Chronic inflammatory disease, lymphoid tissue neogenesis and extranodal marginal zone B-cell lymphomas

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Chronic inflammatory disease, lymphoid tissue neogenesis and extranodal marginal zone B-cell lymphomas

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ABSTRACT

Chronic autoimmune or pathogen-induced immune reactions resulting in lymphoid neogenesis are associated with development of malignant lymphomas, mostly extranodal marginal zone B-cell lymphomas (MZBCLs). In this review we address (i) chemokines and adhesion molecules involved in lymphoid neogenesis; (ii) the autoimmune diseases and pathogens which are associated with development of B-cell lymphomas; (iii) the molecular mechanisms involved in the initiation and progression of MZBCL; and (iv) ‘potential’ mouse models for MZBCL.

Key words: B-cell non-Hodgkin’s lymphoma, extranodal marginal zone B-cell lymphoma, immunoglobulin, B-cell antigen receptor, inflammation, lymphoid tissue neogenesis.

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Lymphoid tissue neogenesis and ectopic germinal center formation

Inflammation is a local response to cellular injury and is initiated by macrophages and local epithelial and/or stromal cells that sense microorganisms and cell damage by pattern recognition receptors, i.e. the Toll-like receptors (TLRs), soluble intracellular NOD-like receptors and RIG-like helicases. The triggered cells respond by secretion of a plethora of inflammatory mediators such as histamine, prostaglandins, leukotrienes, platelet-activating factors and typical pro-inflammatory chemokines and cytokines like IL-1β, IL-6, IL-8 (CXCL8) and TNF. These mediators, and in particular TNF, lead to endothelial activation and vasodilatation followed by a local efflux of circulating leukocytes. The first leukocytes arriving on site are granulocytes which combat the microbial invaders, while monocytes/macrophages clean up dead cells, including apoptotic granulocytes and destroyed tissue. In parallel, dendritic cells (DCs) take up and process antigens (Ag) from the intruder, mature and migrate to a local lymph node to set off an adaptive immune response.

Chronic inflammatory conditions, due to improper eradication of pathogens, auto-immune processes or chronic allograft rejections, are associated with the genesis of organized lymphoid tissue. In recent years, a number of key molecular determinants operating during the generation of tertiary lymphoid tissue, have been identified. In the complex sequence of events, TNF is again one of the key molecules as it induces the production of CCL19 and CCL21 (SLC), which are important for the attraction of B- and T-lymphocytes.

The infiltrating lymphocytes switch on expression of membrane-bound lymphotoxin αβ (mLTαβ) when activated e.g. by Ag. High levels of mLTαβ lead to lymphotoxin receptor (LTβ-R) ligation on stromal cells and/or macrophages and induce CXCL13 (BLC) production. The local production of CXCL13 mediates homing of B cells and induces the arrived B cells to further upregulate mLTαβ and probably also TNF. The enhanced interaction of CXCL13-producing stromal cells with the TNF- and mLTαβ-producing B cells promotes differentiation of resident stromal cells into follicular dendritic cells (FDCs) which start expressing characteristic molecules to trap immune complexes, i.e. the complement receptors CD21 and CD35 and the FcγR-IIB. Subsequent production of CXCL13 by FDCs establishes a positive feedback loop essential for ectopic lymphoid tissue development, similar to embryonic lympho-organogenesis and normal follicle formation (Figure 1). The importance of LT and TNF in this process has been demonstrated by transgenic expression of TNF, LTα and LTαβ in the pancreas and the kidneys, leading to formation of organized lymphoid tissue including FDC-containing follicles. Transgenic expression of CCL21 alone, resulted in extensive lymphoid tissue development in the pancreas. However, ectopic expression of CXCL12 (SDF), CCL19 or CXCL13 leads to attraction of lymphocytes, some compartmentalization but not to the genesis of FDC-containing follicles.

Depending on the type of pathogen, i.e. differences in the Ag presentation mode and the combination of costimulatory molecules and cytokine signals, Ag presenting cells (APCs) guide T-cell differentiation into the direction of T-helper type 1 (Th1) or T-helper type 2 (Th2) cells. A Th1-polarized response depends

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on IL-12 and results in IFN-γ producing T cells which also produce IL-2 and TNF and in their turn stimulate macrophages, natural killer (NK) cells and CD8+ cytotoxic T cells. This type of reaction mainly generates cellular responses against intracellular pathogens like viruses but also evokes T-cell help leading to production of, for example, opsonizing IgG2 antibodies (Ab). A T±2 response is characterized by secretion of IL-4, IL-5 and IL-13, and yields humoral immunity, in particular the generation of plasma cells that secrete high-affinity Abs, mainly of IgG1, IgG4, IgA and IgE classes.

In the ectopic follicles, germinal center (GC) reactions may occur (Figure 1). There are no reasons to assume that the biological processes operating in ectopic GCs differ from those in GCs of secondary lymphoid tissues. In secondary lymphoid tissues, GC reactions are initiated after activation of B cells and T cells by native and processed antigenic determinants, respectively. The B cells bind and internalize antigenic proteins with their membrane-bound immunoglobulins (mlg or B-cell Ag receptors, BCR) and, after intracellular processing, express Ag-derived peptides in MHC-II molecules at their surface. CD4 T cells, that are activated through cognate interactions with these peptide-MHC complexes, recompense the B cells by providing costimulatory signals (T-cell help) through CD40, CD80, CD86 and cytokine receptors.

