CD103, CTLA4 and GITR in iDC-F8 treated mice. We observed a 60% and 61% reduction in the level of inhibitor titres from iDC-F8 mice compared with Neg-Control and iDC-Ctrl mice. Splenic CD4+ T-cell proliferation in response to FVIII stimulation in cells from iDC-F8 mice was suppressed by approximately 90% compared with proliferation of cells from Neg-Control and iDC-Ctrl mice. We also showed that pre-immunized mice treated with four infusions of mFVIII-Fc vector transduced iDCs had a reduction in their inhibitor titres by 54% ($P < 0.05$). No significant change in inhibitor titres were seen in untreated controls or mice given four doses of untransduced iDCs.

Summary: These data indicate that mFVIII gene transduced tolerogenic DCs are useful in decreasing inhibitory antibodies in haemophilic mice. More in vivo studies are in progress to confirm the antigen-specificity and durability of these effects. Our future studies will focus on isolating and characterizing the regulatory T cell populations induced by in vivo transgene of modified iDCs.

Immunogenetics of factor VIII inhibitor development

J. ANZIETZ, IDAN MENASCE, B. BARBARA KRONER, JAMES GOEDERT AND PHILIP ROSENBERG

NIH Clinical Center, Bethesda, MD, USA

Objective: The purpose of our study is to investigate the hypothesis that polymorphisms in immunoregulatory genes and the factor VIII gene are partly responsible for recognition of therapeutic factor VIII as foreign, thereby provoking inhibitor antibodies. As a critical first step in our study we have set out to determine the minimal number of regions on the factor VIII gene which might encode polymorphisms which could define the relevant DNA haplotypes seen in the Caucasian population (CEU) as shown in the HapMap project.

Methods: We searched the CEU dataset in the HapMap genetic database (release 23a, March 8, 2008) as a source for SNPs with potential to define haplotypes ‘tag SNPs’ in the genes for IL1-beta, IL4, IL10, IL13, interferon gamma, TNF-alpha, TGF-beta, zinc alpha-2-glycoprotein I and the complement factor VIII gene. These genes were selected based on their central role in regulation of the immune response, previously published correlations with factor VIII inhibitor risk, or results of studies of factor VIII immunogenetics in animals. Using the Haploview programme we identified tag SNPs in each gene, plus approximately 20 kb of DNA 5’ to 3’ to each gene to assess potential regulatory elements that might influence gene expression. Tag SNPs were picked for each gene at various levels of coverage, based on correlation coefficients ($r^2$) of $>0.8$, $>0.9$, and $>1.0$.

Summary: Our analysis indicates that the total number of tag SNPs required to study the nine genes of interest, with $r^2$ values of $>0.8$, $>0.9$, or $>1.0$, is on the order of 96, 138 and 146, respectively. Thus, a study of these proposed candidate genes is feasible with currently available SNP ascertainment based on multiplex PCR amplification/extension and MALDI-TOF analysis.

Conclusions: With approximately 150 SNPs (at $r^2 = 1.0$) we will be able to determine association between factor VIII inhibitor development and any of seven immunoregulatory genes and two plasma proteins, including the coagulation factor VIII gene. We have extracted genomic DNA from peripheral blood leukocytes obtained from >900 Caucasian haemophilia A patients who participated in the Multicenter Haemophilia Cohorts Studies I & II, and are being asked to consent to their association with risk for development of inhibitors to factor VIII in haemophilia A patients.

Recurrent of inhibitor after orthotopic liver transplantation in severe haemophilia A

SALLY STABLER, BRENDA RISKE, SUE GERAGHTY

University of Colorado at Denver and Health Sciences Center, Aurora, CO, USA; Mountain States Regional Hemophilia and Thrombosis Center, Aurora, CO, USA

Objective: Orthotopic liver transplantation (OLT) treats both hepatitis C-associated cirrhosis and haemophilia A with factor VIII activity increasing into the normal range in most patients. However, the results of OLT in haemophilia patients with inhibitors have been mixed. We report the follow-up factor VIII values after OLT in an inhibitor patent.

Methods: A middle-aged white male with severe haemophilia A, who had been on alphaone for >30 years for a high-responder factor VIII inhibitor underwent OLT. Factor VIII activity and inhibitor titres were followed over the next 9 months.

Results: The patient had received factor VIII 40 to 50 U/kg three times/week for many years, which suppressed inhibitor titres to <2 BU. Higher doses of factor had been used to support him through surgery with good results. At the time of OLT, his inhibitor titre was 0.78 BU. He was treated prior to transplant surgery with 116 u kg^{-1} with smaller doses every 2-4 h during monitoring of the operation. In the next 24 h he was required another 300 u kg^{-1} of factor VIII to maintain activity between 61-122%. On postop days 1 and 2 he received 46 and 35 u kg^{-1} to maintain mean activity of about 50%. Around Day 6 his requirement for factor VIII increased to 70 to 80 U kg^{-1} daily to maintain the same levels. He was treated for possible acute liver rejection with high dose Solumedrol and his requirements for factor VIII decreased so that it was discontinued on day 13 postop. Over the next month, his factor VIII activity increased from 57-91% and he had no bleeding complications. Immune suppression was achieved with tacrolimus, mycophenolate and prednisone, being discontinued three and a half months after OLT. After a peak of factor VIII activity at one month, his level decreased so that it was 52% and 37% at 4 and 6 months, respectively, despite the continuous use of immunesuppression for the OLT. Seven months after transplant, the patient underwent a procedure