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MINUTES OF A MEETING ON THE VIROLOGICAL ASPECTS OF THE SAFETY OF BLOOD PRODUCTS, HELD AT NIBSC ON FEBRUARY 7TH, 1986

Dr. Schild welcomed participants and noted that the meeting was a timely one in view of recent events (to be reviewed by the meeting) involving transmission of virus infection which had indicated that the safety margin for intravenous immunoglobulins, and possibly other blood products, may be less secure than had been believed. He hoped there would be free and constructive discussion and that the meeting would provide for an exchange of information or value to the licensing authority and the manufacturers of blood products.

Professor Weiss presented an update on the T-lymphotropic retroviruses, which had only been discovered five years ago. HTLV I is a known cause of human lymphoma, and can be transmitted perinatally, and also in blood transfusions. However, transmission by transfusion was apparently associated only with transfer of whole blood, and not plasma. There was some evidence that HTLV I infection transmitted through blood transfusions could be associated with leukaemia.

Less is known about HTLV II, which was isolated from a leukaemic patient, and also occurs in drug addicts. It may be a cause of hairy cell leukaemia.

The causative agent of AIDS, LAV/HTLV III, is a lentivirus, the prototype being visna virus or sheep. Several species of monkeys harbour agents closely related to human LAV/HTLV III, but nevertheless distinguishable from the human viruses. These have been designated STLV III. Antibodies which cross-react with STLV III, as well as HTLV III, have been found in some non-symptomatic patients in Senegal, West Africa. STLV III is non-pathogenic in vervet and mangaby monkeys, but causes an AIDS-like

syndrome in rhesus monkeys. In man, HTLV III is capable of infecting chimpanzees, but other primates are apparently not susceptible. STLV III isolated from vervets will infect human T cells, but it is not known whether HTLV III will infect vervets.

HTLV III specifically infects T_4 lymphocytes, and other cells which contain the T_4 antigen, such as macrophages and microglia: the latter accounts for the brain disorders which are being increasingly seen as part of the pathology of AIDS. The virus occurs in saliva, tears and semen, but is probably contained within lymphocytes in these fluids. Similarly, there may be little extracellular virus in blood. Antibodies to the virus in human sera are mostly non-neutralising: this is also the case with monoclonal antibodies.

Dr. Minor gave a brief account of the general methods used to inactivate viruses, including chemicals, heat, pH extremes, detergents, solvents, proteases and irradation. Freeze-drying during plasma fractionation may itself contribute towards virus inactivation.

Retroviruses consist of single-stranded RNA with a lipid envelope, and would be expected to be sensitive to heat, detergents and organic solvents. However, caution should be exercised in drawing deductions from model viruses, since viruses of the same type are not always equally susceptible to inactivation. Furthermore, high concentrations of protein may significantly reduce the effect of inactivation of viruses by physico-chemical methods.

Dr. Garrett described the inactivation of HTLV III, which is relatively easily achieved by heat or chemical means. Infectivity is destroyed by wet heat at 30 mins., or dry heat for 48 hours at 68°C. However, the virus is surprisingly resistant to inactivation when dried and can survive freeze-drying. Some data had been reported that indicated inacti-

vation by ethanol at room temperature, but at the lower temperatures encountered in plasma fractionation, infectious virus survived.

During discussion it was emphasised that measurement of the effect of 'inactivation' procedures on the biological properties of the virus, such as reverse transcriptase (RT), may be unreliable as evidence of inactivation of infectivity. However, RT activity is a useful indicator for virus growth in cell cultures. Studies on inactivation of virus should involve direct measurements of infectivity. Professor Weiss emphasised that at present no measurement or infectivity was sufficiently sensitive to reliably detect virus in blood products.

Dr. Thomas pointed out the limitations of using chimpanzees for studies on non-A non-B hepatitis. Experience in Italy had shown that chimpanzees had failed to develop hepatitis after receiving material which subsequently proved infective in humans.