When properly stimulated at the follicular boundaries, the B cells directly differentiate into short-lived Ab-forming plasma cells or migrate back into the follicle to undergo a phase of brisk proliferation thereby creating the GC dark zone. The rapidly proliferating B cells, termed centroblasts, express high levels of the DNA mutator activation-induced-cytidine-deaminase (AID) and accumulate nucleotide substitutions in their Ig variable (IgV) genes, a process designated as somatic hypermutation (SHM). B cells in the GC are prone to undergo apop-
sis except for those which, based on favorable point mutations, obtain higher affinities of their BCRs for the Ag. These high-affinity B cells are selected in the GC light zone based on successful competition for survival signals elicited by native Ag that is exposed at the surface of FDC, and by CD40L from GC T cells. In addition, the Ag-selected B cells may undergo class switch recombination (CSR) a process during which the switch (S) region sequence upstream of Cµ-Cδ is recombined with any of the other S region sequences located 5’ of each of the constant region genes Cγ, Cα, Cε, and Cα2, thus leading to isotype switching from IgM/IgD to either IgG, IgA or IgE. The Ag-selected B cells, either class-switched or not class-switched, will finally differentiate into memory B cells or Ab-producing plasma cells.

**Marginal zone B cells**

In humans and rodents, distinct populations of recirculating peripheral B cells are being distinguished, i.e. naïve (B2) or follicular (FO) B cells, naïve CD5- B cells, marginal zone (MZ) B cells and class-switched memory B cells (Figure 2). Initial studies in mice and humans indicated that CD5-expressing naïve B cells frequently display poly-/self-reactivity. However, more recent work in humans demonstrated a similar frequency of poly-/self-reactivity between CD5+ and CD5- naïve B cells. MZ B cells particularly respond to T-cell independent type 2 (TI-2) Ags, like large polysaccharides of bacterial cell walls and polymeric bacterial flagellin, which by repetitive antigenic epitopes, are able to crosslink BCRs. Naïve B2 cells are involved in T-cell dependent (TD) GC reactions, generating plasma cells, secreting high affinity IgGs, and CD27+ memory B cells. Recently, we obtained evidence in primary human lymph nodes that isotype-switched memory B cells can re-engage in GC reactions.

The marginal zone (MZ) was originally defined as an anatomical compartment within the spleen located around primary or secondary follicles and containing B cells with distinct phenotypic and functional characteristics. The MZ of the spleen is believed to be positioned in such a way that it primarily encounters blood borne pathogens. Later, primary mucosa-associated lymphoid tissues (MALT) of e.g. Waldeyer’s ring, Peyer’s patches and appendix, locations known for a significant influx of Ags, were also found to contain a marginal zone. MZ B cells in mice and rats express essentially unmutated IgV genes possibly acquired in a T-cell independent manner. After antigen recognition, mature naive B2 cells engage in T-cell dependent germinal center (GC) reactions in which SHM and CSR occur, generating high-affinity class-switched memory B cells and plasma cells (PC).

**Figure 2.** B-cell development in man. Transitional B cells in the spleen potentially mature into three B cell subsets: (i) CD5+ mature naïve B cells; (ii) conventional mature naïve B2 or FO B cells; and (iii) marginal zone (MZ) B cells which contain mutated IgV genes possibly acquired in a T-cell independent manner. After antigen recognition, mature naïve B2 cells engage in T-cell dependent germinal center (GC) reactions in which SHM and CSR occur, generating high-affinity class-switched memory B cells and plasma cells (PC).
genes and are supposed to be non-recirculating. On the other hand, human MZ B cells of both spleen and Peyer’s patches harbor mutated IgV genes and recirculate (Figure 2). Human splenic MZ B cells are IgM+ IgD+ CD27+ and express the B-cell markers CD20, CD22, CD23, CD79a/b, the anti-apoptotic molecule BCL-2, and BCL-6. Human MZ B cells, both in tissues and in the circulation, express high levels of CD1c. Expression arrays of splenic and recirculating IgM+ IgD+ CD27+ B cells revealed similar profiles, including high expression of CD81, CD44 and IL-6, thus confirming the non-resident nature of MZ B cells in the humans.

Where and when human MZ B cells obtain their somatic IgV mutations is still under debate. There is evidence that this occurs outside of GCs as part of an innate diversification program, like in sheep and birds. This is supported by the fact that mutated IgM+ IgD+ MZ B cells are also found in CD40L-deficient, hyper-IgM patients lacking GCs and that the Ig repertoire of MZ B cells is as diverse as naïve B cells, thus not resembling the highly selected Ig repertoire of class-switched IgM+ IgD+ CD27+ B cells. More importantly, AID expression was observed in splenic MZ B cells of children under the age of two years, but not in older individuals. Recent data by Scheeren et al. indicated that MZ B cells, with mutated IgV genes, are already present in human fetuses in which no active immune responses are thought to happen. AID expression was found in fetal liver and mesenteric lymph nodes but not in the fetal spleen. Repopulation experiments with human hematopoietic stem cells in Rag2-/-; µ-/- mice showed that IgV-mutated MZ B cells develop in a T-cell independent manner. Others think that MZ B cells mutate their IgV genes within GCs and argue that some residual GCs may be present in CD40L-deficient patients. GC formation without T-cell help has been described in mice, albeit these GCs were smaller, short-lived and SHM frequencies were low. Moreover, CD40L-deficient patients have significantly reduced numbers of circulating MZ B cells, being ~20-25% as compared to healthy individuals, indicative for at least a partial defect in MZ B-cell development. According to this scenario, MZ B cells would thus not belong to a distinct developmental line, but originate from conventional naïve B2 or follicular (FO) B cells. As currently there are no clues as to the heterogeneity of the MZ B-cell population, the possibility of multiple developmental routes producing hypermutated B cells with an MZ-like phenotype is not excluded (Figure 2).

