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Abstracts
Swiprosins-1 and –2: regulators of B cell signaling and differentiation?

Division of Molecular Immunology, Dept. of Internal Medicine III, University of Erlangen-Nürnberg, 91054 Erlangen, Germany
*Contributed equally

Pre-BCR (B cell receptor) and BCR signals are essential for B cell differentiation, homeostasis and negative selection, which are regulated by strength and quality of BCR signals. Recently, we identified a new adaptor protein, Swiprosin-1 (Efhd2), in lipid rafts of B cell lines that undergo apoptosis after BCR stimulation. During murine B cell development, Swiprosin-1 exhibited highest expression in immature B cells of the bone marrow, but was also expressed in resting and activated splenic B. In contrast, Swiprosin-2 (Efhd1), a very close homologue, is only expressed in pro B cells. Moreover, whereas Swiprosin-1 does not appear to be regulated by the pre-BCR, we could show in several systems that Swiprosin-2 becomes down-regulated through pre-BCR signals, both on mRNA and protein level.

Ectopic expression of Swiprosin-1 in the immature murine B cell line WEHI231 enhanced spontaneous and BCR-induced apoptosis. In contrast, shRNA-mediated down-regulation of Swiprosin-1 impaired specifically spontaneous and BCR-elicited apoptosis, but not BCR-induced G1 cell cycle arrest and up-regulation of the cell cycle inhibitor p27kip1. In accordance, Swiprosin-1 levels regulated net cell growth of WEHI231 cell populations through reciprocal regulation of Bcl-xL, but not Bim-levels, thereby controlling spontaneous apoptosis. In accordance, Swiprosin-1-induced spontaneous and BCR-induced apoptosis were blocked through NF-κB-activating stimuli, namely BAFF, anti CD40 and LPS. This correlated with enhanced BCR-induced IkB-α phosphorylation and degradation in cells expressing a Swiprosin-1-specific shRNA. Finally, ectopic Swiprosin-1 enhanced BCR-induced cell death in primary, LPS stimulated B cells.

Collectively, Swiprosin-1 levels could shift the balance of pro- and anti-apoptotic signals in B cells towards pro-apoptotic signals by virtue of its expression level. Swiprosin-2, on the other hand, may be involved in pre B cell differentiation. Hence, Swiprosin-1 may play a role in establishing central and peripheral tolerance by regulating the life span of immature B cells through induction of a default pro-apoptotic pathway. This could be balanced by BAFF in T2 and mature B cells, and through LPS or CD40 during T-independent or T-dependent antigenic challenges, respectively.
Evolution of GFP by hypermutation and selection in the B cell line DT40

Genome wide mutations and selection for the fittest within a genetically diverse population are the basis of natural evolution. A similar process occurs during antibody affinity maturation when immunoglobulin genes are hypermutated and only those B cells which express antibodies of improved antigen binding specificity are expanded. Protein evolution might be simulated in cell culture, if transgene-specific hypermutation can be combined with the selection of cells carrying beneficial mutations. Here, we describe the optimization of a GFP transgene in the B cell line DT40 by hypermutation and iterative fluorescence activated cell sorting. Artificial evolution in DT40 offers unique advantages and may be easily adapted to other transgenes, if the selection for desirable mutations is feasible.
Objective
Our initial observation was that human peripheral B cells stimulated with CpG DNA oligonucleotides express several molecules that are normally expressed on T cells. Among these we found CD5, CD25 and CD38. Since these molecules are also typical of chronic lymphatic leukaemia (B-CLL) we asked whether TLR-stimulated B cells could also express ZAP-70.

Methods
B cells from murine spleen and from PBMC from healthy volunteers were isolated by CD19+ positive selection with MACS technology (Miltenyi Biotech). Purity of human B cell preparations: 98-99.8%; T cell contamination 0.5% (ranging from 0-1%). Purity of murine B cell fractions: 90-98%. Intracellular ZAP-70 protein expression was calculated by subtracting mean fluorescence intensities (MFI) of the isotype controls from anti-ZAP70 mAb MFI. BrdU incorporation was used to mark proliferating B cells. mRNA expression was determined by real-time PCR analysis (Taqman).

Results
1. Phenotypical analysis of TLR-stimulated human peripheral B cells shows that TLR9 stimulation of human peripheral B cells induces B-CLL markers CD5, CD38 and CD25.
2. Our data show that CpG DNA stimulated human B cells express high levels of ZAP-70 in flow cytometric analysis.
3. ZAP-70 mRNA expression levels in human B cells correlate with beta-actin mRNA expression levels.
4. ZAP-70 protein expression in CpG DNA stimulated murine B cells is dependent on MyD88.
5. In contrast to murine B cells ZAP-70 expression is not upregulated by crosslinking of the BCR with anti-Ig in human B cells.
6. Furthermore, CD40 ligation fails to induce an increase in ZAP70 expression.
7. Our experiments further reveal that ZAP-70 protein expression is highest in the proliferating B cell subset.

Conclusions
Our data show that B cell stimulation via TLR9 induces a MyD88-dependent upregulation of ZAP-70 expression. The results indicate that the upregulation of ZAP-70 in human peripheral blood B cells is linked to B cell proliferation. We can only speculate whether ZAP-70 expression may be involved in postreplicative cellular processes such as activation-induced cell death.
Secondary lymphoid tissues have a common organization composed of T- and B-dependent zones. The B zone consists mainly of naïve B cells and a dense network of follicular dendritic cells. It is organized into several egg-shaped follicles with a diameter of a few 100 micrometer which are called primary lymphoid follicles before immunization. The T-zone is mainly composed of T cells and fibroblastic reticular cells filling the space between the follicles.

The formation of the B cell area and especially the organization into primary lymphoid follicles is studied using three-dimensional computer simulations. The model predicts that a dynamic lymphatic endothelium and an unknown negative regulation of the generation of follicular dendritic cells are critical to achieve stable follicles of proper size. Experiments to define the predicted mechanism are proposed.

Prior to follicle formation an intermediate state of secondary lymphoid tissue appears. A ring structure composed of a shell of B cells around the T zone shows up in agreement with known experimental results. The simulations demonstrate that the major determinant for this morphology is the biomechanics of cell migration. We conclude that, in contrast to the general point of view, the ring structure is a natural result of inherent cellular behaviour and does not depend on regulatory factors acting differently on B and T cells.
MicroRNAs (miRNAs) constitute a growing class of noncoding RNAs that regulate gene expression mainly at the posttranscriptional level. The regulatory function of miRNAs in a variety of organisms play critical roles in central biological processes such as development (*C. elegans*), apoptosis, fat metabolism (*Drosophila*) or insulin secretion (mice). To date, a large number of miRNAs have been identified, but the targets of most of these molecules remain unknown.

The goal of this project is to understand the role that miRNA play during the antigen-induced activation of B lymphocytes. To determine the miRNA pattern in naive B cells and to analyze whether the pattern changes upon antigen activation, mature B cells were isolated via magnetic CD43 beads from spleen and stimulated either with LPS (“simulates thymus-independent activation”) or with a combination of anti-IgM, anti-CD40 and IL4 (“simulates thymus-dependent activation”) for various times. The analysis of isolated RNA using miRNA microarray- and qRT-PCR assays identified 35 microRNAs, most of which were downregulated after stimulation. In silico analyses using various prediction programs revealed a very interesting and promising set of potential transcripts that could be controlled by miRNA. We will now verify biochemically the potential targets and determine their effect on B cell development by ectopic expression of the regulated miRNA in primary B cells using a retroviral expression system.

In addition, establishment of a conditional mouse knock-out model for one of the key enzymes required for miRNA processing should clarify the *in vivo* function of miRNA function during the antigen-dependent phase of B cell development.
In vitro and in vivo activation induces BAFF and APRIL expression in B cells

Van Trung Chu,* Philipp Enghard,† Gabriela Riemekasten† and Claudia Berek*

* Deutsches Rheuma Forschungszentrum, Berlin
† Department of Rheumatology and Clinical Immunology, Charite University Hospital, Berlin

B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) play key roles in peripheral B cell survival, maturation and differentiation. BAFF and APRIL are produced by a variety of cell types such as macrophages/monocytes and dendritic cells. Our analysis shows that BAFF mRNA is also expressed in all B cell subsets isolated from bone marrow, spleen and peritoneal cavity of BALB/c mice. APRIL expression is restricted to the stages of early B cell development in the bone marrow and the peritoneal B1 subset. Stimulation of B2 and B1 cells with LPS or CpG-oligodeoxynucleotides (CpG-ODN) induced MyD88 dependent plasma cell differentiation and intracellular expression of BAFF and APRIL. Furthermore, in vivo activation of B cells with a T-dependent antigen induced BAFF expression in germinal center (GC) B cells. In (NZBxNZW)F1 mice with established autoimmune disease, marginal zone (MZ), GC B cells as well as splenic plasma cells expressed high levels of BAFF. These data suggest that normal B cell development might be supported by an autocrine pathway in which B cells provide their own cytokines BAFF and APRIL. In (NZBxNZW)F1 mice the continuous activation of B cells and thus over expression of BAFF and APRIL may contribute to the development of autoimmune disease.
SWAP-70 is required for B cell development in secondary lymphoid organs, and homing of marginal zone B cells to the marginal zone

1 Michael Chopin, 2 Laurence Quemener, 1-2 Rolf Jessberger
1-Institute of Physiological Chemistry, Dresden University of Technology, Dresden 01309, Germany and 2- Department of Gene and Cell Medicine, Mount Sinai School of Medicine, New York, New York 10029, USA.

SWAP-70 resembles Dbl-homology family RhoGTPase interacting factors, and has been implicated in several cellular functions including in F-actin dynamics, adhesion, B cell activation and migration. SWAP-70 appears to be involved in signaling from several receptors including the B cell antigen receptor. We noted that SWAP-70−/− mice have reduced numbers of lymphocytes in the spleen. By phenotyping the different subsets of splenic B cells, we observed a reduced frequency of the T2 transitional and the marginal zone B cell populations. Immunofluorescence analysis of spleen sections showed an impaired marginal zone but unaltered follicles in SWAP70−/− spleen. To understand the role played by SWAP-70 in the generation of T2 B cells, we analyzed the process of B cell maturation in vivo. We noted impaired maturation of T1 into T2 B cells in SWAP70−/− mice. We also observed that T1 B cells from SWAP70−/− form clusters of cells in the red pulp, leading to improper localization of these cells, and subsequent blockage in maturation. This improper localization is likely due to hyperactivity of the integrins on T1 cells from SWAP-70−/− mice. These results suggest that SWAP-70 provides a key regulatory signal by adjusting the activity of integrins, and proper localization of T1 into the white pulp of the spleen.
The origin of B-1a cells: Analysis in mice with inducible B cell development

1 Sandra Düber, 2 Martin Hafner, 3 Elias Hobeika, 3 Michael Reth, 4 Ari Waisman, 1 Karsten Kretschmer, 1 Siegfried Weiss
1 Molecular Immunology and 2 Experimental Immunology, HZI, Braunschweig, Germany,
3 Molecular Immunology, Max-Planck-Institute for Immunobiology, Freiburg, Germany,
4 I. Medizinische Klinik und Poliklinik, Johannes Gutenberg-Universität Mainz, Mainz, Germany

The origin of B-1a cells, which are suggested to act as a ‘first line of defense’ against mucosal and systemic pathogens, is still controversially discussed. It is unclear, whether these cells are only generated during fetal and neonatal life, or whether the adult bone marrow can also give rise to such B cells. Most of the previous results leading to the controversy were derived from transgenic mice or adoptive transfer experiments. But now we are able to investigate the origin of B-1a cells under more physiological conditions. For this we have generated mice with inducible B cell development. Switching on B cell development in adult mice should definitively answer the question about the involvement of the adult bone marrow in the generation of B-1a cells.

