181

ZETA GLOBIN GENE ORGANIZATION AND EXPRESSION. J. Zhao,* D.H.K. Chui and A.E. Felice. Hemoglobin Research Laboratory, Veterans Administration Medical Center, and Depts. of Cell and Molecular Biology and Pediatrics, Medical College of Georgia, Augusta, Ga., U.S.A., and Dept. of Pathology, McMaster University Medical Center, Hamilton, Canada.

Re-arrangements of the α and ζ globin genes are rather common in Black Americans. Two variants of the -3.7 Kb α globin gene deletion occur with a gene frequency of 0.18 while four variants of a ζ globin gene deletion occur in 3% of the population with or without α-thalassemia. Another type of ζ gene deletion occurs in the context of a [ζα]°-thalassemia in which all α and ζ globin genes are deleted. Further variability of ζ globin genes is due to dimorphism of the ζ1 or ψζ1 genes. Using SST I digestion and a ζ gene probe we find the ζ2-ζ1/ζ2-ψζ1 haplotypes are unequally distributed with only 8/180 chromosomes having a ψζ1 gene instead of the ζ1 gene. With this approach we also found one 7 year old Hb S homozygote who had a triplicated ζ gene haplotype, i.e. ζζζαα/ζζαα. The identification of these different haplotypes in the same population with or without associated α globin gene re-arrangements or hemolytic disease or both offers opportunities to evaluate the genetics and control of the embryonic ζ to α globin gene transition. The presence and quantities of ζ globin in blood of patients is determined by high performance liquid chromatography on reverse phase columns (RP-HPLC) with slight modifications of the method described by Shelton et al. (J. Liq. Chrom. 7: 1969, 1984) and by a radio-immunoassay (Chui et al., PNAS 81: 6188, 1984). SEA α°-thal heterozygotes have minute amounts of ζ globin present in blood. However, two heterozygotes for the Black or Filipino [ζα]°-thal and a third patient with Hb H disease due to the Black [ζα]°-thal and the type I -3.7 Kb α^+-thal lacked detectable ζ chain in blood. Persons with single ζ globin gene deletions also lacked detectable ζ_2 chain whether the α genes were intact or not due to α^+-thal in trans, i.e. ζζ-α/-ζαα. These data suggest that the derepression of ζ genes in adults with α°-thal may occur in cis but not in trans and imply regulatory roles for the inter-ζ gene region or for the region of DNA juxtaposed 3' to ψζ1.

182

AN IMMUNOASSAY TO DETECT HUMAN EMBRYONIC ε GLOBIN CHAINS BY A MURINE MONOCLONAL ANTIBODY. J-Q. Zhao, H-Y. Luo, B.J. Clarke, and D.H.K. Chui. Department of Pathology, McMaster University, Hamilton, Ontario, Canada.

Human embryonic ε globin chain is a β-globin like chain which is expressed during early embryonic development as components of Hb Gower 1 ($\zeta_2\varepsilon_2$) and Hb Gower 2 ($\alpha_2\varepsilon_2$). In this investigation, we have established a sensitive immunoassay for ε globin chains using a murine monoclonal anti-ε globin antibody. Lysates of cultured human K562 cells induced with hemin for two weeks were chromatographed on a DEAE-cellulose column. The hemoglobin fraction Peak II, containing embryonic ε and ζ globin chains was separated by Triton X-100/acid urea/polyacrylamide gel electrophoresis. The isolated ε globin chains were used to immunize Balb/c mice. A murine hybridoma cell line, 7B4, was established capable of secreting large amounts of monoclonal anti-ε globin antibody. The antibody was purified from hybridoma ascites fluid by ammonium sulfate precipitation and protein-A agarose affinity column chromatography. The monoclonal anti-ε antibody is of IgG1 subtype, and does not react against human ζ, δ, $^A\gamma$, $^G\gamma$, β, and α globin chains as shown by solid phase enzyme linked immunosorbant assay and Western blot. A slot blot immunoassay using ^{125}I-labelled monoclonal anti-ε globin antibody 7B4 was established. The assay can detect as little as 0.6 ng of ε globin chains in 1 μg of adult hemolysate. In 20 normal full term cord blood hemolysates, ε globin chains were not present as assessed by this assay. In another 10 hemolysate samples from fetuses of 17-31 weeks gestation, three samples had no detectable ε globin chains, but the other seven samples had a mean ε globin chain content of 0.15% (range 0.09%-0.20%). This specific and sensitive immunoassay for ε globin chains will be useful for a definitive study of the normal ontogeny of ε globin chains during human embryonic and fetal development as well as to search for the exprssion of ε globin chains in various hereditary or acquired hematological disorders.