It has been demonstrated that about 4% of MZ B cells are responsive to bacterial polysaccharides. Still, a large fraction of MZ B cells may thus have other specificities. In one donor, previously vaccinated with Streptococcus pneumoniae polysaccharide Ag, of the 27 Abs (7%) generated out of the MZ B-cell fraction, specifically reacted with this bacterial Ag. Capolunghi et al. showed, by polyclonal activation of naïve MZ and MZ B cells with CpG DNA, anti-S. pneumoniae (PnPS serotype 14) production exclusively by MZ B cells. In children below the age of two years, no or limited responses are detected against these T1-2 Ags. After the age of two, the percentage of IgM+ IgD+ CD27+ MZ B cells in the blood increases, which coincides with the appearance of the anatomical MZ structure in the spleen and with increased humoral responses to T1-2 Ags, such as pneumococcal polysaccharides.

Human IgM+ CD27+ MZ B cells, when compared to IgM+ CD27+ naïve B cells, appear to be selected against poly- and self-reactive BCRs. This selection is associated with a decrease in the average length of the IgVH complementarity determining regions 3 (IgVH-CDR3), which is largely due to deletion of B cells expressing the JH6 gene. Indeed, long IgV-CDR3s have been associated with self- and poly-reactivity. Upon reversion of somatic IgV mutations to their corresponding germline IgV sequences, these Abs did not regain poly- and/or self-reactivity. This indicates that naïve B cells with poly-/self-reacting BCRs are already efficiently excluded from the MZ B-cell pool before the onset of SHM. Also in the rat, selection of naïve B cells into the MZ B-cell compartment is accompanied by a decrease in IgVH-CDR3 lengths. This selection is most likely driven by self antigens as it is also observed in germ-free rats. Notably, no preferential selection of short CDR3s is observed in conventional class-switched IgG+ CD27+ memory B cells. Surprisingly, it has been described that ~50% of IgG+ CD27+ B cells show poly-/self-reactivity which is generally lost when reverting IgV SHM. Thus, the poly-/self-reactivity of IgG memory B cells is due to the accumulation of IgV SHM and not due to intrinsic properties of the CDR3s.

Auto-immune inflammatory conditions associated with B-cell lymphomagenesis

A number of chronic autoimmune conditions, organ-specific as well as systemic, are associated with an increased incidence of non-Hodgkin’s lymphomas (NHL). Among these, Hashimoto’s thyroiditis (HT) and Sjögren’s syndrome (SS) are the best examples with increased relative risks of 3-67,44 and 9-44,43,45-48 respectively to develop extranodal marginal zone B-cell lymphomas (MZBCLs). Moreover, for SS patients a 9-fold increased risk of obtaining a diffuse large B-cell lymphoma (DLBCL) has been reported. These lymphomas may develop either de novo or by transformation out of a prior low-grade MZBCL. Systemic lupus erythematosus patients were reported to have 8-fold and 5-fold higher incidences of, respectively, MZBCL and DLBCL as well. Rheumatoid arthritis (RA) appears to be weakly associated with non-RA treatment related, development of DLBCL and lympho-plasmacytic lymphoma, with reported odd ratios of 1.8 and 2.5, respectively. A recent meta-analysis, however, did not reveal an overall statistically significant association between RA and NHL. Celiac disease is strongly associated with the occurrence of enteropathy-type T-cell lymphoma. It is not understood why some autoimmune diseases do and many others, like e.g. Crohn’s disease, ulcerative colitis, type 1 diabetes, multiple sclerosis, pernicious anemia and sarcoidosis, do not entail increased risks of developing NHLs (Table 1).

Sjögren’s syndrome

Sjögren’s syndrome (SS) is a systemic autoimmune dis-
ease characterized by complaints of dry mouth (xerostomia) and eyes (keratoconjunctivitis sicca). Biopsies of (minor) salivary and lacrimal glands typically show mixed infiltrates consisting of CD4 T cells, some CD8 T cells, macrophages, myeloid and plasmacytoid dendritic cells (DC), B cells (~20%) and plasma cells. In about 20% of the patients the infiltrates contain GCs (Figure 1).54 Compatibly, lymphocyte attracting chemokines such as CXCL12 and CCL21 which mainly attract T cells and CXCL13 which attracts B cells are abundantly expressed in SS.54,55,64 High expression levels of CXCL13 and CCL21 within inflamed tissues correlated with the extent of inflammatory aggregates, the degree of T-cell/B-cell compartmentalization, the number of peripheral lymph node addressins (PNAd)-positive high endothelial venules (HEVs) and the presence of FDC-network containing GCs.64 Salivary gland epithelial cells of both normal and SS patients produce CCL28, which mediates the homing of CCR10-expressing IgA plasmablasts.65 Plasmacytoid DCs are present in SS salivary glands which secrete high amounts of type I IFNs (IFN-α and β) and IL-6, supporting plasma cell differentiation.66 Furthermore, IFN-α induces the B cell and plasma cell survival cytokine BAFF, which was indeed found to be highly expressed in SS patients.66,67 CD4 T cells from SS salivary glands express ~40-fold higher mRNA levels of IL-2, IFN-γ and IL-10, as compared to peripheral CD4 T cells from SS patients or from healthy controls. In agreement with this, the IL-4 and IL-5 mRNA levels of the SS CD4+ T cells were low.70 Thus, in general, the proinflammatory T-helper cell 1 (Th1)-type cytokines IL-2 and IFN-γ are abundant in Sjögren’s sialadenitis.70 Accordingly, the IFN-γ induced inflammatory chemokines CXCL9 (MIG) and CXCL10 (IP-10) are highly expressed in SS salivary gland epithelial cells but not in normal salivary glands.42 A variety of nuclear auto-Ags are humoral immune targets in SS patients. Anti-nuclear Abs, among which are the anti-SSA/Ro and anti-SSB/La Abs, are detectable in 70-85% of the patients. SSA/Ro52, SSA/Ro60 and SSB/La Ags together form a complex with a small cytoplasmic uridine-rich Y RNA.73 Five anti-SSA/Ro human monoclonal Abs of SS patients have been produced, i.e. 2 anti-Ro52 IgM Abs derived from peripheral blood B cells and 3 anti-Ro60 IgG Abs obtained from B cells of affected salivary glands. The IgM anti-SSA/Ro52 Abs were regarded unmutated containing 0 and 3 somatic mutations in their IgVH genes, while the IgG anti-SSA/Ro60 Abs were heavily mutated, containing >20 somatic mutations.73,74 By immunohistochemistry using biotinylated SSA/Ro52, SSA/Ro60 and SSB/La, evidence was obtained that the anti-SSA/Ro and anti-SSB/La Abs are produced by local plasma cells, most likely generated within the ectopic GCs.74 Serum anti-SSA/SSB Abs levels correlated with the presence of ectopic GCs.74 By immunohistochemistry, evidence for AID expression was provided75 and by tissue microdissection formal proof was obtained of the occurrence of clonal B-cell expansion and SHM in the ectopic GCs.76 Also R J Bende et al., unpublished data, 2009). Ig repertoire analysis on cells isolated from crude tissues of parotid and minor salivary glands of SS patients, revealed that most (~80%) of the infiltrating B cells harbored mutated IgVH genes and thus are GC, marginal zone or memory B cells.76 The combined data strongly suggest local generation and affinity maturation of the auto-Abs.

