

THE HEMOPHILIA BULLETIN

August, second issue of 2005. Carol K. Kasper, M.D., Orthopaedic Hospital, 2400 S. Flower St., Los Angeles, CA 90007; carolkasper@hotmail.com. This publication may be copied for educational purposes.

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(for June FDA meeting report, see page 6.)

ISTH in Sydney, August

Such glorious weather, sunny but crisp, with clear blue skies! Such a beautiful venue, on a sparkling harbor! Such great social events, a fascinating Verdi's "Nabucco" at the famous opera house on the classic end, a duet of trumpet and didgeridoo on the popular end, just to mention my favorites. Thank you, Australia!

Gene therapy for hemophilia is progressing mostly in studies in animal models. As you recall, delivery of the factor IX gene in an adeno-associated viral vector to the liver, through the hepatic artery, resulted, in dogs, with elevation of plasma factor IX levels that have persisted now for several years. In humans, the same system resulted in short-term expression of factor IX and a rise in liver enzymes. The cause of that phenomenon now has been pin-pointed, said Kathy High, as an immune reaction to a part of the AAV virus that is highly conserved among AAV serotypes. Finding another serotype is not likely to help for humans, who, unlike dogs, are highly likely to have been infected with AAV previously. No antibody to factor IX had been formed. **The temporary use of immunosuppressive drugs for liver transduction** is a possible future avenue.

The same viral vector construct also was effective when injected into **skeletal muscle of dogs and of humans**. Factor IX was expressed long-term and entered the plasma, but the levels were inadequate. Myoblasts were transfected only in the immediate area of the injections. Transfecting more myoblasts would require an unreasonable number of local injections. How else could more myoblasts be transfected? By **local arterial perfusion**, either isolated limb perfusion through the femoral artery using histamine to increase vessel permeability, or, anterograde lower limb perfusion through the saphenous vein under tourniquet, which does not require use of a vasoactive drug. The latter approach has been used in dogs. In the first three dogs, plasma factor IX levels of about 5 to about 20% have been sustained for 150 to 250 days so far.

Investigators from Beijing, China, have used an AAV-factor IX construct to **transduce gut endothelial cells**, which are rapidly replicating, **by means of colonic infusion**, that is, an enema, in mice. Plasma factor IX levels in 16 mice were sustained at levels between 1.5 to 2.5 % for 35 days so far.

An interesting approach to **hemophilia A gene therapy** was reported from Bob Montgomery's laboratory. In mice, bone marrow cells were transduced using a lentiviral vector with a platelet-specific promoter and a B-domain deleted factor VIII (FVIII) gene. **F VIII was expressed long-term in platelets**. Treated mice survived the hemostatic challenge of tail-clipping. The FVIII-containing platelets of the transduced mice were transfused into hemophilia A mice, who then survived tail-clipping. Bone-marrow transplants from the transduced mice to hemophilia A mice also allowed them to survive tail-clipping, all without any plasma factor VIII. When mice with FVIII inhibitors were transduced to express FVIII in platelets, they also survived tail-vein clipping. This approach has the **potential to offer a solution to pa-**

tients with persistent inhibitors to factor VIII. Factor VIII-rich platelets interact in hemostasis, on-site, despite the presence of such inhibitors in the plasma.

Another approach to manage persistent inhibitors is **transduction of liver cells with an AAV vector expressing factor VIIa**. Hemophilic mice so treated have decreased tail clipping bleeding, said Kathy High. Dogs are now being studied.

Von Willebrand disease got a lot of attention, with updates from the large European and Canadian studies of type 1 VWD. Evan Sadler offered a **new definition**: "**Von Willebrand disease refers to bleeding disorders caused by inherited defects in the concentration, structure or function of von Willebrand factor**" **independent of which gene, or genes, may be involved**. In other words, a mutation in the VWF gene itself is no longer required, as was stated in the 1994 definitions. **Type 1 VWD** is characterized by the following: (1) a decrease in plasma VWF, (2) proportional decreases in factor VIII, VWF:Ag and VWF:RCo, (3) no "significant" decrease in high molecular weight multimers, (4) subunits of VWF may or may not be mutant (multimer bands may or may not show abnormal proteolysis patterns, i.e. sub-bands). Type 1 patients typically respond well to DDAVP.

In the multicenter European study of 154 families with type 1 VWD, a **score for severity of bleeding symptoms** was devised. A negative number reflected absence of bleeding during hemostatic challenges. Giancarlo Castaman reported that in normal control subjects the mean score was -1, in unaffected family members it was 0, in affected family members it was 5 and in index cases it was 9. High bleeding scores were correlated with lower levels of VWF:Ag and of VWF:RCo. **The bleeding score was a better predictor of bleeding after surgery or tooth extraction than was the VWF:RCo level**.

Jeroen Eikenboom reported on linkage analysis in the same study, using three gene markers. **Linkage of the VWD phenotype to the same VWF gene within a family was confirmed in 70% of families**. Some families had complete co-segregation, that is, the phenotype of VWD was expressed in everyone with the implicated gene. Other families had incomplete co-segregation, that is, some persons with the implicated gene did not have VWD (incomplete penetrance) and/or some persons with a VWD phenotype did not

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Have the same genotype (phenocopy). The likelihood of complete co-segregation was related to the VWF:Ag level of the index case, as follows:

VWF:Ag in Index case,%	Complete Co-segregation	Incomplete Co-segregation	Odds ratio
0-15	15 families	6 families	1
16-30	18	9	0.73
31-45	18	10	0.67
>45	12	18	0.24

In the Canadian study of type 1 VWD, David Lillicrap found that linkage could be demonstrated in 61% of the 92 families in which a gene mutation was identified and in 39% of the 58 families in which no mutation was identified. (To me, that means either that mutations exist that haven't been found, or, a bleeding phenotype with somewhat low VWF is prevalent.)

