C.W.

In recent years, the management of haemophilia has been complicated by a number of factors, not least of which is the risk of contamination with the AIDS and hepatitis viruses. A group of scientists and physicians have gathered here in Lisbon to discuss some of the problem areas and the way in which modern medicine is attempting to overcome them. The Congress was organized by the University of Milan under the Chairmanship of Professor Mannucci.

Prof Mannucci

The world of haemophilia has been surrounded by lights and shadows in the last few years. The shadows were particularly the epidemic of AIDS, due to the infection of haemophiliacs through the blood derivitives in factor concentrate with the human immunodeficiency viruses. I think that the monster of AIDS has led to lights, I mean to an improvement in haemophilia care that has been spectacular in the last few years.

PROF. BLOOM

There are a great number of problems. I mean, the main one at the moment is obviously HIV, and this is a problem, not only for the patients and their families, it is also a problem for the physicians as well, and it is going to get more and more so and it really is a tragedy.

CW

The problem of HIV infection in the haemophiliacs aroused a great deal of discussion. Dr Sultan presented data on the incidence of infection in haemophilia in France.

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116

DR Sultan

We could get information from about 2,500 haemophilic patients and from this group of patients, 1000 are HIV positive, so the total incidence is about 42% of the French population of haemophilic patients.

PROF BLOOM

In the UK, about 35% or so of treated haemophiliacs are not HIV positive and of those, a certain proportion are progressing to AIDS or AIDS related complex each year.

C.B.W.

The other major virus infection in the haemophiliacs, is hepatitis. Professor Losowsky from Leeds presented interesting data on this problem.

Professor Losowsky

Hepatitis. viral hepatitis may arise due to a number of different viruses and some of these are well worked out and others are not. The main problem at the moment, is what is termed non-A, non-B hepatitis, which is a confession of ignorance. It indicates that we cannot demonstrate the organism in any way. We are certain now that there is more than one organism, certainly at least three. These have different effects, but we cannot separate them. The only tests we have at the moment are very non-specific tests, looking at liver function tests, but nevertheless from that evidence it is quite clear that non-A, non-B hepatitis is common after the infusion of blood and blood products, that it frequently becomes chronic and that the chronic ones frequently

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go on to severe and life threatening liver disease.

CB Wood

But did this include hepatocellular carcinoma?

Prof Losowsky

Yes, chronic active hepatitis, cirrhosis and hepatocellular carcinoma.

CB Wood

I asked Professor Losowsky, was this a big problem in haemophiliacs?

Prof Losowsky

It is a major problem with the haemophiliacs.

CB Wood

I then asked him what proportion of patients were infected with the hepatitis virus?

Prof Losowsky

There is good information now which shows that the proportion is high, in some figures, anywhere between 30 and 60%, so that it certainly is a major problem in those patients who have been transfused with the older preparations.

CB Wood

So a great deal of effort is now being concentrated on reducing

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the risk of transmitting viruses to the haemophiliac population. I asked some of the delegates, what exactly was being done in this respect?

DR. KERNOFF
The earliest methods of dry heating were probably not much good. The newer methods are probably very good. Similarly, there are different solvent detergent methods. There is a lot more to it than merely time and temperature. There is the question of stabilizers in concentrates, the question of protein content, there is the question of donor source and so on.

DR. LUDLAM.
In factor VIII concentrates available at the moment, I think the risk of them transmitting HIV is very very small indeed. I think it is foolish to say that all the factor VIII's that are currently available are entirely safe, but I think the chances of transmission must be extremely small.

DR. KERNOFF
Undoubtedly, it seems that hepatitis viruses are more difficult to get rid of and the inactivation strategies have to be more stringent and there has been more of a problem getting rid of hepatitis and indeed proving one has got rid of hepatitis.

PROF LOSOWSKY
The major thrust is in terms of making safer concentrates and there is very good evidence that safer concentrates can be made and there is accumulating clinical evidence that they really are safe, it depends to what temperature, it depends for how long you heat and it depends whether you heat dry or wet and there is now

a certain amount of information which indicates that you can select the right situation to minimize the transfer of these non-A, non-B viruses.

CB Wood

There is no doubt that heat treatment can inactivate viruses, but dead virus or virus particles remain in the factor 8 concentrates after heat treatment. But does the presence of residual virus particles have any harmful effect on the patient?

DR LEVINE

There have been multiple studies done by Dr Callow and by Dr Foucci and by others. There have been some studies from the Institute Pasteur as well, that have suggested that virus, not just live virus, but dead virus or pieces of virus are capable of reawakening or up-regulating the production of HIV from resting cell culture and that is worrisome.

