The Proposed Role of XBP-1 and the UPR in Antibody Secreting Cells and Common Variable Immunodeficiency Pathogenesis

Common Variable Immunodeficiency

Primary immunodeficiency disorders (PIDs) are a heterogeneous group of disorders characterized by defects affecting the innate and adaptive immune system. A subtype of PID, Primary Antibody Deficiency, results in reduced biosynthesis of antigen-specific, high-affinity antibodies (Baldovino et al., 2013). While other forms of primary antibody deficiencies, like X-linked agammaglobulinemia, are well understood and congenital, other primary immune deficiencies, like Common Variable Immunodeficiency (CVID) have variable age of onsets and is poorly characterised. This poor characterisation is likely owing to CVID being a diagnosis of elimination, with patients having to undergo multiple tests to eliminate other PID diagnoses (Baldovino et al., 2013).

Common Variable Immunodeficiency (CVID) is the most common primary immune deficiency, and is hallmarked by hypogammaglobulinemia. CVID is a heterogeneous disease, with 40% of individuals experiencing comorbid conditions like gastrointestinal disease, autoimmune disease and inflammation. Most also experiencing recurrent respiratory infections (Wehr et al., 2007). Due to its diverse presentation, CVID presents as a diagnostic challenge to clinicians, and has previously led to misdiagnosis (Buchbinder et al., 2015). Despite its heterogeneity, current methods of subtying are based on the classification of antibody expression or depletion, with little understanding of the underlying molecular and pathogenic mechanisms of the disease. The heterogeneous presentation of this disease warrants the need for subtyping to better understand the disease pathogenesis and allow for the implementation of more adequate detection, treatment and management.

Next generation sequencing (NGS) has recently been applied to identify over 270 different genes associated with Primary Immunideficiency with the aim of aiding diagnosis and identifying subgroups (Nijman et al., 2014, van Schouwenburg et al., 2015). Most cases of CVID are sporadic, with 10% of

patients having first-degree relatives with hypogammaglobulinaemia, resulting in the majority of genetic contributions being left unidentified (Baldovino et al., 2013, van Schouwenburg et al., 2015). However, with strong evidence of famillial clustering, CVID is thought to have a genetic basis. There is insufficient knowledge linking associated genes to molecular pathways, and how they are involved in the disease pathogenesis. So far, only monogenic causes of CVID have been identified in rare, mendelian inheritance cases. Defects have been found in the genes that encode for B cell antigen receptor associated complex (CD19, CD81, and CD21) (van Zelm et al., 2006, van Zelm et al., 2010, Thiel et al., 2012), CD20 (Kuijpers et al., 2009), inducible co-stimulator (ICOS) (Bossaller et al., 2006), and B cell activating factor receptor (BAFF-R) (Warnatz et al., 2009). Moreover, mutations resulting in deficiencies in transmembrane activator and calcium-modulating ligand interactor (TACI), encoded by the tumour necrosis factor receptor superfamily (TNFRSF13B), have been identified in a number of CVID cohorts (Salzer et al., 2009). Mutations in TACI have been observed in 10% of individuals with CVID (Baldovino et al., 2013). TACI is known to have an important function in intercellular signaling cascades and is expressed peripheral B-cells and activated T-cells, although, the functional and molecular impact of TACI mutations in individuals with CVID remains unknown (Salzer et al., 2009).

Few successful efforts have been made to identify subtypes or pathogenic pathways linking the genetic defects to antibody deficiency in CVID. Studies have applied NGS to large cohorts of CVID patients, resulting in a large number of candidate CVID genes being identified without identifying a pathogenic pathway (van Schouwenburg et al., 2015). A more targeted approach that connects genotype to phenotype in CVID can aid in establishing disease mechanisms and can steer potential treatment strategies and aid in diagnosis. Some studies have indicated that the Unfolded Protein Response (UPR) and the *Xbp-1* gene could drive the pathogenesis of a subset of sporadic cases of CVID (Kuribayashi et al., 2008).

The Role of XBP-1

XBP-1 in the Unfolded Protein Response

The UPR occurs in response to stress in the endoplasmic reticulum (ER), and is activated to prevent the accumulation of misfolded or unfolded proteins and resulting cell toxicity (Moore and Hollien, 2012) (see Figure 1). The UPR is driven by three pathways, each of which rely on a different transmembrane receptor that spans the ER lumen into the cytoplasm. These three receptors are IRE1α, PERK and ATF6α (Bettigole and Glimcher, 2015).

