Manfred Kraus: Functional studies on the B cell antigen receptor signaling subunit Igalpha by conditional gene targeting in mice. 2001

In dieser Arbeit wurde Iga, das zusammen mit Igb die Signalkomponente des B Zellrezeptors bildet, in vivo untersucht. Durch gezielte Genveränderungen wurden vier konditionale Iga Mausstämme erstellt, die Modifikationen des mb-1 Gens in einem bestimmten Entwicklungsstadium der B Lymphozyten ermöglichen. Die jeweilige Mutation führt entweder zu einem völligen Fehlen der Iga Ausprägung, zu einer Verkürzung der zytoplasmatischen Signaldomäne oder einem spezifischen Austausch der beiden Tyrosine des Iga ITAMs zu Phenylalanin. Die Analyse dieser Mutanten demonstrierte eine absolute Notwendigkeit für Iga und ITAM Phosphorylierung des B Zellrezeptors in frühen Entwicklungsstadien der B Zellentwicklung. Für reife B Lymphozyten konnte durch induzierte Mutation von Iga gezeigt werden, dass der Verbleib der meisten B Zellen, nachdem sie in die periphere B Zellpopulation selektioniert worden sind, weiterhin von Signalen der B Zellrezeptorkomponente Iga abhängig ist. Von besonderer Bedeutung für Signale des B Zellrezeptors erwies sich die ITAM Phosphorylierung von Iga. Fehlte diese aufgrund einer Mutation, so verringerte dies die Aktivierung der Protein-Tyrosin Kinasen Syk und Lyn, verhinderte die Bildung normaler B1 und MZ B Zellpopulationen und führte zu einer ineffizienten T-abhängigen Immunantwort. Außerdem konnte zusätzlich zu der bekannten aktivierenden Rolle, eine negativ regulatorische Signalfunktion für Iga in unreifen B Lymphozyten gezeigt werden.

The objective of this thesis was to investigate the function of the BCR signaling subunit Iga in B cell development. Four different conditional Iga mutant mouse strains were generated by gene-targeting to allow for Cre/loxP mediated modification of the mb-1 gene at defined developmental stages, which leads to Iga-deficiency, Iga cytoplasmic truncation or a specific replacement of the Iga ITAM tyrosines by phenylalanines. In Iga-deficient mice, B cell development was arrested at the pro-B cell stage in fraction C, presumably due to defective pre-BCR assembly. VDJ recombination was not dependent on Iga function, indicating that signaling-competent Igb monomers or dimers are expressed on the surface of pro-B cells. Inducible deletion of mb-1 exon IV in peripheral B cells was achieved with the help of the interferon inducible Mx-Cre system. Abrupt cytoplasmic truncation of Iga in mature B cells led to their fast disappearance from the periphery and also from the bone marrow. This demonstrates that maintenance of most B cells after they were selected into the peripheral pool depends on BCR mediated signaling via its Iga chain component. To study the function of Iga in B cell tolerance, mb-1 cytoplasmic truncation mutant mice (IgaDc/Dc) which coexpress transgenes encoding hen egg lysozyme (HEL) and HEL specific immunoglobulin were analyzed. In the presence of soluble HEL (sHEL) and depending on the IgaDc mutation, most immature B cells bearing the HEL-specific Ig transgene undergo rearrangements at the endogenous k light chain loci, resulting in loss of HEL specificity. Moreover, immature B cells from IgaDc/Dc mice responded to BCR crosslinking with an exaggerated and prolonged calcium response and induction of protein tyrosine phosphorylation. These data imply a novel negative signaling role for Iga in immature B cells. To investigate the specific function of Iga ITAM phosphorylation in B cell development a mutant mouse strain was analyzed in which the Iga ITAM tyrosines were replaced with phenylalanines (IgaFF/FF mice). In the analysis of B cell development, a strikingly different phenotype for IgaFF/FF mice was observed in comparison to IgaDc/Dc mice. While IgaFF/FF mice had no apparent block in early B cell development, B1 and MZ B cells were reduced in number, and l1 light chain usage increased. Phenotypically, B cells in IgaFF/FF mice showed increased surface BCR expression and also altered surface antigen expression of other coreceptors due to adaptation or selection. The IgaFF/FF mutants responded less efficiently to T-dependent, but normally to T-independent antigens and showed a significant reduction of serum IgG1 immunoglobulin. Signaling studies with IgaFF/FF splenic B cells revealed that upon BCR ligation, the cells exhibited heightened calcium flux, but weaker Lyn and Syk tyrosine phosphorylation in comparison to wild-type controls. Importantly, following BCR ligation Iga still showed

phosphorylation at tyrosine residues, indicating phosphorylation and a signaling related function of non-ITAM Iga tyrosines. When the Iga ITAM mutation was combined with a truncation of Igb, B cell development was completely blocked at the pro-B cell stage, demonstrating an absolute requirement of BCR ITAM phosphorylation for further cellular differentiation.