To make B cell development inducible the recombination activating gene 1 (Rag1) was chosen as target. The coding sequence of Rag1 was initially inverted for gene inactivation resulting in a block of B (as well as T) cell development. Flanking this region with loxP sites in opposite orientation made it possible to revert it by expression of Cre recombinase resulting in restoration of the transcription unit. The temporal control of Cre expression was realized by crossing-in the MerCreMer mouse. These mice express the tamoxifen-inducible MerCreMer recombinase under the control of the B cell specific mb-1 promoter.

Oral administration of tamoxifen to the mice leads to a wave of newly generated B cells and allows their analysis in the periphery. Experiments in which the mice were induced at an age of 8 weeks clearly show that B-1a cells are present in the peritoneum. Also if older mice were used for the induction (5 months old) B-1a cells can be detected in similar numbers. A certain proportion of these cells shows phosphatidyl choline (PtC)-specificity, a typical property of B-1a cells.
Function of Swiprosin-2 during B cell development

Sebastian Dütting, Kai Herrmann, Christiane Lang, Hans-Martin Jäck, Dirk Mielenz
Division of Molecular Immunology, Department of Internal Medicine III, FAU Erlangen-Nürnberg
Supported by GRK592 (Lymphozyten)

During B cell development functional peripheral B cell subsets are produced from hematopoietic stem cells. Hematopoietic stem cells develop into progenitor B cells (pro-B) that undergo IgH chain gene rearrangements and develop into large pre-B cells, synthesising membrane-anchored HC of the µ isotype (µHC). µHC together with λ5 and VpreB are then transiently expressed as pre-B cell receptor (pre-BCR) on the plasma membrane. Signals of the pre-BCR initiate a limited clonal expansion phase of two to five cell divisions that results in the generation of small non-dividing pre-B cells. The expression of the pre-BCR at the transition from the late pro-B to the large pre-B stage is an important checkpoint of early B cell development.

Swiprosin-2 (also: EFhd1/Mitocalcin) is a close homologue of Swiprosin-1/Efhd2, an adaptor protein we showed recently to be involved in proximal B cell receptor signalling. To exclude potential redundancies between Swiprosin-1 and -2, we analyzed the expression pattern of both proteins. In contrast to Swiprosin-1 that was expressed in all murine B cell lines and primary B cells examined, albeit at different levels, Swiprosin-2 protein and mRNA were only expressed in a transformed pro-B-cell line (38B9) and in primary pro-B cells. We thus hypothesized that Swiprosin-2 is down-regulated by the pre-BCR. Using a transgenic tet-off system (Hess et al., 2001) that allows inducible pre-BCR-expression in primary cells, we demonstrated pre-BCR-dependent down-regulation of Swiprosin-2 on mRNA and protein level. We corroborated this result with IL-7 dependent R5B pro-B cells and transformed 38B9 pro-B cells either lacking or expressing a functional surface pre-BCR. A dysfunctional, cytoplasmic µHC did not regulate Swiprosin-2 expression, demonstrating that only signals from the surface pre-BCR induce Swiprosin-2 down-regulation.

Recently, human Swiprosin-2 was described as “Mitocalcin” in differentiated neuronal cells. There, overexpression of Swiprosin-2/Mitocalcin resulted in neurite extension (Tominaga et al., 2006). Complementary, impaired expression of Swiprosin-2/Mitocalcin caused suppression of neurite outgrowth and then cell death. (Tominaga et al., 2006). For B cells we hypothesize that pre-BCR-induced down-regulation of murine Swiprosin-2/Mitocalcin is required for pre-B cell differentiation and/or proliferation. To test this hypothesis we are currently infecting primary pro-B cells with a Swiprosin-2/Mitocalcin-encoding retrovirus that overcomes down-regulation of Swiprosin-2. In the future, we want to identify the function of Swiprosin-2 during B cell development and determine the mechanisms by which Swiprosin-2 potentially affects B cell development.

References:

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Induction of pre-B cell proliferation after de novo synthesis of the pre-B cell receptor.

Tominaga M, Kurihara H, Honda S, Amakawa G, Sakai T, Tomooka Y
Molecular characterization of mitocalcin, a novel mitochondrial Ca2+-binding protein with EF-hand and coiled-coil domains.
A hallmark of B cell antigen receptor (BCR) signaling is the antigen-induced elevation of the cytosolic Ca\(^{2+}\) concentration. Spatial and temporal modulation of intracellular Ca\(^{2+}\) fluxes controls the cellular response of B lymphocytes to antigen stimulation. However, signaling pathways that regulate differential profiles of Ca\(^{2+}\) mobilization in distinct developmental B cell stages remain unclear. We identified the hematopoietic adaptor protein Dok-3 (downstream of kinase-3) as a key component of negative feed-back regulation in Ca\(^{2+}\) signaling from the BCR. Dok-3 localizes at the inner leaflet of the plasma membrane and is a major substrate for activated Src family kinase Lyn. Phosphorylated Dok-3 inhibits antigen receptor-induced Ca\(^{2+}\) elevation by recruiting cytosolic Grb2, which acts at this location as negative regulator of Bruton's tyrosine kinase. This leads to diminished activation of phospholipase C-\(\gamma\)2 and reduced production of soluble inositol-trisphosphate. Alternative Grb2 recruitment by transmembrane adaptors like NTAL impedes this Dok-3 function in mature B cells. Hence, the defined sub-membraneous localization of Grb2 is a critical parameter to orchestrate the interaction efficiency of Ca\(^{2+}\) mobilizing enzymes. Dok-3 and NTAL provide ‘membrane zip codes’ for Grb2 to operate its inhibitory function.
Enhanced signaling of the IgG-BCR is accomplished by a tyrosine-phosphorylation motif within the cytoplasmic mIgG tail

Niklas Engels and Jürgen Wienands
Georg-August-University Göttingen, Cellular & Molecular Immunology, Humboldtallee 34, 37073 Göttingen

Depending on the developmental stage, B lymphocytes express on their cell surface distinct antigen receptor (BCR) classes, which are defined by the isotype of the immunoglobulin (Ig) subunit. All BCR classes utilize Ig-associated Igα and Igβ proteins as common signaling subunits. Yet, the BCR of the IgM class on naïve B cells triggers activation less potently than the IgG-BCR on class-switched memory B cells during secondary immune responses. We now demonstrate that increased signaling by the IgG-BCR is accomplished by inducible phosphorylation of an evolutionary conserved tyrosine residue in the cytoplasmic tail of the membrane-bound IgG (mIgG) heavy chain. Tyrosine-phosphorylated mIgG binds the adaptor molecule growth factor receptor-bound protein 2 (Grb2) and triggers prolonged activation of protein tyrosine kinases as well as intracellular Ca$^{2+}$ mobilization. Hence, the Ig component of the IgG-BCR not only functions as antigen recognition device but actively improves the immune response of memory B cells, which is a fundamental requisite for successful vaccination against pathogens.
The EMPD region of membrane-bound IgE as a possible target for blocking IgE production

1 Stefan Feichtner, 1 Daniela Inführ, 2 Reto Crameri, 1 Gernot Achatz
1 Department of Molecular Biology, Division Allergology & Immunology, 5020 Salzburg, Austria
2 Swiss Institut for Asthmaresearch, 7270 Davos, Switzerland

Regarding the five immunoglobulin isotypes IgM, IgD, IgG, IgA and IgE, the biological function of the latter still has not been clearly defined. So far, IgE is only known for its role in type I hypersensitivity reactions and allergic disorders. Since these disorders have become an increased challenge in industrial countries, various medical and immunological approaches to smoothen the typical symptoms have been established in allergy treatment. Nevertheless, none of these methods is highly effective and reliable, and it would be of great advantage to interfere systemic IgE production instead of treating the chronic symptoms. For this reason we focused on B cells expressing membrane-bound IgE receptors as possible targets for a new systemic anti IgE therapy with a special view on the extracellular membrane proximal domain (EMPD), consisting of 19 amino acids as part of the transmembrane domain. As a result of the isotype specificity of the EMPD region this domain could be used as an appropriate target for the generation of monoclonal anti mlgE antibodies with the capacity to inhibit IgE synthesis. By stimulating mlgE expressing B cells with anti-EMPD monoclonal antibodies we tried to mimic a developmental pathway of early B cell development and to force mlgE positive B cells to enter apoptosis. For the generation of mAbs Balb/c mice were immunised with the EMPD-peptide and their sera were monitored for the presence of antibodies that recognize this region. Mice splenocytes were isolated and fused with myeloma cells to form hybridomas. Secreted antibodies were then tested for their capacity to recognize the EMPD region in FACS. For a functional in vivo approach mice were immunised with recombinant Betv1 and anti-IgE-EMPD antibodies and their sera were monitored for the presence of IgE. Parallel application of the mAbs showed a reduction of specific IgE by 80% at day 21, reflecting the in vivo functionality of the anti-IgE-EMPD antibodies. Our results clearly demonstrated that mlgE positive B cells display a promising approach for systemic anti IgE therapy in the future.
The marginal zone (MZ) and B1 subsets of B cells, which differ from conventional follicular B cells (FO) both developmentally and functionally, are known to be critical for an early response to several infectious pathogens and are thought to act in the early-induced immune response. Whereas FO B cells probably contribute to most T-dependent germinal center-based responses, MZ and B1 B cells are presumed to be critical for T-independent responses. It is also assumed that MZ and B1 B cells are self-renewing and show several similarities in their response upon BCR-stimulation.

Here we present the functional characterization of a novel gene, AL1, which was isolated in a screen for lymphoid-specific genes in the mouse. In vivo, AL1 is present in secondary lymphoid tissues, and the expression of AL1 is most abundant in the MZ and B1 subpopulations of B cells. Minor AL1 expression is detected in FO B cells. Decreasing the amount of AL1 protein in mature K46 B cells by RNAi enhances the proliferation and tyrosin-phosphorylation in the absence of BCR-stimulation. Moreover, we show that the down-regulation of AL1 corresponds to an attenuated intracellular Ca^{2+}-mobilization. Interestingly, AL1 protein interacts with the adaptor-protein RACK1 (receptor for activated C kinase), which was shown to regulate cell cycle progression by altering the activity of src kinases and PKCs. Together, these data suggest that AL1 protein could act as a modulator of the BCR-signaltransduction cascade in B cells, especially in the MZ and B1 subpopulations.
Constitutive alternative NF-κB signaling promotes marginal zone B cell development but disrupts the marginal sinus and induces HEV-like structures in the spleen

Feng Guo, Debra Weih, Elke Meier, and Falk Weih
Leibniz-Institute for Age Research – Fritz-Lipmann-Institute, 07745 Jena, Germany

Nuclear factor (NF)-κB plays a crucial role in B cell and lymphoid organ development. Here, we studied the consequences of constitutive, signal-independent activation of the alternative NF-κB pathway for the splenic marginal zone (MZ). In contrast to nfkb2−/− mice, which lack both p100 and p52, mice that lack only the inhibitory p100 precursor (p100−/−), but still express the p52 subunit of NF-κB2, had markedly elevated MZ B cell numbers. Both cell intrinsic mechanisms and increased stromal expression of vascular cell adhesion molecule-1 (VCAM-1) contributed to the accumulation of MZ B cells in p100−/− spleen. While migration of p100−/− MZ B cells towards the lysophospholipid sphingosine-1 phosphate (S1P) was not affected, CXCL13-stimulated chemotaxis was impaired, correlating with reduced migration of MZ B cells into follicles in response to LPS. Strikingly, p100 deficiency resulted in the absence of a continuous marginal sinus but strongly induced expression of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and glycosylated cell adhesion molecule-1 (GlyCAM-1) and the formation of ectopic high endothelial venule (HEV)-like structures in the splenic red pulp. Thus, constitutive activation of the alternative NF-κB pathway favors MZ B cell development and accumulation but leads to a disorganized microarchitecture of the splenic MZ.
High affinity memory B cells and plasma cells are an important element of effective immune responses to pathogens and vaccines. The affinity maturation that occurs in a T-dependent humoral response is critically dependent on the formation of germinal centers (GCs). Affinity maturation within the germinal center (GC) requires rounds of mutation, proliferation and selection of higher affinity variants. Division and somatic hypermutation is thought to primarily occur within the GC dark zone (DZ), located proximal to the T cell zone. However, it has been suggested that the selection of higher affinity B cells requires contact with antigen in the form of immune complexes deposited on follicular dendritic cells (FDCs) in the light zone (LZ). To reconcile the need for the sequential events of mutation and selection by antigen at disparate locations within the GC, re-circulation of B cells between these zones was proposed. However, this hypothesis could never be tested using conventional histological methods as they are performed on fixed sections and the fate of single cells in the tissue cannot be observed over time.