In a steady state, where proteins are correctly folded, each of the pathways are held inactive by BiP chaperone proteins. In response to the accumulation of unfolded proteins and cellular stress, IRE1α receptor autophsophorylates in to activate an endoribonuclease cleavage domain. This endoribonuclease domain cleaves the *Xbp-1* mRNA transcript to enable its translation to form the XBP-1 transcription factor (see Figure 1b). This transcription factor then upregulates transcription of glycosylases, chaperones and ERAD proteins to correct the stress. Recent research has also suggested that unprocessed *Xbp-1* acts to suppress IRE1 phosphorylation and prevent degradation of secreted heavy μ chain of Ig (Benhamron et al., 2014). If the stress becomes unsolvable, then apoptosis is initiated. IRE1α is the only UPR branch required for robust antibody secretory production in plasma cells (Bettigole and Glimcher, 2015). However, research suggests that the ATF6α pathway is also activated in plasma cell development, but at a later stage in the pathway (Gass et al., 2008, Ma et al., 2010). The significance of the activation of the ATF6α remains unknown. Unlike IRE1α, research indicates that ATF6α is not necessary for high Ig secretion in plasma cells (Aragon et al., 2012).

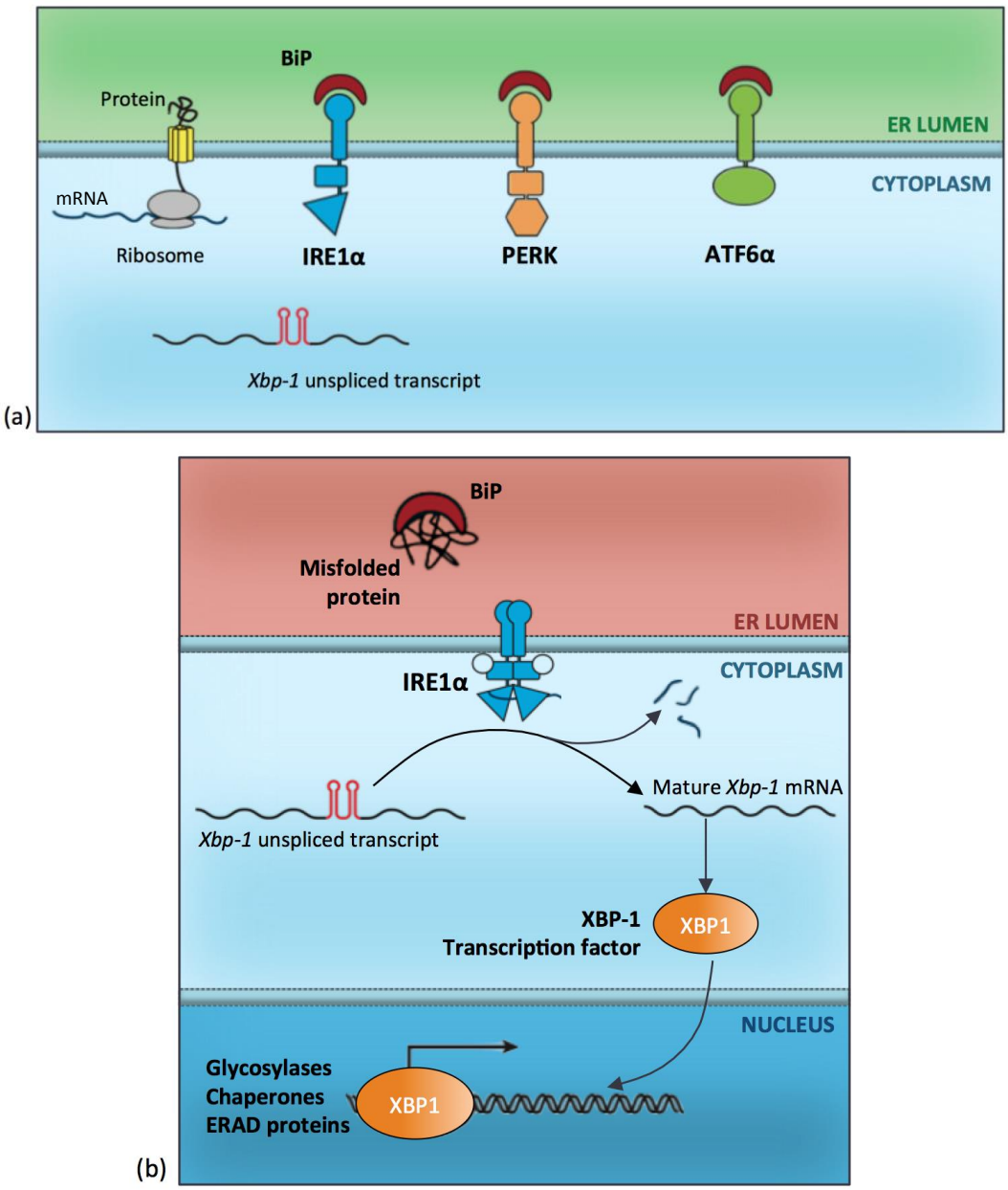

Figure 1 – Endoplasmic Reticulum (ER) stress and the Unfolded Protein Response (UPR). Adapted from Bettigole and Glimcher (2015). **(a)** The mRNA is translated through the ribosomes and the protein is fed through a transmembrane domain where it enters the ER lumen. If these proteins are correctly folded, the cell remains in a steady state, and each receptor (IRE1α, PERK and ATF6α) is held inactive and prevented from initiating UPR by BiP chaperone protein. **(b)** Upon ER stress stress, BiP binds to accumulating misfolded proteins and dissociates from the transmembrane receptors, thereby initiating UPR. In the IRE1α pathway, the BiP dissociation triggers autophosphorylation of the IRE1α transmembrane protein. This autophosphorylation triggers a conformational change, activating IRE1α endoribonuclease activity. The endoribonuclease domain is then able to cleave *Xbp-1* mRNA transcript, enabling its translation into the XBP-1 transcription factor. XBP-1 can then enter the nucleus and transcribe proteins like glycosylases, chaperones and ERAD proteins to correct the stress.