Dr. Perry gave a brief overview of the Cohn plasma fractionation process, and the possible virus load in the various products. Albumin, Factor VIII and Factor IX were regarded as potentially infective before final heat treatment, whereas intramuscular IgG was regarded as safe. Some intravenous IgG preparations have transmitted virus infection, and this has cast doubt on the inherent safety of this group of products. Many factors contributed towards product safety, including plasma quality, distribution of virus during fractionation (much virus may be in waste fractions I and III), inactivation by alcohol and freeze-drying, and terminal treatment, e.g. by heat or chemicals.

The safety of intra-muscular IgG may be due to freeze-drying from an ethanolic solution, although other factors may also contribute to product safety. I.v. IgG products from several companies have given non-A non-B hepatitis when non-virucidal conditions have been used in the downstream

processing of Cohn fraction II. However, products which have undergone one or more of the following processes appear safe:

β-propiolactone

reduction/alkylation

sulphonation

pH_4/pepsin

plasmin digestion

In conclusion, Dr. Perry emphasised the need for the collection of more scientific data on the effects of individual stages of the plasma fractionation process on virus inactivation. Present policy in the UK was not to issue blood products if they were positive for antibodies to LAV/HTLV III or hepatitis B surface antigen, or if they were prepared from a pool that was known to contain a donation that was HTLV III Ab or HBSAg-positive.

Dr. Cuthbertson summarised the published data on inactivation of HTLV III during fractionation. Freeze-drying alone gave 1-2 logs decrease in Factor VIII preparations. Data on heat treatment were variable, e.g. it was claimed by Dr. Prince that 72 hours' dry heat at 60°C was needed to inactivate LAV/HTLV III in Factor VIII preparations, whereas others claimed that much milder conditions were effective. As already pointed out, precise definition of residual moisture content of lyophilised preparations is crucial in the interpretation of results of such studies. One study claimed complete inactivation by 20% ethanol, but another study found little inactivation in ethanol below 0°C. As previously emphasised by Dr. Lane, it is important to use an adequate infectivity assay for these studies.

For i.v. IgG preparations, pH_4/pepsin treatment is regarded as safe, although there are no data on HTLV III. Dr. Minor asked whether pepsin

treatment of pH_4 alone was adequate to inactivate the LAV/HTLV III in the presence of large concentrations of IgG, but Dr. Perry said that the presence of pepsin may enhance viral inactivation and is an essential component of the overall process, at least in the SNBTS product. Dr. Tedder said that i.v. IgG could potentially transmit hepatitis B, but did not do so because of careful screening of donor panels. However, this safety with respect to hepatitis B could not necessarily be extrapolated to other potential viral contaminants.

Dr. Thorpe reviewed the various immunoglobulin products used. Intramuscular IgG's were given in low doses, whereas high doses of the intravenous IgG's were given, and this may be a factor in the safety of intramuscular IgG. In biochemical terms, all products were fairly pure and there was little to distinguish the i.v. from the i.m. preparations. Antibodies to HTLV III have been found in several batches of IgG and also in specific anti-hepatitis IgG. Chemical treatment of IgG could be considered for viral inactivation, but this might affect IgG structure and Fc function.

Dr. Webster described two groups of patients who received regular IgG treatment. About 50 patients in the UK have X-linked congenital deficiency of IgG, and about 600 have acquired deficiency. An increasing number of patients, currently about 30%, is being treated by i.v., rather than i.m., IgG. Since the early 1970's, a small number of these patients has developed signs of cellular immune deficiency, as well as deficiency in the humoral system.

Twelve patients given a newly-introduced i.v. IgG from BPL, Elstree, developed non-A non-B hepatitis. Two of these have since died, and most of the others still had evidence of infection, with high ALT levels. More recently, a virus resembling HTLV III has been isolated from two

patients given i.v. IgG. In one patient, the material had been obtained from Cutter, while in the other case, the i.v. IgG had been provided by Sandoz. One of the patients has a definite AIDS syndrome, but had evidence of cellular immune deficiency before treatment, with low T cells. There have been no other clinical reports of AIDS during the last two years in patients treated all over the world with i.v. IgG.