Anne Goodeve, reporting for the European study, found one or more mutations in 108 (70%) index cases of type 1 VWD and none in 45 (30%) other index cases. The mean level of VWF:RCo in index cases with mutations was 27.9% and in those without mutations was 54.3%. Missense mutations predominated (80%), and, of these, 40% involved loss or gain of a cysteine nucleotide. Mutations were found throughout the gene but more than 40% were in the A domain.

Ian Peake looked at the 154 index cases in Europe and selected a subset of 103 patients with normal multimer structure as defined by the gold-standard method of Ulrich Budde. Mutations could be found in only 60 of these normal-multimer patients. The cohort with (mildly) abnormal multimers had lower levels of VWF:Ag, VWF:RCo and VWF:CB than the cohort with normal multimers. Thus, **the presence of mildly-abnormal multimers correlates with abnormal VWF function**. Mutations could be found in 94% of the abnormal multimer group and in 58% of the normal multimer group. Families with abnormal multimers are more likely to show linkage. In the normal multimer group without mutations, non-linkage was more common than linkage.

In the group with normal multimers, families with mutations and with linkage have the lowest levels of VWF, whereas those with mutations but without linkage, or those with no mutations but with linkage, are less likely to have very low levels of VWF. **More than 90% of cases with no mutation, no linkage and normal multimers have blood group O, and may not have VWD at all.**

Prof. Peake thus distinguishes three groups in this study of supposed type 1 VWD, as follows:

Group	Number of index cases	Multimers	Ratio, VWF:RCo/VWF:Ag	Mutations
1	49 (32%)	Abnormal	< 0.7	Usually found
2	60 (40%)	Normal	>0.7	Found
3	43 (28%)	Normal	>0.7	Not found

He proposes that cases in group 2 obviously do have type 1 VWD, those in group 1 have VWD but perhaps not according to previous definitions of type 1, and those in group 3 may or may not have VWD at all. He warns that few laboratories including his own can distinguish mildly-abnormal multimer patterns as well as that of Ulrich Budde, and that finding an abnormal ratio of VWF:RCo and VWF:Ag depends on assay precision. Because of these difficulties with tests, group 1 is likely to remain within the scope of "type 1" VWD. Prof. Peake also feels that **a search for a causative mutation should be performed in patients who appear to have type 1 VWD, and, if one is found, the diagnosis of VWD can be firm**. He believes that the mutations found so far are not just polymorphisms.

Stefan Lethagen described a group of 77 patients with type 1 VWD. Of these, 54 had identified mutations, for a total of 41 different mutations. One mutation was found in 41 cases, two in 11 cases, three in two cases. They were distributed throughout the gene but found most heavily in the D' to D3 region of the gene and the A' to A3 region, and also in the D4-CK region. The 51 patients with mutations in D1-2, D'-D3, and A1-A3 had relatively low basal levels of VWF, compared to the 10 patients with mutations in D4-CK or the 23 in whom no mutation could be found. The mutation was related to the degree of response to DDAVP. After DDAVP, ten-fold increases in VWF:RCo were seen in 15 patients, of whom five had the C1130F mutation, six had an R1205 mutation and four had other mutations. This group also had a relatively rapid decline in VWF:RCo after two hours. Patients with mutations in D'-D3 domains had relatively low VWF:RCo baselines, high peak levels and rapid half-lives. Patients with mutations in D4-CK had higher baseline levels and high peak levels and they sustained good plasma VWF levels for a relatively long time in all but one instance.

Dr. Lethagen defined "complete response" to DDAVP as a rise of VWF:RCo and FVIII to more than 50% and a "partial response" a rise to a level less than 50% but at least three times the baseline. Of his type 1 patients, 82% had a complete response and 13% a partial response. **Response to DDAVP relative to location of the mutation was as follows:**

Domain of mutation	Complete response	Partial response	No response
D1-D2	100%	0	0
D'-D3	84%	16%	0
A1-A3	43%	43%	14%
D4-CK	91%	0	9%
No mutation found	95%	5%	0

The above result makes me wonder whether the supposed type 1 VWD patients with mutations in the A1-A3 domain (the locus of type 2 mutations) also are likely to have mildly-abnormal multimers and have, in fact, mild type 2 VWD. That correlation has not yet been reported. I await the next SSC meeting.

How does proteolysis contribute to the VWD phenotype? Sandra Haberichter (from Dr Montgomery's laboratory) described the survival of VWF:Ag and of its propeptide (pp). The nor-

mal ratio of pp to VWF:Ag in plasma is expected to be about 1.0 and was measured at 1.13 in normal subjects in her lab. In 20 patients with type 1 VWD, the mean ratio of pp/Ag was 3.7 (range up to 11.5). After DDAVP, pp rose in all patients and VWF:Ag rose in most. The mean half-life of VWF:Ag after DDAVP was 7.8 hours (range, 1.8-33) and of pp was 4.1 hours (range, 1.6-7.2). **A high pp/VWF:Ag ratio correlated with a high response to DDAVP and also with a rapid half-life.** Dr. Haberichter hypothesized that **rapid proteolysis of VWF was a major cause of low VWF levels** in some patients with type 1 VWD

Alessandra Casonato also studied the half-life of VWF after it peaked in response to DDAVP infusion. The shortest half-life was seen in the Vicenza variant. The half-life in type 2 variants was shorter than in type 1, which was shorter than in normal persons. Thus, there's an **inverse correlation between VWF clearance and plasma VWF levels.** In normal persons, the half-life is 20% shorter in blood group O individuals than in those of other blood groups. (I presume that a high baseline pp level, as well as the presence of extra-large multimers, suggest hyper-production of VWF in response to extra-rapid proteolysis, just as the presence of reticulocytes in the circulation suggests extra hemolysis.)