**Hashimoto’s thyroiditis**

Hashimoto’s thyroiditis (HT) and Grave’s disease represent extremes of a clinical spectrum of typical organ-specific autoimmune diseases, histologically character-

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### Table 1. Chronic inflammatory conditions and lymphoma association.

<table>
<thead>
<tr>
<th>Disease or inflammatory condition</th>
<th>Cause</th>
<th>% of patients with ectopic GCs</th>
<th>Affected tissue</th>
<th>Lymphoma association</th>
<th>Lymphoma sub-types and odd ratios</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>Autoimmune</td>
<td>10-35%</td>
<td>Synovial membrane</td>
<td>?</td>
<td>DLBCL (OR: 2), LPL (OR: 3)</td>
<td>7, 48, 53</td>
</tr>
<tr>
<td>Sjögren’s sialadenitis</td>
<td>Autoimmune</td>
<td>~20%</td>
<td>Salivary gland</td>
<td>Yes</td>
<td>DLBCL (OR: 9), MZBCL (OR: 9-44)</td>
<td>43, 45-48, 54</td>
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<tr>
<td>Systemic lupus erythematosus</td>
<td>Autoimmune</td>
<td>Systemic</td>
<td>Yes</td>
<td>DLBCL (OR: 3), MZBCL (OR: 8)</td>
<td>48, 52</td>
<td></td>
</tr>
<tr>
<td>Celiac disease</td>
<td>Autoimmune</td>
<td>a</td>
<td>Intestinal mucosa</td>
<td>Yes</td>
<td>EATCL (OR: 17)</td>
<td>52</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>Autoimmune</td>
<td>a</td>
<td>Intestinal mucosa</td>
<td>No</td>
<td></td>
<td>48, 52</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>Autoimmune</td>
<td>a</td>
<td>Colon mucosa</td>
<td>No</td>
<td></td>
<td>48, 52</td>
</tr>
<tr>
<td>Type 1 diabetes</td>
<td>Autoimmune</td>
<td>30-40%</td>
<td>Pancreas</td>
<td>No</td>
<td></td>
<td>48, 52, 52</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Autoimmune</td>
<td>30-40%</td>
<td>Central nervous system</td>
<td>No</td>
<td></td>
<td>7, 48, 52</td>
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<tr>
<td>Sarcoïdosis</td>
<td>Autoimmune</td>
<td>Systemic</td>
<td>No</td>
<td></td>
<td>48, 52</td>
<td></td>
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<tr>
<td>Psoriasis</td>
<td>Autoimmune</td>
<td>Skin</td>
<td>No</td>
<td></td>
<td>48, 52</td>
<td></td>
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<tr>
<td>Myasthenia gravis</td>
<td>Autoimmune</td>
<td>100%</td>
<td>Thymus</td>
<td>No</td>
<td></td>
<td>7</td>
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<tr>
<td>Hashimoto’s thyroiditis</td>
<td>Autoimmune</td>
<td>100%</td>
<td>Thyroid gland</td>
<td>Yes</td>
<td>MZBCL (OR: 3-67)</td>
<td>43, 44, 55</td>
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<td>Grave’s disease</td>
<td>Autoimmune</td>
<td>~60%</td>
<td>Thyroid gland</td>
<td>No</td>
<td></td>
<td>7</td>
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<tr>
<td>Arthererosclerosis</td>
<td>Autoimmune</td>
<td>~30%</td>
<td>Arteries</td>
<td>No</td>
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<td>Conjunctivitis</td>
<td>Chlamydia psitacci</td>
<td>?</td>
<td>Eye</td>
<td>Yes</td>
<td>MZBCL (OR: ?)</td>
<td>56</td>
</tr>
<tr>
<td>Gastritis</td>
<td>Helicobacter pylori</td>
<td>30-100%</td>
<td>Gastric mucosa</td>
<td>Yes</td>
<td>MZBCL (OR: ?)</td>
<td>57</td>
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<tr>
<td>Hepatitis</td>
<td>HCV</td>
<td>30-85%</td>
<td>Liver</td>
<td>Yes</td>
<td>DLBCL (OR: 2), LPL (OR: 3), MZBCL (OR: 3)</td>
<td>58, 59, 60</td>
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<tr>
<td>Dermatitis</td>
<td>Borellia burgdorferi</td>
<td>?</td>
<td>Skin/synovial membrane</td>
<td>Yes</td>
<td>MZBCL (OR: ?)</td>
<td>61, 62</td>
</tr>
</tbody>
</table>

