the United Kingdom was negligible so that the Regional Transfusion Centres were not obliged to find high titre donors.

In 1971-72, from 26727 outpatients attending the hospital as casualties 224 blood donors and 105 other patients (total 329) volunteered initially to be tested after either booster injection or the final (third) injection of TT ads. In 23.7% levels exceeded 10 IU/ml. The necessary follow-up was time-consuming and laborious and did not justify the work involved. Frequently the individual concerned did not attend for the second or third injection, and blood samples were not always obtained within two to four weeks of the booster or final injections of TT ads. Figure 2 shows the antitoxin levels of some 40 donors 3, 6, 10, 12, and 14 weeks following a booster injection of TT (simple). These donors were divided into five groups—<10, 10-20, 20-50, 50-80, and 80+ IU/ml, according to the antitoxin level found at these times. In 21 of these donors the concentration was less than 10 IU/ml by the eighth week but in a further 10 averaged 20-50 IU/ml in the twelfth week. In six of these donors the concentration exceeded 50 IU/ml in the first six weeks after injection. The notable response in one of these donors is shown in figure 3. This donor had a pre-injection level of less than 5 IU/ml, but accurate assays of the pre-injection levels in the 40 donors were not done as previous experience had shown that less than 5% would be above 1 IU/ml. Wilson (1967) found an average level of 0.7 IU/ml in all blood donor clinic. Accordingly, our assay system was set to detect only those with more than 10 IU/ml, ie, those in whom a relatively high titre of
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Fig 2 Concentrations of tetanus antitoxin in the serum of donors during 14 weeks

antitoxin was likely to be maintained during a 10-12 week period of plasmapheresis.

Discussion

Before the development of immunological tests, such as immunodiffusion and immunoelectrophoresis, tetanus antitoxin was measured by neutralization of tetanus toxin in mice and this is still the official method of assay. Regional Transfusion Centres in Scotland before 1970 often collected plasma from donors with a recent history of injection of tetanus toxoid but did not measure antitoxin levels in each donor. In our experience, the Ouchterlony or Mancini methods of assay, using suitable standards, have proved invaluable for selecting and following up boosted donors.

The Inverness Transfusion Centre serves a scattered population of about 190,000, covering one-third of Scotland throughout the Highlands and Islands. The donor population bled is just over 10,000 annually so that automated screening methods would hardly be justified because of the small number of donors and because selected donors (ie, with over 10 IU/ml) would probably be unable to attend for plasmapheresis regularly due to the long distances between their homes and the centre. There is still a strong community spirit in the donor population, so for schemes involving plasmapheresis, for producing plasma for the preparation of specific immunoglobulins, eg, anti-D, anti-tetanus, anti-zoster, and anti-vaccinia, we have depended on donors in Inverness.

Previous data showed that less than 5% of unselected donors had an antitoxin concentration above 10 IU/ml. Sgouris et al (1966) found that 2.8% of unselected donors had levels of 10-20 IU/ml. Entwistle and Eldridge (1973) reported that 1.5% of East Anglian donors had more than 10 IU/ml, and Nelson (1973) found 12.1% of donors in Melbourne had levels above 3 IU/ml. Other workers have shown the value of boosting previously immunized donors followed by plasmapheresis. At the Conference on Tetanus in Berne in 1967, Fischleowitz and Sturm reported that of Swiss Army personnel with a past history of three injections of tetanus antitoxin given 10-15 years previously, who were given a booster injection of TT ads, 41% produced a peak level of 10-50 IU/ml and 12% reached 50-100 IU/ml. Hässig (1967)
observed that 25-35\% of Swiss Army recruits had concentrations over 5 IU/ml after a second booster. Billaudelle (1967), working in Sweden, observed that approximately the same concentration of antitoxin is evoked by each booster injection so that the same group of selected donors may be used as a source of plasma for many years. Similar findings were reported from Russia where Papko et al (1970) showed that 16-7\% reached 15-20 IU/ml after a dose of 30 units of tetanus toxoid and that doses of 50 and 100 units of TT produced concentrations of at least 50 IU/ml in 34-52\% of subjects.

The response to TT ads is superior to that produced by TT (simple) (Chernaya and Kevtunovich, 1963), and it is probable that peak levels in our donors would have been still more impressive if adsorbed toxoid had been used. The risk of local and general reactions to TT increases with the frequency of the booster injections (British Medical Journal, 1974). For this reason and because we plan to invite these donors to revolunteer, say every fourth year, we decided not to use TT ads for the initial booster dose. By so doing we hope to reduce the number of reactions in later years. It remains to be seen whether a second group boosted with TT ads will show more reactions in three to five years when reboosted.

The use of plasmapheresis enabled us to collect from 33 donors within 14 weeks of a single booster injection over 100 litres of high-titre antitetanus plasma from which would be prepared 5000 × 250 IU prophylactic doses, or a smaller number of 5000 or 7500 IU doses for treatment. Although a minimum level of 2 IU/ml is acceptable for fractionation, the protective dose of 250 IU (at 10\% protein concentration) is then contained in 5-0 ml of immunoglobulin. The higher concentration of antitoxin obtained by the above method permits the dose volume to be much reduced.

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References


