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HIF-1BETA DETERMINES ABCA1 EXPRESSION UNDER HYPOXIA IN HUMAN MACROPHAGES- A POTENTIAL TARGET FOR CARDIOVASCULAR PROTECTION

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Background: ATP-binding cassette transporter A1 (ABCA1) is a plasma membrane protein that is primarily involved in high-density lipoprotein (HDL) production in liver cells and in the elimination of intracellular cholesterol in peripheral tissues. Functional deficiency of this protein leads to low levels of circulating HDL, a clinical condition called Tangier's disease. In peripheral tissues impaired ABCA1 function may lead to local cholesterol accumulation.

Aim: Atherosclerotic lesion associated lipid-loaded macrophages express ABCA1 as a primary reverse cholesterol transporter. These macrophages are exposed to regions local hypoxia inside the lesions, which may have a major impact on ABCA1 mediated reverse cholesterol transport. We studied the effect of hypoxia on ABCA1 regulation and cholesterol efflux in human macrophages.

Methods: Primary human monocyte derived macrophages and hepatocytes as well as monocytic and hepatocytic cell lines were cultivated in vitro in order to analyze the binding of the hypoxia-inducible factor 1 (HIF-1) complex to the ABCA1 promoter and to determine the ABCA1 response to HIF-1 signaling in normoxic and hypoxic conditions. To evaluate the functional effect of hypoxia on ABCA1 mediated lipid efflux total cholesterol was measured from the culture media in hypoxic conditions and after transduction of the cells with constitutively active HIF-1 alpha adenovirus construct. The results were confirmed in human subendothelial fat depositions and pre-eclamptic placentas.

Results: We found that HIF-1 specifically binds to the HIF-1 response element of the ABCA1 promoter, and the HIF-1 complex increases ABCA1 promoter activity along with ABCA1 expression. Primary human macrophages exposed to hypoxia or expressing constitutively active HIF-1alpha respond with a potent change in ABCA1 expression, which shows a strong correlation with HIF-1beta expression (r 0.95-0.91). Moreover, ABCA1-mediated cholesterol efflux was also found to be regulated by HIF-1beta under hypoxia. In vivo, macrophages prepared from human atherosclerotic lesions ABCA1 levels show a strong correlation with HIF-1beta expression. This in vivo regulatory mechanism was confirmed in human pre-eclamptic placentas, a clinical condition with severe local hypoxia.

Conclusions: These results demonstrate that HIF-1beta availability determines ABCA1 expression and cholesterol efflux in macrophages under hypoxia. The individually observed differences in HIF-1beta expression, therefore, may contribute to the interpersonal variability of atherosclerotic lesion progression. HIF-1beta as a potential drug target may open novel pathways for cardiovascular protection therapies.

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AUTOMATIC BLOOD GROUP SEROLOGICAL INSTRUMENT WITH LOW CONSUMABLES STRATEGY

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Background: The ACT-24 semiautomatic serological analyzer is capable of ensuring standard conditions for routine blood group serological methods performed in tube. The machine performs all the steps of tube technique (cell washing, adding AHG reagent, reading results) automatically, so it standardizes all the methods of "carefully made" manual spin tube technique. The measuring principle of the ACT (Automatic Coombs Test) analyzer is particle size analysis of the agglutinations by centrifugal photo-sedimentometry. The strength of the reactions is characterized by sedimentation curves. This is the first blood group serological instrument

(performing also antiglobulin tests) which is equipped with an opto-electronic reading unit.

Aim: The sensitivity and specificity of the automatic spin tube technique can be equal to the new serological techniques (gel, solid phase etc.) using appropriate additives (e.g. Polyethylene Glycol). The full automatic versions of the original ACT-24 semi-automatic centrifuge can be competitive among blood group serological instruments, moreover, it fills a gap.

Methods and results: To ensure the full automatic system, the ACT-centrifuge is completed with a sample/reagent dispensing unit. According to our strategy to decrease the need of consumables, the new advanced centrifuge unit consists of self washed glass cuvettes. Two kinds of instruments are developed: a) The centrifuge is equipped with a blow air incubator. This way the reactions are prepared directly into the centrifuge tubes (cuvettes). To achieve a reasonable sample/hour ratio it is necessary to build in at least a pair of centrifuge units preferably with 36 centrifuge tubes in each. Versions of disposable plastic cuvettes, and self washed glass cuvettes are available. b) Using changeable rotors or round shape microplates, the time consuming preparation procedure and incubation phase takes place outside of the centrifuge unit, accelerating the system this way.

Conclusion: Full automatic blood group serological systems are developed with relatively cheap consumables or without them. The speed of the smallest instrument in case of Coombs/Enzyme screening is 12 patients/h, while the largest one is 64 patients/h.

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PEPTIDE MICROARRAY DETECTION OF BLOOD GROUP ANTIBODIES

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Background: Blood group serology has traditionally exploited agglutination for detecting antibodies. Glass or polycarbonate tubes replaced slide and tile assays but as the number of tests increased, so newer assay formats were developed, including microplates and gel cards. Both these solid-phase assays still rely on the interaction of antibodies with red cells to cause agglutination. Blood group Mimotopes are short synthetic peptides capable of mimicking the natural epitopes of the individual blood group antigens on red blood cells. These small peptides normally bear no sequence similarity to the expressed antigen but form a secondary structure that allows binding to the corresponding antibody thereby mimicking the action of the native antigen.

Aim: The use of these peptides in microarrays to specifically bind anti-blood group antibodies raised against clinically significant blood group antigens could negate the disadvantages associated with red cell reagents in traditional antibody screening methods and confirm their potential application as a novel method of blood group antibody screening.

Methods: Mimotopes were determined by panning random phage display libraries with specific antibodies. Peptides were custom synthesised, either as the "native" sequence or with covalent terminal modifications. The mimotopes were contact printed onto activated glass slides to generate a multi-specificity antibody-screening microarray, offering high binding capacity and low Background signals. Microarrays representing the clinically important blood group antigens RhD, RhE, Fy^a and Fy^b were printed in a variety of formats including multi-functional spots containing mimotopes for pairs of antigens. Slides were printed and stored at temperatures ranging from 4°C to 55°C and sampled at regular time points to determine thermal degradation properties of the mimotopes. Microarray slides were blocked and probed with antibodies at empirically determined concentrations. Antibody binding was detected using fluorescently conjugated secondary antibodies and visualised using a GeneTAC UC-4 scanner.

Results: Mimotopes for a number of clinically significant blood groups have been incorporated into the basic microarray system and probed with both their cognate "panning" antibodies and antibody-positive plasma