*In these inflammatory bowel diseases, it is difficult to distinguish between lymphoid neogenesis and hyperplasia of normal mucosa-associated lymphoid tissue; aEarly onset myasthenia gravis.*
ized by chronic lymphocytic infiltration. In Grave’s disease, inflammation is generally mild and accompanied by the production of thyrotropin receptor stimulating Abs, resulting in hyperthyroidism. In HT, the infiltrates are more severe and progressive, ultimately causing destruction of the thyroid parenchyma and hypothyroidism. It has been shown that in all HT patients the lymphocytic infiltrate is well-organized containing GCs. T-cell attracting chemokines CXCL12 and CCL21 are produced by the follicular epithelium surrounding HEVs. Ectopic expression of CCL21 in the thyroid of mice leads to the development of lymphoid tissue containing FDCs, resembling HT. In this model, also the IFN-γ inducible inflammatory chemokines CXCL9, 10 and 11 were expressed. Cultures of CD4 and CD8 T cell clones retrieved from human HT patient thyroids yielded high levels of TNF and IFN-γ, whereas hardly any clones produced IL-4. Quantitative RT-PCR on patient thyroids revealed high levels of IFN-γ and IL-2, confirming that the T1 cytokines are highly expressed in HT.

Auto-Abs induced in HT are specific for thyroglobulin (Tg), thyroid peroxidase (TPO) and the thyroid stimulating hormone receptor (TSH-R). The Abs against Tg and TPO are not detected in all patients. Auto-Abs specific for the TSH-R are capable of blocking the activation of this receptor and thus can contribute to the impairment of the thyroid function. Numerous anti-Tg and anti-TPO human monoclonal Abs have been reported, isolated from peripheral blood B cells, thyroid tissue and cervical lymph node tissue. All these human monoclonal Abs were heavily mutated containing >10 mutations per IgV gene. A correlation has been found between the levels of CCL21, CCL22 and CXCL13 in the inflamed thyroid and the titers of thyroid-specific auto Abs. Biotin-labeled Tg and TPO were shown to bind immunohistochemically to ectopic GCs in HT. The combined data support local generation and affinity maturation of anti-thyroidal Abs.

**Infections indirectly provoking B-cell lymphomagenesis**

*Helicobacter pylori*-infection related gastric MZBCL is the most commonly mentioned example of bacterium-driven tumorigenesis but in fact is the only undisputed example. Cutaneous MZBCL has been linked to chronic *Borrelia burgdorferi* dermatitis (Lyme’s disease) in a minority of European patients, but not in cases from Asia or the United States. Recently, an association of *Chlamydia psittaci* and ocular adnexal MZBCL was found by PCR in studies from Italy, South Korea, Germany and Austria. Immunohistochemistry, laser assisted microdissection PCR and electron microscopy further provided evidence that *C. psittaci* was present in monocytes/macrophages within the MZBCL. Moreover, *C. psittaci* was cultured *in vitro* from conjunctival swabs and/or PBMCs from 25% of ocular adnexal MZBCL patients. However, the association between *C. psittaci* and ocular adnexal MZBCL could not be confirmed in studies from The Netherlands, Japan and The United States (Florida), suggesting geographical differences regarding this link. Hepatitis C virus (HCV) infection has been inferred in the development of malignant B-cell proliferations, in particular splenic and extranodal MZBCLs and DLBCLs. In a large intercontinental study, the relative risks for HCV patients to develop MZBCL, DLBCL or lymphoplasmacytic lymphoma were calculated as 2.5, 2.2 and 2.6, respectively (Table 1).

*H. pylori* is a gram-negative bacterium able to persist during lifetime in the gastric mucosa and is present in ~50% of the world population. *H. pylori* binds tightly to epithelial cells via multiple bacterial surface components. *H. pylori*, like most intracellular bacteria, evoke T1 immune responses characterized by high IFN-γ levels. IFN-γ targets genes with microbicidal properties such as enzymes that generate NO and O2 radicals. To circumvent the negative effects of these radicals *H. pylori* produces radical-scavenging enzymes. In addition, *H. pylori* secretes urease to neutralize the local low pH. Most strains of *H. pylori* possess the cag pathogenicity island, including the *CagA* gene. The CagA protein leads to a massive influx of neutrophils by inducing high production levels of the chemotactic factor CXCL8 (IL-8) by epithelial cells. Later, during the chronic phase, also T cells, B cells, plasma cells and macrophages are recruited and secondary mucosa-associated lymphoid tissue (MALT) is formed within the gastric mucosa. *In situ* hybridization and immunohistochemistry, CXCL13 was found to be expressed in the ectopic primary follicles and mainly in the mantle zones of ectopic GCs. *H. pylori* induces a strong Ab response which does not lead to eradication, but instead may contribute to the tissue damage. Two human anti-*H. pylori* single-chain Ig variable fragment (Ig-Fv) isolated from peripheral blood B cells of a *H. pylori*-infected patient have been reported, each displaying mutated IgV genes (>7 mutations). Lymphoid aggregates with GCs have also been observed in *B. burgdorferi*-induced skin and synovial lesions, and in the liver of ~60% of the patients suffering from chronic HCV infections. In the ectopic GCs of *B. burgdorferi*-induced synovitis, B-cell expansion and IgV diversification have been demonstrated.

