Project Proposal for PhD position granted by the International Graduate School in Molecular Medicine Ulm

1. **Institute address**

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2. **Project title**

Molecular consequences of a point mutation of phospholipase C-γ2 identified as a potential protective factor in late-onset Alzheimer’s disease

3. **Keywords**

Microglia-mediated innate immune responses; innate immune receptor signaling; genome-wide significant associations; receptor-mediated Ca²⁺ signaling; phospholipase C-γ2

4. **Research training group**

The main focus of the project clearly deals with an important neurobiological question. However, ageing is the most important risk factor for Alzheimer’s Disease and TREM2-DAP12/DAP10 deficiency has been proposed accelerate microglial senescence by progressively disabling the capacity of microglia to respond to degeneration. Finally, the project deals with a pathophysiologically highly relevant immunomodulatory process.

5. **Project description**

5.1 **Project background**

Many extracellular signaling molecules elicit intracellular responses by activating inositol phospholipid-specific phospholipases C (PLCs), which hydrolyze phosphatidylinositol 4,5-bisphosphate (PtdInsP₂) to inositol 1,4,5-trisphosphate (InsP₃) and diacylglycerol (DAG). These two second messengers modulate intracellular events through the regulation of intracellular free Ca²⁺ and protein kinase C isozymes, respectively. At the same time, PLCs decrease the local or general plasma membrane abundance of their substrate PtdInsP₂, which serves as an intracellular messenger in its own right by regulating multiple cellular processes. The mammalian PLC isoforms can be divided into six major families: PLCβ, PLCγ, PLCδ, PLCɛ, PLCζ, and PLCη. The PLCβ and PLCγ subclasses are regulated through G-protein-coupled and protein-tyrosine-kinase-linked receptors, respectively. The mechanisms by which the other PLC isoforms are coupled to membrane events are less well understood.

PLCγ2 is an important regulator of a variety of cell functions in immune effector and related cells such as B lymphocytes, mast cells, NK cells, dendritic cells, macrophages/monocytes, osteoclasts, and microglial cells of the CNS (Sims et al., 2017). For example, PLCγ2-deficient mice have decreased mature B cells, a block in pro-B-cell differentiation, and B1 B cell deficiency. Importantly, some of these changes are also manifested in mice deficient in the tyrosine kinase Btk, the adaptor protein Bink, or Rac2, suggesting that these signaling components may lie together with PLCγ2 within the same signal transduction pathways of B cells downstream of the B cell antigen receptor. Our laboratory was first to show that interaction of PLCγ2 with Rac amplifies the B-cell-receptor (BCR)-induced Ca²⁺ signaling by increasing the sensitivity of the cells BCR ligation, augmenting the BCR-mediated Ca²⁺ release from intracellular stores, enhancing the Ca²⁺ entry from the extracellular compartment, and
facilitating the nuclear translocation of the Ca\textsuperscript{2+}-regulated nuclear factor of activated T cells, NFAT (Piechulek et al., 2005; Walliser et al., 2015).

Very recently, a consortium of 161 institutions (International Genomics of Alzheimer’s Project, IGAP) identified three rare coding variants in (i) the human gene encoding PLC\textgamma\textsubscript{2}, \textit{PLCG2}, as well as (ii) the gene encoding type 3 interactor of the c-Abl proto-oncogene product corresponding to the Abelson murine leukemia virus oncogene \textit{v-abl}, \textit{ABI3}, and (iii) the gene encoding the type 2 innate immune receptor triggering receptor expressed on myeloid cells, \textit{TREM2}, with genome-wide significant (GWS) associations with late-onset Alzheimer’s disease (LOAD) (Sims et al., 2017). The fact that the three gene products are highly expressed in microglial cells suggests that they contribute to a protein-protein interaction network and that derangements of the corresponding microglia-mediated innate immune response may contribute directly to AD development. The mutation in \textit{PLCG2}, causing a replacement of P522 by R, is protective, whereas the changes in the other two genes increase the risk. Upon ligand binding, \textit{TREM2} transmits intracellular signals through one of two adaptors, DAP12 or DAP10, which recruit the protein tyrosine kinase Syk through their cytosolic immunoreceptor tyrosine-based activation motifs (ITAMs) (Colonna and Wang, 2016; Yeh et al., 2017). Activated Syk initiates a cascade of signaling events, including protein tyrosine phosphorylation, phosphoinositide 3-kinase (PI3K) activation, increase in cytosolic Ca\textsuperscript{2+}, and mitogen-activated protein kinase (MAPK) activation. Together, these signals are integrated to promote survival, proliferation, phagocytosis, enhanced secretion of cytokines and chemokines, and augmented activation of integrins, causing remodeling of the actin cytoskeleton to control adhesion and migration (Colonna and Wang, 2016).

Although PLC\textgamma isozymes have been postulated to be involved in TREM-2 signaling as early as 2006 (Ford and McVicar, 2006), the molecular mechanisms mediating this interaction are still poorly understood. In mouse bone marrow preosteoclasts, crosslinking of TREM-2 caused the recruitment of PLC\textgamma\textsubscript{2} to DAP12 as well as its tyrosine phosphorylation (Peng et al., 2010). Enhanced tyrosine phosphorylation was also observed for the Rac guanine nucleotide exchange factor (GEF) Vav3. Both PLC\textgamma\textsubscript{2} and Vav3 are known to be activated by tyrosine phosphorylation. The relative importance of these two processes, protein tyrosine phosphorylation and Vav3-mediated Rac activation, for \textit{TREM2}-mediated activation of PLC\textgamma\textsubscript{2} and the involvement of signaling proteins known to be crucial for receptor-mediated activation of PLC\textgamma\textsubscript{2} in other cells, e.g. Tec family kinases and their adaptor proteins, is essentially unknown.