Long-term prophylaxis in VWD, reviewed by Thomas Abshire from U.S. statistics, is being carried out in 0.2% of 4040 type 1 patients, 1.8% of 998 type 2 patients and 23.9% of 305 type 3 patients. The most common symptom for which prophylaxis is given is joint bleeding, followed by oral/nasal bleeding, then by gastrointestinal bleeding, and then by menorrhagia. One protocol being used consists of a dose of FVIII-VWF concentrate once weekly, at 50 VWF:RCO units/kg, and if that is insufficient, escalating to twice or thrice weekly. For menses, that dose is given the first day, and, if one dose does not suffice, then it is repeated on subsequent days.

Dr. Abshire pointed out that **range of motion loss in type 3 VWD in the USA. is about equivalent to that seen in patients with moderate classic hemophilia.** A target joint is seen in 18% of type 3 patients, with the ankle being the most-affected joint. Among adults, 17.6% use a cane intermittently, 5.7% use a wheelchair intermittently and 30% must restrict work activities. He referred to a study of 1234 VWD patients in Italy in which 70% of those with type 3 had joint bleeding, and to a series from Iran in which 37% of type 3 patients had joint bleeding.

Stefan Lethagen reported that **37 VWD patients in Sweden** (20 female, 17 male) **are on long-term prophylaxis**, including 28 with type 3, 3 with type 2B, 3 with type 2A and three with type 1 VWD. The most common reasons for prophylaxis were as follows: both nose and mouth bleeding 29%, both nose and joint bleeding 27%, joint bleeding only 22%, menorrhagia 8%, gastrointestinal bleeding 11%. In children, the predominant indication is epistaxis, whereas in adults, it's joint bleeding. He noted that patients who started prophylaxis young, for nosebleeds, also didn't get joint bleeding, but, those who started as teen-agers or adults for joint bleeding already had suffered joint deterioration. Thus, **early prophylaxis may be ideal in type 3 VWD.**

Prophylaxis started "late" in severe hemophilia may still be worthwhile, says Kathelijin Fisher of The Netherlands. She looked at 61 patients born between 1944 and 1974 who started prophylaxis between the ages of 17 and 52 years, median 26 years. She distin-

guished two groups, those 25 patients who had been on short-term prophylaxis, median 1.3 years, mostly commonly for rehabilitation, frequent joint bleeding or special events, versus those 36 patients on long-term prophylaxis, median 18 years, most often for frequent joint bleeding. Long-term prophylaxis reduced the frequency of hemorrhages by about two-thirds in these patients. Progression of joint disease was measured by comparing Petterson scores from one examination to the next, over years. **Long-term prophylaxis slowed the progression of joint disease as measured by sequential Petterson scores**, but short term prophylaxis did not.

Can we predict which patients with hemophilia will get inhibitors? Gil White reviewed the interaction of immune mechanisms with genetic mutations. A person's major histocompatibility complex (MHC) molecules bind specific peptide sequences and present those peptides to CD4T lymphocytes, leading to B cell stimulation and eventually to antibodies. **MHC molecules are selective about which peptides they recognize. A given person with hemophilia may have MHC molecules that do not recognize his mutant FVIII or foreign (transfused) FVIII molecules.** Or, they may recognize some but not all FVIII molecules. Dr. White also pointed out that we have been presuming that certain nonsense mutations are null, that is, that no FVIII molecule is formed, but the phenomenon of exon-skipping may result in a non-null phenotype. The exon with a mutation may be skipped and the resulting mutant FVIII molecule may contain the message from the exons both before and after the skipped exon. Thus, the fetus is exposed to a fairly complete molecule, as is the case with missense mutations. We do not yet know how many mutations may behave this way. His explanation left me feeling that we are trying to study small groups of patients who are genetically very heterogeneous, in ways we already know something about, never mind all the ways we don't yet know about. It's no wonder that the results of one small study may not match the results of another similar small study.

Is infusion of factor VIII in the first few months of the life of a baby with hemophilia A more likely to provoke inhibitor formation than exposure starting later? A report from Spain (Lorenzo et alia, Br J Haematol 2001, 113:600) that exposure at an early age was associated with a higher inhibitor incidence has led to other studies. Marijke van den Berg analyzed detailed data on patients with severe hemophilia A in **all the PUP trials of recombinant FVIII concentrates done to date.** She looked at 236 evaluable patients, all of whom had at least 50 exposure-days (EDs). She looked only at "relevant" inhibitors, that is, measurement of at least 0.6 Bethesda units (BU) on at least two occasions, together with a decreased *in vivo* recovery. Among the 236 evaluable patients, 67 (28.4%) developed relevant inhibitors, 44 of which were more than 5 BU. The median age at inhibitor development was 16 months at a median of 10 ED. Inhibitors developed in 12/26 (46%) of PUPs with a family history of inhibitors, and 23/88 (26%) of those without such a history. Inhibitors developed in 50/196 (25.5%) white patients, in 11/22 (50%) black patients, in 6/11 (55%) Hispanic patients, and in none of 7 patients of other racial/ethnic groups.