Here, we report the use of multi-photon (MP) intravital microscopy to determine migration patterns of GC B cells. We are able to track the movement of GFP-labeled antigen-specific GC B cells within the popliteal lymph node of anesthetized mice in vivo. Additionally, we have developed a method for in vivo labeling of FDC dendrites and hence can define the different GC zones. Analysis of time-resolved images has revealed unexpected patterns of movement as well as GC B cell morphology. Most motile B cells within the GC migrate toward, from or along the outer zone (OZ) that defines the peripheral border of the GC. Moreover, few movements across the DZ–LZ interface are observed. Cell track trajectories indicate cells are predominantly recirculating within individual LZ and DZ compartments. The results suggest a revision to our views of B cell circulation within GCs and the functional relationship of its two major compartments.

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1alpha,25-dihydroxyvitamin D3 regulates B cell differentiation

The cooperation of antigen-specific and non-specific signals results in shaping of B cell differentiation upon activation by antigen or pathogen-associated molecular patterns. Here we show that in this context, 1alpha,25-dihydroxyvitamin D3 (Vitamin D), an anti-inflammatory seco-steroid hormone, modulates expression of IgE and interleukin-10 by human B cells. Vitamin D selectively blocks the induction of Ig-epsilon germline transcripts by anti-CD40 and interleukin-4, most likely through inhibition of NF-kappaB activation. In the presence of Vitamin D, IgE production of ex vivo activated B cells is reduced by 85.5±9.7%, while expression of other antibody classes (IgA, IgG and IgM) remains unaltered.

Vitamin D is known to promote expression of interleukin-10 by dendritic cells and T cells. Here we show that Vitamin D also enhances interleukin-10 production of B cells, when induced by CD40L, interleukin-4, and antigen or CpG-DNA. We demonstrate direct binding of the Vitamin D receptor/Vitamin D complex to the promoter of interleukin-10, but (additional) indirect effects of Vitamin D, through regulation of calcium-influx, on the expression of interleukin-10 cannot be ruled out yet.

B cell activation and differentiation is regulated late by Vitamin D, after activation of the B cell and the initial calcium-influx. This is underlined by the observation that expression of the receptor for Vitamin D is induced upon B cell activation within hours. Moreover, B cells also start to express CYP1 upon activation, enabling them to synthesize Vitamin D on their own and consecutively downregulate immune responses in an autocrine and paracrine fashion.

1, 2 Guido Heine, 2 Andreas Radbruch, 1 Margitta Worm
1 Allergie-Centrum-Charité, Klinik für Dermatologie und Allergologie, Charité - Universitätsmedizin Berlin, Charité Campus Mitte, 2 Cellular Biology, Deutsches Rheumaforschungszentrum Berlin
Early B cell maturation is controlled by signals emanating from the immature pre-B cell receptor (pre-BCR) consisting of a μ heavy chain (µHC) and the surrogate light chain (SLC). SLC was proposed to enable the release of a µHC from the ER-resident chaperone BiP, which enables the transport of a pre-BCR to a signaling-competent compartment at the cell surface. This paradigm was challenged by the finding that a transgenic µHC can reach transport and signaling competence independently from SLC. We show here in a retroviral-based cell culture system that ~60% of cloned splenic µHCs initiate differentiation and expansion signals independent from SLC. In contrast, ~40% of the tested µHCs could reach the cell surface and signaling capability only in the presence of a SLC (SLC-dependent µHCs). Importantly, co-immunoprecipitation assays clearly showed that the degree of transport and signaling competence was inversely correlated to the affinity of µHC idiotype to BiP. Therefore, we suggest that SLC increases antibody diversity by first controlling the degree of clonal duplication via the cell surface density of a pre-BCR and by rescuing functional SLC-dependent µHC idiotypes. However, the representation of a µHC idiotype in the final antibody repertoire is determined by its differential affinity to BiP.

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Siglec-G is a novel B1-cell inhibitory receptor which controls B1 cell expansion and regulates calcium signaling specifically in B1 cells

B1 cells are an important cell population for production of natural antibodies and for anti-bacterial immunoglobulin responses. Here we describe the cloning and characterization of the mouse protein Siglec-G, a member of the family of Sialic acid binding immunoglobulin-like lectins (Siglecs), as a novel B1 cell inhibitory receptor. Siglec-G is expressed in a B-cell restricted fashion with high levels in B1 cells. When over-expressed in B cell lines, it can inhibit BCR-mediated Ca^{2+} signaling. Siglec-G-deficient mice show a massive expansion of B1a cells, which is a B-cell intrinsic phenotype, as shown by adoptive transfer studies. This is accompanied by increased levels of natural IgM antibodies, but interestingly does not result in a higher penetrance of IgG autoantibodies. Siglec-G-deficient mice show selectively a strongly enhanced Ca^{2+} signaling in B1 cells, but not in B2 cells. These results demonstrate a novel negative regulatory pathway in B1 cells which may explain the naturally muted signaling response of B1 cells.
The CD40 receptor plays a central role in thymus-dependent immune responses. The interaction between CD40 expressed on B cells and its ligand CD40L, induced on activated T cells, leads to B cell activation, proliferation and survival. Transgenic mice expressing a constitutively active CD40 receptor in B cells show splenomegaly and enlarged lymphnodes due to hyperproliferation and prolonged survival of B cells. At the age of one year more than 50% of those mice develop tumors.

Currently we are investigating which signaling pathways are active in B cells expressing a constitutively active CD40 receptor in vivo and which of those signaling pathways are crucial in driving proliferation and survival of CD40-activated B cells. Furthermore signaling pathways active in B cell lymphomas will be analyzed and compared to those active in pre-malignant B cells.
Regulation of B lymphopoiesis by EBF proteins

M. Kieslinger, G. Dobreva, S. Folberth and R. Grosschedl
Max-Planck-Institute of Immunobiology, Dept. of Cellular and Molecular Immunology, 79108 Freiburg, Germany

Lymphopoiesis is dependent on the interaction of lymphoid progenitors with stromal cells of the lymphoid organs. Several transcription factors have been shown to regulate the generation and differentiation of lymphocytes. In particular, EBF1, which is expressed in lymphoid progenitors and B lymphocytes, synergizes with the E2A transcription factor to regulate the generation of pro-B cells. EBF1 and E2A proteins also regulate the expression of several B cell-specific genes, including Pax5. To gain insight into the regulatory network underlying the expression and function of EBF1, we are studying the effects of signaling pathways and transcription factors that regulate the Ebf1 gene.

Another member of the EBF family of transcription factors, EBF2, is expressed in non-hematopoietic cells of the bone marrow, such as adipocytes and osteoblasts. To examine the potential function of EBF2 in regulating the stromal compartment, we analyzed EBF2-deficient mice and found that adult, but not fetal, lymphopoiesis is markedly impaired. Adoptive transfer experiments indicated that this defect is cell-non-autonomous. EBF2-expressing osteoblastic cells are in close contact with immature haematopoietic cells and the frequency of haematopoietic stem cells (HSC) is reduced in EBF2-deficient bone marrow. Gene expression profiling of EBF2-expressing and EBF2-nonexpressing osteoblastic cells revealed that EBF2 regulates both genes already implicated in the maintenance of HSC and novel genes. Osteoblastic cells have been implicated in the generation of a niche for hematopoietic stem cells and EBF2 participates in the regulation of genes that support the maintenance of progenitor cells.
The so called “Tet off” or “Tet on” constructs make use of doxycycline (dox) dependent gene regulation and are among the most frequently applied exogenous regulation systems in mammals.

We have increased the regulatory range of “Tet on” by screening for trans-activator mutants with decreased basal expression and increased effector sensitivity in order to be able to control expression of apoptosis inducing genes. Ultimately these constructs shall be used as safety devices for cell based gene therapy. The new construct allows the construction of a stable HeLa cell line with caspase 2 under Tet control. Induction of caspase 2 expression results in killing of more than 90% of the cells. However, it has been impossible to obtain a cell line with tBid under Tet control with this setup. To further reduce the basal expression level we have used a combined transactivator/transrepressor system, in which both transregulators are dox responsive, the transrepressor comes off and the transactivator binds to DNA when triggered. This combined setup apparently reduces the basal expression level enough to allow the construction of stable Tet-tBid cell lines. Upon induction these cell lines show a killing efficiency of larger than 99%.

A similar setup of two transregulators was used to construct a genetic switch that can be stepwise induced independently of the effector concentration. For this purpose, a transrepressor mutant with relaxed inducer specificity was used, that can be induced by dox analogs which do not effect the transactivator activity. Since all necessary elements are combined in one plasmid, it should be feasible to efficiently obtain stably transformed cell lines or transgenic animals. A respective HeLa cell line expressing Gfp exhibits four stable expression levels that can be adjusted at will by several chemically distinct effectors. Cell sorting indicates that one intermediate expression level shows variance in individual cells, while the others express Gfp homogeneously.