**Extranodal marginal zone B-cell lymphoma**

Extranodal marginal zone B-cell lymphoma (MZBCL) of mucosa-associated lymphoid tissue, also designated as MALT lymphoma, appears as heterogeneous infiltrates containing small centrocytic and monocytoid B cells, plasma cells and in some scattered immunoblasts and centroblasts. The growth characteristics of MZBCLs resemble those of the normal MZ of, for example, Peyer’s patches. MZBCLs typically expand around ectopic GCs and are able to invade the epithelium to form lymphoepithelial lesions. Colonization of ectopic GCs by tumor B cells contribute to a pseudo-follicular growth pattern. Also immuno-phenotypically, MZBCL cells are reminiscent of normal MZ B cells of the spleen and Peyer’s patches. They express the pan-B cell markers CD20, CD22 and CD79a/b, the memory B-cell marker CD27, the complement receptors CD16/CD11b, CD21 and CD35, the anti-apoptotic molecule BCL-2, and CD1c/CD1d. MZBCLs do not express CD5 and CD23, nor the GC molecules CD10 and BCL-6.101 There are as yet no markers by which MZBCLs can be unequivocally identified. MZBCLs have a low tendency to disseminate systemically, a feature which explains why the majority of these
malignancies can be controlled by local treatments alone. About 30% of MZBCLs disseminate which, due to expression of the mucosal homing integrin α4β7, most often involves other mucosal sites or regional lymph nodes. \[11,12\] Interestingly, in both lymph nodes and spleen, MZBCL cells also tend to expand peri-follicularly, in accordance with their MZ B-cell properties. \[13,14\]

As outlined, the chronic inflammatory conditions enabling MZBCL development are generally of T1 type, characterized by high IFN-γ levels. IFN-γ receptor binding leads, via STAT1 activation, to induction of genes encoding microbicidal proteins and to induction of the transcription factor t-Bet which in its turn induces, among other genes, expression of the chemokine receptor CXCR3. \[15,16\] CXCR3 is the specific receptor for the IFN-γ-induced chemokines CXCL9 (MIC), CXCL10 (IP-10) and CXCL11 (TAC), Indeed, extranodal and splenic marginal zone B-cell lymphomas almost invariably express CXCR3 and t-Bet. \[16,17\] High expression of CXCL9 has been demonstrated in histiocytes, fibroblasts and endothelial cells of thyroid and gastric MZBCLs. \[12,13\] We recently reported that within the group of MZBCLs, most cutaneous MZBCLs are distinct as they arise in a T2 background and, in accordance with this immunological context, lack CXCR3 and t-Bet and carry isotype-switched Igs. Interestingly, a few cases of cutaneous MZBCL did express IgM and CXCR3 like other MZBCLs, and were thus most likely established in a typical T1 inflammatory environment. Notably, the few B. burgdorferi-infection associated MZBCLs we have studied also co-expressed IgM and CXCR3. \[12,13\]

Genetic alterations in low- and high-grade extranodal marginal zone B-cell lymphomas

Recurrent chromosomal translocations identified in MZBCLs are t(11;18) (API-2/MALT1), t(14;18) (IgH/MALT), t(1;14) (BCL10/IgH) and t(3;14) (FOX-P1/IgH) (Table 2). \[12-18\] Except for the t(11;18), these translocations involve IgH loci, like most translocations in other mature B-cell lymphomas. \[19\] The t(11;18) is extraordinary since it does not involve the Ig locus and encodes a fusion protein which is constituted by the amino-terminal portion of API-2 and the carboxyl-terminal of MALT-1. The overexpression of either BCL-10 or MALT-1, but also the API2-MALT1 chimeric protein, cause constitutive activation of the canonical NF-κB signaling pathway. \[14,16\] The t(14;18) is found in 5-15% of pulmonary, salivary gland and ocular adnexae MZBCLs. About 5% of intestinal and pulmonary MZBCLs harbor the t(1;14) (Table 2). \[12,13,14,16\] More recently, three novel IgH translocations in non-gastric MZBCL involving ODT2, CNN3 and JMD2C have been described, of which ODT2 and JMD2C were found recurrently. \[14\]

The t(11;18) is present in ~40% of pulmonary- and in ~20% of gastric- MZBCLs and is virtually absent in MZBCLs of the salivary gland (Table 2). \[11,12,14\] Gastric MZBCLs harboring the t(11;18) were found to be associated with CagA-positive strains of H. pylori. CagA induces activation of neutrophils releasing reactive oxygen species. It has been hypothesized that these are the genotoxic conditions which are instrumental in generating the t(11;18). \[13,14\] The assembled literature indicates that t(11;18)-carrying MZBCLs generally possess a limited degree of additional chromosomal imbalances, are non-responsive to H. pylori eradication therapy and are not prone to transform into high-grade DLBCLs. \[10,13,14,17-19\]

T(11;18)-negative gastric MZBCLs with a high degree of genomic imbalances were also associated with H. pylori independency. \[16,19\] Trisomies of chromosome 3, 12 and 18 are observed in t(11;18)-negative gastric (20%), pulmonary (40%), ocular adnexal (40%) and salivary gland (60%) MZBCLs. \[13,16,18\] Interestingly, in MZBCLs, concurrent gains at 8q24, 9q34, 11q11-18 and 18q21 are frequent. \[16,19\] The gains of these loci appear to target genes whose products stimulate the NF-κB pathway (i.e. TRAF2 and CARD9 at 9q34, RELA at 11q11-13 and MALT-1 at 18q21) and the cell cycle (Cyclin D1 at 11q12-13) (Figure 3). \[16\] Gain of 6p and loss of 6q25 was specifically found in ocular adnexal MZBCL in ~25% of the cases. \[16,19\] High resolution tile-path array CGH indicated that 6p gains were centered at the TNF locus at 6p21.33 and 6q25. \[16\] FISH assays further confirmed the occurrence of A20 deletions in MZBCLs of the ocular adnexa (19%), salivary gland (8%) and thyroid (11%) but not in MZBCLs of lung, stomach, skin and small intestine. A20 is a potent inhibitor of NF-κB signaling which is required for termination of TNF- and TLR- induced NF-κB activation. A recent study showed that both MALT1 and API2-MALT1 can inactivate the A20 inhibitor by proteolysis, which further implicates A20 in the pathogenesis of MZBCL. \[14\]

