UNITED STATES OF AMERICA

DEPARTMENT OF HEALTH AND HUMAN SERVICES

PUBLIC HEALTH SERVICE

FOOD AND DRUG ADMINISTRATION

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TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

ADVISORY COMMITTEE

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MEETING

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Friday, June 2, 2000

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The Advisory Committee met at 8:30 a.m. in the Ballroom, Holiday Inn-Gaithersburg, Two Montgomery Village Avenue, Gaithersburg, Maryland, Dr. Paul W. Brown, Chairman, presiding.

MEMBERS PRESENT:

PAUL W. BROWN, M.D., Chairman

ERMIAS D. BELAY, M.D.

DAVID C. BOLTON, Ph.D.

DEAN O. CLIVER, Ph.D.

BRUCE M. EWENSTEIN, M.D., Ph.D.

LISA A. FERGUSON, D.V.M.

PETER G. LURIE, M.D.

MEMBERS PRESENT (Continued):

J. JEFFREY McCULLOUGH, M.D.

PEDRO PICCARDO, M.D.

SHIRLEY JEAN WALKER

WILLIAM FREAS, Ph.D., Executive Secretary

TEMPORARY VOTING MEMBERS PRESENT:

SUSAN F. LEITMAN, M.D.

LAWRENCE B. SCHONBERGER, M.D.

F. BLAINE HOLLINGER, M.D.

PAUL R. McCURDY, M.D.

EDMOND C. TRAMONT, M.D.

GUESTS PRESENT:

LOUIS KATZ, M.D.

ROBERT G. ROHWER

MERLIN SAYERS, M.D., Ph.D.

ROBERT WILL, M.D.

INVITED SPEAKER:

FABIO MONTRASIO, Ph.D.

ALSO PRESENT:

DAVID ASHER, M.D.

CHARLES DURFOR, Ph.D.

JAY EPSTEIN, M.D.

JONG-HOON LEE, M.D.

JARO VOSTAL, M.D., Ph.D.

C-O-N-T-E-N-T-S

P-R-O-C-E-E-D-I-N-G-S

(8:33 a.m.)

CHAIRMAN BROWN: Good morning. This morning we are asked to consider the issue of leukoreduction and whether or not, one, it's a good thing and, two, if it is a good thing, what is it good for?

And then finally, we're going to have just an update on the matter of dura mater, which came before the committee on two occasions in the past couple of year.

If the speakers adhere to their time as well as yesterday's speakers did, I am optimistic that we can adjourn this meeting before lunch, and if the committee members have no objection, I will, therefore, not interrupt the morning session for lunch and then come back, but we'll see if we can't get everything done before lunch.

The first speaker today, as yesterday, will be Dave Asher, and he'll provide us a little background and our charge, and the questions which today will be less complicated than yesterday.

Dr. Asher.

DR. FREAS: Dr. Brown, before Dr. Asher begins I just need to make a short announcement that

the conflict of interest statement read into the record yesterday still pertains to all of the topics being discussed today.

Also, I would like to remind everybody that the next tentatively scheduled meeting of this committee will be July 27th, and more information will be posted on the FDA Advisory Committee hand line -- excuse me -- on the Advisory Committee information line, and that is on the public agenda, the next to the last page.

And also a plea to all speakers both yesterday and today. Please provide us copies of your slides. They are to be put up on your Web page, and the address of the Web page is also on the next to the last page of the public agenda.

Thank you.

DR. ASHER: Good morning. I'll try and keep it very short today. Yesterday I unfortunately encroached.

Is it on?

Okay. Yesterday I unfortunately encroached on the territory of two of our speakers. I'll keep it very short today and attempt not to do that.

Yesterday we asked the TSE Advisory

Committee to address the management of risk in attempting to assure a safe source of blood. We asked you to consider deferral policies that might reduce the theoretical possibility that donor blood was contaminated with the agent of new variant Creutzfeldt-Jakob disease.

Today we are asking you to consider another means to reduce risk, and that is process, a process that offers some potential for reducing the amount of TSE infectivity in blood, and that is leukofiltration.

In 1998, based on a risk-benefit analysis, the FDA's Blood Products Advisory Committee, the BPAC, advised the FDA that leukoreduction of blood should be recommended to reduce non-hemolytic transfusion reactions, transmission of cytomegalovirus, and other reasons that will be reviewed for you shortly.

The BPAC intentionally did not address the possible beneficial effects of leukofiltration to reduce the TSE infectivity that at least theoretically might be present in the blood of some donors, and they left that to you to do today.

In the experimental TSEs of rodents discussed yesterday, a large fraction, perhaps the major part of the small amount of total infectivity in

blood is found in the buffy coat, the fraction containing nucleated cells, but a substantial part has also been detected in plasma.

Intuitively you would think that even if you can't get it all, that removing a substantial part of the TSE infectivity from blood by filtration would be a useful thing to do, especially since the procedure has already been recommended for other reasons, but just how useful is leukofiltration likely to be in reducing the theoretical risk of transmitting CJD to recipients of blood components or plasma derivatives? Is it useful enough for the FDA to recommend as an additional reason to encourage implementation of universal leukoreduction? Is it useful enough to relax or even replace the blood donor deferral policies that you agonized over yesterday?

And several European countries have already done that or are apparently considering it.

Next slide, please.

We ask the committee today to discuss the scientific evidence suggesting that leukoreduction might be expected to reduce the theoretical risk of transmitting CJD and new variant CJD by human blood and blood components and plasma derivatives.

We ask you to consider whether the

reduction in risk of transmitting CJD and new variant CJD is likely to be substantial enough to have practical value and consequently whether universal leukoreduction of blood and blood components should be recommended by the FDA for that purpose.

Next, please.

To assist the committee in these deliberations, we've invited several speakers to present information on four topics: the background, recent recommendations, and prospects for implementation of leukoreduction; techniques, theoretical applications of leukoreduction to remove TSE agents from blood; possible role of leukocytes in experimental pathogenesis of TSEs in rodents and implications for human blood, and TSE infectivity in blood of experimentally infected rodents and implications for CJD and new variant CJD.

Next slide, please.

The questions to be answered today are these:

One, can leukoreduction be expected to reduce significantly the infectivity theoretically present in the blood of donors during the course of CJD and new variant CJD? If so, for which components?

Next overhead, please.

If the answer to question number one is yes, should leukoreduction be recommended by the FDA for blood components for transfusion? Should leukoreduction be recommended for plasma for further manufacture?

I look forward to the presentations and your discussions today.

Thank you.

CHAIRMAN BROWN: Thank you, Dr. Asher.

We'll now proceed immediately to Mr. Lee's presentation, which is a background overview of recent recommendation prospects for the implementation of leukoreduction.

Dr. Lee.

DR. LEE: Thank you, Dr. Brown.

This morning I'll try to briefly go over with you the introduction, background, recent recommendations and future prospects for implementation of the routine use of leukocyte reduction in manufacturing blood components.

This is a subject that has been discussed in detail in the recent past, and here we are now discussing whether or not this has additive value in efficacy against the potential reduction of -- specifically for the reduction of theoretical risk of

transmitting new variant CJD.

Unlike your typical pharmaceutical product, the manufacturing of blood is different in many ways, including the fact that much of the safety measures that you build into manufacturing really occurs at the identification of the source material, and obviously in this case that happens to be the donor. So all of the testing that goes in, all of the donor screening questions that we discuss, all of those are measures to identify the right donor or, in a broader sense, identifying the right raw source material from which to manufacture.

And beyond that, there is little that we can do to further assure the safety of the blood product during manufacturing. When we collect a unit of blood, we do so from the safest donor possible. Then after that is a matter of making sure that you maintain the efficacy that's contained in the blood and you process it and store it in a way to preserve as best you can what you have.

And measures to further reduce the adverse effects that can be associated with a unit of blood are few in number, and one of them happens to be leukocyte reduction. That is, blood transfusion has progressed from initially the collection and

transfusion of whole blood to component therapy, and component therapy is obviously tied in with the idea of product purity.

In other words, you isolate the cellular product that carries the efficacy, and you remove all other blood components of that blood because those might be associated with adverse effects rather than efficacy.

So in red cells and platelets, we provide component therapy, and we hope to increase the purity and safety of the product by removing the contaminant residual white blood cells in them.

Whether or not this needs to be done for every transfusion recipient has been debated for a long time, and much of the debate, the full blown debate began with the FDA workshop in March of 1995 where, at that workshop, the leukocyte reduced blood components has been recognized as a special class of blood components, which is in a sense more pure than the leukocyte replete counterparts.

However, whether or not this should be provided for every transfusion recipient, that's a question that is ongoing, and that question was discussed, but was settled in the following way.

It was decided that the indications for

using leukocyte reduced blood or a unit of blood that is as pure as you can make it under current available technology in terms of residual light cell contaminant content, that decision was left up as medical practice. In other words, the physicians will recognize which patients will benefit from this class of product that has been made more pure than its counterpart. And indications were not really considered under the jurisdiction of the FDA.

As an outcome of the workshop, the FDA issued a blood memorandum in 1996, which laid out recommendations for manufacturing leukocyte reduced blood and not recommendations on how to use leukocyte reduced blood as that was left up to the prescribing physicians consistent with the discussion at the '95 workshop.

So in the 1996 memorandum, FDA recommended using a threshold level of 5.0 times ten to the sixty residual leukocytes per blood unit as the threshold level that the leukocyte reduction process must meet. It should be that number or fewer for every unit.

And FDA also recommended that the efficacy portion of the unit that is being leukocyte reduced be maintained at a level of at least 85 percent of the original therapeutic cellular content or better.

So those were recommended at two product specifications, if you will, efficacy at 85 percent recovery or better, and also purification, if you will, to a level below 5.0 times ten to the sixth residual cells per unit or fewer.

In addition, these product specifications were recommended to be achieved through a rigorous application of good manufacturing practice principles; that they be standardized in terms of operating procedures. They are to be manufactured by well trained personnel, and all of the equipment that goes into the leukocyte production be validated and controlled, and end product of the leukocyte reduced products be subject to quality control testing to assure that all of your controlled measures are actually working as intended.

And in the way or recommending the quality control testing measures, FDA recommended testing four units per month or one percent of the manufactured units per month, whichever is greater, towards making sure that all of your controlled processes are working properly.

And if you were to discover one red cell unit that does not meet or any blood unit that is leukocyte reduced that does not meet the product

specifications, then you are to investigate further, correct, and proceed, and beyond that, it did not make any further concrete recommendations as to how to investigate a potential failure as detected by a unit that fails quality controlled testing.