Taken together, a number of novel dox dependent gene regulation devices with increased versatility are presented. Since each protein will have its own biologically relevant expression window, these devices expand the range of addressable problems. A few examples from the literature are shown to indicate the type of question that can be addressed using dox dependent gene regulation.
B cell receptor (BCR) signals are essential for B cell differentiation, homeostasis and negative selection, which are regulated by strength and quality of BCR signals. Recently, we identified a novel adaptor protein, Swiprosin-1/EFhd2, in lipid rafts of B cell lines that undergo apoptosis after BCR stimulation. During murine B cell development, Swiprosin-1 exhibited highest expression in immature B cells of the bone marrow. However, Swiprosin-1 was also expressed in resting and activated splenic B cells. Overexpression of Swiprosin-1 in the immature murine B cell line WEHI231 enhanced spontaneous as well as BCR-induced apoptosis by lowering the BCR signal threshold. In contrast, shRNA-mediated down-regulation of Swiprosin-1 impaired specifically spontaneous and BCR-elicited apoptosis, but not BCR-induced G1 cell cycle arrest and up-regulation of the cell cycle inhibitor p27Kip1. In accordance, Swiprosin-1 levels regulated net cell growth of WEHI231 cell populations by controlling spontaneous apoptosis. Furthermore, Swiprosin-1 downregulation in WEHI231 cells led to increased IκB-α phosphorylation after BCR stimulation. In contrast, we observed basal MAPK phosphorylation in Swiprosin-1 overexpressing WEHI231 cells. These data suggest that Swiprosin-1 levels are setting the course of BCR signaling for proliferation or negative selection in immature B cells. In support of the idea that Swiprosin-1 serves as a molecular switch in the BCR signaling pathway, we observed co-clustering of Swiprosin-1 with the B-cell receptor in WEHI231 cells expressing Swiprosin-1-EGFP fusion protein. Finally, Swiprosin-1 overexpression induced cell death in primary, LPS stimulated immature B cells isolated from the bone marrow, but not in LPS stimulated splenic B cells. Hence, Swiprosin-1 determines life span and BCR signals in immature B cells and may regulate the decision between immunity and tolerance.
The adaptor function of Syk controls the late Ca response and development of B cells

Signal transduction from the B cell antigen receptor (BCR) is a highly controlled process that involves the release of BCR signalling elements from auto-inhibition and their translocation to place in the cell where they meet other signalling partners allowing signalling to progress. This regulation is exemplified by the cytosolic protein tyrosine kinase Syk which plays an important role in B cell activation. Due to auto-inhibition Syk is not a very active kinase. We have studied the molecular mechanisms that mediate auto-inhibition and activation of Syk and found that both the tandem-SH2 domains and the inter-domain B region of Syk play an important role in this process. Furthermore we found that upon B cell activation Syk not only function as a kinase but also as an adaptor protein interacting with several signalling molecules. Interestingly, one of the protein binding to Syk is the adaptor protein SLP-65, the immediately downstream target of Syk. Upon BCR and Syk activation the SH2 domain of SLP-65 binds to a tyrosine at the Syk tail region. This interaction appears to have a dual function. First, binding of SLP-65 to Syk increases Syk-kinase activity presumably by counteracting Syk autoinhibition, and second, the Syk/SLP-65 complex regulates the late calcium response in B cells. This was shown by introducing a mutant form of Syk (SykF3) lacking the tail tyrosine into the Syk-deficient B cell line DT40 as well as primarily Syk-deficient pre-B cells. These B cells have a reduced calcium influx. Furthermore SykF3-expressing pre-B cells display a severe B cell developmental defects. Thus the novel Syk/SLP-65 interaction seems to play an important role in the development and activation of B lymphocytes.

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A/T mutagenesis in hypermutated Ig genes strongly depends on PCNA$^{K164}$ modification

To generate high affinity variants of their Immunoglobulin, B cells use translesion DNA synthesis (TLS) to introduce somatic mutations around genetic lesions caused by activation induced cytidine deaminase. Monoubiquitination at lysine$^{164}$ of proliferating cell nuclear antigen (PCNA$^{K164}$) stimulates TLS. To determine the role of PCNA$^{K164}$ modifications in somatic hypermutation, PCNA$^{K164R}$ knock-in mice were generated. PCNA$^{K164R/K164R}$ mutants are born at a sub-Mendelian frequency. While PCNA$^{K164R/K164R}$ B cells proliferate and class switch normally, the mutation spectrum of hypermutated Ig genes alters dramatically. A strong reduction of mutations at template A/T is associated with a compensatory increase at G/C - a phenotype similar to polymerase eta and mismatch repair deficient B cells. Mismatch recognition, monoubiquitinated PCNA and Pol$\eta$ likely cooperate in establishing mutations at template A/T during replication of Ig genes.

1 Petra Langerak, 2 Anders OH Nygren, 1 Peter HL Krijger, 1 Paul CM van den Berk, and 1 Heinz Jacobs
1: The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands
2: MRC-Holland bv, 1057 DN Amsterdam, The Netherlands
Construction of an “IgE-only” mouse as tool to study the biological function of the IgE immunoglobulin

1 Stefan B. Lenz, 1 Gernot Achatz
1 Department of Molecular Biology, University of Salzburg

Our interest is focused on the in vivo function of immunoglobulin E (IgE). To be able to study IgE+ B cells in an animal, we plan to construct an “IgE-only” mouse mutant, by means of embryonic stem cell targeting and subsequent tetraploid embryo complementation. The thus generated mouse should give us important data not only about the in vivo function of the membrane bound receptorform of IgE (mIgE), but also about the significance of this immunoglobulin isotype in B cell development. IgE, like IgG, belongs to the immunoglobulins which have a 28 amino acid cytoplasmic tail, compared to the three amino acid KVK tail of IgM and IgD. According to data gained from a mouse mutant (KVKΔtail) previously constructed in our laboratory, we suppose a distinct function of the cytoplasmic tail of mIgE in altering or modifying B cell antigen receptor signalling.

Still an open question remains if mIgE can substitute for all other isotypes in B cell development. The ability to mediate B cell development in absence of IgM has already been shown for IgD (Lutz, C., et al), IgG1 (Martin, S.W. and C.C. Goodnow) and IgG2b (Kurtz, B.S., P.L. Witte, and U. Storb).
CD25+ regulatory T cells induce peripheral B cell tolerance against non-lymphoid tissue autoantigens

I. Ludwig-Portugall, E. Hamilton-Williams, Felix Heymann and C. Kurts
Institute of Molecular Medicine and Experimental Immunology, University of Bonn, Bonn, Germany,

To study mechanisms of peripheral B cell tolerance against non-lymphoid tissue autoantigens we generated transgenic RIP-OVA/HEL (ROH) mice expressing the model antigens, OVA and HEL in pancreatic islets. T cells in these mice were not centrally tolerant to OVA, because double transgenic ROHxOT-I mice produced OVA specific CD8+ T cells (OT-I cells) and became diabetic. When ROH mice were immunized with OVA, we observed OVA specific IgG titers at day 21, suggesting that CD4+ cells able to switch Ig subclass were present. Nevertheless, titers in ROH mice were much lower than in nontransgenic controls, indicating specific tolerance. When we depleted CD25+ cells with the PC61 mAb, autoantibody production was completely restored, whereas titers against the foreign model antigen βGal were unaffected. These findings suggested that regulatory tolerance suppressed autoantibody production, rather than deletion of autoreactive B or T cells.

To investigate whether this tolerance was induced in the periphery, we injected naïve HEL-specific B cells from the transgenic IgHEL donor mice into ROH mice and analysed their phenotypical changes. After 3 days we detected neither proliferation, nor changes in IgHEL cell numbers, nor B cell receptor (BCR) downregulation. After 22 days numbers of IgHEL cells in transgenic recipients were reduced, and surviving cells showed reduced expression of the BCR. IgMa produced by IgHEL cells rapidly vanished in transgenic mice, even when specific help was provided. Thus, IgHEL cells were peripherally tolerized to a non-lymphoid tissue autoantigen. We conclude that peripheral B cell tolerance against autoantigens in nonlymphoid tissues can be maintained by CD25+ Treg.
Impaired B Cell Development in the Presence of a Non-Coding IgM mRNA

Johannes Lutz\(^1\), Werner Müller\(^2\), Chander Raman\(^3\) and Hans-Martin Jäck\(^1\)
\(^1\)Division of Molecular Immunology, Department of Internal Medicine III, Nikolaus-Fiebiger-Center of Molecular Medicine, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany.
\(^2\)Bill Ford Chair of Cellular Immunology, Faculty of Life Sciences, University of Manchester, Manchester, UK
\(^3\)Departments of Medicine and Microbiology, Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, USA.

To establish allelic exclusion (AE) at the Ig heavy chain (IgH) locus VDJ recombination of the second locus must be terminated once a productive VDJ rearrangement has been made. Many studies have demonstrated that the suppression of further VDJ rearrangements is mediated by the newly synthesized IgM heavy chain (µHC) through pre-B cell receptor (pre-BCR) signals. However, it is still unclear how rearrangement of the second IgH allele is prevented during the time required for the synthesis of the µHC and the initiation of pre-BCR signals. Here we present evidence that a stable µHC transcript, which reflects the presence of a productive rearrangement, can already exert a suppressive effect on VDJ recombination in the absence of a µHC signal. B cell development was impaired at the pro-B to pre-B cell transition with an increased frequency of µHC-negative pro-B cells in transgenic mice expressing a non-coding µHC transcript. This transcript contains a premature translational stop codon at position +3 and resembles rather a stable productive than an extremely unstable non-productive µHC mRNA. Providing a productive transgenic µHC restored B cell development, which indicated that the non-coding µHC transcript interfered with no other processes than VDJ recombination at the IgH locus. These observations suggest a new role for µHC transcripts by indicating the presence of a productive VDJ rearrangement and temporarily suppressing VDJ recombination of the second IgH allele until the initiation of signals provided by a µHC.

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Autoreactivity in IgG+ memory B cells from healthy donors and patients with Systemic Lupus Erythematosus

Diversity in the antibody repertoire is generated by random V(D)J recombination during early B cell development and by somatic mutation in response to antigen-mediated activation. A hallmark of the autoimmune disease Systemic Lupus Erythematosus is the production of high affinity serum IgG autoantibodies. However, where and when during B cell development and differentiation B cell self-tolerance is broken in SLE has not been clearly defined. By cloning and in vitro expression of antibodies obtained from single isolated human B cells at different stages during their development we could previously show that SLE is associated with an early defect in the establishment of B cell self-tolerance leading to high levels of autoreactive mature naïve B cells as compared to healthy controls. We have now extended our study to the IgG+ memory B cell pool. Surprisingly, we find low-level self-reactive antibodies already frequently expressed by IgG+ memory B cells under normal conditions and the majority of these antibodies gained self-reactivity by somatic mutations. Low-level autoreactive antibodies are not enriched in the IgG+ memory B cell pool of SLE patients, but highly specific strongly reactive antibodies were detected. The characteristics of IgG+ memory B cells antibodies from SLE patients as compared to healthy individuals will be discussed.
Immunoglobulin Class Switching in Extrafollicular Responses Occurs in T Zone Preplasmablasts

Jennifer L. Marshall, Lalit Pallan, Mei-Chi Hsu, Francesco Falciani, Ian C. M. MacLennan, Kai-Michael Toellner  
MRC Centre for Immune Regulation, University of Birmingham Medical School, Birmingham, B15 2TT, United Kingdom

B cells differentiate via two pathways in antibody responses: either as extrafollicular plasmablasts that differentiate into early non-affinity matured plasma cells or as germinal centre B cells in follicles leading to high affinity antibody and memory.

To characterize the earliest B cell differentiation stages after antigen contact mice with 5% of B cells expressing a transgenic NP-specific B cell receptor were immunized with NP-Ficoll. This leads to a strong extrafollicular responses and the induction of large germinal centres. Antigen-specific B cells were studied during the first three days after immunization. NP-binding B cells move to the T zone within 8 hours after immunization and start proliferating by 30 hours. Only during the third day do the responding B cells form CD138−, Blimp-1−, B220int plasmablast foci at the T zone-red pulp junction and AID+, Bcl-6+, B220high germinal centre founding cells.

No clear separation of precursor plasmablasts or germinal centre founders has yet been identified among the B blasts in the T zone, but the following features characterize the B blast phase: Single cell PCR shows that before immunization 40% of B cells express Bcl-6 mRNA. This is not expressed as protein. Frequency and expression levels of Bcl-6 mRNA is reduced during the B blast phase. B blasts are Blimp-1− and CD138−. A proportion does express AID mRNA two days after immunisation. These AID+ B blasts are exclusively Bcl-6−. A proportion of B blasts start to undergo switch recombination as shown by the appearance of recombined heavy chain transcripts. Switched cells have become plasmablasts expressing IgG3 protein by 72 hr. At this time plasmablasts have become CD138+, express high levels of blimp-1 and completely lose Bcl-6 and AID mRNA expression. The lack of AID expression in plasmablasts means that switching in extrafollicular responses occurs at the T zone B blast stage.