XBP-1 in Plasma cell development and Ig secretion

B-cells are produced in the bone marrow where they are activated by antigen-activated helper T-cells. These activated B-cells undergo clonal expansion in the germinal centre, where they differentiate into memory B-cells or antibody secreting plasma cells (Nutt et al., 2015) (see Figure 2). Two genes that are known to be important in the differentiation process are *Pax5* and *Blimp-1*. *Pax5* prevents B-cells from differentiating into plasma cells and is thought to suppress *Xbp-1* expression, while *Blimp-1* enables the differentiation of mature B-cells into the plasma cell (Nutt et al., 2011).

While earlier studies have suggested that *Xbp-1* was also essential for plasma cell differentiation (Reimold et al., 2001), recent studies using B-cell conditional *Xbp-1* knockout mice have demonstrated that plasma cells can develop relatively normally in the absence of XBP-1 (Taubenheim et al., 2012). While there is consensus in *Xbp-1* being necessary for antibody secretion, the role of XBP-1 in later stages of development is still being debated (Taubenheim et al., 2012, Todd et al., 2009). Both Taubenheim et al. (2012) and Todd et al. (2009) analysed *Xbp-1* B-Cell conditional knockout mice using qRT-PCR, ELISA/ELISpot and electron microscopy to find that XBP-1 depletion does not effect *Blimp-1* or *Pax5* expression, but does reduce Ig secretion and alter ER morphology, however, differential conclusions were drawn. Based on the data that XBP-1 is not expressed alongside the gene network involving *Blimp-1* and *Pax5* that govern plasma cell differentiation, Taubenheim et al. (2012) formed their conclusion that XBP-1 is not involved in B-cell differentiation. Todd et al. (2009), however, based their conclusions on previous reports that XBP-1 is essential to for differentiation, combined with findings that XBP-1 alters the morphology of the ER in the plasma cell, and suggested a late yet-to-be-defined stage in plasma cell differentiation occurs after the expression of Blimp-1 and CD138 and before Ig secretion. Further research is still required to determine whether the observed abnormal ER morphology appears before or after the development of the mature plasma cell. While it is currently unknown if XBP-1 is required for plasma cell development, results have consistently demonstrated that XBP-1 is necessary for normal antibody secretion in plasma cells.

