incidentally this particular product has been withdrawn from the market, and of course Baxter now working on the second generation product.

There are basically two issues that we deal with, our work sort of, you know, focus on, and these are really issues or rather problems that we encounter because of the uniqueness of this product. One of them, one of the primary problems is really we are dealing with a rather unique product.

Hemoglobin, once you take the hemoglobin out of the red cells, you can't really control it. That's primarily because the hemoglobin, unlike any plasma-derived product, contains an active product which is the heme or rather the iron. These are transition metals. They continually change, undergo changes, so the hemoglobin that we really would like to keep in circulation of course is the ferrous functional form, the iron in two states.

Hemoglobin outside the red cells will uncontrollably go to the ferric, non-functional form. The heme now turns into the ferric form or the 3+. In some instances where there are oxidants in the tissue, hemoglobin can even turn to even higher oxidation state, the ferryl, which is rather toxic. In brief, the hemoglobin, this particular product can go from functional to non-functional and in some instances downright toxic.

The other issue that we deal with, because of the uniqueness of this product, is the locality or the neighborhood where the hemoglobin actually finds itself, blood cells, and that is primarily the vasculature. And what I'm showing you here, basically a cartoon section of the vascular wall. This is the inside of the blood vessels. This is the vessel wall. And all we have learned in recent years about the endothelial cells that line the blood vessels, generate an important factor which is endothelial relaxing factor, which we know is a simple nitric oxide. And this nitric oxide activate a number of enzymatic reaction, ultimately lead to relaxation of blood vessel. That's under normal conditions.

Other, additional important things that transpire from research in recent years, that nitric oxide, besides its vasodilatory action, it actually has some antioxidant property. In conditions where the vasculature is compromised, in conditions such as diabetes or hypertension or a number of other conditions, and/or when you have hemoglobin floating around close to the vascular system, close to the nitric oxide's production side, the reaction is of course immediate and very rapid. That would lead to vasoconstriction instead of vasodilation, and of course the hemoglobin now has to face these unwanted oxidants which normally the nitric oxide suppressed when we have a higher

level of nitric oxide.

This is the hemoglobin molecule, and really I am showing this just to show you where we are focusing in terms of research. And we wanted to know, or rather to define, the interplay between the hemoglobin and these oxidants or antioxidants, or we define them as ligands. The area that we focus, of course, is where the heme resides in the protein. We call it the heme pocket, where the actions take place; all these reactions actually take place here.

But also, the second reason is that when you modify the hemoglobin as they do, the manufacturers, even if your reagent is here or on the surface, you are influencing this very crucial area. So we spend a great deal of time and energy, really, to look at these dynamics and the chemistry of the heme pocket and whether we can actually influence the reactions there at that level.

This is just two close-ups of the heme pocket. I am showing you in normal hemoglobin, this is the heme, this is the oxygen, a number of key amino acids. So we have reason, we said if we can actually in some instances make it a little bit more crowded or more space for ligands to get in, we can actually control some of these reactions.

And what we did, basically we use a variety of prototypes, variety of proteins. The major proteins that we use are simpler components than the hemoglobin, which is the

recombinant, in collaboration with a number of outside people, particularly Rice University, and we were able to change key amino acid in the heme pocket, and we were able to see actually some reduction in the rate of hydrogen peroxide reaction and nitric oxide reaction.

We also use chemically modified hemoglobin. In fact, some of these example of hemoglobin that we have taken directly from industry. They were kind enough to give us very little of those compounds. And we have also looked at the dynamics in respect of these unique chemical modifications on these reactions.

We have also developed an endothelial cell model that these reactions can be tested, and our hypothesis, that if we can genetically engineer or chemically alter the heme pocket, we can influence the reaction at the level of endothelial cells and consequently the toxicity.

In brief, what we did over the years, over the last nine years or so, we determined length between the redox chemistry of a given protein and its toxicity. We have also developed ways and means to actually suppress and control some of the side reactions of hemoglobin. We developed a number of endothelial cell models that mimic ischemia or perfusion, an important phenomena in particularly those patients who receive blood substitute, and also we developed endothelial cell model in which to

mimic the lowered antioxidant capacity of the individual, such as what you encounter particularly in those patients that receive these candidate products.