On the third day also germinal centre founder cells appear which are located in follicles. These express high levels of AID mRNA, coexpressed with high levels Bcl-6 mRNA in the same cells.
Signals from the B cell antigen receptor (BCR) and its precursor the pre-BCR regulate the development and selection of B cells. Here, we compare the effects of surrogate light chain (SLC) and conventional LC expression on receptor-induced Ca2+ flux in B cells that express an inducible form of the adaptor protein SLP-65. We found that SLC expression strongly enhanced an autonomous ability of μ heavy chain (μHC) to induce Ca2+ flux independent of additional receptor cross-linking. In contrast, LC expression reduced this autonomous μHC ability and resulted in antigen-dependent Ca2+ flux. While these data confirm ligand-independent pre-BCR aggregation and signaling, they suggest a negative role for LCs in BCR-aggregation and a novel mechanism for B cell selection.
Objective/Introduction: Plasma cells provide both, protective humoral immunity and autoimmunity. Specific therapeutic targeting of plasma cells, to improve efficacy of vaccines or treat autoimmunity, cannot be achieved so far. A better understanding of the biology of plasma cells is needed to this end. Here we compare the diversity and functional heterogeneity of human plasmablasts (PBs) and plasma cells (PCs) in apparent immunological quiescence (“steady state”) and activation, by Tetanus Toxoid (TT) vaccination.

Methods: Human ASC were detected cytometrically, according to expression of the marker combination Lin-/CD19+/CD27high/CD20-, or high expression of intracellular immunoglobulin, before and at various times after secondary systemic immunization with TT. Subsets of blood and bone marrow (BM) plasmablasts and plasma cells were analyzed for their ability to migrate towards chemokine gradients in vitro, and transcriptomes of PC subsets were analysed (Affymetrix genome-wide HG-U133). Proliferative activity was determined according to Ki-67 expression, apoptosis according to annexinV staining.

Results: 1) Among 50 healthy individuals only 3 showed more than 0.5% of PB/PC frequencies among peripheral blood mononuclear cells (PBMC). The frequencies of PB/PC in the others, i.e. in steady state was less than 0.5%. Among PBMC of 23 TT-vaccinated individuals the frequencies of PB/PC varied between 0.1% and 2% on days 6 and 7 after vaccination.
2) In steady state, most PB and PC expressed IgA (80% IgA, 16% IgG, 4% IgM). Seven days after secondary TT vaccination, the frequencies of IgA-secreting and alpha4beta7 integrin+ PB/PC remained constant, while additional IgG-secreting PB/PC were detectable.
3) Steady state IgA PB/PC expressed the intestinal adhesion molecule alpha4beta7 integrin and/or the mucosal chemokine receptor CCR10 and some migrated towards CCL28 (4 donors, mean 27% migratory of total blood CD19+/CD27high cells). At the same time, IgA-secreting PB/PC were able to navigate towards CXCL12 (~30% migration), suggesting they might have an option to migrate into the BM.
4) About 40% of human bone marrow plasma cells (BMPC) secreted IgA, 50% IgG and 10% IgM PC. A subset of BMPC expressed alpha4beta7 integrin, indicating a mucosal origin of the respective cells.
5) In steady state, dividing (Ki-67+)/HLA-DR-high PB and non-dividing (Ki-67+)/HLA-DR-low PC were found. On days 6 and 7 after TT vaccination, TT-specific PB but not PC were detectable in blood.
6) After vaccination, peripheral PB/PC expressed CXCR4, but only HLA-DR-high PB migrated towards its ligand CXCL12. Comparison of transcriptomes of blood PB and PC, after TT vaccination, with BMPC revealed expression of genes of cell proliferation and DNA replication in PB, not in PC. BMPC had upregulated expression of genes protecting from apoptosis, like bcl-2.

Conclusions: The frequencies of PB and PC in normal human blood is very low and their phenotype suggests that they are derived from mucosal immune reactions. Only on days 6 to 8 after systemic immunization with an antigen like TT, PB specific for the vaccine are detectable in the blood. At the same time PC appear in the blood, which are not specific for the vaccine and are not migratory. Their transcriptome resembles that of BMPC, except for distinct differences, e.g. downregulation of the anti-apoptotic gene bcl-2. These data demonstrate the tightness of systemic humoral memory in steady state and support the concept of competition between newly generated plasmablasts and old plasma cells as the basis of memory homeostasis.
Extensive immunoglobulin production sensitizes myeloma cells for proteasome inhibition

Silke Meister 1, Ulrich Schubert 2, Kirsten Neubert 1, Kai Herrmann 3, Renate Burger 4, Martin Gramatzki 4, Sabine Hahn 2, Sandra Schreiber 2, Martin Herrmann 4, Hans-Martin Jäck 4, Reinhard E. Voll 1.5

1 IZKF N2, NFZ, Univ. of Erlangen, 2 Institute of Clinical and Molecular Virology, Univ. of Erlangen, 3 Div. of Molecular Immunology, Dept. of Int. Medicine 3, NFZ, Univ. of Erlangen, 4 Div. for Stem Cell Transplantation and Immunotherapy, 2nd Medical Dept., Univ. of Kiel, 5 Dept. of Int. Medicine 3, Univ. of Erlangen

Multiple myeloma (MM), an incurable plasma cell neoplasia, is characterized by overproduction of large amounts of monoclonal immunoglobulins (Ig). The recently clinically approved proteasome inhibitor bortezomib (Bz) acts directly on MM cells to cause cell death along with inhibiting their growth and survival, supposedly by blocking the key survival protein nuclear factor (NF)-κB. However, the exact mechanism by which Bz acts is still under investigation. Extensive synthesis of Ig in MM cells results also in defective ribosomal products (DRiPs) and unfolded proteins degraded by the proteasome. Therefore, we hypothesized that the pro-apoptotic effect of Bz is due to the accumulation of unfolded proteins and DRiPs along with inhibition of NF-κB. Using the human MM IgG-secreting cell line JK-6L and murine µH-chain-transfected Ag8.H myeloma cells, proteasome inhibitor treatment induced markedly more apoptotic cell death in subclones producing high compared to low amounts of Ig. Unexpectedly, bortezomib did not markedly alter NF-κB activity. In contrast, Ig positive MM cells showed a highly induced AP-1 DNA binding activity upon Bz treatment. Importantly, in Ig-high MM cells Bz triggered production of reactive oxygen species (ROS) as well as strong activation of endoplasmic reticulum (ER) stress components involved in apoptosis such as CHOP and caspases. Additionally, cells harbouring Ig showed enhanced expression of the pro-apoptotic factor Bax and reduced expression of the anti-apoptotic protein Bcl-2 due to Bz treatment. Moreover, proteasome inhibition results in formation and accumulation of IgG-derived DRiPs. Hence, we conclude that proteasome inhibition preferentially affects cells producing high amounts of proteins. Bz-induced cell death in MM cells is most likely mediated by ER stress induced through accumulation of unfolded proteins/DRiPs.

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The immuno-deficient phenotype of Ii-/- mice is partially explained by a dysfunction of H2-DM

Jürgen Neumann, Angelika König and Norbert Koch
Section of Immunobiology, Institute of Genetics, University of Bonn

The CD4+ T cell compartment and the development of B cells are largely reduced in Ii-/- mice. The T cell phenotype was attributed to a declined MHCII surface expression by antigen presenting cells. Hence, selection of CD4+ T cells in the thymus is reduced in Ii-/- mice. However, very low expression of transgenic Ii on an Ii-/- background almost completely reconstituted the immune competent phenotype of the mice. The low amount of Ii however, does not elevate MHCII surface expression to a normal level. We show here, that co-expression of MHCII and DM subunits results in the absence of Ii to retention of DM in the endocytic reticulum. MHCII subunits associate aberrantly with DM chains and inhibit export of DM heterodimers from the ER to endocytic compartments. In splenocytes from Ii-/- mice the half life of H2-DM is strongly reduced, because H2-DM and IA subunits form aggregates, which are retained in the ER and subsequently degraded. In the lack of functional H2-DM, IA molecules remain free of peptide, yielding a deficiency to present self antigens. In the presence of low tg Ii functional H2-DM heterodimers are formed. This result indicates that Ii is a chaperone for the assembly of H2-DM.
Proteasome inhibition ameliorates lupus symptoms in NZB/W mice

Kirsten Neubert¹, Silke Meister¹, Damian Maseda¹, Kerstin Amann², Martin Herrmann³, Joachim R. Kalden³, Reinhard E. Voll¹
¹IZKF, Nachwuchsgruppe 2, Nikolaus-Fiebiger Center for Molecular Medicine, University of Erlangen-Nuremberg, Erlangen, Germany
²Institute for Pathology, University of Erlangen-Nuremberg, Erlangen, Germany

Systemic lupus erythematosus (SLE) is an autoimmune disease which is characterized by circulating IgG autoantibodies predominantly directed towards nuclear antigens such as DNA and histones. The proteasome inhibitor bortezomib (Bz) is used for the treatment of multiple myeloma, a disorder in which malignant plasma cells (PC) accumulate. One major mechanism of Bz might be the blockade of the key transcription factor NF-κB, which is important for survival of B lymphocytes especially mature B cells. In addition, NF-κB is a key transcription factor in the immune and inflammatory response. Therefore, we investigated the effects of NF-κB inhibiting agents such as Bz in a mouse model for SLE.

To address this question, we treated NZB/W mice with Bz twice weekly over 10 months. Bz-treated mice have significantly prolonged survival time and decreased proteinuria compared to the control mice. Histologically, there were no or only minor signs of glomerulonephritis in Bz-treated mice. The IgG anti-dsDNA and anti-Histone antibodies were strongly reduced during the whole treatment. The IgG concentrations in sera from both Bz-treated groups were significantly decreased during the first 7 or 5 months of treatment, respectively. After the first month of treatment the IgG serum concentrations increased again and reached the levels of control mice at the end of the experiment. Interestingly, the serum IgM and IgA concentrations remained reduced also after 8 or 6 months of treatment.

Flow cytometric analyses of the lymphocyte compartment from NZB/W mice, which were Bz-treated over 8 weeks, revealed a strong reduction of T and B cell numbers. Interestingly, Bz had no significant effect on the B cell numbers in the bone marrow.