The 1996 memorandum also discussed the registration process. That is, the leukocyte reduction process is considered product manufacturing even for a blood center that does not originally collect, but obtains a unit collected at another facility, but then proceeds to leukocyte reduce at that facility. Then that step is considered product manufacturing, and therefore, that facility must register with the FDA within five days of initiating that activity, and if that facility were to be interested in distributing this product in interstate commerce, then that facility must apply for and obtain licensure to distribute products that are labeled as leukocyte reduced.

The term "leukocyte reduced" has been discussed, and that name was specifically selected to the exclusion of other similar terms, such as leukocyte removed or leukocyte poor, as that specific term now means very specific things in terms of product specifications, as I mentioned earlier.

At this workshop, it was recognized that there are many indications for which physicians may prescribe leukocyte reduced blood, and one of them is the reduction in the incidence of febrile non-hemolytic transfusion reaction, and this is an indication that was most widely accepted as being efficacious in terms of leukocyte reduction.

And, in fact, this became the only indication which became accepted by the FDA through FDA's review and approval of a product insert that accompanies the blood product. In the case of blood products, the product insert happens to be the circular of information for the use of human blood for transfusion, and this is a product insert that's jointly written by the major members of the blood industry, including the AAB, American Association of Blood Banks, the American Red Cross, and America's Blood Centers.

Beyond febrile non-hemolytic transfusion reaction; there were plenty of other indications for which physicians may prescribe leukocyte reduced blood, and that includes the reduction in the incidence of transmitting CMD infection; the reduction, potential reduction in the incidence of recipient allo-immunization against HLA antigens which

may complicate future transfusion therapy; the potential for transfusion related immunomodulation, and that might be better termed transfusion related immune suppression, as it is known that transfusion may have an immunosuppressive effect on the recipient, and it was first recognized that in the case of renal transplant patients that immunosuppressive effect is actually beneficial towards preserving the transplanted graft.

However, in the typical patient, that immunosuppressive effect is expected to have an adverse effect in terms of increasing the potential for bacterial infections post surgical procedures or, in the case of a surgical oncology patients, perhaps an increase in the incidence of tumor recurrence because of the immunosuppressive effect.

So these three, CMV indication, HLA indication, and the potential to reduce the immunosuppressive effect of transfusion, were three other major considerations in addition to the well accepted indication of febrile non-hemolytic transfusion reaction.

There are others beyond these three, such as the reduction in the potential for reperfusion injury post cardiopulmonary bypass procedures; the

potential to reduce bacterial contamination; the potential to reduce the storage lesion, but these are all measures that, indications that are progressively weaker in comparison to the ones that are listed on this slide.

Nonetheless, the '95 workshop was useful in generating product criteria and process controls, and it resulted in a memorandum which specified those recommendations from the FDA, including the regulatory framework of registration and licensure.

Next overhead.

So that got us started, and after that workshop we now have two classes of products, those that are leukocyte reduced and those that are not, but although it was left up to the prescribing physician in terms of deciding which indication to pursue for a leukocyte reduced product, it became clear that this is highly effective against the reduction of CMV, and this question was brought up before the Blood Products Advisory Committee in September of 1997 towards the aim of achieving public consensus that the product insert of the circular of information for blood and blood components be allowed to state that leukoreduction is effective in reducing transfusion transmitted CMV.

And the questions that were brought to the BPAC in relation to CMV were three, as listed on this slide. First of all, is it effective against CMV, to which the BPAC voted eight to one in favor of yes.

And, secondly, if it is effective, then is it equivalent to a CMV seronegative unit in terms of its decreased potential to transmit CMV, and to this question the data were insufficient and the committee voted seven to one in favor of no.

A follow-up question to these were if it is effective, if leukocyte reduction is effective against CMV, then are all the different methods that are used to achieve leukocyte reduction, are they equivalent, provided that they meet the product specifications outlined earlier of five times ten to the sixth residual leukocytes per unit or fewer.

And, again, in this case there were insufficient data to make a clear statement, and the committee voted nine votes to none in favor of no.

However, it was clear that this could be the basis for revising the circular of information or the product insert for blood and blood products to state that CMV is effective in -- leukocyte reduction is effective in reducing CMV.

Next overhead.

As you might anticipate, this conversation about increasing the general acceptance in terms of product labeling to include CMV indication could be further extended to include HLA indication, the immunosuppressive effect indication, and so forth for every indication that leukocyte reduced blood may be provided, may be prescribed to a transfusion recipient.

Rather than going through each one of those indications in a public forum, the whole process was sort of accelerated. When the European countries began to leukocyte, routinely leukocyte reduce blood, for many reasons, one of the major reason being the potential to reduce transfusion transmitted CJD.

So the next question that was discussed in public was not a specific indication, but to include all of them and say: is the benefit/risk ratio associated with leukocyte reduction sufficiently great to justify the universal leukocyte reduction of all non-leukocyte transfusion blood components irrespective of the theoretical considerations for transfusion transmitted CJD?

In other words, there are plenty of indications for which leukocyte reduction has been used, and although transfusion transmitted CJD

indication triggered this meeting, that data for that is complex, and it appeared that transfusion transmitted CJD need not be considered to answer this question, and hence the phrasing of the question, as you see, on this slide.

And to this question, the committee voted in overwhelming favor of yes, 13 votes to none, with three abstentions. Both the consumer and the industry representatives agreed with the yes vote.

It should be pointed out thought that the voting members were reminded the cost/reimbursement considerations were not to be considered consistent with the charge to the FDA.

Next slide, please.

I'm reminded I only have one minute.

So this question was then further discussed in the December 1999 FDA workshop, and given the BPAC recommendations, the implementation -- another workshop was held at which the consensus emerged where if universal leukocyte reduction were to be implemented, then it should be implemented within two years of an FDA statement to allow time for blood centers to prepare for this major transition. The implementation plan were to be center specific and designed by the center, and while doing so, this is a

good opportunity update the previous 1996 memorandum to include updated recommendations on quality controlled testing to more rigorously assure that the leukocyte reduced blood is actually performing as you intended per product specifications, and was also recommended that the licensing procedure be streamlined so that there is less of an obstacle for centers interested in interstate commerce.

Next slide.

And at the end of that workshop, all of the discussion and all of the discussion that occurred prior to that led to this current FDA thinking. The advances in the understanding of the role of leukocytes is ongoing, and this results in a growing list of indications for using leukocyte reduced blood, and it may include its efficacy against reducing the variant CJD risk.

Consistent with these advances, FDA favors the routine use of leukocyte reduced blood components, but will continue to recognize both leukocyte reduced and non-leukocyte reduced components, the potential for physicians to continue to use non-leukocyte reduced blood.

However, the major obstacle and the only obstacle against this transition is reimbursement, and

this reimbursement issue has been discussed at the DHHS level with the Public Health Service Advisory Committee as the focal point so that the potential adverse impact, if there any because of reimbursement concerns, could be minimized by careful implementation of the transition to the routine use of leukocyte reduced blood.

So at the end of deliberations at this committee today, the outcome of those deliberations will, may very well hasten the process towards transition to using leukocyte reduced blood routinely or it may not, but regardless of the outcome, the FDA's position has been this, and it may be accelerated, but not reversed.

That's the current thinking that we are at at this point, and I'm out of time.

Thank you.

CHAIRMAN BROWN: Thank you, Dr. Lee.

(Applause.)

CHAIRMAN BROWN: The next presentation is by Dr. Vostal, who will present you some data on leukoreduction of blood as a result of experimental studies that he has and is in the process continuing to study.

Dr. Vostal.

DR. VOSTAL: Good morning. Thank you very much for the invitation to come present

I'm going to follow up on Dr. Lee's presentation. He gave you the indications for leukoreduction. So I'm going to cover some of the techniques involved in preparing leukoreduced products and touch on some of the theoretical applications to TSE, the removal of TSE agents in blood.

Next slide, please.

Well, leukoreduction is considered the process of reducing the total number of leukocytes in a transfusion component to less than 1,000 to the sixth. Now, this is the European standard, and the current U.S. standard is 5,000 to the sixth cells.

The major methods involved right now are filtration and apheresis, and I'm going to start by covering filtration.

Next slide, please.

Now, first we should consider some of the physical properties of human blood cells that allow us to differentially isolate or insolate different components by differential centrifugation and by filtration. Here are the different cell types. Here is the -- this column has the density of the different cell types, and you can see they go from the red cells

being the highest down to platelets being the lowest, and these are the cells that would be found in the buffy coat.

Now, these next three characteristics, size, deformability, and adhesiveness, are important in terms of selection by filtration. You can see that there's a range of size of the different cell types, and they also have range in the deformability, red cells being the most deformable, with decreased in deformability in the leukocytes and in the platelets.

And also important is the adhesiveness of the cells either to themselves or to some of the materials used in filtration. You can see the red cells are the least adhesive, with the white cells and platelets being more adhesive.

Next slide, please.

Now, let's look at some of the characteristics of the filters involved in leukoreductions. They're made of synthetic fibers of different kinds, like cellulose acetate, polyester and microfiberglass. These fibers are randomly oriented and compressed to a specific density so that there is a uniform, microporous, uniform size of the micropores in the filter.

The mechanism of how the cells are

retained by the filters involve barrier attention or more what could be considered a size exclusion of the cells which can't fit into the micropores; cell adherence of the cells to the material, the fiber material or to themselves; and cell-to-cell interaction, which is either the leukocytes to themselves or leukocytes to the platelets.

Next slide, please.

Now, let's go over the factors that affect filter efficiency. For red cells, the efficiency depends on a temperature at which the product is filtered. Red cells are highly deformable, and they stay deformable even at four degrees Celsius.

However, leukocytes, as they have a nucleus, have more trouble being deformable at four degrees. So they are more easily trapped by the filters. So it's very important to filter at least for some filters that the product is filtered at the proper temperature.

Flow rate, the rate at which the blood product goes through the filter, can affect the efficiency of the filter by either not allowing enough time for the cells to adhere to the filters or producing sheer forces that can force the leukocytes through it.

Leukocyte load is important in terms of platelet filtration. Some of the filters have a certain capacity, and if you exceed that, then leukocytes pass through the filter.

Some of the other products, other factors are protein count of the medium, whether if the product is still in plasma or if it's been diluted by a storage medium or the age of the cellular component in terms of how long it's been stored, and also important is the sickle cell trait of the donor. The red cells of these donors are less deformable and have a difficult time going through some of the filters.

Next slide, please.

Now, this is a typical scheme for a whole blood component isolation. Whole blood is collected into plastic bags. It's then given a soft spin to separate out the three main fractions. So here you have red cells, the buffy coat, and platelet rich plasma. These can then be expressed into another bag, and so we have packed red cells, and then you have platelet rich plasma.

This is then given a hard spin to pull down the platelets and produce plasma which can be frozen to fresh frozen plasma and then later on processed into liquid plasma and cryoprecipitate.