Dr. Van den Berg could not substantiate an effect of exposure at an early age to inhibitor development. Inhibitors developed in 34% of patients first exposed before age 6 months, in 25% of those first exposed at age 6-12 months, and 28% of those

first exposed after 12 months of age, which figures were not significantly different. **Intensity of exposure did appear to matter.** If the child was more than 36 months old before 50 ED were achieved, risk of inhibitor formation was the lowest, but if 50 ED were achieved within 18-36 months, the risk of inhibitor formation was 2.7 times greater. If 50 ED were achieved in less than 18 months, the risk of inhibitor formation was 5.5 times that of the patients who did not achieve 50 ED until after 36 months of age. Babies who had had intensive periods of therapy for three or more days had 1.6 times as many inhibitors as those who had not had any intensive therapy ($p=0.05$). Babies who had undergone a surgical operation were more than twice as likely to develop inhibitors as those who had not. She also looked at the cumulative dose in the 5 ED before inhibitor development in patients who developed inhibitors and in the same ED in patients who did not develop inhibitors; those whose cumulative dosage over the five ED was >250 U/kg had 1.9 times the chance of developing an inhibitor as those whose cumulative dosage was <175 U/kg. She was not able to judge the effect of "early" prophylaxis because it was started after at least 10 ED, thus, many inhibitors already had emerged. She reported no data on mutations.

Elena Santagostino has a carefully-analyzed series of patients from Italy. **Younger age at first exposure to FVIII was associated with a higher rate of inhibitors but when the series was adjusted for genetic background, no association with young age remained.** She has data, on small numbers of patients, suggesting that prophylaxis tends to prevent subsequent inhibitor formation.

Elizabeth Chalmers looked at the **UK experience** retrospectively and could find **no difference in inhibitor development related to age of first FVIII exposure within the first 18 months of age.** When she looked just at those patients with intron-22 inversion, she still found no difference. Although the incidence of inhibitors was higher in non-white vs. whites, and in those with a positive family history of inhibitors vs. those without, the differences were not significant. The incidence of inhibitors was 27% among those 172 patients treated with recombinant FVIII at first exposure vs. 14% among those 132 treated with plasma-derived FVIII at first exposure, a highly-significant difference, but, **the incidence of high-titer inhibitors in the recombinant group, 15%, was NOT significantly different from that in the plasma-derived group, 10%.** The mutation was known in 231 patients, and was relatively minor (missense, small deletion or insertion, splice-site) in 90 patients, of whom 13% had inhibitors of any level and 7% had high-titer inhibitors. Relatively major mutations (large deletions, inversion, stop codons) were present in 141 patients, of whom 29% had inhibitors of any level and 18% had high-titer inhibitors, differences that were statistically significant vs. the minor mutation group.

Samantha Gouw reported on a co-operative European study ("CANAL") of the issue of age at first exposure. Patients were born between 1990 and 2000, with $<2\%$ FVIII (a generous allowance because of assay problems), treated on at least 50 ED and treated at a hemophilia center from the first exposure to FVIII onwards. "Relevant" inhibitors were defined as Dr van den Berg described. Complete data are available on 301 patients, of whom 87 developed inhibitors (28.9%), 69 high-titer (>5 BU). The median age at inhibitor development was 15 months, at a median 14 ED. **She found a highly-statistically-significant correlation between age at first exposure and inhibitor incidence:** 41% if first exposure was at less than one month of age, 30% if at 1-6 months, 23% if at 6-12 months, 20% if at

12-18 months, and 17% if at more than 18 months. The probability of the inhibitor being high-titer also was higher if the patients were exposed at lower ages. The rates of inhibitor formation have not been adjusted for genetic factors. Overall, inhibitor rates with (supposed) null mutations were 32% and with lesser mutations were 12%.

She also looked at **the effect of intensive treatment.** If intensive treatment was given for more than five consecutive days at the first exposure to FVIII, then 55% of patients developed inhibitors; if intensive treatment was given for 3-5 consecutive days at first exposure, then 21% developed inhibitors; if there was no intensive treatment at first exposure, then 19% developed inhibitors. She also looked at the total amount of FVIII given in the five ED before inhibitor development vs. the same five ED in non-inhibitor patients, and found that **the risk of inhibitor increased with the dose** in units/kg, being 2.8 times higher in those receiving a cumulative dose of >250 units/kg vs. those receiving <175 units/kg.

What do we make of all this? **In babies, early treatment and intensive treatment may be associated with a greater likelihood of inhibitor development. Not all studies support this claim, on the other hand, no studies suggest that early treatment is protective.** Early, intensive treatment may be given for urgent medical reasons. **Can we avoid early treatment?** Georges Rivard of Montreal tried treating babies with activated factor VII for hemorrhages in order to delay the first dose of FVIII, but, slightly more than half had to switch to FVIII for better efficacy. He no longer advocates that strategy. We can try to avoid elective surgery such as circumcision, we can avoid over-dosing, which happens so easily, given that it's hard to get small-quantity vials of FVIII, and the more easily-available sizes contain far more units than a small baby needs. When I'm frustrated with too-high-quantity vials, perhaps the only size available in a developing country, and don't want to waste the precious material, I have sometimes advised doctors to reconstitute the vial, withdraw the amount needed to treat a hemorrhage, and freeze the remainder for later use. Sometimes one just has to do what's practical.