PROF MANNUCCI

I think there is some indirect evidence, mainly stemming from in vitro studies, that by giving pure and sterile concentrates, you avoid the reactivation of the lymphocytes where the virus is probably dormant in many of the patients, and that should hopefully stop the progression, the shedding of the virus out of the cells, infection of additional cells and the passing of an asymptomatic state towards full blown AIDS.

DR LEVINE

There are numerous experiments in the laboratory in which one can culture HIV virus stabley in either lymphocytes or monocytes and having done that, and having a resting culture system of infected

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cells, if one exposes the culture system to certain proteins such as albumin, nothing much happens. The development of virus or the proliferation of virus does not increase. If however, you expose these cells to foreign antigenic proteins or to viruses, either dead pieces of virus or living viruses, including hepatitis B, cytomegalovirus, herpes viruses and a whole family of others, then the production of virus from these resting cultures remarkably increases by many logs, so that based on that, it would be a bad idea to be constantly bombarding the relatively quiet T4 cells containing this virus with all these foreign proteins dead and alive.

CB Wood

Recent developments in the modern manufacture of highly purified factor VIII concentrates, involves the use of monoclonal antibody affinity chromatography. One process uses monoclonal antibodies to Von Willebrand factor which capture the factor VIII complex. Pure factor VIII is then removed from the Von Willebrand factor by altering calcium concentration of the eluting fluid. Further purification steps produce a factor VIII with a specific activity in excess of 3,000 units/mg of protein. In addition, the monoclonal antibody purification steps, together with the other column purification procedures, physically remove virus. this may be illustrated by experiments which show the removal of five to six logs of model viruses and HIV during the chromatography stages.

C.B.W

A variety of virus inactivation steps are used to back-up the

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physical removal processes. For example, experiments have shown that solvent detergent processes inactivate 4.2 logs of virus, dry heat treatment at 60°C for 30 hours ~~removes~~ inactivates 7.8 logs, whereas pasturisation at 60°C ~~removes~~ inactivates 11 logs of virus.

DR MADHOK
Well, from the data that I have seen, the monoclonal purified preparation reduces the log dose of the virus to that that has been suggested by the CDC, which is what, six logs or greater than a five log reduction, and they do achieve that.

CB Wood
Numerous publications have suggested that multiply treated haemophiliacs have an abnormal immune response. Dr Madhok presented additional work on this important subject.

Dr Madhok
Well, in the in vivo tests, we have an indication that the haemophiliacs who were HIV antibody negative had an impaired ability to manage a response to a new antigen and that this probably correlated with the amount of clotting factor concentrate that they had had in the previous six years.

CB Wood
I asked Dr Madhok, what were the results of his studies?

Dr Madhok
We therefore took the negative approach to it and looked at the effects of factor concentrates on the ability of normal

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lymphocytes to proliferate in vitro, and what we found was that intermediate purity factor VIII concentrate impaired the ability of normal lymphocytes to proliferate.

CB Wood
You have also looked at one of the monoclonal antibody purified factor 8 concentrates, what response did you get with that?

DR MADHOK
With the highly purified, first of all the reason why we looked at the highly purified preparation was that we had to determine whether it was just a coagulant ~~pro??~~ Protein having an effect on the lymphocytes and with the availability of the highly purified monoclonal preparation that was an ideal situation of looking at it where we could distinguish the effects of all the other antigens or potential antigens in intermediate purity factor VIII concentrate. To answer your question now. The monoclonal purified factor VIII concentrate had little or no effect on the tests that we looked at, at the doses that would be achieved after an infusion.

DR LEVINE
Starting approximately three years ago, we enrolled a small group of patients who had previously been infected with the HIV virus in a study where we treated them only with the monoclonally purified material. These patients were compared to the rest of our haemophilia population who were receiving intermediate purity materials. Over this period of time, what we have seen is that the T4 counts of these patients as an entire group, all the patients we follow in the clinic have drifted down somewhat as

9

111

has been the experience elsewhere. The patients on the monoclonal material appear to have drifted a little less than the rest of the group. We have also been able to show that we can restore cutaneous hypersensitivity reactions in these patients. The patients getting only the purified material are much more likely to redevelop their ability to respond to antigens implanted into their skin, than other patients who continue to receive the intermediate purity material.

CB Wood

I then asked Dr Levine to tell us the results of the randomised control trial that he had just initiated, of intermediate purity products versus the monoclonal purified factor VIII.

Dr Levine

We have so far entered a little over 60 patients in the trial, approximately 30 randomised to the monoclonally purified and 30 to the intermediate purity. There is very little data since the study has only just begun, but interestingly with regard to the skin test results, even at three months into the study, you can see restoration of normal skin test responses in those treated with the ultra pure material and no such restoration in thos receiving the less pure materials, so it is hopeful, but much too soon to be certain about.