In terms of the white cell load, for whole blood there's approximately five times ten to the ninth cells present in a unit of whole blood. When you make the split into red cells and platelet rich plasma, they each have about ten to the ninth leukocytes present, and that gets carried off into the plates.

Next slide, please.

So the question is: when is the optimal time to leukoreduce your product?

It really depends on what kind of product you're interested in making. For example, you can make -- you can leukoreduce whole blood. However, these filters tend to retain a lot of platelets. So if your objective is to make platelets, it's probably better to leukoreduce later on when you have specific components, such as red cells, platelet rich plasma, or platelets.

Of interest is to note that plasma is not currently leukoreduced.

Next slide, please.

Now, the different cell types have different conditions for storage, and this is important in terms of timing of the leukoreduction and what kind of filter is used.

For red cells, storage is done. Red cells can be stored up to 42 days. The storage temperature is one to six degrees Celsius, and when you compare this to platelets, they have a short half-life of five days and they're stored at room temperature, and plasma can be stored up to one year, and it's frozen to minus 20.

I mentioned the leukocyte load. There's quite a bit in red cells and platelets. Plasma, because of the hard spin to produce plasma, there's less cells, approximately ten to the fourth cells are present in a unit of plasma, and this number we got from doing some of our own counting on plasma prepared by the standard methods.

What happens to leukocytes while they're being stored? Well, at four degrees, they're relatively dormant. So they're not producing any cytokines. This is not the case when it comes to platelets. They are at room temperature, and these leukocytes, given enough time, they can produce significant amounts of cytokines which can be associated with or produce transfusion related reactions.

And in terms of leukocytes and plasma, of course, they're frozen at minus 20. So what happens

to them? Well, it turns out that leukocytes initiate apoptosis/necrosis reactions, and they start to disintegrate within three to five days either at four degrees or at room temperature in platelets, and here, of course, they get lysed with a freeze-thaw cycle.

Next slide, please.

Here I wanted to show you some data that was recently published by Dr. Frabetti. He looked at the apoptosis and the number of leukocytes in red cells in terms of storage. Apoptosis here is expressed as a percent of the leukocytes that are undergoing apoptosis, and you can see that relatively rapidly these cells do go into apoptosis, and by five to seven days, a significant proportion of them are already disintegrated, and you can see that the cell numbers are already quickly decreasing with red cell storage.

Next slide, please.

Dr. Frabetti followed up his study with platelets. These are single donor platelet concentrates, and they are, again, looking at leukocyte apoptosis and number.

And here's storage at room temperature up to four days. Apoptosis is starting to take place after day two, and you can see that the leukocyte

count, the leukocyte number in the platelet product is also decreasing.

Next slide, please.

So it's important to be able to time your filtration to be able to access cells that have not broken down yet. So in terms of red cells, storage is -- when they're isolated from a donor you can store them up to eight hours at room temperature, and then they go into cold storage, and so pre-storage leukoreduction is considered to be in the first three days.

There is -- you can filter after they've been stored for a long time, called post storage or bedside filtration, and this can be done at any time, at the time of when they're issued or up to 42 days.

Next slide, please.

Now, a similar slide for platelets. Here platelets are being stored for five days at room temperature, and filtration up to three days after collection is considered to be pre-storage, and filtration from three to five days is considered to be post storage or bedside leukoreduction.

Next slide, please.

Now, there is important differences between pre-storage leukoreduction and post storage

leukoreduction. As Dr. Lee mentioned, pre-storage leukoreduction can be done under GMPs. There is adequate quality control because this is done in the laboratory.

You remove the leukocytes before they undergo breakdown. The filters don't have much effect on removing cell fragments, and you prevent the cytokine production during storage. So you're decreasing the transfusion related reactions.

In comparison, bedside leukoreduction there's no quality control because the product goes directly into the patient. So you don't have a chance to count the leukocytes. Frequently by the time the product is transfused, the leukocytes are disintegrated. Filtration may not remove some of the cell fragments or some of the cell associated pathogens.

There is a chance for a cytokine build-up, and also there has been reports of hypotensive episodes, especially with platelet products that are used at bedside, and that's because there's bradykinin production with the plasma going through the platelet filter and directly into the patient himself.

Next slide, please.

So that briefly covered filtration, and

now we're going to move on to apheresis, and this is the other major way to reduce leukocytes in products, especially platelets.

Apheresis is defined as the selective removal of one or more components of whole blood and returning of the remaining components to the donor. So basically you can do a differential centrifugation and select out the appropriate product that you're interested in and return everything else to the donor.

The advantage of that is that you can pull out more of the product that you're interested in without harming the donor.

Next slide, please.

Some of the components that are collected by apheresis currently are red cells, platelets, granulocytes, peripheral blood stem cells, monocytes, and plasma.

Now, some of the newer apheresis instruments have been designed to produce a platelet product that is already leukoreduced when it comes out. So there's no need to filter this product. It already is less than five -- has less than five times ten to the sixth leukocytes per unit.

Next slide please.

Now, a word about failure rates. This was

a study done by Dr. Kao. He's part of the TRAP Study Group that looked at allo-immunization in platelets, and they followed transfusion reactions to platelets and also had a red cell transfusion.

In part of that study they were looking at the number of failure rates they got after filtration. The failure rate was defined as greater than five times ten to the six leukocytes per unit.

And you can see that in apheresis platelets these are apheresis platelets that were initially isolated by apheresis and then filtered or pooled platelets. So this would be random donor platelets.

The failure rate was about seven to five percent, and when you looked at red cells, the failure rate was about 2.7 percent. So this indicates that the failure rate does -- failure does occur and that there is need for a quality control to assure that the process in your laboratory is actually working at removing the leukocytes.

Next slide.

A word about plasma. Plasma is considered to be the acellular portion of blood because it's given the hard spin during the preparation step. However, in reality it's not really cell free,

although it most likely has less than the five times ten to the sixth leukocytes that is the defining cutoff for leukoreduced products.

The amount of leukocytes present in plasma was not clear. Probably there is a great deal of variability between lab to lab, and I think additional studies will need to be done to quantitate what is the routine amount of leukocytes present in plasma, and because it is considered acellular, it's not routinely filtered.

The next slide, please.

Well, now, the next two slides deal with leukoreduction as a means to reduce TSE infectivity and some of the theoretical aspects. Some of these things are already mentioned by Dr. Loewer yesterday, and in theory if leukoreduction works the way we want it to be, there could be a lot of benefits from that. We could hope that leukoreduction removes the infectious agent or that it removes the cells that carry the infectious agent or that it removes the cells that support the peripheral propagation of the infectious agent or any combination of these three.

However, there could be a negative side to this, and while there isn't any data to support either one of these, we should consider this while we're

designing and interpreting studies, and some of the negative effects could be rupture of the cells that carry infectious agent and the release of the cells or the removal of the cells that could neutralize the infectious agent.

Next slide, please.

Now, also in terms of interpreting and designing studies, we should consider some of the things, some of the similarities and differences between rodent leukocytes and human leukocytes, and the question is: do the rodent leukocytes adequately model human leukocytes?

For example, do the same cell types carry infectivity in the rodent and human blood? Do rodent leukocytes have the similar physical characteristics so that they will follow the same isolation pattern or that they will be removed by the leukoreduction filters that were optimized for human blood?

So these are some of the things we should think about when we design future studies because there certainly is inadequate data to address these things, these issues currently.

Next slide, please.

Okay. In summary, I covered some of the cellular blood products, red cells and platelets.

Without leukoreduction they contain about ten to the nine leukocytes per component. With leukoreduction, we can reduce that down to less than one times ten to the sixth leukocytes.

I mentioned that pre-storage filtration is better than bedside filtration because of the quality control in the early removal of leukocytes, and I talked about plasma being different from the cellular components in that it probably already has a number of leukocytes that's less than the cutoff for leukoreduction.

Thank you very much.

(Applause.)

CHAIRMAN BROWN: Thanks, Dr. Vostal.

The next presentation is a follow-up presentation by Dr. Montrasio from the Hospital of Zurich, who will talk about the infectivity of nucleated blood cells from experimentally infected rodents.

DR. MONTRASIO: Well, good morning, everybody. So today I would like to give you some insight into the role of immune system in peripheral pathogenesis of mouse model, and this would be also an opportunity to compare mouse models with human models and also the danger of potential contamination for

humans being due to transfusion.

So in the first slide, I want just to summarize how peripheral pathogens work in mouse model. So we have normally prions comes into one organism from peripheral sites. That means, for example, in our model we inject a (unintelligible) directly into the pretonural (phonetic) cavity, but if we think about BSE or potential contamination of human by BSE contaminated food, their oral would be preferred. Then we have in mouse model the first passage will be transport of infectious agent from the periphery to the spleen. So the spleen is the first replication site of prion agents in mouse model and other rodents model.

And then we have replication or accumulation of prion agents within the spleen, and then in a second phase we have transport of infection agent from the infrareticular (phonetic) system to the CNS, and we are thinking that the peripheral nerve system can play an important role in TSE transport.

So in our work in the last years, we focused in which cell with in the immune system is involved in prion accumulation and in prion replication.

When we analyze the distribution of

infectious agents within the immune system of infected wild type mouse, we found that both in the spleen, lymph node we can find infectivity and also in prior purchase (phonetic). Whereas when we analyze peripheral blood laical (phonetic) site we didn't found any infectivity and accumulation of PrP scrapie.

So here we see that there is a difference between compartment of the immune system. So in the spleen you have both B lymphocyte, T lymphocyte, and other immune cells which are classified as non-BT cells, and all of them or at least B cells and T cells are positive for infectivity and PrP scrapie within the spleen.

But when we analyze peripheral blood laical sites, which are mainly composed by B and T cells, we were not able to detect any infectivity. So that's indicate that there is some difference between cells which are in the spleens and cells which are in the blood streams.

And what we are also interested is which kind of cells contain infectivity in the stroma fraction of the spleen, and there are indications that T cells are right resistant and PrP expressed in post mytotical (phonetic) cells.

So the question we wanted to address in

our study was to determine which cell within the spleen is involved either in prion accumulation or replication and also to see whether one of these cell types can be involved also in the transport of prion from the periphery to the central nervous system, and we focus our attention to three cell types.

So first, T cells, B cells, and follicular dendritic cells, and the second question was if the expression of PrP, the prion protein on those cells is also important, and so you probably all know when you remove or you ablate expression of PrP in mice or you create a PrP knockout mice, those mice are completely resistant to prion protein, indicating that PrP expression is necessary for prion replication.

But we still do not know if PrP expression is sufficient to confer cell type capability of prion replication.

Just to summarize some previously experiments carried out in our lab, so by using a different set of immunodeficient mice where in different mice where either B cells depleted or T cells depleted, we found out that for prion peripheral pathogenesis, B cells are very important, but T cells are not.