These data indicate that Bz prolongs the survival and ameliorates the clinical parameters of lupus-like disease in NZB/W mice. We suggest that both T and B lymphocyte subsets are affected by Bz, potentially due to inhibition of NF-κB activation along with induction of terminal endoplasmic reticulum stress leading to apoptotic cell death of lymphocytes.
Interaction with F-actin and phosphorylation of SWAP-70, a protein involved in B cell activation, polarization and migration

Glen Pearce, Stefanie Seifert, Rolf Jessberger
Institute of Physiological Chemistry, Dresden University of Technology, Dresden 01309, Germany

The actin-binding, Rac-interacting protein SWAP-70 is strongly expressed in activated B cells. SWAP-70 deficiency in B-cells results in their reduced migration into secondary lymphoid organs. SWAP-70 was required not for integrin expression or chemotaxis, but rather for the regulation of integrin-mediated adhesion and cell attachment. The actin binding domain (ABD) of SWAP-70 is required during B-cell polarisation, as a mutant of SWAP-70 lacking the 12 C-terminal amino acids of the ABD acts as a dominate negative mutant. We noted that following B-cell activation SWAP-70 becomes phosphorylated. The kinase activity was then purified and identified by mass spectroscopy as belonging to Syk kinase. Using a variety of bacterial expression constructs a tyrosine at position 517 was identified as the residue phosphorylated by Syk. Since, this residue is close to the ABD of SWAP-70 its phosphorylation might play an important role in regulating cell polarity.
Epstein-Barr virus-positive, but not EBV-negative Burkitt lymphoma cells are resistant to Interferon-γ

Judith Reschke, Martin Schlee, Gerhard Laux, Berit Jungnickel, and Georg W. Bornkamm
Institut für Klinische Molekularbiologie und Tumorgenetik
GSF-Forschungszentrum für Umwelt und Gesundheit
Marchioninistr. 25, D-81377 München, bornkamm@gsf.de

EBV-positive Burkitt lymphoma are not recognized by antigen-specific cytotoxic T CD8-positive cells even though they carry the EBV genome and express foreign viral antigens. We have generated a cellular model system in which non-recognition of viral antigens in BL cells can be recapitulated in vitro. Starting from primary human B cells we have generated a cell line conditionally immortalized by EBV (EREB2) in which the viral master regulator EBNA2 is fused to the hormone binding domain of the estrogen receptor and is rendered regulatable by estrogen. Introduction of a constitutively active (A1 cells) or tetracycline-regulated c-myc gene into this cell line (P493-6 cells) made cell proliferation independent of EBNA2 and LMP1 and rendered the cells invisible to cytotoxic T cells. We have shown that in this in vitro system overexpression of c-MYC contributes actively to immune escape by negatively regulating the NF-κB and the interferon response. Virtually all genes involved in immune recognition are co-regulated by NF-κB and interferons, i.e. MHC class I and class II, adhesion molecules and activation markers, the peptide transporter TAP1 and TAP2, and the inducible components of the immune proteasome. Studying the interferon response in EBV-positive and –negative cell lines we have made the unexpected observation that EBV-positive, but not EBV-negative BL cells are resistant to IFNγ as revealed by tyrosine 701 phosphorylation of STAT1. STAT1-Y701 is, however, normally phosphorylated after treatment of EBV-positive BL cells by type I interferons. We initially suspected that a viral gene product of EBV would render the cells unresponsive to IFNγ as observed in many other viral systems, this is however not the case. In EBV-loss variants of Akata and Mutu BL cells the IFNγ response is not restored. Genetic defects in the IFNγ pathway could also be excluded by comparing subclones of the BL line Mutu. Cells freshly established in culture exhibit a restricted antigen expression pattern and have the same phenotype as EBV-positive BL (group I Mutu BL cells). Upon prolonged in vitro cultivation part of the cells switch on the viral latency program and adopt the phenotype of EBV-immortalized B cells (group III Mutu BL cells). Comparing Mutu group I and group III cells we could show that group III cells are sensitive to IFNγ, whereas group I cells are resistant. Responsiveness to IFNγ has thus been restored in group III Mutu cells. The clonal relationship of Mutu group I and group III cells was verified by sequencing the VDJ joint. These data point to an epigenetic defect in the IFNγ signalling pathway in EBV-positive BL group I cells. No difference was observed in the expression of the IFNγ receptor by FACS and quantitative RT-PCR analysis between EBV-positive and EBV-negative BL cell lines, a significant difference was, however, seen studying expression of JAK2. EBV-negatively consistently expressed JAK2, whereas the majority of IFNγ resistant EBV-positive group I BL lines lacked JAK2 expression. Reintroduction of JAK2 restored IFNγ responsiveness.

Our data suggest that immune escape in BL cells to viral antigens is mediated by tow mechanisms: the immunesuppressive action of c-MYC and the unresponsiveness of BL cells to IFNγ. We propose a model in which cross priming of phagocyted BL cells may stimulate IFNγ production by activated T cells and that IFNγ is able to overcome the immune suppressive action of c-MYC. IFN-γ resistance may thus be a necessary step in the development of EBV-positive BL in vivo.
Although the entire mouse genome has been sequenced, there remain challenges concerning the elucidation of particular complex and polymorphic genomic loci. In the murine IgH locus, different haplotypes exist in different inbred mouse strains. For example, the IgHb haplotype sequence of the Mouse Genome Project strain C57BL/6 differs considerably from the IgHa haplotype of BALB/c, which has been widely used in the analyses of antibody responses. We have sequenced and annotated the 3’ half of the IgHa locus of 129/Sv, covering the CH region and approximately half of the VH region. This sequence comprises 128 VH genes, of which 49 are judged to be functional. The comparison of the IgHa sequence with the homologous IgHb region from C57BL/6 revealed two major expansions in the germline repertoire of IgHa. In addition, we found smaller haplotype specific differences like the duplication of 5 VH genes in the IgHa locus. We generated a VH allele table by comparing the individual VH genes of both haplotypes. Surprisingly, the number and position of DH genes in the 129/Sv strain differs not only from the sequence of C57BL/6 but also from the map published for BALB/c. Taken together, the contiguous genomic sequence of the 3’ part of the IgHa locus allows a detailed view of the recent evolution of this highly dynamic locus in the mouse.

1) Helmholtz Centre for Infection Research, Department of Experimental Immunology, Inhoffenstraße 7, 38124 Braunschweig, Germany

2) Torrey Pines Institute for Molecular Studies, 3550 General Atomics Court, San Diego, CA 92121-1122, USA

3) current address: INSERM U399 Immunologie et Genetique des Maladies Parasitaires, Faculte de Medecine La Timone, Marseille, France and Laboratoire de Parasitologie Mycologie, Faculte de Medecine La Timone, Universite de la Mediterranee, Marseille, France

4) Helmholtz Centre for Infection Research, Department of Genome Analysis, Inhoffenstraße 7, 38124 Braunschweig, Germany

5) University of Manchester, Bill Ford Chair of Cellular Immunology, B.1238 Michael Smith Building, Oxford Road, Manchester M13 9PT, UK
Epstein-Barr virus (EBV) is a \( \gamma \)-herpes virus which preferentially infects the B lymphocytes of humans. It is highly adapted to persist in B lymphocytes since it encodes for proteins mimicking several cellular proteins playing an important role in B cell biology. Thus, the viral Latent Membrane Protein 1 (LMP1) and the cellular CD40 receptor are considered to be functional homologues. The interaction between CD40 and its ligand CD40L, mainly expressed on activated T cells, is essential for germinal centre (GC) formation during a T cell dependent immune response. However, B cell specific expression of LMP1 in transgenic mice could not substitute for CD40 in the formation of GC; it even blocked GC formation in the presence of CD40. This might be due to the constitutive activity of LMP1 or to differences in the signalling domain. To compare CD40 and LMP1 function in vivo, we generated two transgenic mouse lines conditionally expressing (1) the fusion protein of the transmembrane domain of LMP1 and the signalling domain of CD40 (LMP1/CD40), and (2) the ligand-binding and transmembrane domain of CD40 and the signalling domain of LMP1 (CD40/LMP1). Mice expressing either LMP1/CD40 or CD40/LMP1 in a B cell specific manner from a pro/pre B cell stage were analyzed and results will be discussed.
Naive B-cells generate regulatory T-cells in the presence of a mature immunological synapse

Peter Reichardt¹, Bastian Dornbach¹, Song Rong², Stefan Beissert³, Faikah Gueler², Karin Loser³, and Matthias Gunzer¹*
¹Helmholtz Centre for Infection Research (HZI), Junior Research Group Immunodynamics
Inhoffenstraße 1, D-38124 Braunschweig, Germany
² Department of Nephrology, Hannover Medical School, D-30625 Hannover, Germany ³Department of Dermatology and Interdisciplinary Center of Clinical Research (IZKF), University of Münster, D-48149 Münster, Germany

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Naïve B-cells are ineffective antigen-presenting cells and considered unable to activate naïve T-cells. However, antigen-specific contact of these cells leads to stable cell pairs that remain associated over hours in vivo. The physiological role of such pairs is not evaluated. We show here that antigen-specific conjugates between naïve B-cells and naïve T-cells display a mature immunological synapse in the contact zone which is absent in T-cell-dendritic cell (DC) pairs. B-cells induce substantial proliferation but contrary to DC no loss of L-Selectin in T-cells. Surprisingly, while DC-triggered T-cells develop into normal effector cells, B-cell stimulation over 72 hours induces regulatory T-cells inhibiting priming of fresh T-cells in a contact-dependent manner in vitro. In vivo, the regulatory T-cells home to lymph nodes where they potently suppress immune responses such as in cutaneous hypersensitivity and ectopic allogeneic heart transplant rejection. Our finding might help to explain old observations on tolerance induction by B-cells, identifies the mature immunological synapse as a central functional module of this process, and suggests the use of naïve B-cell-primed regulatory T-cells, “bTregs”, as useful approach for therapeutic intervention in adverse adaptive immune responses.
The relative contribution and repertoire of B-1 and B-2 cell derived IgA in the gut immune system is still a controversial issue. The lack of an exclusive B-1 cell marker has made it difficult to delineate the B-1 and B-2 cell derived IgA producing plasma cells. We have used the L2 mouse line which is transgenic for $\lambda_2^{315}$ light chain and virtually consists of B-1 cells exclusively. This mouse line has been extensively characterized before. A particular feature of these mice is the presence of a few B-1 cell derived specificities at dominating frequencies in the peritoneum. These specificities, detectable as V_H sequences, can be taken as unalterable molecular markers for B-1 cells. Here, we used such markers to investigate the participation of B-1 cells in IgA production at the intestinal mucosa.

Analysis of IgM V_H sequences derived from Peyer’s patch (PP) B cells of L2 mice showed that 10% of these IgM V_H chain sequences were identical to IgM V_H sequences derived from peritoneal cavity (PEC) B-1 cells of these mice. This was a confirmation for the presence of B-1 cells in the PP of such mice. Also, some commonality amongst the IgM V_H sequences derived from the lamina propria (LP) B cells, and PEC B-1 cell associated sequences from L2 mice could be observed.

On the other hand, analysis of IgA V_H sequences derived from the lamina propria (LP) and PP B cells showed no match with B-1 cell associated Ig V_H sequences. Additionally, IgA V_H sequences derived from the LP associated plasma B cells was rather heterogeneous and showed N/P nucleotide addition in the CDR3 region and somatic hypermutation throughout the V_H region at a frequency comparable to that of non transgenic (NT) littermates. The antibody repertoire associated with these cells were completely different from that of B-1 cells from peritoneal cavity. Histology done for the intestine showed a decreased number of IgA+ plasma cells in the lamina propria of L2 mice in comparison to NT control mice. Consistent with this, there was a decrease in the levels of secretory IgA in the intestinal lumen of L2 mice. BrdU incorporation assay showed that the life span of IgA+ cells in the LP of L2 and NT mice was comparable.

In addition, IgA expressing B cells, majority of which was B-1 cells (IgA+CD43+) could be observed in the PEC of L2 as well as NT mice. Analysis of PEC B-1b cell derived IgA VH sequences of normal mice showed the presence of nucleotide exchanges throughout the V_H sequences at a high frequency.

Altogether, these data suggest that IgA producing B cells might be a highly selected population and PEC B-1 cells might diversify due to antigen driven selection in the GALT.
Rheumatoid arthritis (RA) is a chronic inflammatory process, which leads to swollen joints and finally to joint destruction. A hallmark of the disease is a massive infiltration of inflammatory cells, T and B lymphocytes and macrophages. Laser Capture Microdissection (LCM) opens a new way to study ex vivo the immune processes taking place in the inflamed synovial tissue. Frozen tissue sections were stained with the plasma cell (PC) specific antibody Wue-1. We distinguished between those PCs (a) diffusely distributed, (b) in aggregates, (c) associated with lymphocytic infiltrates or (d) in the vicinity of blood vessels. Of two patients with RA, PCs of the different subsets were microdissected by LCM, the RNA extracted and immunoglobulin genes sequenced. The data suggest that both naïve and memory B cells immigrate the inflamed synovial tissue. However, only memory B cells differentiate to PCs and spread into the synovial tissue. Whether the activation of memory B cells is induced by antigen or polyclonal is presently being investigated.
Comparative analysis of the impact of BAFF and CD40L costimulation on TLR-induced human B cell activation

1 Uwe Scheuermann, 1 Klaus Heeg, 1 Isabelle Bekeredjian-Ding
1 Dept. of Med. Microbiology and Hygiene, University Hospital Heidelberg, Germany

Objectives
Our previous data show that CD40L synergizes with TLR7 and TLR9 ligands in terms of B cell proliferation and cytokine secretion. Since BAFF, like CD40L, has been shown to induce a T cell-independent class switch recombination and both BAFF and APRIL have been demonstrated to stimulate the non-canonical NFkB pathway we wanted to address two questions: 1. whether BAFF and APRIL stimulation can modulate TLR-mediated B cell activation; and 2. whether BAFF, APRIL and CD40L stimulation differ in the qualitative outcome of B cell function in this setting.

