INTRODUCTION

The human immune system is charged with the daunting task of protecting us from ubiquitous and harmful microbes without causing excessive damage to the host. As part of this hard job it has to recognize pathogens and mount appropriately sized responses, tailored to the threat faced, while also avoiding cross-reactivity with self-derived molecules. A series of regulatory mechanisms guarantee the perfect functioning of the immune response in healthy individuals. Primary immunodeficiency disorders (PIDD) refers to a group of monogenic diseases affecting diverse components of the immune system resulting in an increased susceptibility to infections, immune hyper-reactivity, autoimmunity, or a combination of these. Despite the rare nature of these disorders, they can teach us valuable lessons about human immunology in natural, outbred populations, which sometimes contrast sharply with findings in mammalian models. The study of diagnostic, genetic or mechanistic aspects of human primary immunodeficiencies, in particular defects of cell death, is the unifying theme of the current thesis proposal.

Chapter 1 provides a general introduction to this thesis. An overview of the processes mediating lymphocyte apoptosis is presented in the first half of the chapter, followed by a revision of the human inherited disorders of apoptosis, including the autoimmune lymphoproliferative syndrome (ALPS).

Chapter 2 presents the current classification and diagnostic criteria of the most well studied human genetic apoptosis defect: ALPS. This consensus document arose from an international ALPS meeting held by the NIH in 2009 and attended by experts from all over the world. Extensive modifications to the classification scheme and criteria are discussed, along with suggestions of flow cytometry and apoptosis protocols to be used for the clinical diagnosis of ALPS.

Chapter 3 describes the discovery that a mutation in NRAS can cause an ALPS-like syndrome. The heterozygous somatic Gly13Asp activating mutation of the NRAS oncogene did not impair FAS-mediated apoptosis, but augmented RAF/MEK/ERK signaling which markedly decreased the pro-apoptotic protein BIM and attenuated
intrinsic, nonreceptor-mediated mitochondrial apoptosis. Use of farnesyl-transferase inhibitors or ERK inhibitors \textit{in vitro} corrected the apoptotic defect, suggesting possible therapeutic targets.

\textbf{Chapter 4} presents the findings that somatic mutations in \textit{KRAS} can also cause an ALPS-like disorder in humans. The activating \textit{KRAS} mutations impaired cytokine-withdrawal induced T cell apoptosis through the suppression of the pro-apoptotic protein BIM and facilitated proliferation through p27\(^{kip1}\) downregulation. These defects could be corrected in vitro by MEK1 or PI3K inhibition. The use of the term RAS-associated autoimmune leukoproliferative disease (RALD) was suggested, to differentiate these disorders from ALPS.

\textbf{Chapter 5} describes the discovery that biomarkers can help to predict the presence of \textit{FAS} mutations in patients with ALPS symptoms. The combination of CD3\(^+\)CD4\(^-\)CD8\(^-\)TCR-alpha/beta\(^+\) (\(\alpha\beta\)-DNT) cell counts, sFasL and vitamin B12 or IL10 plasma levels were strongly linked to the presence or absence of a \textit{FAS} mutation. The biomarkers described should aid in the selection of patients with findings of ALPS for further diagnostic workup. In addition, the presence of a combination of markers strongly suggestive of a \textit{FAS} mutation in the setting of a negative genetic test should prompt a search for somatic mutations in sorted \(\alpha\beta\)-DNT cells.

\textbf{Chapter 6} presents our mechanistic work demonstrating that one form of cell death, induced by the reactivation of T cells, can be mediated by two unrelated apoptotic pathways. Briefly, a marked increase in the expression of BIM, a pro-apoptotic Bcl-2 family protein known to mediate lymphocyte apoptosis, was noted upon TCR re-stimulation. Knockdown of BIM expression rescued normal T cells from TCR-induced death to as great an extent as FAS disruption. The data thus implicated BIM as a critical mediator of apoptosis induced by restimulation as well as growth cytokine withdrawal.

\textbf{Chapter 7} describes the mechanistic studies showing that haploinsufficiency is a common disease mechanism in ALPS patients with \textit{FAS} mutations affecting extracellular domains. It was demonstrated that most extracellular-region \textit{FAS} mutations induced low FAS expression due to non-sense mediated RNA decay or protein instability resulting in defective DISC formation and impaired apoptosis. The apoptosis defect could be corrected by FAS overexpression in vitro. These findings defined haploinsufficiency as
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an alternative to dominant negative interference as a disease mechanism in ALPS patients.

Finally, in the **Concluding Remarks** the author briefly discusses his views on the future developments in the field.