So if you have mice in which B cells, B

lymphocytes are depleted, you are complete abolishment of prion accumulation in the immune system, and you have also delay in complete block of neural invasion. Whereas if you deplete T cells, those mice are as quick as compared to wild type mice.

So then we analyze the importance of PrP expression on B lymphocyte. So we reconstituted by fetal liver sera B cells in immunodeficient mice with fetal liver cells of PrP knockout mice, and just by reconstituting those mice, we are able to restore the wild type phenotypes, indicating that PrP C expression of B cells is not required to restore prion replication in narrowing invasion in B cells deficient mice.

So then the second question was now we have seen that B and T cells within the spleen carry infectivity, and they also carry PrP scrapie. So are those cells able to replicate by itself the prion agent?

So to address this question we generated transgenic mice in which PrP expression was specifically restricted to either T cells or B cells, and what we found was that both of those transgenic mice were not able to replicate prion within the spleen. So this is indicating to us that in the wild

type situation, we have some other mechanism involved, and in those transgenic mice just having expression of PrP on B and T cells is not sufficient.

So there is a missing link between our transgenic animals and the wild type situation.

So just to summarize or to give you an overview, follicle is build in the spleen. So we have here of primary follicles where we have the T cell area, and then we have FD follicular dendritic cells, and if you enlarge an overview of this area, we have really tight contact between follicular dendritic cells and B cells.

So the idea now would be that perhaps follicular dendritic cells are involved in prion replication, and that the contact between follicular dendritic cells and B cells can be very important for transfer of prion from T cell type to B cells, as we observe in the wild type situation.

So what we decide to do to address this question was to try to shut off FDCs by interfering with one pathway which is very important for the maintenance of major FDC within the spleen, and we focused our attention on the lymphotoxin beta receptor pathway.

And it was previously shown by

administration of a fusion protein, which is built from part of the lymphotoxin beta receptor pathway fused to an IgG FC terminal could be related to the disappearance of major and function FDCs.

And upon long treatment with this fusion protein, you have also destruction of B follicle and also a modification of splenic architecture and also marginal zone macrophages.

So what we decide to do was to try to infect mice which were depleted from FDCs and to see whether the depletion of FDCs lead to abolishment of prion replication within the spleen and also to a delay in neural invasion.

So just an overview of our pathway, we decided to treat wild type mice with our fusion protein, and we decide to use two approaches. One was to treat the animal before inoculation with prion, and the second approach was to treat animals one week after interperitoneal inoculation with mice, and what we wanted to observe was the PrP scrapie accumulation in spleen which would lead us to have an idea whether there is accumulation or infectivity in the spleen, and of course, to measure infectivity within the spleen by bioassay, and also to see whether a depletion of follicle dendritic cells can lead to a

delay in neural invasion.

So in this picture I want to show you that the treatment with this fusion protein really leads to a specific depletion of follicular dendritic cells. In the right panel you can see the wild type situation of mice who do not receive any treatment. You can see very well the staining for the FDCs and one marker, which is an antigen present on follicular dendritic cells, and in the left panel you can see just already one week after treatment with this fusion protein all follicular dendritic cells are depleted, and this was also real for the two week standpoint, but sometimes we were able still to have some positive cell which may be a residual FDCs which are not completely deleted upon the treatment, or it can be some macrophages which can also cross-react with our antigen.

When we analyze PrP scrapie accumulation in mice who received the treatment, we were able to see that -- we did this analysis eight weeks after inoculation -- we can see that accumulation of the pathogenic isoform of the prion protein in the spleen of treated mice was completely abolished as compared to control mice.

That indicates to us that by depleting

specifically follicular dendritic cells, we are able to interfere with either accumulation of PrP or replicate prion or with the prion replication within the spleen.

Then we went to measure infectivity titer because I have to remind to you that not also PrP scrapie presence correlates with infectivity titers. So it's not so easy to say if we have PrP scrapies is the same as to have infectivity. So the best way is to check both sites of the presence of the pathogenic form and the infectivity within one organ.

So what we did was to remove spleen, prepare homogenate, and then transmit to indicator mice, and as you can see in this panel, when we look at eight weeks time point, the control mice will receive just control IgGs of higher titer as expected. So the prion replication was ongoing, but in our treated mice the infectivity titer was completely abolished.

And this was true for both treatment protocol one with the treatment one week before or one week after inoculation, and the same was true for the three week time point. So treated mice one week before inoculation, the infectivity titers were reduced as compared to control mice, and for the mice

who received the treatment one week after inoculation, some mice has reduced titer and some others not.

This indicates to us that during the first week where the mice were not treated with the compounds, there was enough time to start the replication of the prion agent, but upon depletion, the titer was abolished. So there is a clearance mechanism.

So then we looked at mice which were depleted for FDCs for a period of two months. Then we stopped the treatment, and we wanted to see whether the process of transport from the periphery to the central nervous system and to the development of scrapie symptoms was delayed.

And when we compared treated mice with mice we received just contra immunoglobulin (phonetic), we can see that we have a delay in the development of the disease. So untreated mice has inoculation time of about 210 days, whereas mice who received the treatment one week after inoculation, they have about 20 days' delay. Whereas when we started the treatment one week before inoculation, we have about 60 days' delay. In one mouse in this table was indicating that it was living longer than 314 days, and now this mouse is still alive, and now we

are about 550 days.

So in one case we completely rescued the disease.

What are the implications now? Just to summarize our result though is that our treatment leads to complete disappearance of follicular dendritic cells, and then the other important point, that by the specific depletion of follicular dendritic cells we abolished accumulation of PrP scrapie within the spleen, and we also found that FDC'd depletion lead to a complete abolishment of prion replication within the spleen, and also the onset of the disease in treated mice is delayed.

Our conclusions are that follicular dendritic cells at least in the mouse model, it's really essential for the deposition of PrP scrape, for the generation of infectivity within the spleen, and that follicular dendritic cells contribute directly or indirectly to neural invasion.

So what are the implications now for humans? So what we can think about now is the new variant CJD strain which has some characteristic indicating that this agent has some lymphotropism. So this is very similar to what we found in our rodent models.

And what we can think about is that if we can diagnose in humans very early on new variant CJD either by tonsilar biopsy or by some other indication that human beings are infected with the new variant CJD infectious agent, and then we can think about if we apply this substance to human being, we can perhaps retard progression of the disease.

And this may be also important if we find out that there is infectious material in blood of new variant CJD cases, and then if we can find out that the prion agent is in white cells or in some other blood component and some recipient of blood donors develop the disease, can theoretically have the risk of developing new variant CJD. We can think about to treat this recipient of contaminated blood with this compound and hoping to have a retardation of the progression of the disease.

What I want to point out is really that we are working in mouse model, and the new variant CJD agent is really not characterized. So we need to really further characterization of this infection agent to find out really which cell within the immune system or within an organism are really infected by this agent and to find out whether we can have similarities between the new variant strains and our

prion strains which are using in our approach.

So thank you very much for your attention.

(Applause.)

CHAIRMAN BROWN: Thank you, Dr. Montrasio.

The last presentation before we begin our discussion is by Dr. Rohwer, who will complement these studies with information of his own rodent models and infectivity in the blood.

DR. ROHWER: If we can go to the first slide, I'll just quickly review some points that Dr. Loewer made yesterday, which bear on this talk. The first is that when we consider TSE diseases and blood borne infectivity, it's important to remember as he reviewed that in the natural disease we have no unequivocal demonstration that there is infectivity in blood.

That doesn't mean that there isn't. It just means that the measurement possibly has not been made sensitive enough to detect it. Certainly the measurements have never been made with the sensitivity that we use to make these detections in rodent blood, which I'm going to show you in just a moment.

In the experimental disease, we do have rodent adapted strains that definitely do have infectivity in their blood. The older literature has

given us some very variable results from those models. I think with the newer methods of assay we're using now, we're getting a much more consistent picture of what's going on in rodents.

Finally, and importantly, in terms of blood borne PrP RES or PrP scrapie, as our previous speaker referred to this amylodotic protein, none has yet been demonstrated in either experimental or natural disease in any system in circulating blood. This is something that still remains to be shown, that there is such a thing in circulating blood.

Next.

I'm going to talk about two different rodent strains today. One is a hamster adapted scrapie strain, 263K, which we use in our laboratory, and the other is a mouse adapted GSS, is Gerstmann-Straussler-Scheinker syndrome, which is a familial variant of Creutzfeldt-Jakob disease, the Fukuoka strain, which has been used in studies by Paul Brown, and one of those studies in collaboration with us and then in another study which we'll discuss.

Go on.

The topics I want to talk about is, one, how much infectivity are dealing with. What are we trying to get rid of in blood? What's the

distribution of that infectivity? Is it in the right places that we could expect leukoreduction to work?

And then there are a couple of attempts to actually look at this directly. This is data from other laboratories. One experiment from Paul Brown, and another one from the Scottish National Blood Transfusion Service, which I just received by fax last night. It's not published yet, and they've been generous enough to share it with us since it will touch on both of those.

Next.

In our laboratory, we've been working with the hamster model, and we've developed a means of measuring the infectivity in the blood of individual hamsters or in blood pools, pooled blood from hamsters, and in general, we get titers as indicated. These are individual measurements here, indicated here, ranging from about four infectious doses per milliliter of blood up into the 20 infectious doses per milliliter of blood, and we'll come back to this in a minute, but go on to the next slide.

I want to show you how we make these measurements. What we do is you have to inoculate an animal, which is traditionally usually done by the IC route because it's expeditious, but we have now

explored several other routes.

You bleed it at some point during its infection. Much of this has been done in clinical disease, but we have some preclinical measurements as well, and then the question is: how do you assay that blood for infectivity?

We know from the earlier work that there's very little infectivity there. So how are we going to see it? The method that we've developed is something that I call limiting dilution titration. By limiting dilution I mean it's a dilution at which not all of the animals inoculated at that dilution get sick. Only some of them.

And so what we do is, for example, to inoculate one milliliter of blood, we inoculate 20 hamsters 50 microliters each by the intracerebral route. This is the intracerebral route because we're asking the question: is there infectivity in the blood? And this is the most efficient way to assay for infectivity in the blood, is the intracerebral route.

Some of these animals will die. So, for example, if six of them die, at the end of this experiment we can say, well, there were approximately six infectious doses in that on mL of blood. The

titer of that blood is approximately six infectious doses per mL.

That has to be corrected for coincidence. Some animals may have received two infectious doses. You do that by applying the Poisson distribution, and so you get a corrected number from the Poisson that gives you an estimate of the titer.

Typically in some of the later experiments we have been using inoculating five mLs into 100 hamsters. This gives us a better number because instead of six out of 20, we would not have five times six or 30 out of 100, and the statistic is better.