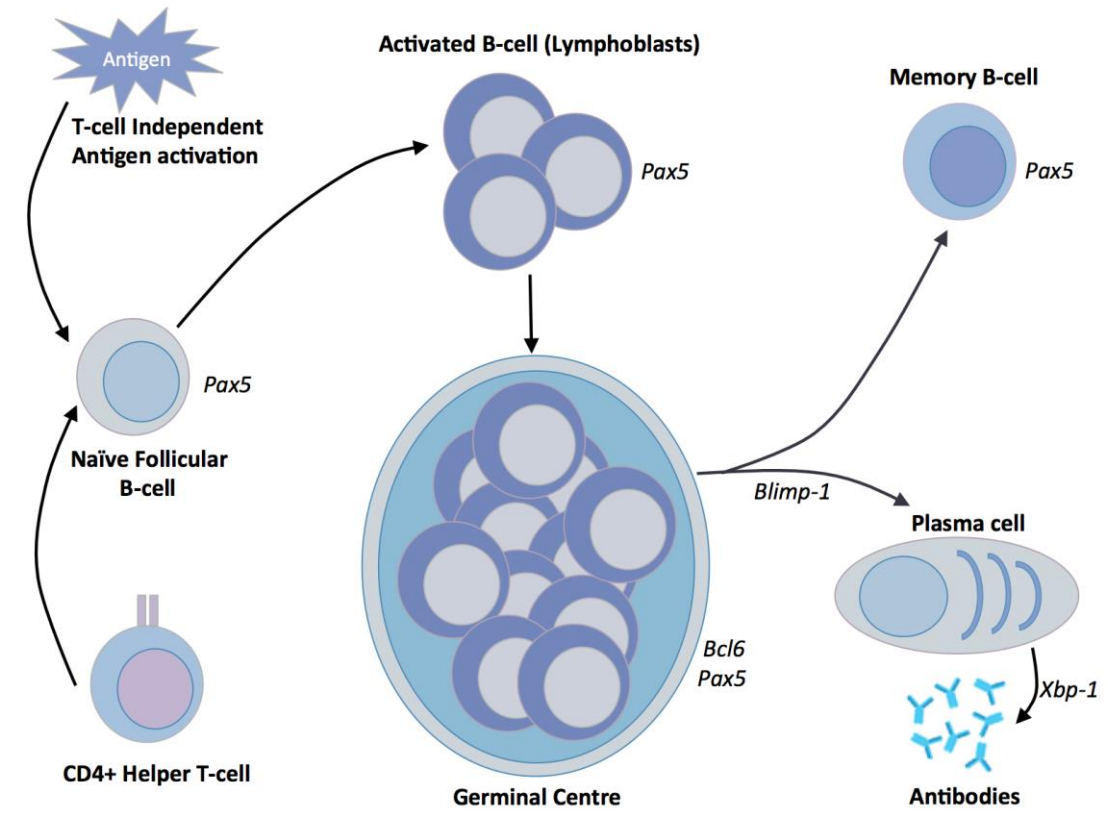

Figure 2 – Terminal B-cell differentiation. Follicular B-cells make up the majority of mature B-cells and are located in the follicles of lymphoid organs. All mature B cell subsets express the transcription factor PAX5. Naïve follicular B-cells, when activated directly by antigens or by helper T-cells, form activated B-cells (also termed B-lymphoblasts). These activated B-cells can then rapidly proliferate and undergo immunoglobulin class-switch recombination. Activated B-cells can also upregulate *Bcl6* during the germinal centre reaction to form the germinal centre, where B-cells with high-affinity antigen receptors exit and differentiate into either memory B cells or long-lived plasma cells, which express high levels of *Blimp-1*, to allow for differentiation, and XBP1, to produce large quantities of antibody.