Dr. Malkovsky showed Southern blots of the viruses isolated from the above patients. The genome was apparently distinguishable from that of classical HTLV III virus by restriction mapping, but the HTLV III viruses as a group are thought to exhibit polymorphism.

In discussion, Dr. Thomas said that, of 200 patients in the US who had been followed up after receiving i.v. IgG, only one was found to be positive for HTLV III antibody, and in this patient there was no control blood sample. Dr. Lane commented that the Swiss-made IgG could well be positive, since HTLV III Ab positive plasma was not excluded from the pool for IgG fractionation, and the incidence of HTLV III Ab positivity was 1 in 4000 Swiss donors.

Dr. Forbes gave data on patients with haemophilia A and B, who together number 2 per 10,000 population, or a total of 4,500 in the UK. Clinical AIDS had occurred in haemophiliacs in several countries, including the UK, since 1983, and was associated with the use of US Factor VIII concentrates.

In a survey of 2,609 UK patients from 81 haemophilia centres, 46% of haemophilia A patients were positive for HTLV III antibody, whereas only 6% of haemophilia B patients and 5% of von Willebrand's disease patients were seropositive. The incidence of antibody positivity was related to the severity of the defect and hence to the intensity of concentrate therapy: of 1,268 severe haemophilia A patients (< 2 iu/dl), 59% were

seropositive, whereas of 270 mild haemophiliacs (> 10 iu/dl), only 9% were positive. There was also a relationship with the type of material used, as shown in the Table 1.

There also appeared to be a relationship with age, the peak occurrence of antibodies being in the 15-30 group.

TABLE 1

Material	% of Haem. A patients seropositive
NHS Factor VIII Cryoprecipitate only	1.2
NHS Factor VIII concentrates only	10
Commercial Factor VIII concentrates only	45
NHS + commercial	63

A major question was whether any seronegative patients had seroconverted after treatment with heated concentrates, which were introduced in January 1985. Dr. Forbes said that three patients had in fact recently seroconverted, but it could not be excluded that this was the result of a long incubation period of the virus following the use of unheated concentrates. The mean incubation period before seroconversion was 84 days, with a range of 21-280 days. Dr. Schild commented that this might be attributed to the presence of non-infectious virus in the Factor VIII preparations which might be capable of stimulating antibodies when administered in repeated doses.

In discussion, Dr. Tedder confirmed that, in a group of patients studied from 1978 to 1984, seropositivity rose rapidly from 33% in 1980 to 64% in 1982 in patients on commercial concentrates, whereas patients on NHS concentrates remained seronegative during this time, rising to a 1% inci-

dence in 1983 and 11% in 1984.

Dr. Schild asked if anyone knew the reasons for the low incidence of clinical AIDS (around 1%) in seropositive haemophiliacs. Drs. Craske and Forbes said that the true incidence was probably much higher, due to a tendency to suppress reporting. Dr. Tedder said that the type and titre of antibodies occurring in haemophiliacs were indicative of genuine infection, rather than passive acquired immunity.

In response to a question, Dr. Tedder said there appeared to be no evidence of a risk associated with transmission of HTLV I by blood products.

Dr. Craske described the situation regarding transmission of hepatitis by Factor VIII and Factor IX concentrates. During 1980-1984, the overall incidence was about 2%, about 30% of cases being hepatitis B and the remainder non-A non-B. The overall effect of introducing screening for hepatitis B had been to delay its appearance in the haemophilic population rather than reduce its incidence. No viruses for NANB have yet been isolated, although the epidemiology suggests at least two causative agents. There is increasing concern that results in chimpanzees may be a misleading guide to behaviour of products in man. A prospective study in 'virgin' haemophiliacs had demonstrated 100% infectivity rate with NANB, and so a clinical trial with a control group would be unethical. Recent studies with a variety of heat-treated products had given somewhat discouraging results:

Travenol	-	84% of 17 patients developed NANB hepatitis
Armour	-	5 of 7 patients developed NANB hepatitis
Alpha	-	Although some batches appeared to be free of infectivity, one batch was now associated with NANB in two patients.
Immuno	-	NANB occurred in all 4 patients given the product

BPL, Elstree - No cases of NANB hepatitis so far in patients given the new '8Y' material.