And, in fact, a nice feature of the Poisson is it gives you a standard error, which is just the square root of that number. So the square root of 30 versus the square root of five. These are actually quite good numbers.

Next.

If we come back to this data, what have we seen? These points here are from clinical disease, animals that are affected by disease. One of them, this one here, I believe, is from a pool of 250 mLs of blood. There's another pool here in one of these limiting dilution inoculations, and these two samples, for example, were taken halfway through the incubation

period.

These two samples were taken at limiting dilution, from a limiting dilution donor. What do I mean by that? This is -- next -- this is to address a potential important artifact in all of this blood work that's preceded us and one which we've been concerned about, which is that if you inoculate an animal with a huge dose of infectivity, this is a typical ten percent brain homogenate by the intracerebral route, and then later in the disease, bleed the animal and assay infectivity. Are you really looking at infectivity that has arisen de novo in the blood or are you just reisolating this inoculum.

This is a concern for this particular agent because it's so stable. In fact, by doing this, limiting dilution, what we've done is we've inoculated the donor animal with only ten to 100 infectious units or less. In some of these, these were animals that were taken from previous blood experiments. So they probably only got one infectious dose.

We know that that infectivity could not have been -- that could not have been the source of infectivity which we later obtained from the blood, and so we have answered the question, I believe, that

at least some of the infectivity arises de novo in the course of the infection.

Next.

One last point I want to make with this type of experiment is that on this graph right here, I've got two graphs superimposed. Let's look at this faint one here on the side. This is the typical dose response for this infection. This is a log scale over here starting at ten to the tenth down to zero infectious units, and you can see there's a regular increase in the dose in the incubation time, depending on the dose that an animal gets, that ends in this particular model out at around 180 days.

However, when you inoculate at limiting dilution as we've done here, the infections start in this latter region of the dose response and they extend randomly out to 400 days or more. Actually the longest incubation that we've seen so far in these studies is about 420 days.

And there doesn't seem to be any particular rhyme or reason to whether you're a high dose donor or a low dose donor. These are transfusion transmissions, which I'll mention in a minute. They're randomly assorted over this period of time.

Incubation time cannot be used as an

indication of titer in these types of experiments.

Okay. Next.

Now, this data is also consistent with a couple of studies that have been done in the mouse system that Paul Brown has initiated, and in this most recent experiment here, the data are not exactly comparable because they measured buffy coat concentrations as opposed to whole blood concentrations, which I just described.

But if we make some assumptions, which I think are reasonable ones based on what we've learned in the hamster, we get an estimate of approximately the same range of titer for whole blood in the mouse system, and we had to make a different set of assumptions in an earlier experiment that we collaborated on, but, again, we could estimate that we had somewhere in the range of approximately ten infectious doses per mL of whole blood.

Next.

So what have we learned from this? There is infectivity in blood. It's present even when the donor received a small dose. It's not an artifact of the inoculation. It's present after two different routes of inoculation in our hands. It's present during the preclinical disease, and it has been seen

in two distinct TSC rodent models, and the titer has been four to 24 infectious units per mL.

Next.

I'm going to say something very -- I don't want to get into much detail about transfusions, but it bears on the leukoreduction question in one important way, and I just want to mention that we have done now over 100 transfusions of infectivity from one hamster to another, transfusing approximately two mLs out of the six or seven total blood volume of the hamster. These are big transfusions on the basis of body volume in these animals, and the fate of those experiments is summarized in the next slide.

Next.

We've so far seen three transmissions out of a total 100 transmissions performed, slightly over 100, over 200 mLs of blood transfused total, and out of that 200 mLs of blood at six to 20 infectious doses per mL, we have transfused in that time 800 to 400 infectious -- intracerebral infectious doses.

That's quite a bit of infectivity, and even if we account for the fact that there's a reduction in the efficiency of transmission because of the intravascular route as opposed to the intracerebral route, if we consider this only in terms

of intravascular doses, we'd still have 60 to 300 intravascular doses.

We should have killed almost all of these animals by the transfusion route if blood borne infectivity behaved the same way that brain derived infectivity would behave in these same animals.

Next.

In fact, it looks like intact whole blood is far less virulent than comparable CNS infectivity, and this makes me wonder if lysis of blood wouldn't release the infectivity and put it in a form in which we would have seen far more infections from this group of inoculations than we saw by a pure transfusion.

Lysed blood may be more infectious than intact blood. Blood products, if that's true, may be more infectious intact whole blood, and the point I want to make with respect to leukoreduction is to the extent that leukoreduction disrupts cells, it may increase the infectiousness of blood rather than decrease it.

Next.

Now, I want to talk a little bit about the distribution of blood borne infectivity into various components. If we extrapolate this ten infectious doses per mL or four to 20 infectious doses per mL to

a unit of blood, we'd expect -- and human blood behaves the same way as rodent blood, which may be a stretch. We don't know yet -- we would have somewhere between 4,000 and 10,000 infectious doses in a unit of human blood -- next -- from an infected individual.

I'm going to talk -- this is data that has been extracted out of a much larger experiment, and I'm going to focus just on the component separations from this experiment and not the plasma fractionation. So I've modified this figure that some of you have seen before a number of times. We're taking whole blood, separating it into a buffy coat, a plasma, and a red blood cell fraction. We're going to treat the plasma further by spinning it to remove platelets and then do a high speed centrifugation to see if we can get all of the infectivity out.

Next.

This is a plot of incubation time down here on the bottom along this axis right here going from zero days out here to 400 days. And these are various components and how the infections fell for inoculations of five mLs of that component, except for the pellets where we inoculated about a 60 mL blood equivalent for these pellets.

But for these liquid components right

here, we've inoculated a five mL aliquot into 100 hamsters, and in this case, here's two separate blood experiments from blood pools. You can see they're similar, and we're getting titers again, and those numbers were on that original plot that I showed you. There are six to ten infectious units per mL.

Here's the red blood cell component right here. Here's the platelet rich plasma. This is the first spin. Whether there are appropriate terms for rodent blood or not is debatable, but this is following the typical speed time parameter for a bag separation.

Platelet rich plasma here and platelet poor plasma. They's still infectivity in this plasma. Because we were originally very surprised to see this both Dr. Brown and myself independently looked at this by doing a high speed spin on this material, and in both the mouse model and the hamster model there's stuff that survives.

In this case it was 20,000 G for a half hour, still in this supernatant, the supernatant carefully collected from the sacrificing supernatant to pellet so we couldn't contaminate the pellet.

Here's cryo-poor plasma. So even after cryoprecipitation we still have a significant titer in

plasma. The buffy coat has a lot of infectivity in it, but remember there's a small volume here, a large volume here, and these things add up to be about the same in terms of the total impact of infectivity, this platelet fraction and the high speed pellet.

So pellets do bring things down. They just don't bring down everything.

Next.

Here's a table summarizing the analysis of this data, and basically what we've taken here is this is the blood fraction we've looked at, the total volume inoculated, the total number of animals, the total infections observed, the infectious units per mL. A concentration can be calculated from that.

The total mass of that fraction, the mass times the concentration gives us the total infectivity in that fraction. If we normalize that to whole blood, we get a fractional distribution over here for the infectivity in the blood, and so, for example, if we look at the platelet rich plasma, we inoculated 5.6 mLs into 112 animals. We had 20 infections. That gives 3.9 infectious doses per mL for a total mass inoculated, a total infectivity of 405 infectious doses and compared to a total in the 250 mLs of blood we started with.

That would give us about 25 percent of the infectivity distributed into that fraction.

Next.

Now, here's perhaps an easier way to look at it just in a bar graph here. This was the distribution of the RBC components, platelets and the buffy coat. This added up to about 80 percent of the infectivity we started with. So we're missing 20 percent, but still this is a remarkably good recovery for any type, for even a virus type of experiment.

Next.

This can be normalized either to whole blood or to the sum of the components, but in general what we're seeing is about 20 to 25 percent of the infectivity in the red blood cells, about 25 to 30 percent in the platelet rich plasma. Here's the buffy coat, and here's the platelet poor plasma, 12 to 15 percent, and the high speed supernatant still has about, you know, 12 to 15 percent of the infectivity in it.

Next.

The mouse data is giving very similar results. Again, it was given as concentrations rather than fractional recoveries, and there was no whole blood determination to which to normalize.

Nevertheless, what we can say from the mouse experiments is that there was a significant concentration in the plasma, and that survived a 17,000 G spin.

Next.

Here's a summary of the data from the Fukuoka mouse strain. These are concentrations now, infectious units per mL, not fractional distribution, but the buffy coat, there's a significant amount in the buffy coat wherever it's been looked at, and the plasma, however, which is most important, I think, for considering leukoreduction and its potential effectiveness contains ten, 20, 30 infectious doses per mL in this system.

Next.

So what are our expectations for leukoreduction given these distributions? Well, plasma associated infectivity is not -- we don't expect that to be removed by leukoreduction. It's not cell associated apparently.

Something that hasn't been answered by these experiments is what is the stability of the cell association that is there. Is this infectivity sort of superficially associated with the cells? Will it be washed off in the course of a filtration?

It certainly isn't clear to me that this is a stable association. Disruption of cells, if disruption occurs, and it is a stable association, would nevertheless liberate infectivity and potentially increase the virulence of the product.

The one way in which leukoreduction might work in spite of all this is if the infectivity is, because of its inherent stickiness, if it was retained adventitiously by the filter. We know this happens with common bacterial filters and things like that, depending upon the milieux in which we put the spike. It's often rather surprising, the kinds of things that will remove infectivity from these materials.

But we can't presume that this will happen without an experiment. So let's talk about some experiments.

Next. Next.

We have three direct pieces of evidence that bear on leukoreduction that I see. One, we have these high speed centrifugations which I just discussed where approximately ten percent of the infectivity is not removed. This is the equivalent of the apheresis situation in a way, and it's telling us that there is going to be a fraction here which isn't going to be susceptible to leukoreduction regardless.

Okay?

And then we have two filtration experiments. Both of these are -- have imperfections, but they're what we have to date, and they bear looking at. Let's go.

The filtration of endogenous infectivity. This is an experiment that was conducted by Dr. Brown and his colleagues using a Pall filter, 28 millimeter filter, and these, they conducted three experiments. This is summarized from this paper, and since Paul is right here, he can -- he may want to comment on this, but basically this summarizes the data from the table in that paper.

They used a frozen and thawed plasma from clinically affected mice and saw a frozen and thawed plasma from preclinically infected mice and a fresh plasma from symptomatic mice, from clinically affected mice.

And this is the challenge to the filter. This is the filtrate, challenge, filtrate, challenge, filtrate. This is the range of values. This is really the range of the standard error for the measurement in each one of these measurements.