In agreement with Taubenheim et al. (2012) and Todd et al. (2009), research has consistently shown that XBP-1 is a key transcriptional regulator in the secretory pathway through its role in ER remodelling. Using expression profiling, B-cell lines that expressed *Xbp-1* showed upregulation in genes involved in vesicle trafficking and translocating proteins to the ER when compared to *Xbp-1* deficient cell lines (Shaffer et al., 2004). Shaffer et al. (2004) also demonstrated that *Xbp-1*-expressing B-cells exhibited expanded the ER and increased cell size when compared to XBP-1 deficient cell lines, highlighting the important role of XBP-1 in plasma cell ER structure. It has been hypothesised that XBP-1 depletion reduces Ig secretion as a result of *Xbp-1*'s functional role in ER remodelling, which enables high levels of antibody secretion. This hypothesised function of XBP-1 has been reflected in studies of plasma cell

differentiation which demonstrated altered ER morphology in *Xbp-1* knockout B-cells compared to wild-type B-cells. Studies using electron microscopy have reported large vesicle-like structures and less extensive ER in Xbp-1 deficient B-cells that were not characteristic of wild-type plasma cells (McGehee et al., 2009, Taubenheim et al., 2012). Most of the XBP-1–deficient plasma cells displayed enlarged and disorganized ER (Taubenheim et al., 2012, Todd et al., 2009). This proposed function of XBP-1 in ER remodeling could provide an explanation and a molecular pathway to how XBP-1 depletion in plasma cells results in reduced secretion of antibodies.

The current issue

Research has strongly suggested that XBP-1 and related UPR pathways are critical elements of effective antibody secretion in plasma cells. With hypogammaglobulinemia being a primary characteristic of CVID, XBP-1 dysfunction presents itself as a candidate in the underlying pathway of CVID in a subset of patients. As it stands, the role of XBP-1 in the plasma cell developmental pathway and CVID pathogenesis remains unknown. The current study therefore aims to decipher whether dysfunctions in *Xbp-1* or related genes account for a subgroup of individuals with CVID and explain the underlying disease pathogenesis in this subgroup. This improved knowledge can then guide potential treatment strategies and aid in diagnosis of this subgroup in future.

Research questions – How do malfunctions in *Xbp-1* or related genes in the UPR pathway cause breakdowns in the plasma cell functioning (what is the molecular pathway)? Does this account for a subgroup of individuals with CVID and account for the underlying disease pathway in this subgroup?

References

Aragon, I. V., Barrington, R. A., Jackowski, S., Mori, K. and Brewer, J. W. (2012) 'The specialized unfolded protein response of B lymphocytes: ATF6alpha-independent development of antibody-secreting B cells', *Mol Immunol* 51:3-4, 347-355.

Baldovino, S., Montin, D., Martino, S., Sciascia, S., Menegatti, E. and Roccatello, D. (2013) 'Common variable immunodeficiency: crossroads between infections, inflammation and autoimmunity', *Autoimmun Rev* 12:8, 796-801.

Benhamron, S., Hadar, R., Iwawaky, T., So, J. S., Lee, A. H. and Tirosh, B. (2014) 'Regulated IRE1-dependent decay participates in curtailing immunoglobulin secretion from plasma cells', *Eur J Immunol* 44:3, 867-876.

Bettigole, S. E. and Glimcher, L. H. (2015) 'Endoplasmic reticulum stress in immunity', *Annual Review Immunology* 33, 107-138.

Bossaller, L., Burger, J., Draeger, R., Grimbacher, B., Knoth, R., Plebani, A., Durandy, A., Baumann, U., Schlesier, M., Welcher, A. A., Peter, H. H. and Warnatz, K. (2006) 'ICOS deficiency is associated with a severe reduction of CXCR5+CD4 germinal center Th cells.', *J Immunol* 177:7, 4927-4932.

Buchbinder, D., Baker, R., Lee, Y. N., Ravell, J., Zhang, Y., McElwee, J., Nugent, D., Coonrod, E. M., Durtschi, J. D., Augustine, N. H., Voelkerding, K. V., Csomos, K., Rosen, L., Browne, S., Walter, J. E., Notarangelo, L. D., Hill, H. R. and Kumanovics, A. (2015) 'Identification of patients with RAG mutations previously diagnosed with common variable immunodeficiency disorders', *J Clin Immunol* 35:2, 119-124.

Gass, J. N., Jiang, H. Y., Wek, R. C. and Brewer, J. W. (2008) 'The unfolded protein response of B-lymphocytes: PERK-independent development of antibody-secreting cells', *Mol Immunol* 45:4, 1035-1043.