CB Wood

And are you at a stage where you can postulate why that might be?

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Dr Levine

Yes. We feel that since the ultra pure materials are, more than 99% are clotting factor concentrate and less than 1% extraneous proteins, whereas the intermediate purity products are more than 99% extraneous proteins and less than 1% pure, that that explains the difference, and that the large amount of foreign protein that is constantly being received by people in the less pure products has long been known to be associated with immune abnormalities. We showed that as an independent variable to HIV some years ago in explaining the absence of skin reactivity.

DR PARAPIA.

Yes, we are presenting our initial experience in the United Kingdom using the monoclonal purified factor ~~8~~ VIII and this is particularly in relevance to a patient that we have had who has reacted to conventionally produced intermediate purity factor 8 and we are also giving some immunological data on the first naive patient ~~???~~ ~~patient ??~~ in the United Kingdom.

CB Wood

What results have you found with your patients?

DR PARAPIA.

Well, the patient representing reacted to all the conventionally prepared intermediated purity factor ~~8~~ VIII and we found that this particular patient has now received 100,000 units of factor ~~8~~ VIII without reacting.

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CB Wood

I asked Dr Parapia what he thought was the reason for this difference?

Dr Parapia

Well, we postulated that it is the foreign protein that is causing the reaction in this patient, particularly the immunoglobulin components which are present in considerable amounts in the conventionally intermediate purity factor VIII

CB Wood

So we have heard that repeated infusions of impure factor VIII concentrates may result in an impaired immune response. How then can we overcome this potential problem?

PROF BLOOM

Basically, people, haemophiliacs are deficient in factor VIII, they are not deficient as a general rule in other proteins, and therefore, I think the longterm aim must be just to replace factor VIII, but how long this is going to take, remains to be seen. I think it is essential that we go on studying and using and developing the highly purified materials. I think it would be a mistake if everyone just stopped at a lowest level of purity, even if the materials seem to be reasonably effective and safe. I think the ultimate aim, as medicine progresses is to produce pure factor 8 for the treatment of haemophilia.

DR. PARAPIA

Well, I am a great believer that you replace the substance that is deficient in the haemophilic and I have yet to be convinced

12

that the impurities that exist in the conventional intermediate factor VIII concentrates are of any benefit to the haemophiliac.

CB Wood

I asked Dr Parapia if there were any additional benefits to the monoclonal antibody purified factor VIII?

Dr Parapia

In terms of patients and doctors, it is easier to handle, it is in smaller amounts, it desolves instantaneously, it is easier for home administration, it needs less storage space and overall we have found that it is more acceptable to the patient.

CB Wood

There has been some concern about the possible development of inhibitors to highly purified factor VIII concentrates, but how much of a problem is this?

DR. LEVINE.

We have seen in the virgin studies we are doing, three out of thirty three for a total instance of 9% which is below the expected incidence of approximately 15%.

DR KERNOFF.

No problems have shown up. In all these studies of high purity products, whether they be recombinant or monoclonal purified, that of course is something people are looking at, but the evidence now is that the rate of new inhibitor development is really no different between these products and conventional ones.

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I am not too bothered about that at the moment.

CB Wood

So monoclonal antibody purified factor VIII concentrates are now in wide spread clinical use, but should they be used in all patients or a selected group of haemophiliacs?

PROF. MANNUCCI.

I am prepared to use these concentrates at lease in HIV positive patients even though the 100% evidence is not available because I think that after having infected these patients, of course without knowing, I don't think we can be blamed, but I think we cannot really spend too much time in discussing the day when the theoretical evidence is solid enough. I think that in some instances like this, we have ethical, moral and also scientific reasons to move to the use of these pure concentrates.

DR. PARAPIA

If patients are reacting to the conventionally intermediate purified factor VIII, then they should certainly be given the opportunity to use the monoclonal purified factor VIII.

CB Wood

What then were some of the comments made about the monoclonal antibody purified factor 8?

DR. SULTAN

Well, I think it is a big progress because patients with haemophilia A will receive just what they need, that means factor VIII and not the other proteins of the plasma.

PROF NILSSON.

I think the most important point is to use safe products. This is the very most important product and I feel institutions would prefer to use more purified concentrates, at least at our centre.

DR LEVINE

Based on that reasoning from the laboratory, it seems to me, and given our state of imperfect knowledge right now, that at least there is good laboratory rationale to use the purer products, and then of course there is the intuitive thought that if we didn't have all this history behind us and one offered us either a product that was 99% pure, a product that was less than 1% pure, that it would seem sensible to use the product that was more than 99% pure.