So starting with 18 to 58 infectious doses per mL, they ended up with four to 30. There's really

no clear indication that anything was removed here.

Here the same thing. Both numbers are in the same ballpark, both numbers in the same ballpark. There really in this experiment was no evidence of removal. I think the caveats associated with this experiment are self-evident. This is frozen-thawed plasma. It's not clear that this is a product that would be subjected to leukofiltration. This is fresh plasma and a better challenge.

I think one thing we can take home for sure from this is that there isn't -- there isn't anything about these filters that will pick up, intrinsically pick up the infectivity. You know, it's not binding by this mechanism that I said we might get lucky and have the infectivity removed by some adventitious association with the filter.

Next.

This next study was conducted by Chris Prowse at Scottish Laboratory in collaboration with Andrew Bailey at Q-One Biotech, and they have a paper, which I don't know whether it's in press or it's been submitted, but it's entitled "Leukodepletion and Removal of Prion Agent, a Cautionary Tale."

This experiment is distinguished from the previous experiment because this experiment was done

not on endogenously infected blood, but on a microsomal fraction from a brain homogenate which was introduced as a spike. The relevance of this experiment is in question. The authors are fully aware of that, but this is something they could do.

Next.

And the assay -- the reason they did it this way is their assay system was not infectivity. It was the Western Blot for PrP amyloid, and by using the brain homogenate, they've got enough signal there that they can actually detect something on both sides of the filter.

They tested four different filters, all of which are being considered apparently in Europe for leukoreduction, and basically the experiment was to take 500 mLs of whole blood, spike it with ten mLs of a microsomal fraction of ten percent brain homogenate from the hamster model.

Next.

And this is the results. They are in this case not assaying infectivity, but rather the intensity of the Western Blot signal on both sides of the filter. So there are several, and they looked at not only just PrP RES removal, but also at leukocyte removal both in a controlled sample, which was not

spiked, and in the brain spike sample.

The precaution that they mention in their study is that brain, the presence of brain spiked into -- at the level at which they spike, which I believe was -- I'd have to go back -- it was 500 mLs and ten mLs. So it's a fairly small spike. They're what, a half percent here, .2 percent?

Nevertheless that was sufficient brain to have a rather drastic effect on the leukocyte removal by these filters, which they attribute to, which they're guessing now, but their suspicion is that this is due to the high lipid content of the brain interfering with the activity of the filter.

And as a consequence of that, they're not sure about the significance of this result, but nevertheless this is what's out here and this is what you need to know. You've got the whole story here.

They saw essentially no removal of PrP RES from this source by these filters.

Next.

Their conclusions are that there was no evidence of removal, and they acknowledged the questionable relevance of the experiment, both due to the -- oh, they also saw hemolysis, a peculiar form of hemolysis because it was not stable. It settled, and

they're not sure what that was, but there was some sort of red cell fragmentation caused by the presence of brain, and there was -- leukocyte reduction was impaired by the brain.

I believe that's the end. No, I had a summary. Sorry. I have two more slides here.

In summary, ten percent or more of the blood borne TS infective is not cell associated. Infectivity per se, that is, free infectivity, doesn't just stick to these filters. We're not lucky in this case. It doesn't seem -- these filters don't seem to be able to pick up the infectivity by adsorption.

As a consequence, leukofiltration cannot be presumed to remove TSE infectivity without proof. That's my personal opinion, and the method must be validated.

One more slide.

What would you have to do to validate a leukofiltration? I think it has to be conducted with endogenously infected blood. It must be performed on a validated scale-down or full scale, and by validation, you have to show that the cellular separation actually works on the blood that you're using.

And unfortunately, this type of test will

require limiting dilution titration at a significant level so that you can make sense of the numbers, and it will, therefore, be costly. There's no way to get around that.

On the other hand, it may be possible to justify that cost if you are considering using TSE removal as a justification for using leukofiltration. Then the $300 million yearly expense of implementation probably more than adequately justifies the cost of an expensive experiment like this.

Quick, one more, but I think that's the end. See, I got it right that time.

(Applause.)

CHAIRMAN BROWN: Thanks, Bob. That was a very interesting and thorough presentation of both the hamster and the mouse data.

I think we can go right into the discussion if people have questions of any of the previous speakers or comments or whatever.

Yes, Paul.

DR. McCURDY: This question, I think, is for Jaro.

In the pheresis platelets which are separated by centrifugation essentially by density, is there a difference in the yield of young versus old

platelets, and does that have an effect on platelet survival and/or function?

CHAIRMAN BROWN: Jaro, if that microphone is as poor as it was yesterday, just let's bypass everything and -- okay. Let's try it.

Jaro, just take one of the table mics. Let's not mess with this again today.

DR. VOSTAL: After all that, I don't really have a very good answer.

(Laughter.)

DR. VOSTAL: It's true that --

CHAIRMAN BROWN: Do you want to go back to the other mic?

(Laughter.)

DR. VOSTAL: It's true that young platelets are bigger, but I don't know if there's any data that looked at the isolation of young versus old platelets during apheresis.

CHAIRMAN BROWN: Yes, bob.

DR. ROHWER: I have a question for Dr. Montrasio.

One thing that continues to puzzle me about these experiments is the effect of splenectomy on the incubation time. At least in the hamster, we've looked at this by three different routes of

inoculation, and the effect is really minimal, and I wonder if you could comment on that.

I mean if you take the spleen out by your general pathogenetic model, it should have a really dramatic effect on the course of the infection.

DR. MONTRASIO: (Inaudible.)

CHAIRMAN BROWN: Let's, for the rest of the morning -- I don't want to mess with these things anymore. We had it all day yesterday, and we're not going to have it all day today. Just use one of the table mics, please.

DR. MONTRASIO: So what we observed in our experiment is that there are alternative pathways ongoing. Also in the experiment I show to you this morning, also by depleting FDCs we have still mice developing the disease. So that means that there are alternative pathway which was involved.

So our hypothesis is that we have a major pathway which is caused at least in our mouse model via the spleen, and then from the spleen via probably the peripheral nervous system to the CNS.

But once we block the major pathway, there are still other pathways which are probably not involved in the immune system, but can go through other pathways directly to the CNS, but are slowly as

compared to the major pathway.

And this may be true for other rodents model where we can take out the spleen that you don't have that delay on the neural invasion.

CHAIRMAN BROWN: I think what Bob is getting at, if I'm not mistaken, is that everybody agrees there are almost certainly major and minor pathways, but if you are going to chemically remove the spleen, which is essentially what you're doing, versus surgically removing the spleen, intuitively you would expect a similar effect, and you don't get it.

DR. MONTRASIO: Well, if I remember correctly in mice, you have a delay if you remove the spleen, but is it something else in hamsters?

DR. ROHWER: Well, the reports are variable, but in the hamster we're certainly not seeing it, and because we're sensitive to this, these are really clean splenectomies, and the results have been variable in the mouse as well.

So I would just think considering how dramatic the effect you're seeing with the drug, how dramatic that effect is, that if you just took out the whole thing, it would be, you know, a complete block, and that's not happening.

DR. MONTRASIO: Well, that factor with the

drug is real clear. So it's a very cut off result. If we have to compare with entire splenectomy, I cannot really give a good explanation for that.

CHAIRMAN BROWN: Would one explanation be that the -- well, one explanation would be theoretically at least that the drugs that you're using to chemically remove the spleen, so to speak, or make it dysfunctional are not limited to the spleen, and they may be affecting other parts of the immune system which would function as the minor pathway.

DR. MONTRASIO: Yes. We know that this drug is also effective in lymph nodes. So if you observe the lymph node structure, we can also see that follicular dendritic cells are depleted in lymph node, and from some recent result in our lab, we know that probably the lymph node can play a very important role in peripheral pathogenesis.

So this might be the real explanation that when you remove the spleen, you have still a lymph node we can make the major job.

CHAIRMAN BROWN: Blaine, you had a question.

DR. HOLLINGER: Yeah. Bob, given the fact that there is cell lysis that occurs in the processing or collection of plasma, and whether or not then there

is a high speed centrifugation afterwards really wouldn't matter.

Is that an explanation or could that be an explanation of why you still see infectivity in plasma?

DR. ROHWER: It may be a contributing factor. On the other hand, that high speed centrifugation is done on plasma that's already been collected after a low speed centrifugation. So, you know, getting to that step, you do your first whatever it is, 2,400 G spin, and then you do your 3,800 G spin or 4,200 G spin, and then you take that plasma, what we're calling PPP, appropriately or not, and that's what was subjected to the high speed spin.

So most of the cells are gone, you know, have been removed by gentler methods before you go to this higher speed spin.

The nature of that material is still mysterious to me. Something I didn't present here, but in collaboration with Jaro Vostal and Carl Holada, they've made a very strong case that it might be in platelets because platelets at least in humans have a very high amount of PrP C associated with them, and it seemed like if there was actually propagation of infectivity in blood, which I personally doubt, that

platelets might be a good place to look for it.

We made -- we took a 20 mL blood -- we made a highly purified 20 mL blood equivalent of platelets, washed them thoroughly after purification out of ficol, and then inoculated the whole thing in hamsters, and we only killed one hamster. The starting blood would have had 120 infectious units or so of infectivity in it.

It's not the platelets. It's got to be something else, but it could be cell debris that's, you know, just really tiny stuff that doesn't come down yet, but filtration actually is one of the things we'd kind of like to look at to see if we can figure out what that stuff is. You know, if we could size it some way, that would be helpful in terms of its characterization.

CHAIRMAN BROWN: Along the same lines, am I correct, having taken down the figures that you put up for fractional recovery of infectivity? You indicated that normalized to whole blood, the buffy coat had about one-third of the total infectivity, .35.

DR. ROHWER: That's, yeah, normalized to whole blood.

CHAIRMAN BROWN: Yeah.

DR. ROHWER: It was .38, I think.

CHAIRMAN BROWN: Well, okay. We're in the same

range anyway.

DR. ROHWER: Yeah.

CHAIRMAN BROWN: It was about a third-plus, and then --

DR. ROHWER: Normalized against the components. If you add up the components and say that, then it comes out closer to 50 percent.

CHAIRMAN BROWN: And that platelet rich plasma, as opposed to platelet poor plasma. Platelet rich plasma had about -- well, the figure I took down was .26. Platelet poor plasma, the figure I took down was .12.

Subtracting that you would get approximately .14 or .15 for a derived value for the platelet component of buffy coat. What I'm getting at is your infectivity measurements imply that buffy coat -- that the white cell, that is, the leukocyte component of buffy coat, has a little more than twice as much total infectivity as does the platelet component of buffy coat, and whether it's twice or two and a half or whatever, but the implications, I think, of your figures in the hamster indicate that within the buffy coat, leukocytes have significantly more infectivity total than do the platelets.