Kuijpers, Taco W., Bende, Richard J., Baars, Paul A., Grummels, Annette, Derks, Ingrid A.M., Dolman, Koert M., Beaumont, Tim, Tedder, Thomas F., Noesel, Carel J. M. van, Eldering, Eric and Lier, René A.W. van (2009) 'CD20 deficiency in humans results in impaired T cell–independent antibody responses', *Journal of Clinical Investigation* 120:1, 214-222.

Kuribayashi, J. S., Bombardieri, C. R., Baracho, G. V., Aliberti, J., Machado, F. S., Kalil, J., Guilherme, L., Kokron, C. M., Rizzo, L. V. and de Camargo, M. M. (2008) 'Slower rescue of ER homeostasis by the unfolded protein response pathway associated with common variable immunodeficiency', *Mol Immunol* 45:10, 2990-2997.

Ma, Y., Shimizu, Y., Mann, M. J., Jin, Y. and Hendershot, L. M. (2010) 'Plasma cell differentiation initiates a limited ER stress response by specifically suppressing the PERK-dependent branch of the unfolded protein response', *Cell Stress Chaperones* 15:3, 281-293.

McGehee, A. M., Dougan, S. K., Klemm, E. J., Shui, G., Park, B., Kim, Y. M., Watson, N., Wenk, M. R., Ploegh, H. L. and Hu, C. C. (2009) 'XBP-1-deficient plasmablasts show normal protein folding but altered glycosylation and lipid synthesis', *Journal of Immunology* 183:6, 3690-3699.

Moore, K. A. and Hollien, J. (2012) 'The unfolded protein response in secretory cell function', *Annu Rev Genet* 46, 165-183.

Nijman, I. J., van Montfrans, J. M., Hoogstraat, M., Boes, M. L., van de Corput, L., Renner, E. D., van Zon, P., van Lieshout, S., Elferink, M. G., van der Burg, M., Vermont, C. L., van der Zwaag, B., Janson, E., Cuppen, E., Ploos van Amstel, J. K. and van Gijn, M. E. (2014) 'Targeted next-generation sequencing: a

novel diagnostic tool for primary immunodeficiencies', *Journal of Allergy and Clinical Immunology* 133:2, 529-534.

Nutt, S. L., Hodgkin, P. D., Tarlinton, D. M. and Corcoran, L. M. (2015) 'The generation of antibody-secreting plasma cells', *Nat Rev Immunol* 15:3, 160-171.

Nutt, S. L., Taubenheim, N., Hasbold, J., Corcoran, L. M. and Hodgkin, P. D. (2011) 'The genetic network controlling plasma cell differentiation', *Semin Immunol* 23:5, 341-349.

Reimold, A. M. , Iwakoshi, N. N., Manisk, J. , Vallabhajosyula, P. , Szomolanyi-Tsuda, E. , Gravallese, E. M. , Friend, D. , Grusby, M. J. , Alt, F. and Glimcher, L. H. (2001) 'Plasma cell differentiation requires the transcription factor XBP-1', *Nature* 412, 300-307.

Salzer, Ulrich, Bacchelli, Chiara, Buckridge, Sylvie, Pan-Hammarström, Qiang, Jennings, Stephanie, Lougaris, Vassilis, Bergbreiter, Astrid, Hagena, Tina, Birmelin, Jennifer, Plebani, Alessandro, Webster, A. David B., Peter, Hans-Hartmut, Daniel Suez, 6 Helen Chapel, McLean-Tooke, Andrew, Spickett, Gavin P., Anover-Sombke, Stephanie, Ochs, Hans D., Urschel, Simon, Belohradsky, Bernd H., Ugrinovic, Sanja, Kumararatne, Dinakantha S., Lawrence, Tatiana C., Holm, Are M., Franco, Jose L., Schulze, Ilka, Schneider, Pascal, Gertz, E. Michael, Schäffer, Alejandro A., Hammarström, Lennart, Thrasher, Adrian J., Gaspar, H. Bobby and Grimbacher, Bodo (2009) 'Relevance of biallelic versus monoallelic TNFRSF13B mutations in distinguishing disease-causing from risk-increasing TNFRSF13B variants in antibody deficiency syndromes', *Blood* 113:9, 1967–1976.