Methods
Human peripheral blood B cells were isolated from healthy donors by density centrifugation and CD19+ MACS positive selection. B cell proliferation was measured by CFSE dilution and B cell survival was determined by Annexin V/Propidiumiodide staining. Intracellular IgM and IgG expression were determined by FACS analysis. Cytokine and Ig secretion was measured by ELISA.

Results
1. Receptor expression
   - Human peripheral blood B cells express BAFF-R, BCMA and TACI

2. Proliferation and survival
   - CD40L and BAFF synergize with TLR-ligands in terms of B cell proliferation
   - CD40L and BAFF support the survival of TLR-stimulated and unstimulated human B cells

3. Plasma cell differentiation
   - TLR9-induced plasma cell differentiation is independent of BAFF and CD40L but enhanced by APRIL
   - BAFF, APRIL and CD40L do not trigger TLR2-induced plasma cell differentiation or immunoglobulin secretion
   - BAFF and APRIL stimulation do not alter TLR9-induced immunoglobulin expression

4. Cytokine secretion
   - B cell-derived IL-10 and IL-6 production upon TLR7 and -9 stimulation are enhanced by BAFF and CD40L
   - TLR-triggered B cell-derived IL-12 production requires CD40L costimulation; BAFF can only partially be substitute for CD40L in this context

Conclusion
BAFF and CD40L synergize with TLR ligands in regards to B cell survival and proliferation. Increases in immunoglobulin synthesis and numbers of CD138+ plasma cells are most likely due to better survival and proliferation as well as increased IL-10 and IL-6 secretion in the presence of CD40L or BAFF. In comparison to CD40L BAFF is only a very weak inducer of B cell-derived IL-12 production.
Multifunctional HAX-1 interacts with membrane-bound IgE directly influencing receptor-mediated internalisation

1 Doris Schmid, 1 Iris Oberndorfer, 1 Gernot Achatz
1 Department of Molecular Biology, University of Salzburg, A-5020 Salzburg, Austria

BACKGROUND:
Signalling through the B cell antigen receptor (BCR) controls various physiological processes in B cells, such as affinity maturation, memory induction and differentiation into plasma cells. In previous work we found that truncation of the cytoplasmic tail of membrane bound IgE (mlgE) in mice led to lower levels of serum IgE, decreased numbers of IgE secreting plasma cells and the absence of specific secondary responses correlating with a defect in the selection of high-affinity antibodies during the germinal center reaction. Based on these findings we concluded that the cytoplasmic domain of mlgE has an important function in BCR-mediated processes.

METHODS AND RESULTS:
We present the multifunctional protein HAX-1 (HS1-associated protein X-1) as interaction partner of the cytoplasmic tail of mlgE. The ability of HAX-1 to interact with mlgE was confirmed in different independent experiments, both in vitro (ELISA, surface plasmon resonance analysis (SPRA), coimmunoprecipitation) and in vivo (coimmunoprecipitation). In protein silencing experiments (using the RNA interference technology) with mlgE− myeloma cells we detected a correlation between the level of HAX-1 expression and the ability of the respective cell clone to internalise antigen via the BCR. A decrease in HAX-1 protein levels reduces the efficiency of BCR-mediated internalisation of antigen.

CONCLUSION:
We provide evidence that the cytoplasmic tail of mlgE has the capacity to interact with HAX-1 in vitro and in vivo. We found that the expression level of HAX-1 influences the ability of B cells to internalise antigen via receptor-mediated endocytosis. Since HAX-1 also interacts with HS1, a protein playing a major role the modulation of actin assembly in hematopoietic cells, we suggest a model in which HAX-1 physically links the mlgE molecule to the cytoskeleton via its interaction with HS1. As HS1 also influences the fundamental processes of clonal expansion and deletion in lymphoid cells and HAX-1 can act as an inhibitor of apoptosis, we suppose that HAX-1 is of importance for the survival of (mlgE−) B cells. In order to investigate the importance of HAX-1 in B lymphocytes in general, and in mlgE− ones in particular, we are generating a B cell-specific inactivation of HAX-1 in mice.
Research in our group aims to understand the locus specificity of AID mediated immunoglobulin (Ig) gene diversification. In this context we examine cis-acting regulatory DNA elements and DNA binding factors, which might take part in recruiting AID to the DNA locus.

The used model system is the chicken B cell line DT40. Antibody diversity in chicken is generated slightly different from mouse and human as the pre-immune Ig gene repertoire is not produced by V(D)J recombination but by pseudo V-gene templated gene conversion (GC). Another source for diversity is Ig hypermutation (HM) which introduces point mutations into the rearranged VJ segment. After deletion of the pseudogenes in the rearranged Ig light chain locus DT40 switches from GC to HM (Arakawa et al., 2004). The hypermutating variant of DT40 can be used to investigate the mechanism of HM. GC and HM both require the B cell specific enzyme AID which is believed to deaminate cytosines to uracil. Many of the AID-induced uracils seem to be further processed by UNG causing an abasic site, which can be repaired by GC or by error-prone repair leading to HM. A lack of AID leads to a complete stop of the all Ig diversification in DT40 (Arakawa et al., 2001).

AID expression is restricted to B cells, but constitutive expression for example in fibroblast cell lines leads to HM of highly expressed genes (Yoshikawa et al., 2002). We therefore presume that there are B cell specific cofactors and cis-acting regulatory sequences which limit HM and GC to the Ig loci. One of these factors seems to be the E2A encoded transcription factor E47, whose expression was found to enhance GC and HM in DT40 (Conlon and Meyer, 2006; Schoetz et al., 2006).

We are continuing to investigate the mechanism of action of E47 and the role of potential cis-acting regulatory elements in the light chain locus of DT40. For example we have designed a reporter construct in which a RSV promoter drives expression of a GFP gene. When this construct is inserted into the rearranged Ig light chain locus in the opposite orientation of the Ig gene, the GFP coding region appears to accumulate hypermutations as indicated by the loss of GFP expression in expanding subclones. Deletion of the Ig locus until the downstream carbonic anhydrase gene stops the loss of GFP during cell proliferation. We are now introducing stepwise deletions of various lengths into the Ig light chain locus to screen for the presence of DNA elements important for HM. We are also continuing our search for transacting factors which influence HM and GC in DT40.

References:
Krueppel-like factor 2: A negative regulator of pre-B cell expansion and B cell activation

Pre-B cell receptor signals are required for pre-B cells to divide 4-6 times before they differentiate into small, resting pre-B cells. However, the mechanisms limiting pre-B cell expansion remain elusive. To address this issue we are using a mouse model system in which expression of a transgenic µHC and subsequently pre-BCR-mediated clonal expansion can be controlled by tetracycline. By Affymetrix microarray analyses we identified krueppel-like factor 2 (LKLF/KLF2), a transcription factor involved in maintenance of quiescence in T cells, as a novel pre-BCR-induced target gene. Moreover, upregulation of KLF2 was detectable in primary small, resting pre-B cells compared to large, proliferating pre-B cells. Retroviral expression of KLF2 inhibited pre-BCR-induced expansion and resulted in G1 arrest. Additionally, we found that KLF2 is expressed in resting splenic B lymphocytes and is rapidly downregulated upon stimulation mimicking either T cell-dependent or T cell-independent activation. Retrovirally expressed KLF2 blocked LPS-induced cell growth as well as LPS-induced differentiation into plasmablasts. Therefore, we conclude that KLF2 not only terminates the cell cycle of clonally expanded small pre-B cells in the bone marrow but also maintains quiescence of mature B cells in the spleen.

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Development of therapeutic concepts for transplant recipients with allo-reactive memory B cells

1 Sybill Thomas-Ecker, 2 Petra Reinke, 1 Hans-Dieter Volk
1 Institute of Medical Immunology, Charité Universitätsmedizin, Berlin, Germany
2 Nephrology and Medical Intensive Care, Charité Universitätsmedizin, Berlin, Germany

Current research in transplantation medicine is predominantly focused on the clinical introduction of tolerance inducing principles. In this regard, the therapeutic aim is to minimize side effects caused by immunosuppressive drugs. To avoid dose-dependent damage of the transplant, a specifically targeted monitoring of the patients both pre- and post surgery is mandatory. It is known, that memory B cells are an integral part of immunologic memory and may have already “stored” antigenic information from a previous exposure to allo-antigen, for instance by a pregnancy, a blood transfusion, or a previous transplantation. It is generally thought that the allo-antigenic information stored in memory B cells contributes to a later humoral rejection response. The existing tests are inapplicable to monitor the status of allograft specific memory-B-cells.

We have developed an antigen-specific B-cell Elispot, whereby it is possible to detect antigen-specific and unspecific Ig secretion. In our system we are able to stimulate PBMCs polyclonal with a high yield of T- and B-cell clones (ratio 70:30). Thereby, memory B cells are differentiating to early plasma cells. The frequency of Ig secreting B cells is depending on the coated antigens (CMV: TT: ). Flow cytometry measurements are showing that 40% of CD19⁺ B cells (lymphocytes live gate) are differentiates into CD19⁺/CD27low/CD38high cells after in vitro stimulation of 7 days.

Subsequent, an allo-specific-B-cell-Elispot should be developed to detect mentioned allograft specific memory-B-cells.

The aim of this study is to develop a new clinical method for monitor allospecific-B-cell-Elispot in humans to improve the long term survival of transplanted patients.
Basic Krüppel-like factor promotes B cell differentiation towards the marginal zone lineage.

Gleb Turchinovich, Sonja Schmid, Jan Kranich, Jörg Kirberg
Max-Planck-Institute for Immunobiology, Freiburg im Breisgau, Germany

Basic Krüppel-like factor (BKLF) belongs to the SP/KLF superfamily of transcription factors. Its biological function is poorly understood. BKLF was identified as a proviral integration site in the A-MuLV induced pre-B cell line 18-81. Unlike other pre-B cell lines, 18-81 constitutively undergoes somatic hypermutation (SHM) and is capable of class switch recombination (CSR) upon stimulation. To study the role of BKLF in B cell development and function we generated BKLF Tg mice, where BKLF expression is driven by the CD19 promoter.

BKLF overexpression does not induce SHM in resting B cells, however, BKLF Tg B cells show increased CSR rates upon stimulation with LPS and IL-4 in vitro. Unexpectedly, BKLF transgenic mice show a 3-10 fold increase in the number of marginal zone (MZ) B cells. Using competitive bone marrow or foetal liver reconstitution experiments we demonstrate that BKLF overexpression promotes MZ B cell development cell autonomously and does not lead to defective production of follicular B cells. The accumulation of MZ B cells in BKLF Tg mice is not due to alterations in the expression of adhesion molecules, migration, or BCR signalling. Moreover, BKLF overexpression is able to promote differentiation towards the MZ lineage of B cells expressing a defined BCR that normally does not favour MZ B cell development. Injection of BKLF Tg mice with a presenilin inhibitor demonstrated that BKLF overexpression is unable to protect MZ B cell compartment in the absence of Notch signalling.

