Project 18

Applicants: **Dr. F. Lederbogen, Dr. P. Schloss, Prof. Dr. M. Deuschle, Dr. M. C. Harmsen**

1. Title: **Culture-derived platelets and endothelial progenitor cells: Impact of serotonin metabolism on differentiation and function**

2. Aims of the Study

1. To identify key mechanisms involved in the regulation of human culture-derived (CD) platelet serotonin metabolism by exposure to specific pharmacologic agents (e.g., corticosteroids, serotonin reuptake-inhibitors)
2. In CD platelets, to study the effect of serotonin metabolism changes on surface adhesion molecule expression (e.g., P-selectin, PAC1)
3. To analyse the influence of candidate polymorphisms (e.g., 5HTTLPR polymorphism) on serotonin metabolism and differential protein expression
4. To examine the impact of altered serotonin metabolism on platelet cross-talk with endothelial progenitor cells in co-cultivation experiments
5. To extend these experiments into clinical science using platelets from specific patient populations (acute coronary syndrome, acute depression, sepsis, normal controls)

3. State of the Art (including Preliminary Studies)

Platelets play a pivotal part in recruitment of CD34+ endothelial progenitor cells (EPCs) toward vascular lesions, using glycoprotein receptors (e.g. P-selectin/PSGL-1, beta1- and beta2-integrin) to mediate adhesion and EPC homing. Recent data indicate that serotonin conjugation is used to augment the retention of these adhesive proteins on platelet surface. Further details of this mechanism came from mice rendered selectively deficient in tryptophan hydroxylase, the rate limiting enzyme in serotonin synthesis. In this animal model, the serotonin-deficient platelets exhibited a reduced adhesion due to a blunted secretion of adhesive α-granular proteins. Platelet serotonin content can also be lowered by selective serotonin reuptake-inhibitors, substances used to treat depression, anxiety and other psychiatric disorders. Interestingly, the use of these substances is associated with a lower rate of cardiac events in depressed subjects, but rarely, also with an increased bleeding tendency. As the technique of platelet culture has become available recently, we propose to analyse the impact of altered serotonin metabolism on surface adhesion molecule expression in functional culture derived platelets. In a second step, co-cultivation experiments are planned to determine the effect of manipulating platelet serotonin metabolism on EPC function.

Our previous work has covered various aspects of serotonin metabolism, platelet and EPC function:

1. We have extensively studied serotonin metabolism in cell culture and found evidence of a surface serotonin transporter downregulation following continuous exposure to the serotonin reuptake-inhibitor citalopram
2. We have analysed platelets with respect to aggregability and surface adhesion molecule expression (e.g., P-selectin and GP53), and demonstrated a close correlation between platelet serotonin transporter density and P-selectin expression
3. We have thoroughly investigated EPC function *in vivo* and *in vitro* in the context of vascular insults (acute myocardial infarction and kidney disease), and of inflammatory disease (systemic lupus erythematosus).

4. Experimental Design / Methods of Procedure

Work on this project will be divided into two segments, taking advantage of the researchers' complementary focus of laboratory techniques. The first segment will be accomplished in the Mannheim lab and includes experiments on serotonin metabolism and surface adhesion molecule expression in culture derived platelets. The second segment will take place in Groningen, focusing co-

cultivation experiments to determine the effect of manipulating platelet serotonin metabolism on EPC function.

4.1. Work at the Mannheim lab:
First, the graduate student is introduced in platelet culture and analysis technique: For preparation of CD platelets, CD34+ hematopoetic progenitor cells are recovered from sodium citrate anticoagulated whole blood by immunoaffinity selection. Differentiation into megakaryocytes is induced by thrombopoetin, interleukin and stem cell factor. CD platelets are harvested by centrifugation and washing procedures. In a first set of experiments, the graduate student then investigates serotonin metabolism regulation. For that purpose, cell cultures are exposed to specific agents, e.g. with known influence on serotonin transporter density (serotonin reuptake-inhibitors, glucocorticoids). These experiments are run both before and after differentiation of megakaryocytes into mature platelets, to assess the effect of protein synthesis during megakaryopoesis. Surface adhesion molecule expression (e.g., P-selectin, PAC1) is determined by flow-cytometry. The influence of specific polymorphisms (e.g., 5HTTLPR polymorphism) with known influence on serotonin metabolism and protein expression is incorporated into the experiments. Parts of the experiments will be performed by the help of research students, dependent on the complexity of the methods employed.

4.2. Work at the Groningen lab:
The graduate student transfers knowledge and technique of working with CD platelets to the Groningen lab. The student establishes platelet cultures and extends work to co-cultivation experiments. To determine the effect of manipulating platelet serotonin metabolism on *in vitro* cultured EPCs, EPC number is determined by flow-cytometry (CD34, CD14 among others), culture (colony assays) and transcript analyses (RT-PCR). EPC function is assessed by anti-coagulatory characteristics, migration and others. Finally, these experiments are repeated by using blood donated from different patient populations.

5. References

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