Dr. Mortimer described the various ELISA tests currently available for the detection of antibodies to LAV/HTLV-III, and the results of PHLS studies on their relative sensitivities and field performance in the screening of blood products. He divided the solid phase assays into three main groups, as shown in the following Table:

TABLE 2

Type	Solid phase	Sequential additions
I	antigen	(1) specimen (2) anti human immunoglobulin-enzyme conjugate (3) enzyme substrate (direct binding assay)
II	antigen	(1) specimen (2) anti LAV/HTLV-III enzyme conjugate (3) enzyme substrate (competitive assay)
III	anti-human immunoglobulin (γ or μ chain)	(1) specimen (2) antigen (3) anti-LAV/ HTLV-III-enzyme (4) enzyme substrate

Weak reactions in ELISA could be attributed to:

1. Low titre antibody - found at seroconversion and in terminal stages of some cases of AIDS;
2. technical failure in performing the assay;
3. insensitive assay (but most commercial tests are sensitive enough for

diagnostic work);

4. non-specific reactions.

Eighteen sera were tested in eight type I, three type II and one type III ELISAs and by immunoblot assay. Four were gp41, p24 positive: 11 were p24 positive: 3 were negative, i.e. lacked gp41 and p24. A type II assay based on a monoclonal antibody was the most sensitive of the 18 tested. Then, ranked in decreasing order of sensitivity, the other assays: Dupont; Compria; Pasteur and Wellcome; Abbott; Behring; Ortho/Litton; Labsystems; Organon; EN1. All except the last three were at least as sensitive as the immunoblot.

Based on titration of a positive serum, the ranking was: Abbott; Ortho; Behring; Wellcome; Dupont; Pasteur; EN1; Organon; Labsystems.

In a small group of commercial immunoglobulin preparations examined by ELISA, four (from an Italian manufacturer) were positive, 19 batches of normal immunoglobulin, one of hyperimmune globulin and one of antihepatitis B immunoglobulin (from BPL, Elstree) were negative. Varicella-zoster immunoglobulin (GZ32A and GZ32B from BPL) were positive in the ELAVIA (Pasteur) and weakly positive in the COMPRIA assay. However, it was emphasized that the various ELISA tests were developed primarily for the screening of blood donors and not for the detection of antibodies in blood products.

Dr. Gunson reviewed the national experience of testing for LAV/HTLV-III antibodies in the NBTS. All blood donations collected from 14 October 1985 onwards in England and Wales were screened for antibodies to LAV/HTLV-III. In 21 test centres, 16 used Wellcozyme tests and five used the Organon kit.

Dr. Gunson was concerned about the apparent lack of standardisation of kits and, particularly, about the low sensitivity of recent batches from

Organon. Organon gave a consistently higher rate of repeat positives than did Wellcome. The low positive reference serum provided by the PHLS gave results around the cut-off value in both tests but Organon frequently failed to record it as positive. Accordingly, Dr. Gunson's laboratories used a cut-off value 20% below that defined by Organon to record equivocal results.

Blood donations which show repeatably positive/equivocal results which cannot be confirmed are not used but the donors' records are flagged and repeat tests are performed on the next donation. From over 600,000 blood donations, 13 were confirmed positive - an incidence (1:46,000) much lower than that reported from blood transfusion centres of other countries.

In discussion of Dr. Gunson's paper, Dr. Thomas commented on the low incidence of LAV/HTLV-III positive sera in the UK blood donations. Most other countries had reported an incidence of 1:1000 to 1:5000 confirmed positives in their blood donors. He was concerned that the screening tests used in the UK might be less sensitive than those used in other countries - a possibility that Drs. Mortimer, Tedder and Craske considered unlikely. Those members of the meeting who were involved with the screening of blood donations were of the opinion that the low incidence of seropositive individuals in the UK was a reflection of the efficient removal of high risk donors from the transfusion panels and the provision of alternative centres for screening, confidentially, people in high-risk groups, rather than any lack of sensitivity in the test. Other factors are a lower prevalence of LAV/HTLV III in the population and higher false positivity rates in other countries.