In summary, BKLF is a potent regulator of MZ B cell development. We show that BKLF overexpression does not interfere with BCR signalling, B cell homing, and/or Notch signalling, factors known to be involved in MZ B cell lineage commitment. Whether BKLF acts downstream in one of the above mentioned pathways or represents a novel modulator of MZ B cell differentiation is currently being investigated.
The unique tail of lambda-5 controls clonal expansion but is dispensable for differentiation of pre-B cells

1 Christian Vettermann, 1 Kai Herrmann, 1 Harald Bradl, 2 Michael Bösl, 1 Hans-Martin Jäck

1 Division of Molecular Immunology, Department of Internal Medicine III, Nikolaus-Fiebiger-Center, University of Erlangen & Nürnberg
2 Max-Planck-Institute for Neurobiology and Biochemistry, Martinsried

The precursor-B cell receptor (pre-BCR) induces proliferation of pre-B cells, which diversifies the antibody repertoire, since each expanded pre-B cell clone can rearrange a different immunoglobulin (Ig) light (L) chain and thus a different Ig receptor specificity. However, it is unclear how proliferative expansion signals from the pre-BCR are initiated. The pre-BCR consists of an Igμ heavy chain, the signal transducers Igα-Igβ and the surrogate light chain (SLC) components VpreB and λ5. It has been proposed that the basic amino acids in the N-terminal non-Ig-like (so-called unique) tail of λ5 mediate pre-BCR aggregation in order to enhance signaling strength. Here, we show that developmental transition through the pre-BCR checkpoint is impaired in genetically modified mice expressing mutated λ5, in which the unique tail was either abrogated or modified by substitution of all basic residues to alanine. Flow cytometry revealed a diminished frequency of cycling pre-B cells in mice with mutated λ5 compared to wildtype mice, which was paralleled by reduced pre-B cell growth in vitro. In contrast, the pre-BCR-induced differentiation signals leading to suppression of SLC and to induction of IgL-chain remained largely intact. Further, we show that a pre-BCR interacts via the basic amino acid residues of λ5’s unique tail with the stroma cell ligand heparan sulfate, indicating a putative role for ligand-mediated amplification of pre-BCR signals. Hence, proliferative signals are amplified via pre-BCR aggregation mediated by the unique region of λ5, which results in clonal expansion of pre-B cells with a functional μHC.

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Classical Hodgkin lymphoma (HL) is a hematological malignancy mainly derived from germinal center (GC) B cells and is characterized by several deregulated signaling pathway, including the constitutive activation of STATs and NF-kappaB. Recently, we demonstrated that the kinase inhibitors AG17 and AG490 blocked cellular proliferation and STAT3 phosphorylation. RNA-interference directed against STAT3 in HL cells demonstrated its essential role for cell proliferation of these HL cells. Differential gene expression profiles of HL cell lines after STAT3 silencing was described performing microarray analysis. Several genes were described as affected by overexpression of constitutive IkappaB-alpha. Furthermore, genes were affected by STAT3 silencing resembling a CD40-stimulation signature of GC B cells and NF-kappaB mediated HL signature.

In over 50% of cases the Epstein Barr Virus (EBV) can be detected in the malignant cells of cHL. It is suggested that the EBV latent membrane protein 1 (LMP1) plays a central role in the transformation of B cells. To identify cellular genes potentially involved in LMP1-mediated transformation of GC B cells a differential gene expression profile of human tonsillar GC B cells transfected with LMP1 was investigated. LMP1 expression in GC B cells showed an overlapping but also divergent pattern of modulated genes and demonstrate that LMP1 might contribute to the survival of B cells that should usually undergo apoptosis.

Our data provide new insights into the process of B cell rescue in the germinal center reaction that might lead to the transformation of these cells.

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A common complement receptor 2 haplotype is a disease modifier for human systemic lupus erythematosus

1 Sven Wach, 1 Ute Wellmann, 1 Thomas Winkler
1 Institute for Biology, Hematopoiesis Unit, University Erlangen-Nürnberg

Systemic lupus erythematosus (SLE, OMIM 152700) is a complex autoimmune disease characterized by autoantibody production against nuclear antigens. Recent studies suggested, that a polymorphism in the complement receptor 2 gene (CR2) is associated with increased susceptibility to SLE in lupus prone mice (1). On the surface of B-cells, CR2 forms a complex with CD19 and acts as a coreceptor for the B-cell receptor. On follicular dendritic cells CR2 is involved in the presentation of antigen during affinity maturation (2). Since CR2 has been proposed to be a potential susceptibility gene for human SLE, we tested common polymorphisms in CR2 for their association with SLE in a case-control study. We genotyped 29 single nucleotide polymorphisms (SNPs) in the CR2 gene and found, that strong linkage disequilibrium extends over large portions of the CR2 gene. Furthermore, we discovered, that the reconstructed haplotypes could be partitioned into two main clades and a haplotype-tagging SNP (htSNP) was defined in order to distinguish between the main clades of haplotypes. There was no evidence of association of rs17615 genotypes with SLE susceptibility in three independent case-control cohorts. When we analyzed our data for association of rs17615 genotypes with clinical phenotypes of SLE in a German cohort, we found, that patients homozygote for haplotypes of the minor clade show a delayed time point of SLE diagnosis, median 40 years vs. 28 years for patients homozygote for haplotypes of the major clade (P=0.02). This finding could be confirmed in an independent cohort of North Americans with European descent (EurAm) with median 41.5 years vs. 33 years (P=0.01), but not in a cohort of North Americans with African descent (AfAm). These findings suggest, that common haplotypes of CR2 as distinguished by rs17615 are not associated with SLE susceptibility in a case-control study. We could demonstrate, however, that CR2 haplotypes modify the course of SLE, resulting in a delayed onset of the disease in patients homozygote for haplotypes of the minor clade.

1. Boackle et al. (2001) Immunity.: 15
Plasmablasts migrate from secondary lymphoid tissue to the bone marrow to survive in this microenvironment to produce memory antibodies. Contacts made with other cells and stroma have a major impact on proliferation, differentiation, survival, and migration of plasma cell precursors as well as on the lifespan of the antibody-secreting cells. Here, we track plasma cells and their precursors during a specific memory immune response against the protein antigen ovalbumin (Ova). Ova-specific plasma cell precursors enter the bone marrow via small capillaries. At that time they make contact to B78+ endothelial cells that constitute the vascular bone marrow niche, known to provide cytokines and other stimulating factors important for the regulation of hematopoietic stem cell differentiation. Following differentiation into a more mature phenotype Ova-specific plasma cells migrate apart from the vascular niche and adhere to mesenchymal (CD45-) stromal cells. Importantly, at all stages of development, plasma cells and their precursors are co-localized with Gr-1+ myeloid cells which are likely attracted by the chemokine CCL3 that is produced by plasma cells. We assume that GR-1+ myeloid cells constitute a mobile part of niches for plasma cell differentiation in the vascular niche and for survival at the mesenchymal stromal niche, contributing to these environments through the production of cytokines. The cytokine expression profile of Gr-1+ myeloid cells, B78+ endothelial lineage cells and mesenchymal stromal cells is currently under investigation, with a particular focus on IL-5, IL-6, TNF-alpha, APRIL and BAFF. We and others have demonstrated already earlier that cytokines are important survival factors for long-lived bone marrow plasma cells.
Cytomegalovirus (CMV) viremia and disease are a major cause of morbidity and mortality in immunosuppressed patients e.g. after bone marrow or stem cell transplantation. CMV replication in these patients arises as a result of lack of immune control. Thus, bridging the period of immunodeficiency by passive transfer of immunity is a major goal not only in transplant medicine. Neutralizing antibodies as a product of the humoral arm of the immune system contribute to CMV protection by elimination of disseminating virus. Using non-infectious hCMV particles in mice we have recently shown that reactivation of virus-specific memory B cells is independent of cognate or bystander T cell help. These findings raised the possibility that adoptive transfer of memory B cells into immunodeficient hosts can protect from viral dissemination and rapid lethality.

To test this we switched to an infectious animal model using mouse CMV (mCMV). Memory B lymphocytes from mCMV-infected C57BL/6 mice were adoptively transferred into B and T cell deficient RAG1⁻/⁻ mice. In the recipient mice strong IgG anti-CMV titers developed within 4-6 days after mCMV infection whereas RAG1⁻/⁻ mice transferred with B cells from naïve donors did not develop detectable anti-mCMV antibodies upon infection. A 100-1,000 fold decrease in viral titers and a 1,000-10,000 fold decrease of viral DNA load in spleen and lung was measured in mice which received mCMV specific memory B cells when compared to the naïve B cell recipients. In further experiments we used a recombinant mCMV (mCMVde1157luc) that cannot be controlled by NK cells and leads to rapid lethality of infected C57BL/6 RAG1⁻/⁻ mice. In this virus a luciferase reporter-gene has been integrated allowing in vivo bioluminescence imaging of the course of the virus infection. In addition, C57BL/6 CD8⁻/⁻ donor mice were used to completely rule out involvement of cytotoxic T cell contaminations of adoptively transferred memory B cells. Also in this experimental setting we could demonstrate that adoptive transfer of memory B cells in the absence of CD8 cells and cognate or bystander T cell help is sufficient to protect from viral dissemination and rapid lethality. This protection was long-lasting, as recipient Rag⁻/⁻ mice were still alive after 100 days in contrast to the naïve B cell control groups. We also showed that transfer of sera from mCMV-immune animals has a comparable protective effect against mCMV infection, indicating that indeed antibodies represent the protective principle. We also were interested whether adoptive memory B cell transfer could rescue animals after an established lethal mCMV infection. Transfer of memory B cells into mice that were infected 3 days earlier with mCMV prevented further dissemination and lethality and induced long-lasting protection. Our data indicate an applicability of adoptive memory B cell transfer to bridge the period of immunodeficiency.

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The major birch pollen antigen (Bet v1) is one of the main causes of respiratory allergy. Birch pollen preparations consist of a mixture of different Bet v1 isoforms (Bet v1a, Bet v1b, Bet v1c, etc.). Although the isoforms show a high degree of amino acid sequence homology, their immunologic properties are different. Serum IgE from birch-pollen allergic patients reacts with only a subset of Bet v1 isoforms (Bet v1a, e, f and j), whereas the hypoallergenic isoforms Bet v1d, g and l are not recognized. On the other hand, a basic T lymphocyte reactivity is demonstrated for all isoforms, with only minor variations in the T cell restriction to particular isoforms (Ferreira et al., 1996). In our present study, we focused on the differences between immunogenicity and allergenicity of recombinant Bet v1a and Bet v1d in mice. Therefore, mice were immunized with Bet v1a, Bet v1d or with mixtures of defined ratios according to their relative presence in birch pollen. The levels of specific serum IgE and of the other Ig classes IgG1, IgA and IgG2a were determined. We could show that in Bet v1d immunized mice a clearly higher titer of specific IgG1, IgA and IgG2a could be detected whereas Bet v1a immunized animals exhibited a higher concentration of specific serum IgE as determined by beta-hexosaminidase release assays. We think that based on these results, hypoallergenic Bet v1 isoform might be a promising tool for use in specific immunotherapy in order to shift the immune response from IgE production towards production of protecting IgG and IgA antibody classes.

1 Nadja Zaborsky, 1 Fatima Ferreira and 1 Gernot Achatz
Department of Molecular Biology, Division of Immunology and Allergology, 5020 Salzburg, Austria