Rolf Ljung mentioned that **in Malmo, the induction of immune tolerance protocol has been modified** to use mycophenolate mofetil (CellCept®) instead of cyclophosphamide, to which many clinicians objected, together with daily high dose dexamethasone as immunosuppressive agents. He also said that for children, he prefers to use the Bonn protocol.

Angiola Rocino studied **induction of immune tolerance using recombinant FVIII**, in Milan and Naples. Of the 26 patients, 22 were newly-diagnosed. Recombinant FVIII had been the product used in 21 patients, but five others, primarily those with long-standing inhibitors, previously had used plasma-derived FVIII. All had baseline inhibitor levels under 10 BU or waited until the inhibitor fell to that level. Patients who had used rFVIII before inhibitor development used the same brand for tolerance induction. The overall rate of achieving complete tolerance (normal *in vivo* recovery and half-life) was 73%, partial success was achieved in 8%, and 19% failed. **(This rate of success is similar to ours in the 1980's using plasma-derived FVIII.)** Among 17 patients with intron 22 inversion, 12 succeeded in achieving tolerance, two had partial success and three failed. Among five patients with nonsense mutations, 4

achieved tolerance and one failed. A patient with intron 1 inversion achieved tolerance, another with a large deletion failed. (I give details because I'm interested in the issue of gene mutation and the probability of successful tolerance induction. If some genotypes are relatively resistant, we ought to know about it.)

Alessandro Gringeri reported on a prospective trial of **tolerance induction with a plasma-derived FVIII-VWF concentrate**, the Spanish Fanhdi® (similar to Alphanate®), **in patients with high-responding inhibitors who had two or more risk factors for resistance to tolerance**, namely, age over six years, delay of starting tolerance for more than two years after diagnosis of inhibitor, an historical peak inhibitor level of >200 BU, an inhibitor level at outset of >10 BU, or previously failure of tolerance induction carried out for at least 12 months (four patients, three who had been on rFVIII, one on pdFVIII). To date, they have achieved complete tolerance in 10/17 (56%) patients, partial in five more, and two patients are still being treated. Three of four patients who "failed" previous attempts at immune tolerance did achieve tolerance on this protocol. (I don't consider the first two supposed risk factors to be truly risky, and, as Dr. Gringeri says, one really needs a prospective, randomized control trial comparing pdFVIII-VWF to rFVIII. I almost despair of controlling all the variables in such a trial.)

Uri Seligsohn has used **low doses of FEIBA® and NovoSeven (rVIIa)®, together**, for patients resistant to rVIIa alone, for >250 treatments, with a good rate of success and much lower cost.

There are some other **new ideas for the treatment of bleeding inhibitor patients**. Kazuhiko Tomokiyo reported development in Japan of a new **concentrate of factor VIIa and factor X**, more potent than VIIa alone in generation of thrombin, for potential use in patients with inhibitors. Saulius Butenas, of Kenneth Mann's lab, synthesized **peptides that inhibit activated protein C and tissue factor pathway inhibitor**, another potential alternative treatment for bleeding inhibitor patients.

Rainer Seitz reported that a **PCC** (used domestically in one European country) **associated with thrombotic events contained more than three units of prothrombin per unit of factor IX**, a ratio higher than that found in other PCCs. Spiking non-thrombogenic PCCs with prothrombin led to excess thrombin generation. Levels of prothrombin in PCC should be controlled.

Guenter Auerswald described the **treatment of patients with factor X deficiency**. CNS hemorrhage occurs in >20%, especially in early life. Joint bleeding also is common, especially in patients with <1% factor X. He has treated 34 patients for bleeding or for surgery with **Faktor IX HS®** made by ZLB Behring, which **consists of factor IX**, about 1200 U/vial, and **with factor X**, about 800 units/vial, but **without prothrombin or factor VII**. His dose for hemorrhages is 10-20 factor X units/kg, and, for surgery, 15-20 factor X units/kg as a loading dose and 10-15 factor X units/kg post-operatively, once daily. For CNS hemorrhages, he gives the product twice daily. For prophylaxis, he gives twice or thrice weekly doses.

Uri Seligsohn reported that **the probability of surgical bleeding in patients with severe factor XI deficiency was strongly related to the site of surgery**. Excessive bleeding in patients who had

not received plasma products occurred in 55/110 oral and dental surgeries, in 29/48 tonsillectomies or nasal surgeries or prostatectomies (areas with a lot of fibrinolysis going on), but only 8 of 121 operations in other areas. He tailors his treatment to the surgical site. He uses tranexamic acid alone for tooth extractions, tranexamic acid plus factor XI coverage for seven days (seeking a trough factor XI level of 45%) for surgeries in the fibrinolysis-heavy areas, and, for the least risky areas, he advocates use of fibrin glue.

Sam Schulman prospectively studied **whether a low level of plasminogen activator inhibitor (PAI) was a risk factor for bleeding**. For each patient coming in for study because of a history of excessive bleeding, he found an age and sex-matched normal control, often the patient's friend. Although levels of PAI were more likely to be below one unit/ml in patients (23%) vs. controls (10%), a low PAI level did not seem to aggravate symptoms in patients with other hemostatic defects. **He feels that PAI levels are unimportant**. He mentioned that higher body mass index was correlated with higher PAI levels. Connie Miller commented that there is great variation among commercial kits and poor sensitivity at low levels.