DR. ROHWER: Oh, definite. But, yeah, I don't think there's any question about that. However, if you'll notice, I was very tentative about the stability of that association, and I am -- we have some indications that it's not a very stable association and that it may be possible quite easily to separate a significant amount of that infectivity from the cells.

CHAIRMAN BROWN: Right. Okay. Yes, Jeff.

DR. McCULLOUGH: This is a question for Dr. Montrasio.

Your work really involves the role of the dendritic cell in the infected animal. Do you think there are any implications for this regarding dendritic cells in a blood component?

DR. MONTRASIO: Well, we have to stress out that what we look at are not the dendritic cells, but are follicular dendritic cells, which are only present within the spleen and lymph node. They are not present in blood.

CHAIRMAN BROWN: Jeff, does that answer your questions or not? Does it?

DR. McCULLOUGH: Yes.

CHAIRMAN BROWN: Okay. Bruce, you had a question earlier.

DR. EWENSTEIN: Well, I think you've sort of provided one explanation, which I was thinking about, and that is that the peripheral lymph nodes in different species may be more important than in other species and may take the place of the spleen, and that may be especially important if you're talking about peripheral IV inoculation rather than intra-abdominal.

But I guess the two questions I had, you know, for any of the speakers would be, one, and maybe the first one for Dr. Montrasio is, what about immunodeficient patients? I mean as sort of my naive perhaps take on your data would be that severely affected AIDS patients, for example, might behave in the same way because of the nature of the part of the immune system that you're hypothesizing mediates the propagation of the infection.

And the other question maybe for any of the speakers would be is it possible to predict from what we know about the chemical nature of PrP RES what materials should be, you know, added to or built into a new kind of filter that would perhaps just nonspecifically, if you will, absorb the particle.

CHAIRMAN BROWN: Yeah. Dr. Montrasio, the first question, and, Robert, do you have any ideas on the

second one?

Dr. Montrasio.

DR. MONTRASIO: So just to answer the question about immune deficient patients, as HIV infected patients, so in these patients are mostly lymphocyte which are depleted, and we show that what we have to deplete are follicular dendritic cells, and this is not the case in HIV patients.

And what we can point out, that these compounds lead to immune suppression. So we have to think about if it's worth to use the potential to treat humans because we can have side effect.

CHAIRMAN BROWN: Thank you.

Bob or maybe Jaro Vostal, anybody in the room, I suppose. This is a fairly technical question. What kind of elements do you add to a filter to make it more sticky for PrP RES and let the filtration biologically remain effective?

DR. ROHWER: PrP RES is well known for its adherent properties, and those adherent properties have frustrated attempts to purify the agent and things like that for decades, and so there's a lot of potential there, and it's an approach that we're very interested in and we're working on, but that's all I'd like to say

about it now.

CHAIRMAN BROWN: Dr. Montrasio?

DR. MONTRASIO: I would just specify once again that PrP scrapie is not on the same as infectious agent. So the first thing probably we have to do is really to find out what is the real infection agent. We know that at the moment PrP scrapie normally correlate with infectivity, but it is not said that PrP scrapie is infectivity.

CHAIRMAN BROWN: Yeah, you reminded me to ask you or confirm through you something that I spoke to Dr. Aguzzi about, and that was the failure to demonstrate infectivity in the blood, and I believe the method that you use to assay infectivity in the blood used a comparatively small number of animals, six or eight. So in view of what, you know, we've been talking about this morning, that should not be taken as an equation that there is no infectivity in the blood, circulating blood.

Certainly you demonstrated there's a lot less, for sure.

DR. MONTRASIO: Yes. We have, too, said that in our lab we didn't use a limiting dilution experiment by using 100 of indicator mice. So we use a small amount

of mice, and I think we should repeat our experiment by using more indicator mice to see whether we can detect some infectivity in our experimental model.

But what we showed is that at least with our system we can detect at least 20 infectious units for ten to the sixth cells. So this is our detection limit, and from what I heard today, what is shown by the other experiment is that there are less than 20 infectious units for a milliliter.

CHAIRMAN BROWN: Yeah, you might have as little as four or five. You might have as many as 20, but you might not, and the range as you saw ranges between about five and 20 infectious units per whole milliliter of blood. It's higher in buffy coat.

But other questions. Yeah, Dr. Belay.

DR. BELAY: This question is for Bob Rohwer.

I was curious about one issue. Are there any studies that are planned or ongoing like the ones that have showed in other animal models, such as monkeys, for example?

DR. ROHWER: There are. We just recently got our USDA approvals to repeat these experiments in the 301v model of mice, which is the BSE new variant model of mice, and that's underway.

There are, I believe, monkey experiments that Dr. Brown could more appropriately tell us about.

CHAIRMAN BROWN: Yes. There's a decent size study of both new variant and sporadic CJD inoculated into squirrel monkeys, and they include groups of animals inoculated with buffy coat separated and with plasma separated, and in one of the experiments there is a comparison of this material inoculated intracerebrally as opposed to inoculated intravenously.

We've tried to make the maximum use out of about 75 monkeys. I mean we would have liked to have done about a 500 monkey experiment, but that was just out of the question. So we sort of tried to shrewdly guess where we would get most of the information, and if we guessed right, we'll get a lot of information. If we didn't we'll get much less.

But that experiment is now about a year down the road. So in view of earlier experiments having nothing to do with new variant, using squirrel monkey's brain, which was also inoculated both from patients with new variant and from sporadic, we will expect to begin to transmit disease after about two years roughly, ranging from about one and a half years to three years.

And we also know that materials which have much

less infectivity than the brain will take longer. So alas, alas, if that had been done five years ago we might have very solid information today. We do not, and we probably will not be able to say that blood does not have infectivity, for example, from patients with new variant CJD for four or five years.

If the experiments turn out positive, then we'll have information much sooner.

Yes, susan.

DR. LEITMAN: I have two questions. The first is for Jaro. It has to do with at least the definition of pre-storage leukodepletion for both red cells or whole blood and platelets.

You commented that leukodepletion up to three days of storage qualified as a pre-storage leukodepletion. Platelets have a life span on the shelf of five days. So I consider three days mid-storage leukodepletion. In my facility we define pre-storage as within 24 hours of collection. So that sufficient cytokine released from white cells doesn't have time to occur.

Is that an FDA definition, pre-storage, you know, filtration up to three days of storage? Where did that come from?

DR. VOSTAL: As far as I know, I mean, 24 hour is what we recommend. However, it's on the books that it can be done up to three days for platelets. So we encourage filtration within 24 hours.

DR. LEITMAN: That's not an FDA definition though, is it? This is just recommendations?

DR. VOSTAL: Yes, as far as I know, but I think some filters are actually approved for up to three days' leukoreduction in platelets, you know, and defined as pre-storage.

DR. LEITMAN: In terms of efficacy of leukoreduction, defined efficacy.

DR. VOSTAL: Right. We usually rely on the data provided by the manufacturer, you know, in terms of efficacy of filtered leukoreduction.

DR. LEITMAN: Okay. The second question is for Bob Rohwer.

You commented several times in your talk that you thought perhaps that lysed cells were more infectious than intact leukocytes, but I fail to follow the evidence that led to that comment.

DR. ROHWER: Not that the lysed cells themselves would be more infectious, but rather than lysis of the cells would release infectivity to the medium, and that

infectivity would be more likely than to pass through a filter.

And the evidence for that, the principal evidence that I cited were the transfusion results. In other words, there is an enigma associated with these transfusions. We have many of those same bloods -- well, several of those same bloods were actually assayed for infectivity by limiting dilution. The remaining two mLs were inoculated into animals, and so we know they were infected. We know that, we have good reason to believe that all those bloods that were transfused had infectivity in them.

We should have seen many, many more infections than we saw had we been inoculating the same level of infectivity from a brain homogenate by the same route, and we didn't.

So one way to account for that is that the infectivity is somehow locked up or, you know, tied up in some dead end pathway in the blood system, and it may be in the process of being cleared, and by disrupting the blood and releasing that infectivity back into the, you know, fluid contents, my expectation is it will be found to be more infectious by the IV route.

We'd like to do that experiment directly. There's a technical problem with doing it, and that is inoculating that much lysed blood is lethal, and so we haven't got around that yet, but that's my expectation of what we would find.

Does that answer your question?

CHAIRMAN BROWN: One experiment that I didn't do, I don't think Bob has done, and I'm not aware that anyone has done it, and it's really too bad because it would be a critical experiment for at least one answer to one of these questions, is the leukofiltration or leukoreduction filtration of whole blood for the purpose of, for example, preparing packed cells.

The filtration we did was plasma, and I think, Bob, you also did leukofiltration with plasma.

DR. ROHWER: I have done no leukofiltration experiments to date.

CHAIRMAN BROWN: Oh, okay.

DR. ROHWER: And we will not be able to do them without support. I mean they're expensive experiments.

CHAIRMAN BROWN: Yeah. No one has done the leukofiltration with whole blood, and obviously it's one of the concerns this morning. If you are going to approve the idea of leukofiltration to reduce

infectivity, you'd obviously like -- you would think that it would be a good strategy to get rid of white cells because we know white cells have a lot of infectivity at least in the rodent models as opposed to using it to leukofiltrate or reduce the infectivity in plasma for which we have good evidence is pointless, but nobody has done the experiment.

So we're just theorizing about its usefulness to reduce infectivity because we know that in rodent models infectivity is largely associated with buffy coat. But the actual hard data, the experimental data to support that intuition are missing.

Yes, Jeff.

DR. McCULLOUGH: I want to clarify your answer, Bob. The data on which you're basing this then is the reduced infectivity of the blood, lower than what you would expect from brain, but not any increased infectivity you've seen in any infiltration experiments which haven't been done, right?

DR. ROHWER: That's correct.

DR. McCULLOUGH: Okay. Then can I follow up with a question. Maybe this is also for Dr. Vostal or anyone. What is known about the extent of hemolysis or lysis of buffy coat that occurs during filtration of

blood components?

CHAIRMAN BROWN: Jaro, do you have any information about that or does anybody? If Jaro doesn't, does anybody in the room have information, experimental information or factual information about the propensity of one or another filtration to cause destruction and lysis?

Do you have any information?

DR. LEITMAN: Jeff, lysis of red cells or lysis of white cells?

CHAIRMAN BROWN: Anything.

DR. McCULLOUGH: Either, both.

DR. LEITMAN: There's a fair amount of evidence that there's very, very little lysis of red cells. The loss of red cells can be accounted for by the red cells retained in the dead space or volume of the filter easily and completely. So there's very, very -- I mean, there's almost no red cell lost through lysis.

DR. McCULLOUGH: That's why I'm asking the question.