183

THE PROMOTER OF THE α-GLOBIN GENE DOES NOT FUNCTION IN AN ENHANCER-INDEPENDENT FASHION. X.X. Zhu* and G.F. Atweh*. (Intrd. by R.F. Todd III) Ann Arbor VA Hospital and University of Michigan School of Medicine, Ann Arbor, MI

The expression of the α and β globin genes is coordinately regulated in adult erythroid cells to result in balanced synthesis of the respective globin chains. Previous studies have shown that transfection of a cloned α-globin gene into HeLa or COS cells results in a constitutively high level of expression of α-globin mRNA, regardless of the presence of a linked viral enhancer. The β-globin gene, on the other hand, can only be expressed at high levels in such cells when linked to a viral enhancer. The difference in the constitutive expression of these two genes may be a result of true enhancer-independence of the α-globin gene promoter, or alternatively, a result of the presence of an endogenous enhancer in the α-globin gene. We designed the following two experiments to distinguish these possibilities. When a hybrid gene consisting of the promoter of the α-globin gene linked to the coding sequences of the β-globin gene is introduced into HeLa or COS cells, no increase in β-globin expression is seen over that of a wild-type β-globin gene introduced into similar cells. When a construct containing the promoter of the β-globin gene linked to the coding sequences of the α-globin gene is introduced into HeLa or COS cells, α-globin expression is decreased when compared to that of a wild type α-globin gene. The level of expression of this hybrid gene is equivalent to that of a wild type β-globin gene. These experiments suggest that the α-globin gene promoter does not function in an enhancer independent fashion since it does not result in a high level of expression of linked β-globin sequences. They also suggest that the α-globin gene does not contain an endogenous enhancer since a β-globin promoter linked to an α-globin gene is also not expressed at high levels. We therefore conclude that sequence elements may be present within the body of the α-globin gene or its 3' flanking sequences which can specifically activate or "enhance" gene expression from the α-globin gene promoter and not from that of the β-globin gene.

184 MYELOID AND MONONUCLEAR CELLS

RAPID DEACTIVATION OF NADPH OXIDASE IN NEUTROPHILS: CONTINUOUS REPLACEMENT BY NEWLY ACTIVATED ENZYME SUSTAINS THE RESPIRATORY BURST. L.P. Akard*, D. English* and T.G. Gabig. Indiana University School of Medicine, Indianapolis, IN.

The cell-free system for activation of the neutrophil NADPH oxidase has allowed us to examine activation of the oxidase in the absence of its NADPH-dependent turnover. The covalent sulfhydryl-modifying reagent N-ethylmaleimide completely inhibited the activation step (Ki = 40 uM) in the cell-free system, but had no effect on turnover of the preactivated particulate NADPH oxidase (up to 1 mM). When N-ethylmaleimide was added to intact neutrophils during the period of maximal O_2^- generation in response to stimuli that activate the respiratory burst, O_2^- generation ceased within seconds. Study of components of the cell-free activation system indicated that the cytosolic cofactor was irreversibly inactivated by NEM whereas the membrane-associated oxidase could be activated by arachidonate and cytosolic cofactor even after NEM treatment. Thus inhibition of oxidase activation by N-ethylmaleimide unmasked a rapid deactivation step that was operative in intact neutrophils but not in isolated particulate NADPH oxidase preparations. The specificity of N-ethylmaleimide for oxidase activation and lack of effect on turnover of the NADPH oxidase suggested that sustained O_2^- generation by intact neutrophils is a result of continued replenishment of a small pool of active oxidase. The probable existence of an inactive pool of NADPH oxidase molecules in certain particulate preparations from stimulated neutrophils was supported more directly by activating these preparations again in the cell-free system. Thus we conclude that continuous activation of new oxidase enzyme is required to sustain the respiratory burst.