Rezan Kadir studied **women with VWD** and found that 74% had heavy menstrual blood loss as indicated by a pictorial bleeding assessment chart (PBAC) score of >100, 25% had menses lasting more than eight days, and 64% were iron-deficient (vs. 34% of normal females). Hysterectomies had been performed in 23% of women with type 2 or 3 VWD and in 26% of women with type 1 VWD, vs. 9% of normal women. She finds that DDAVP alone often fails to control hypermenorrhea but the combination of DDAVP and tranexamic acid is effective. Endometrial ablation can be performed by several methods, but control of menses often is only short-term and hysterectomies are eventually performed. **The prevalence of other gynecologic disorders was higher in women with VWD** (#102) vs. normal women (#88), as follows: ovarian cyst, 52% vs 22%; endometriosis 30% vs. 13%, fibroids 32% vs. 17%, endometrial hyperplasia 10% vs. 1%. Dr. Kadir prefers to test for VWF and FVIII in the first week of the menstrual cycle, when levels are lowest, especially in women not on oral contraceptives.

Connie Miller is studying 172 women with a history of menorrhagia and a PBAC score >100 who have any coagulation test abnormality. The efficacy of oral contraceptives versus DDAVP plus tranexamic acid in controlling menses will be evaluated. Definite VWD has been diagnosed in 5.2%, possible VWD in 6.2%, definite deficiencies of PAI in four, fibrinogen in two, and FVIII or factor XI in one patient each. Of her 172 women, 68% are white, 23% are black, and the remainder are from other racial groups. **VWD is diagnosed three times more often in whites than in blacks but platelet aggregation abnormalities are twice as common in blacks than whites**. Platelet aggregation must be studied in fresh platelet-rich plasma, that is, on-site, soon after phlebotomy. American systems of re-imbursalment for laboratory tests discourage small, high-quality laboratories and favoring off-site bulk "reference" laboratories. You can't mail in platelet aggregation. Let's hear some support for small quality labs!

June meetings: PPTA, FDA

The Plasma Products Therapeutics Association, representing companies that produce biological products from human plasma, met near Washington, D.C., just prior to an FDA workshop on “**Biological Therapeutics for Rare Plasma Protein Disorders**”. The two meetings covered much of the same ground.

In the past quarter-century, prescription drugs, including biologicals, taken at home, have become an ever-larger share of treatment modalities and of health-care costs, replacing much hospital and doctor’s office treatment. Payment for such drugs, however, was not designed as part of US payer systems, that is, insurance and government programs. It has been difficult to add such drugs to the existing systems, as witness the clumsy recent extension of Medi-Care. As payers try to limit spending, more and more costs are being shifted onto the individual consumer in the form of co-payments or restriction of choice among drugs or pharmacies or home-care providers, all of which are protested by patient groups.

I sat next to a group of Canadians, who were bowled over by an exposition of complex new Medi-Care re-imbursment rules. They said, thank God we’re Canadians, our national health system isn’t perfect, but we have good access, no worries over payment, and no such labyrinthine rules. They also felt that American consumers’ pleas for payment and for drug choice were made less effective when they also pleaded, with equal urgency, for nurses coming to the home to administer I.V. drugs, a seeming luxury. I agree. At times, that’s appropriate, but it’s also over-used.

Plasma products constitute a 6-billion-dollar-a-year industry, small compared to the 300-billion-dollar industry for chemically-derived and other non-blood-derived drugs, (pills, antibiotics etc.) The most expensive part of plasma product production, about 40%, is the raw material, plasma, which now costs over \$100 per liter. Tests considered necessary for viral safety (serology and NAT, nucleic acid tests) are growing in number and are expensive. In the chemical-derived pharma industry, the biggest expense, some 30%, is marketing.

Plasma-derived biologicals serve relatively small patient groups. A commercial company must be able to make a profit on a product, and it’s hard to do so with a drug for a rare disorder. **The most daunting aspect of development of such a drug, said industry personnel, is the clinical trial.** It may be necessary to involve many different treatment centers in order to enroll enough patients, which means that many different Institutional Review Boards must give approval, a slow and sometimes expensive process. Today, it’s common for IRBs to demand payment to review any proposal involving a pharmaceutical company. Keith Hoots pleaded for a national IRB in the USA.

Pharmacokinetic studies are relatively easy, but proof of efficacy by means of some clinical end-point may be a challenge. A direct end-point might be the efficacy of a clotting factor to control bleeding in a surgical procedure, or, efficacy of an anti-thrombotic factor (such as anti-thrombin or protein C) to prevent thrombosis in a surgical procedure. Either of these end-points require comparison to another treatment method, or, perhaps, with the FDA’s consent, to historical control data. Surrogate endpoints such as plasma factor levels after infusion may not suffice for the FDA. Plasma products not related to

coagulation, such as C-1 esterase inhibitor for hereditary angioedema and alpha galactosidase for Fabry’s disease, have posed more difficult end-point problems.