It was agreed that deterioration of the antigen-coated test wells of the ELISA kits may occur on storage and this suggested a need for study of

the stability of kits. Wellcome produced kits in small batches and considerable batch-to-batch variation was observed at times. Monitoring of batches was required and regular in-house assessment of the performance of all kits was recommended. The sensitivity of Organon kits had declined in recent months and it was suggested that this deterioration should be brought formally to the notice of the manufacturers. Dr. Thomas pointed out that no requirement existed in the UK for manufacturers to submit their diagnostic kits for routine testing.

It was agreed that manufacturers of blood products should be asked to provide the Licensing Authority with details of the ELISAs used in their screening tests.

Dr. Thorpe outlined the principles of immunoblotting and the procedure adapted by NIBSC for detecting antibodies to LAV/HTLV-III antigens. Lysed LAV/HTLV-III-infected cells were used as antigens which, after separation on PAGE, were transferred to nitrocellulose and treated with antiserum. Antigen-antibody complexes were detected by reaction with an I^{125} labelled monoclonal antibody directed against part of the γ chain of human IgG. High specificity and sensitivity was achieved; no reaction with host (H9) cells was observed - a characteristic Dr. Thorpe attributed to the specificity of the labelled antibody. The dominant antigens in all sera tested so far were p24 and p55; gp 110/120 was detected occasionally and patterns varied according to the sera tested. Dr. Tedder suggested that sensitivity of the immunoblot system should refer to dilution of the test serum in normal serum not to the final dilution or the test serum in the blocking medium.

In general discussion, the following points were raised:

a) A continuing need exists for close monitoring of recipients of blood products, with particular reference to seroconversion for HTLV III and evidence for liver damage from viral hepatitis. While it was recognised that the haemophilic population was already being carefully followed, patients receiving immunoglobulins and other blood products, including platelets and leucocytes, should also be monitored for possible long-term adverse effects.

b) The apparent recent decline in performance of the Organon diagnostic test kits should be drawn to the attention of the manufacturer, and also to the Regional Blood Transfusion Centres. Further consideration needs to be given to the reliability of sequential batches of commercial kits.

c) Manufacturers have been asked by the Committee on Safety of Medicines to demonstrate that their plasma fractionation procedures remove or inactivate viruses and, in particular, HTLV III virus. It was agreed that standard procedures should be established for testing for viral removal or inactivation, and that these procedures should be adopted by the UK manufacturers.

d) It was agreed that there were as yet insufficient grounds for routine screening of blood donors for HTLV I.

e) Blood products manufactured in the UK that were found to contain anti-HTLV III antibodies are not being released for distribution. NIBSC would not recommend the release of commercial blood products that were found to be positive for anti-HTLV III, either by ELISA tests or immunoblotting. Currently, no batch of product is distributed if it is determined subsequently that a positive donor (either HTLV III or hepatitis B) had contributed to the plasma pool.

f) There was general agreement that a closer scientific liaison should be established between the Blood Products Laboratory, Elstree, the Protein Fractionation Centre, Edinburgh, and NIBSC.

RECOMMENDATIONS

1. A working group should be established between the Blood Products Laboratory, Elstree, the Plasma Fractionation Centre, Edinburgh, and NIBSC, which would meet periodically and provide a forum for the exchange of technical and scientific information pertaining to the safety of blood and blood products, especially in relation to virus contamination and the evaluation of the capacity of manufacturing procedures to inactivate or eliminate viruses.
2. Further attention needs to be given to the reliability, stability and sensitivity of the diagnostic test kits currently used for screening blood donors, particularly in view of the evidence for batch-to-batch variation in some test kits.
3. The meeting noted and endorsed the recent recommendation of the Committee on Safety of Medicines that all blood products found to be positive for anti-HTLV III antibodies, either by screening procedures or immunoblotting, should not be released for clinical use.
4. The Licensing Authority should ask manufacturers of blood products to provide them with full details of the screening procedures used on blood donors, including the nature of the tests employed.