CHAIRMAN BROWN: What about platelets and/or leukocytes? When you subject blood to leukoreduction filtration, is there any evidence during that process that platelets may be disrupted or white cells may be

disrupted that you know of?

DR. McCULLOUGH: I assume the manufacturers know this, and they just don't happen to be here, but I agree with Susan. In terms of red cells, it's not considered there's any meaningful lysis that occurs. I don't know. I assume the different filters might have different propensity to lyse and release material from either the leukocytes or platelets when they pass through the filters. Somebody knows this, I'll bet.

DR. SAYERS: Just one thing in that regard. I suspect that if there was significant white cell lysis then you'd see those complications attributable to cytokines increased in those individuals getting filtered product, and actually you at least theoretically see a decrease of that complication.

CHAIRMAN BROWN: Yes. No, Blaine, did you?

DR. HOLLINGER: Just a question for anyone here also. I think you probably answered this, Bob, and I just may have missed it, but what is the charge -- or anyone here -- the charge characteristics of the prion protein in terms of PI and so on?

You know, we talk about using filters for perhaps removing things like this, you know, free prion particles, but when you put serum with it, that often

negates often any charge advantages you might have, but do we know anything about the charge characteristics of the prion protein or anybody?

DR. ROHWER: The prion protein in its unglycosylated form is highly basic. It has a histone-like basicity. With the glycosylations intact, it has a range of isoelectric points which range all the way down almost to neutrality in some people's hands.

But, again, I think it's important here to emphasize a point that Dr. Montrasio just made a few minutes ago, and I would like to second the point that he made, which is that it's not proven that this protein is the infectious agent. At least it's not proven in some of our minds, my mind in particular, and as a consequence, until that correlation is made absolutely, it may be a little risky to base everything on the distribution of the amylodotic protein.

And until we've actually demonstrated that this protein exists in blood, I think it's kind of dangerous to presume that it's even an appropriate target for assay.

CHAIRMAN BROWN: Some of the studies that are published using PRP as a surrogate for infectivity have been validated by parallel titrations of both

infectivity and PrP. I'm thinking particularly of the Bayer study, and where you have a model that's been validated and shown to have parallel reductions, you're in good shape.

But you can't, as Bob said or anybody else, necessarily translate that to every other model you're using. There are circumstances where there's a big disparity between the amount of PrP and the amount of infectivity, but there in those models that are validated, then I think you can trust PrP as a good surrogate.

CHAIRMAN BROWN: Dr. Epstein.

DR. EPSTEIN: I just wanted to add a comment on the subject of biochemical properties. It's been suggested that the prion of new variant CJD may be quite different than the prion of sporadic CJD, and that, therefore, it may be very difficult to extrapolate from any of the known data whether it be pertinent to filtration or clearance in fractionation.

Also I want to comment that we did not bring to this meeting any data on clearance of new variant prion in the fractionation process because we're not aware that anyone has generated any to date, at least not published, either using human material or using BSE

material.

And that question may or may not be pertinent in different people's thinking to whether one can ignore residual infectivity in plasma because it's handling fractionation or whether more aggressive measures are needed to try to eliminate it from plasma for fractionation.

And just on that point, I would just remark that in our system in the United States, we have two different sources of plasma for fractionation, some of which comes from further processing of whole blood as Jaro described, but some of which comes directly from apheresis and then is directly frozen.

So, you know, a good part of the plasma that's fractionated has been freeze-thawed up front with presumably lysis of whatever residual leukocytes were present.

So just two caveats really. One is that we're sort of lumping all discussion of prions, but, in fact, their biochemistry may not be the same. Cell distributions may not be the same, et cetera.

And, secondly, that we don't have data on disposition of infectivity of BSE or variant CJD in fractionation.

CHAIRMAN BROWN: Yes. That's certainly correct. As usual, the committee will be making decisions without any information, and we certainly still do not have the necessary information about the differences, if any, at a fundamental level between the agent that causes the new variant of CJD and classical CJD.

I think when that information becomes available, we're going to find that the agent that causes new variant CJD is A versus A prime rather than A versus X.

But wait a second. I think Larry had a comment.

DR. SCHONBERGER: Yeah, this is for Bob.

I'm trying to get a clarification on what your views are on the net effect of leukoreduction on infectivity. Can I assume that because the leukocytes are thought to contain about a third of the infectivity, but that you cannot account for perhaps 20 percent of the infectivity, that leukoreduction would not have the net effect of increasing infectivity of the final product, but may not simply reduce it to the extent that you might otherwise expect?

Is that a fair interpretation, or do you actually think the net effect could be to increase the infectivity of the final product?

DR. ROHWER: No, I don't think it will increase

the net infectivity because when we measure the infectivity in blood, we lyse the blood before we measure it to make sure that we get rid of any cell associations which may interfere with the assay, and we do it by means that are far harsher than anything that's going to happen during leukofiltration.

The only point I was going to make is that especially if you're considering a transfusion product, the evidence from these transfusion experiments that we've done is that there's some inherent safety built into the transfusion process as long as the blood remains intact.

If it's just simply a matter of moving whole blood from one animal to another, we're not getting the level of infection that we would expect on the basis of the infectivity that's present in those bloods, and if you start to manipulate that blood, and if you manipulate it in the extreme, which we've done before we inoculate it by the intracerebral route, you see that infectivity is there.

And so until you've actually tested the system for the effect of this manipulation, I don't think you can say, you know, how much of that infectivity will show up in your subsequent use of the product after

this process.

In that regard, I do have to say something about fragmentation. I'm a little uncomfortable with this because it's not my data, and it's not my product, but at a CHI blood safety meeting two years ago there was a presentation, a technical presentation on the use of one of these filters that's one of the more commonly used ones in which the technical rep. was showing FACS analysis of these cells that come through the filter.

And there was a lot of debris in that FACS profile. I didn't know what it was, and I clarified it with a question at the end of the presentation, but that's what that stuff was.

And so I think that's really what has provoked me to get concerned about the idea that if there is fragmentation of these cells on the way through these filters, that could be a problem.

CHAIRMAN BROWN: Go ahead, Jeff.

DR. McCULLOUGH: I'm sorry to beat this to death, but I want another question about the dendritic cells. I'm sorry that I don't know enough about the difference between the follicular dendritic cell and the circulating dendritic cell, but given the discussion about the sensitivity of the assays and the

small number of animals, are you fairly confident that the lack of infectivity that you've showed with the peripheral blood is -- how confident are you in that?

And biologically is there enough difference between a circulating dendritic cell and a follicular dendritic cell to believe that these might function differently?

DR. MONTRASIO: So in our experiment, as I told you before, we just use a very small number of indicator mice when we analyze infectivity in blood leukocyte. So I think we have to increase the number to see whether we can find traces of infectivity. So I want to clarify that.

And about the difference between follicular dendritic cells and dendritic cells, there are two types, two different types of cells. So they have different cell function, different location, and until now nobody has really found out whether dendritic cells within the blood stream are carrying infectivity. So I think we have to look it up.

CHAIRMAN BROWN: What sort of proportion of follicular dendritic cells are running around in the blood stream? I naively thought they were all located in the spleen.

Are there circulating follicular dendritic cells?

DR. MONTRASIO: No, there are no circulating follicular dendritic cells.

CHAIRMAN BROWN: That's what I thought. So there are no circulating follicular dendritic cells.

DR. McCULLOUGH: But there are dendritic type cells in the circulation, my understanding.

CHAIRMAN BROWN: Are they coming from the spleen? I'm not a hematologist. Who can answer this question?

DR. TRAMONT: Monocytes and macrophages in dendritic cells are all the same lineages. In fact, dendritic cells is felt to come from the blood into the local site along mucosal surfaces and then given the name "dendritic cells."

CHAIRMAN BROWN: Yes.

DR. TRAMONT: But they have all the same markers, and that's how they're defining them.

DR. McCULLOUGH: But I guess that's why I'm asking. I thought this was kind of a continuum of cell and that there wasn't a great distinction between the biology of a similar kind of cell that would be in the circulation versus those that are in the follicular area.

CHAIRMAN BROWN: Yes, Dr. Montrasio.

DR. MONTRASIO: I have to specify that nobody really knows where follicular dendritic cells are coming from. So the cell lineage of those cells is not really now identified, and there are also some kinds of strange cells because they have antigens on the surface which are coming from lymphocyte specific ones or T lymphocyte dendritic cell macrophages. So they are really a special cell type.

And what is believed until now is that they are not from hemopoietical regions, but there are other studies which claim that they can reconstitute follicular dendritic cells up in bone marrow reconstitution, but nobody really knows from where the cells are coming from.

DR. LEITMAN: Can I try answering that? I thought a dendritic cell was a tissue based cell by definition. One can take a circulating mononuclear cell and convert it in tissue culture with various cytokines to appear in that manner, and there's evidence, you know, that a premature hematopoietic CD34 positive stem cell can be transformed in the laboratory to a dendritic cell.

CHAIRMAN BROWN: Yeah, Bruce. I don't know if it's worthwhile continuing to beat this particular one to death because I think the bottom line is that the developmental biology of the follicular dendritic cell is still somewhat mysterious and its related cells. Would that be a fair statement?

DR. EWENSTEIN: I was going to try to go back to Dr. Epstein's two points and just ask the committee to sort of consider it. It seemed like on the first point that we used a fair amount of freeze-thawed plasma. The implication would be, I think, that leukoreduction would not be as effective because the cells had already been lysed and probably the filters would not do as much good.

On the other question, I guess the implication would be if there are some types of prions, if you will, that have a propensity for white cells, such as lymphoid tissue, more than those that are being used in the current animal models, and we may be underestimating the effectiveness of leukofiltration.

So, you know, I don't know if that's the way the rest of the committee would interpret his comments.

CHAIRMAN BROWN: Well, I interpreted Jay's comments as meaning that we'd forgotten a little bit that we're supposed to be thinking about new variant as well as classical CJD, and we don't know anything about new variant other than that -- that is, with respect to classical CJD from the point of view of this committee -- other than that lymphoid tissues contain more PrP in new variant disease than in classical disease.

That much we know. That's a solid fact. Beyond that we're in total darkness, and as far as freeze-dried or freeze -- frozen and thawed plasma and fresh plasma, that is correct, and our experiments that Bob showed on the slide indicated that it didn't matter whether there was fresh frozen, frozen, thawed or fresh; that if you started with plasma after the two preliminary spins that are in customary use, it didn't matter whether it was frozen or fresh. It didn't have any effect on the residual infectivity in plasma.

But if you start with plasma, whether it's fresh plasma or frozen and thawed plasma, if you start with plasma and leukoreduce by filtration the plasma, you don't do anything, at least not in the rodent models and not controlled by measurements of the