Both the EMEA (the regulatory agency in the European Union) and the FDA have mechanisms (called conditional or accelerated licensing) to give **rapid approval** to an orphan drug with the understanding that further clinical trials (phase 3, not phase 4) will continue, but it’s those further trials that industry dreads. Don’t make them go on forever, they said, don’t make them too daunting, do accept foreign data. The FDA says, companies tend to slack off after licensure. The EMEA has an additional, easier track for super-orphan drugs, intended for very few patients, a track that does not have an FDA parallel. **Overall, the EMEA is more accommodating**

The FDA steers between Scylla and Charybdis. On the one hand, they are asked to approve new drugs quickly, don’t make it too hard, especially for new drugs for dreaded infections like HIV, or for rare disorders. On the other hand, if there’s an unexpected side-effect, as with Vioxx®, everybody’s down on them, politicians rant and rave. And the USA is litigious. This year’s definition of a split-second was the interval between the news of cardiac side-effects of Vioxx® and the announcement of a class-action lawsuit by an eager attorney.

So what are the prospects for orphan coagulation product concentrates? What are we dealing with? Dr. Mike Soucie of the CDC reported the following **numbers of patients with rare deficiencies of clotting factors in the USA**, as reported by hemophilia treatment centers; (his numbers may include some heterozygotes):

	Number of patients
Factor II	64
Factor V	149
Factor VII	527
Factor X	86
Factor XI	539
Factor XIII	106

Are plasma-derived products likely to serve these needs? Dr. Don Baker of Baxter said, recovery and purification techniques have improved a great deal; **manufacture of therapeutics present at the microgram-per-ml level can be commercially viable.** (That’s just manufacture; it’s the expensive clinical trials that may not make sense, for commercial endeavors.) Development of a plasma-derived product may hindered by fear that someone else will develop a recombinant that will be deemed safer, and supercede the plasma-derived product for orphan drug status.

The largest group of patients with a rare coagulation factor deficiency requiring fairly frequent replacement therapy is those with factor VII deficiency. An opportunity was missed when Baxter acquired Immuno, which had a plasma-derived factor VII concentrate, viral-inactivated by vapor heat, produced in an FDA-compliant fractionation plant. That concentrate is still made, but not for the USA. BioProducts Laboratory in the UK and LFB in France

also make factor VII concentrates. The US market has been left to recombinant activated factor VII, NovoSeven®, but I'd prefer a non-activated product for congenitally-deficient patients.

For another major group, those with **factor XI deficiency**, there's not much immediate hope in the USA. BioProducts Laboratory in England, established originally by the National Health Service but since privatized, makes a factor XI concentrate. The company's management has decided not to make it available in any way in the USA because Americans are too litigious. **Both the British and the French (LFB) factor XI concentrates can be thrombogenic if dosage recommendations are exceeded.**

LFB (Laboratoire Francais du Fractionnement et les Biotechnologies) is still part of the French national health system, entirely supported by the government. It does export its products when they are in excess of French needs. **Its long-existing factor VII concentrate and factor XI concentrate are "grandfathered" into European approval.** There may have been no formal studies of safety and efficacy in the past. Performing such studies for a US license would be daunting

There has never been a concentrate of factor V, and, no concentrate of factor X alone. Factor X is available only as one part of prothrombin complex, or, with factor IX in ZLB Behring's product.

A plasma-derived factor XIII concentrate (Fibrogammin P®) is made by ZLB Behring in Germany and exported but not to the USA.

Diane Nugent reported on a new **recombinant factor XIII concentrate**, made in yeast cells, by NovoNordisk. FXIII is composed of an A and a B subunit; the recombinant consists only of A subunits but a few patients do lack the B subunit. A major problem in studies of FXIII is the lack of a sensitive assay at low levels. She's using an ELISA method that's not ideal. Increases in clot strength and resistance to fibrinolysis can be demonstrated with thromboelastograms. To study the new product, patients were brought from wherever they lived to one center (Children's Hospital of Orange County, California), on two occasions, for initial studies in a clinical research inpatient unit. Further blood samples were obtained by a phlebotomist at the patients' homes. Doing studies at one center was efficient and cost-effective. Only one IRB was involved. The pharmacokinetic studies were completed in 4.5 months. It may take a very long time to demonstrate efficacy. It's interesting that this rare disorder was deemed worthy of drug development. Now that assays exist, however imperfect, it has been found that mild deficiencies, e.g. around 20%, may be associated with an increased rate of miscarriage. Other problems of mild deficiency may be identified. Uses for FXIII other than congenital deficiencies may be found.

The development of several other products, for conditions other than bleeding disorders, was instructive. Amy Shapiro described a rare and miserable condition, **plasminogen deficiency, which causes ligneous conjunctivitis** (a fibrous gluing together of the eyelids leading to blindness) or ligneous cervicitis (causing infertility) or gingivitis or other involvement of mucous membranes. **A fibrinolytic agent containing human plasminogen, Eminase®,** is licensed in the USA but no longer marketed here. It's available in Europe. The manufacturer decided that it was not feasible to update the biologics license application at the FDA when the market is so small. There is no con-

centrate of plasminogen alone. Dr. Shapiro has four affected patients. She showed a picture of a baby whose eyelids were sealed with crusty lesions, and then a picture of the same child after treatment with Eminase®. The end-point is good: it's visible and obvious. Whole plasma also can be used but does not work as well, a situation reminiscent of hemophilia.