And some of these, as a final outcome of what we have done, some of these suggestions that we publish in the literature luckily found itself in the second generation of blood substitutes, and of course this is quite pleasing and rewarding, and also in the fact that that will also give us, based on that, a little bit know-how and a nitty-gritty type of knowledge of what the genetic engineering or chemical modification of second generation hemoglobin might entail.

And I think that's all I have in my time, which is 10 or so minutes. Thank you.

DR. HOLLINGER: Are there any questions of Dr. Alayash?

[No response.]

DR. HOLLINGER: Okay. Dr. Scott?

DR. SCOTT: Abdu and I want to thank you for staying to listen to us, because I know it's getting late. I'm just going to briefly give you an overview of one of my projects, and quite briefly, in a whirlwind fashion, summarize some of the others that I've been working on in the lab.

The first one is involving antibody subclass efficacy in specific immune globulins. Just to remind you,

we regulate a number of human specific immune globulins which are used to prophylax against infectious agents, and I have just listed them here for you. We're expecting a couple of others to come down towards us in the next year.

And the question that I got interested in finding out the answer to was, could we develop techniques to assess whether or not the activity of specific immune globulins resides within a particular subclass of IgG?

Just to show you a snapshot of the different subclasses of IgG, all of these are contained in most immune globulins, except that some processing removes a lot of IgG4, and these are the subclasses IgG1, 2, 3 and 4. And what you can notice is that they have somewhat different structures, different numbers of disulfide bonds. There are other differences which aren't reflected here, and especially IgG3 has this long hinge region which makes it very susceptible to proteolytic degradation. They have different chemical characteristics, and based on these, they can be separated from a preparation of immune globulin.

So there are some potential regulatory implications or at least some efficacy implications if there is a selective subclass that works against some of these pathogens. First of all, if you knew that there was a particularly efficacious subclass, you might want to select donors that had that best antibody subclass against the

pathogen. And in cases where donors were to be immunized for a specific immune globulin, you might wish to select an immunization method or an adjuvant which resulted in production of the best antibody subclass. Also, in manufacturing one may be able to enrich for an antibody subclass, and certainly one would wish to avoid processes that removed the desired subclass.

It's going to be a little difficult, but you can leave off the top. What I started with was HIV immune globulin. We had access to these preparations through NIH. And this is just almost a prototype of the sort of project that I would like to continue, and I used protein A Sepharose and a pH gradient to sequentially elute the IgG subclasses and then tested these for binding of viral antigens in the solid phase, and also in bioassays, in collaboration with Hanna Golding's lab at FDA, which looked at neutralization of HIV, both HIV as a free virus and neutralization of cell-to-cell viral transmission.

You have the data, I think, in the package you received, but I'm just going to summarize the two major findings. And one was that all of the IgG subclasses that we looked at, which were 1, 2 and 3--this preparation has a chromatography step which removes almost all of the IgG4, which is a very minor component anyway--all of the other subclasses bound well to HIV antigens in ELISA and on

Western blot, and the rank order of binding was, IgG1 was often slightly better than or similar to the total preparation, and both of those bound better than IgG2 an IgG3 in ELISAs. However, even though IgG3 typically was the worst at binding in ELISA, and even by Western blot, it was superior in preventing infection by cell-free virus and in preventing cell-to-cell transmission of HIV.

So, very briefly, I just want to emphasize that now we have the ability to separate antibody subclasses. We have been able to develop this, and I have shown you at least in one case that there is evidence for selective efficacy of a particular subclass. And we have also looked at RSV immune globulin with Judy Beeler, and it appears that in that case IgG1 by far is the most effective in bioassays. And, as I pointed out, we may be able to enhance efficacy of specific immune globulins just by knowing the subclass, how well the subclass functions, although obviously this isn't an in vivo challenge, and we plan to study other specific immune globulins.

Also, I would just note that there was a discrepancy between binding to solid phase antigens, such as in ELISAs, and bioassays. And to us this stresses the importance of selecting biologically relevant tests which predict efficacy in the case of specific immune globulins.

And for HIV immune globulin in particular, one

could imagine that you could take some of this information, it would be nice to test it in an in vivo model first, but this has some implications because one might improve its efficacy by enriching and stabilizing IgG3, which only comprises about 4, 2 to 4 percent of that total preparation. And also I would point out that one might wish to change the dosing interval if you really thought that IgG3 was by far more important than the other subclasses, because IgG3 has a very short in vivo half life. It's only 7 days, compared to the others which are 21 days.