The t(3;14)(FOX-P1/IgH), deregulating expression of the forkhead box P1 (FOX-P1) transcription factor, was initially reported by an Austrian study in as much as 4 out of 20 (20%) ocular adnexae MZBCLs and in 8 out of 6 (50%) thyroid MZBCLs. \[17\] However, in more recent studies by North American and German groups, this translocation was not detected in series of 133 and 122 MZBCLs, respectively. \[12,13\] Also others did not detect the

Table 2. Chromosomal translocations of MZBCL

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Stomach</th>
<th>Lung</th>
<th>Salivary gland</th>
<th>Intestine</th>
<th>Ocular adnexa</th>
<th>Skin</th>
<th>Thyroid</th>
</tr>
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<tbody>
<tr>
<td>t(11;18)</td>
<td>29%</td>
<td>40%</td>
<td>2%</td>
<td>35%</td>
<td>3%</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>t(14;18)</td>
<td>2%</td>
<td>9%</td>
<td>1%</td>
<td>7%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>t(3;14)</td>
<td>1%</td>
<td>2%</td>
<td>0%</td>
<td>7%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
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</table>

*The t(14;18) IgH/MALT was only detected by Streubel et al. in 7 of 51 cutaneous MZBCL. The t(3;14) FOX-P1/IgH was only detected by Streubel et al. in 4 of 20 ocular adnexae MZBCL in 2 of 20 cutaneous MZBCL and in 3 of 6 thyroid MZBCLs.*
of genetic damage, B cells may undergo active proliferation. Direct antigenic stimulation can be accomplished by auto-Ags like IgG-containing immune complexes, by bacterial or other, unknown, Ags. Indirect stimulation is provided by H. pylori-specific T cells. Due to the acquisition of genetic damage, B cells may obtain growth advantage. Gastric MZBCL with t(11;18) grow autonomously, do not respond to H. pylori eradication, but rarely progress to DLBCL. Gastric MZBCL with trisomy 3 and/or 18 and/or having extra copies of the MAL gene have a more aggressive clinical behavior. Following inactivation of the tumor suppressor genes TP53 or CDKN2A or due to mutation in oncogenes, possibly by aberrant SHM, MZBCL may transform to DLBCL.

Figure 3. Scenarios of multistep development of gastric MZBCL. Chronic H. pylori (HP) infection induces lymphoid tissue neogenesis. As a result of both direct and indirect stimulation infiltrating B cells will undergo active proliferation. Direct antigenic stimulation can be accomplished by auto-Ags like IgG-containing immune complexes, by bacterial or other, unknown, Ags. Indirect stimulation is provided by H. pylori-specific T cells. Due to the acquisition of genetic damage, B cells may obtain growth advantage. Gastric MZBCL with t(11;18) grow autonomously, do not respond to H. pylori eradication, but rarely progress to DLBCL. Gastric MZBCL with trisomy 3 and/or 18 and/or having extra copies of the MAL gene have a more aggressive clinical behavior. Following inactivation of the tumor suppressor genes TP53 or CDKN2A or due to mutation in oncogenes, possibly by aberrant SHM, MZBCL may transform to DLBCL.

The molecular mechanisms underlying MZBCL progression are as yet ill-defined. A number of genetic alterations has been associated with histological transformation such as allelic loss and mutation of TP53 and hypermethylolation or deletion of CDKN2A (p16-INK4A, ARF). Furthermore, several chromosomal gains and losses are associated with transformation. Since most MZBCLs express mutated IgV genes with intra-clonal sequence variation, proving previous and suggesting continued exposure to the SHM machinery, a role for the DNA mutator AID in MZBCL transformation cannot be excluded. However, immunohistochemical expression analyses showed that AID is detectable only in a minority of the cases. Accordingly, several investigators demonstrated variable, but generally low AID mRNA expression levels in MALT lymphomas. On the other hand, in ~50% of DLBCL, several proto-oncogenes, including PIM1, PAX5, RhoH/ITF and cMYC are targeted by aberrant SHM. Sequence analysis of MALT lymphomas revealed that 75% of low-grade MZBCLs and 100% of low-grade MZBCLs with a DLBCL component contained mutations in one or more of these oncogenes. In the latter group, higher frequencies of aberrant SHM were found as compared to pure low-grade MZBCLs, supporting the concept of AID-mediated lymphoma progression.

BCR specificity of marginal zone B-cell lymphomas

The general idea is that MZBCLs still depend on environmental stimuli and on antigen-receptor ligands. IgV and IgV, gene sequence analyses have revealed that in spite of high mutation loads the overall structure of the Ig is being preserved. Apparently, selective forces prevent the outgrowth of BCR- MZBCL mutants. Nearly 80% of early stage H. pylori-associated gastric MZBCLs, but also a proportion of cutaneous and ocular adnexal MZBCLs is curable by bacterial eradication alone. Similarly, IFNα-2b treatment can cause regression of HCV-associated MZBCL.