5.2 Scientific objectives

The main objectives to be pursued in this project are: (i) to determine the molecular mechanisms of PLC\textgamma\textsubscript{2} regulation by \textit{TREM2}-DAP12/DAP10 and (ii) to establish the functional effects of the PLC\textgamma\textsubscript{2} P522R mutation in immune effector cells with endogenous expression of \textit{TREM2}-DAP12/DAP10.

The fact that \textit{TREM2} variants have also been found to be associated with other neurodegenerative diseases, such as frontotemporal lobar degeneration and Parkinson disease, and that functional \textit{TREM2} improves the outcome of stroke and autoimmune encephalitis in animal models suggests that that functional deficiency of the \textit{TREM2}-DAP12/DAP10-PLC\textgamma\textsubscript{2} pathway may be a general risk factor for microglial dysfunction and neurodegeneration and that the pathway as such may assume a more general role in controlling the microglial response to pathological changes in the CNS (Colonna and Wang, 2016; Yeh et al., 2017). The pathway components may thus represent potential candidate targets for future drug treatment of several CNS diseases.
5.2 Preparatory work

Mutations of the human PLCG2 gene have previously been found to be involved in dominantly inherited diseases of the immune system such as PLAID (PLCγ2-associated antibody deficiency and immune dysregulation) and APLAID (autoinflammation and PLCγ2-associated antibody deficiency and immune dysregulation) and to mediate resistance of chronic lymphocytic leukemia (CLL) cells and Waldenström Macroglobulinemia tumor cells to the irreversible Bruton’s tyrosine kinase inhibitor ibrutinib. We have thus far functionally analyzed all (~20) disease-related PLCγ2 mutants currently reported in the literature (Schade et al., 2016; Walliser et al., 2016; Walliser et al., 2017, in preparation), together with two mutants identified in two mouse models of autoimmunity and autoinflammation, designated Ali5 and Ali14 and caused by gain-of-function point mutations of PLCγ2, D993G and Y495C, respectively. In addition, we have performed, in collaboration with Drs. Ilker Kacara and A. Ramirez, Bonn, preliminary experiments on the P522R mutant of PLCγ2. The results showed that the P522R mutant displays several-fold higher basal activity and enhanced sensitivity to stimulation by activated Rac2^{G12V}. The results are unlikely to be due to differences in enzyme expression, since wild-type and P522R mutant PLCγ2 was expressed and functionally analyzed in stably transfected Flp-In™ 293 cells.

5.2 Work program

The work program comprises three sections. First, wild-type PLCγ2 and PLCγ2^{P522R} will be reconstituted with epitope-tagged TREM2 and DAP12 in cultured mammalian cells to examine the effects of TREM2 ligation by epitope-reactive antibodies on PLCγ2 activity at the level of [3H]inositol phosphate formation. The functional properties of PLCγ2^{P522R} will be compared to those of other PLCγ2 mutants with known mechanisms of action, in part identified in other human pathologies. PLCγ2 mutations mediating resistance to activation by tyrosine phosphorylation (4F) or Rac (F897Q) will be used in addition to the P522R mutation to investigate the relative contribution of the two enzyme activation mechanisms. Second, PLCγ2 will be introduced by lentiviral gene transfer into cultured cells already known to harbor TREM2 as an endogenously expressed protein, such as mouse RAW264.7 or human U937 monocyte/macrophage cells (Daws et al., 2001). Here, anionic and zwitterionic lipids known to associate with fibrillar Aβ in lipid membranes and to be exposed on the surface of damaged neurons to be sensed by TREM2 such as phosphatidylcholine and sphingomyelin (Wang et al., 2015) will be used to trigger [3H]inositol phosphate formation or, using fluorescent indicators, increases in cytosolic Ca^{2+}. Third, the CRISPR/Cas9 technology will be used to introduce the P522R mutation into the PLCG2 gene of a suitable, TREM2-expressing cultured human microglial cell line (e.g. HMC3) to examine TREM2-mediated wild-type versus mutant PLCγ2 activation. To this end, the native TREM2-PLCγ2 coupling will be examined prior to gene editing. Changes of intracellular Ca^{2+} will also be tested at the single cell level, as previously established by us for cultured B lymphocytes reconstituted with the Rac-resistant PLCγ2 mutant F897Q (Walliser et al., 2015). Experience in using this gene editing methodology in cultured cells is available in the laboratory through PD Dr. Panagiotis Papatheodorou, a guest researcher currently working in the Institute (Czulkies et al., 2017). The specific contribution of activated Rac to TREM2-mediated PLCγ2 stimulation will be examined using the specific Rac inhibitot EHT 1864 (Walliser et al., 2016).

5.3 Time table and milestones

All three sections of the work program will be initiated at the beginning of this doctoral work to allow completion of parts expected to be challenging and time consuming within three years time.
5.4 Cooperation partners

Dr. Ilker Karaca and PD Dr. Alfredo Ramirez, Functional Genomics of Cognition, Department of Psychiatry and Psychotherapy, University of Bonn, Sigmund-Freud-Straße 25, 53105 Bonn

5.5 Funding

This is a new project based on a new and unexpected finding. The project leans on project A8 "Mechanistic basis of chronic lymphocytic leukemia cell resistance to targeted tumor therapy in pathophysiologically relevant cellular contexts" funded within SFB 1074 "Experimental Models and Clinical Translation in Leukemia", but follows a different train of thoughts. This laboratory currently receives no funds through IGradU.

References