Shaffer, A. L., Shapiro-Shelef, M., Iwakoshi, N. N., Lee, A. H., Qian, S. B., Zhao, H., Yu, X., Yang, L., Tan, B. K., Rosenwald, A., Hurt, E. M., Petroulakis, E., Sonenberg, N., Yewdell, J. W., Calame, K., Glimcher, L. H. and Staudt, L. M. (2004) 'XBP1, downstream of Blimp-1, expands the secretory apparatus

and other organelles, and increases protein synthesis in plasma cell differentiation', *Immunity* 21:1, 81-93.

Taubenheim, N., Tarlinton, D. M., Crawford, S., Corcoran, L. M., Hodgkin, P. D. and Nutt, S. L. (2012) 'High rate of antibody secretion is not integral to plasma cell differentiation as revealed by XBP-1 deficiency', *J Immunol* 189:7, 3328-3338.

Thiel, J., Kimmig, L., Salzer, U., Grudzien, M., Lebrecht, D., Hagena, T., Draeger, R., Voelxen, N., Bergbreiter, A., Jennings, S., Gutenberger, S., Aichem, A., Illges, H., Hannan, J. P., Kienzler, A. K., Rizzi, M., Eibel, H., Peter, H. H., Warnatz, K., Grimbacher, B., Rump, J. A. and Schlesier, M. (2012) 'Genetic CD21 deficiency is associated with hypogammaglobulinemia.', *Journal of Allergy and Clinical Immunology* 129:3, 801-810.

Todd, D. J., McHeyzer-Williams, L. J., Kowal, C., Lee, A. H., Volpe, B. T., Diamond, B., McHeyzer-Williams, M. G. and Glimcher, L. H. (2009) 'XBP1 governs late events in plasma cell differentiation and is not required for antigen-specific memory B cell development', *Journal of Experimental Medicine* 206:10, 2151-2159.

van Schouwenburg, P. A., Davenport, E. E., Kienzler, A. K., Marwah, I., Wright, B., Lucas, M., Malinauskas, T., Martin, H. C., Consortium, W. G. S., Lockstone, H. E., Cazier, J. B., Chapel, H. M., Knight, J. C. and Patel, S. Y. (2015) 'Application of whole genome and RNA sequencing to investigate the genomic landscape of common variable immunodeficiency disorders', *Clinical Immunolgy* 160:2, 301-314.

van Zelm, M. C. , Reisli, I., van der Burg, M., Castaño, D., van Noesel, C. J., van Tol, M. J., Woellner, C., Grimbacher, B., Patiño, P. J., van Dongen, J. J. and Franco, J. L. (2006) 'An antibody-deficiency syndrome due to mutations in the CD19 gene.', *New England Journal of Medicine* 354:19, 1901-1912.

van Zelm, M. C., Smet, J., Adams, B., Mascart, F., Schandené, L., Janssen, F., Ferster, A., Kuo, C. C., Levy, S., van Dongen, J. J. and van der Burg, M. (2010) 'CD81 gene defect in humans disrupts CD19 complex formation and leads to antibody deficiency.', *Journal of Clinical Investigation* 120:4, 1265-1274.

Warnatz, K., Salzer, U., Rizzi, M., Fischer, B., Gutenberger, S., Böhm, J., Kienzler, A. K., Pan-Hammarström, Q., Hammarström, L., Rakhmanov, M., Schlesier, M., Grimbacher, B., Peter, H. H. and Eibel, H. (2009) 'B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans.', *PNAS* 106:33, 13945-13950.

Wehr, C. , Kivioja, T., Schmitt, C. , Ferry, B., Witte, T., Eren, E. , Vlkova, M., Hernandez, M., Detkova, D. , Bos, P. R. , Poerksen, G. , von Bernuth, H. , Baumann, U. , Debre, P. , Jacobs, R. , Jones, J. , Bateman, E. , Litzman, J., van Hagen, P. M. , Plebani, A. , Schmidt, R. E. , Thon, V. , Quinti, I. , Espanol, T. , Webster, D, Chapel, H., Vihinen, M., Oksenhendler, E. , Peter, H. H. and Warnatz, K. (2007) 'The EUROclass trial: defining subgroups in common variable immunodeficiency', *Blood* 111:1, 77-85.