So this is just to illustrate how such findings might be applied in the future. I'm just not quite finished with that. Thanks, though. I wanted to say that right now I'm pursuing studies to determine whether the IgG efficacy in the HIVIG is due to preferential recognition of important HIV neutralizing epitopes, or whether it's due to the IgG3 hinge structure, and I think I set that out also in the report, but I'd be glad to talk about it.

The other major project that I've been working on is understanding immune responses to inactivated Gram-negative bacteria, and the preparation I use is a heat inactivated or a heat killed Brucella abortus, but this represents sort of a class of immunogens which have both toxic effects but also may be used in a way as a vaccine adjuvant. And so really there are two kind of separate arms

to this study, and one is understanding the toxicity of these inactivated bacterial components.

And the reason I would like to do that is that it enhances my expertise in understanding inflammatory responses to bacterial constituents, and this might be very relevant to the side effects that occur from products containing trace levels of inactivated bacteria. And we already know that, for example, immune globulins contain bacterial DNA, which is a known immune stimulant, and they often may contain very low levels of LPF, certainly lower than the amount that is specified as a cutoff.

What I have been able to show is that such microbial preparations induce proinflammatory cytokines like TNF and IL-1, but in particular and most recently, interleukin 12, and this interleukin 12 comes from dendritic cells. They also cause migration of dendritic cells to T cell areas of spleens, and all of the preparations that I have looked at seem to elicit to a greater or lesser degree an anti-inflammatory cytokine as well, interleukin 12, and they are toxic in vivo to mice who are deficient in IL-10.

So I'll go to the next one. Besides understanding the proinflammatory responses to these, it's also possible to harness the immune responses to microbial substances, because in our case, the ones that we have looked at, and in particular the heat-killed Brucella but some others can do

this, as well, induce potent T and B cell responses to antigens that are covalently linked to them. So these can be used as vaccine adjuvants, if you can get over the proinflammatory initial response.

And it has been shown by myself and by Dov Golding that you can use such an adjuvant to induce certain subclasses of complement fixing antibodies in mice, and we believe on several lines of evidence that this could be achieved in humans, as well. And so understanding the adjuvant activity of such preparations is potentially useful in promoting subclass specific responses for the generation of specific immune globulins, which relates back to what I talked to you about a minute ago. And also the studies that I've done in IL-10 suggest that it might be useful to evaluate the induction of IL-10 as a safety factor for adjuvants that might be given to humans.

And finally, just to very briefly say I do have a third project that I've been working on, which is the study of proinflammatory cytokine release, particularly, IL-6, IL-1 and TNF, by immune globulins, and by what mechanisms this may occur. And I've been able to develop an in vitro model which demonstrates that many immune globulins from different manufacturers can reliably cause release of TNF by human cells, and I'm very interested in finding out how this works.

The final project that I have just begun to begin is one where I would like to look at the induction of tolerance in prevention of Factor VIII inhibitor antibodies in hemophiliac mice which we have just gotten and we are breeding to be able to address this in a mouse model.

So that's a summary of the research projects that I've been working on, and I think you'll find as an add to this package that we have a list of our recent publications.

DR. HOLLINGER: Thank you, Dr. Scott.

Any questions of Dr. Scott?

[No response.]

DR. HOLLINGER: If not, that really concludes the open public portion of this review, and we are now going into closed committee deliberations, so I'll have Linda tell us what we have to do at this point.

DR. SMALLWOOD: For this particular session, only those members of the committee that are permitted to participate in a closed session and the members of the FDA with their identifying identification are permitted to remain here in the room. We would ask those other individuals, that we thank you for your participation, and if you could move swiftly and quietly, we can proceed. Thank you.

[Whereupon, at 2:45 p.m., the Open session was adjourned.]

CERTIFICATE

I, **ELIZABETH L. WASSERMAN,** the Official Court Reporter for Miller Reporting Company, Inc., hereby certify that I recorded the foregoing proceedings; that the proceedings have been reduced to typewriting by me, or under my direction and that the foregoing transcript is a correct and accurate record of the proceedings to the best of my knowledge, ability and belief.

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