Rainer Seitz described EMEA's marketing authorization under exceptional circumstances for **Ceprotrin®, a plasma-derived protein C** made by Baxter Bioscience. A robust efficacy study could not be conducted. Only 17 homozygous cases exist in an international database. Some double heterozygotes exist. Patients with a dominant, symptomatic heterozygous condition exist in up to one in 16,000 persons. Severely-affected newborns have purpura fulminans. Adults have skin necrosis when oral anticoagulation therapy with vitamin K antagonists is started. Pharmacokinetic data and acute safety data are available from eight symptomatic patients. Retrospective studies are underway among patients who were able to use the product under compassionate use rules. The product is **licensed for congenital deficiencies but also is being used off-label for acquired deficiencies**, for example, disseminated intravascular coagulation (DIC) in sepsis. Baxter's David Gelmont commented that **although a greater number of patients with acquired deficiency use the drug than do patients with congenital deficiency, the congenitally-deficient patients use a much larger total quantity of the product.**

GTC Biotherapeutics has made a **recombinant antithrombin, ATryn®, produced in the milk of transgenic goats.** (Hooray for transgenics!) Dick Scotland described the travails of its clinical trials in congenitally-deficient patients. Lack of any registry of such patients has slowed enrollment. He identified 82 patients in 55 centers in 14 countries and was able to enroll 14 patients (17% of known patients). For the EMEA in Europe, safety and efficacy data could be obtained for 12 patients, over 28 months, and licensure is expected soon. For the US product license application, he is trying to get historical data from medical charts on the efficacy and safety of plasma-derived antithrombin. He hopes to compare plasma-derived and recombinant antithrombin in 17 patients. The pharmacokinetics of the two types of product differ.

Genzyme developed an enzyme, **Fabrazyme®, for Fabry's disease**, an X-linked deficiency of alpha galactosidase that causes progressive accumulation of globotriaosylceramide in multiple tissues. It affects about 5000 patients world-wide **Their challenge was to choose an end-point.** Patients suffer from pain in early adulthood, peaking around age 20, after which it decreases, but pain is hard to quantify. They chose to study renal accumulation of globotriaosylceramide in renal biopsies before and after 20 weeks of therapy. Disappearance of the deposits on non-blinded therapy was impressive. A conditional license was granted by the FDA but further studies were demanded, using a placebo control. The EMEA approved the drug 18 months sooner than the FDA and would not permit placebo controlled trials. (As an aside, another company completed their product license application to the FDA for a similar drug within two weeks of the Genzyme application. The other company had used evaluation of pain as an endpoint, and a different dose. The other company was turned down by the FDA but licensed by the EMEA. My comment is, how orphan is this disorder, or how unprofitable, if two companies were chasing it?) it will be much more expensive to treat Fabry's disease than to treat se-

vere hemophilia. As we find ways to treat more and more chronic disorders, the total cost to society of medical care will go up. We have to plan for the long-term effects of our discoveries.

How can development of products for rare disorders be made more likely, in a capitalist system? Ideas included the following: (1) give exclusivity through patents and orphan drug status (which confers 7 years of exclusivity), (2) give grants and tax credits for clinical trials, (3) give assistance with protocol design (which the FDA is willing to do, to make sure it's adequate for them), (4) waive the application fee to the FDA (which normally is \$600,000), (5) have accelerated review so some product can be sold and bring in revenue while trials continue, (6) give Business Innovation Research and Small Business Technology Transfer grants, which are appropriate for some small companies, but, the industry folks say this is only useful in some instances. One attendee said, a grant of a million dollars over three years sounds like a lot, but it might constitute 20% of the cost of a small clinical trial.

Industry says, please simplify approval processes and harmonize with EMEA. Please allow surrogate endpoints. Clinical trials are difficult, it's useful to **have registries of patients with rare disorders**, so one can start to estimate the true number of persons affected and find patients for trials. The number of affected patients may be much larger than first anticipated. Robert Sandhaus said that only some 200 patients were known to be candidates for treatment when the first alpha-1-antitrypsin drug was developed in the 1980's, but some 100,000 persons are now identified in the USA. The number of known affected persons increased as test methods improved and as clinicians learned to recognize all the symptoms. Prevalence figures

For Primary Immune Deficiency also are moving upwards as public-awareness campaigns lead to diagnosis of less-obvious cases. In addition to the congenitally-deficient patients for whom the drug was developed originally, many more patients with acquired disorders are seen as candidates for its use.

Many rare disorders were represented at the FDA conference by organizations founded by affected persons or their relatives. Such groups lobby for drug development and organize registries of affected persons, and make their numbers and concerns known. Those registries also facilitate clinical trials. A recent issue of the magazine "The Scientist" (2005, vol 19: # 14, page 45) has an article by Ted Agres on "Venture Capital, with a Twist". He describes the **foundation of a nonprofit biotechnology company** by a man, Jamie Heywood, whose brother had amyotrophic lateral sclerosis. The company screened potential drugs for efficacy in mouse models and selected two drugs which are being further developed. Heywood said **"There are no orphan diseases, there are only decisions to go to clinical trials based on a risk/reward profile"**. According to Agres, "disease advocacy foundations are moving down the drug development pipeline, establishing clinical networks, sponsoring preclinical and clinical trials, and partnering with biotech and pharmaceutical companies. They have even established venture capital funds..." **Such foundations may help under-write the cost of clinical trials**, which were obviously the biggest concern to industry at the FDA meeting. Some of these organizations have a conceptual difficulty in moving from funding of research on the disorder to funding clinical trials. The disorder represented by the most business-like thinking is most likely to get its needed drug development — and licensure!

MEMORIAL

Prof. Takeshi Abe, colleague and friend, died in April. He had traveled widely in Europe between the world wars. I treasure his insightful essays on European customs from those travels. He appreciated the utility of exchange of ideas among scientists of all nations. He promoted visits of foreign scientists to Japan, and studies by young Japanese scientists in other countries. He brought modern hemophilia care to Japan. I miss his enthusiasm and energy!