In vitro culture experiments with gastric MZBCL cells have revealed that the tumor B cells do not respond to H. pylori directly, but instead depend for their survival on stimuli provided by intra-tumoral, H. pylori-specific T cells. We have recently produced soluble recombinant Abs derived from gastric and other MZBCLs, and indeed did not observe any reactivity with H. pylori bacteria. Alternatively, it appeared that ~10% of gastric, and as much as ~40% of salivary gland MZBCLs expressed Vh-69/JH4- and V3-7JH3- encoded BCRs with strong IgV-CDR3 amino acid sequence homology to canonical rheumatoid factors (RF). Among an extensive panel of B-NHLs, this RF homology was unique for MZBCL. Indeed 7 out of 10 recombinant MZBCL-derived Abs showed strong in vitro binding activity to immobilized human IgG. MZBCLs with high affinity IgG-specific BCRs may thus be continuously stimulated by Ab-Ag immune complexes, like IgG-opsonized H. pylori in chronic gastritis or IgG-chromatin and/or IgG-SSA/SSB-RNA in Sjögrens sialadenitis. The IgG-reactive BCRs may also capture and internalize Ab-Ag complexes and activate TLR9 and/or TLR7 by autologous or bacterial CpG DNA or by autoantigen-associated RNA, consequently...
potentiating the NF-κB pathway (Figure 4). Synergistic effects of BCR and TLR9 or TLR7 engagement have originally been shown in the mouse by T-cell independent activation of IgG-reactive B cells, using IgG-chromatin or IgG-RNA complexes.171,172

Intriguingly, none of 8 previously published,119 nor 12 newly analyzed MZBCLs that harbored the t(11;18), express BCRs with RF homology or reactivity170 (also H. Inagaki, written communication, Department of Pathology, University Graduate School of Medical Sciences, Nagoya, Japan, April 4, 2008). Moreover, the frequency of RF-BCRs being <40% of salivary gland, ~10% of gastric and <1% of pulmonary MZBCLs, inversely correlates with the t(11;18) frequencies found in these entities (Table 2).119,128,132,133 This tentative inverse relation suggests that t(11;18)+ MZBCLs do not depend on BCR (and perhaps neither on CD40 and TLR7/9) signals for their expansion since constitutive NF-κB activation is already guaranteed due to the expressed fusion protein (Figures 3 and 4). The facts that: (i) t(11;18)+ gastric MZBCLs are resistant to H. pylori eradication therapy; and that (ii) within the overall group of MZBCLs, t(11;18) and trisomy 3 harboring cases were from patients without underlying autoimmune diseases, support this hypothesis.146,147 Of note, the finding that t(11;18)+ MZBCLs lack RF-BCRs, indicates that this genetic aberration occurs independently of the selection process favoring this specificity.

Mouse models of marginal zone B-cell lymphoma

As yet a limited number of mouse models have been generated aimed at MZBCL development. In general, four categories of potential’MZBCL’ models can be distinguished: (i) mice with transgenic expression of genes involved in lympho-organogenesis; (ii) mice chronically challenged with Helicobacter species; (iii) mice carrying MZBCL-specific gene alterations; and (iv) mice with chronic or uncontrolled T-cell mediated B-cell activation. As delineated in the first chapter, transgenic expression of the key molecules LT and TNF results in augmented lymphoid tissue neogenesis.10,11 Similarly, transgenic expression of B- and T-lymphocyte-attracting chemokines initiates formation of ectopic lymphoid tissues, presumably also via the LT/TNF axis. Although it could be argued that in these transgenic animals the continued ectopic lymphoproliferation would ultimately lead to cellular transformation, development of MZBCLs has not been described. This may be due to the fact that in these mice chronic antigenic stimulation has not been assayed.

In mice infected with Helicobacter species, the occurrence of organized lymphoid tissues in and beyond the gastric mucosa has been described.174,175 Oral infection of A/J mice with Helicobacter sp leads to development of hepatic inflammatory lesions containing HEVs, the production of CCL21 and CXCL13 and influx of B and T cells.176 Infection of BALB/c mice with Helicobacter felis resulted in a massive influx of B cells and lympho-organogenesis in the stomach.177,178 It was reported that after 23 months of infection, 25-75% of these mice developed low- or intermediate-grade MZBCLs. In these mice, regression of the infiltrates after anti-bacterial therapy was also demonstrated.174,175 Other investigators have infected BALB/c mice with different ‘H. heilmannii’ isolates originating from human and animal hosts. MZBCLs developed in ~25% of the infected mice. The lymphoma prevalence was dependent on the origin of the infecting isolates and the duration of infection.180,181 Finally, infection of C57BL/6 mice with Candidatus H. heilmannii resulted in the development of gastric MALT lymphoma in 100% of the mice after six months.182 It is noted that in all these infection protocols, the diagnosis of MZBCLs was based on morphological grounds alone, i.e. the presence of centrocyte-like cells and lympho-epithelial lesions, but not
on the assessment of monoclonality by Ig gene rearrangement assays nor on any other molecular genetic analyses.\(^{174,175}\)

Transgenic FVB mice expressing the API2-MALT1 fusion gene, driven by the SR\(\alpha\) promoter under control of the \(\beta\)2m heavy chain enhancer, specifically triggered the expansion of splenic MZ B cells. However, the expression of the API2-MALT1 fusion protein alone was not sufficient for the development of lymphomas over a period of 50 weeks.\(^{176}\) Immunization of these mice with complete Freund’s adjuvants induced the loss of splenic compartmentalization and a poly- or oligo-clonal lymphoid hyperplasia which gradually disappeared after cessation of the antigenic stimulation.\(^{170}\) P100-/- mice with signal-independent activation of the non-canonical NF-\(\kappa\)B pathway had markedly elevated MZ B-cell numbers and a disturbed spleen microarchitecture.\(^{181}\) BAF overexpression, activating the non-canonical NF-\(\kappa\)B pathway in BAF-Tg and BAF-Tg/TNF-/- mice, resulted in increased survival and accumulation of transitional T2 and MZ B cells.\(^{102,181}\) Interestingly, the BAF-Tg/TNF-/- mice showed a high incidence of B-cell infiltrates with histological features of extranodal MZBCL, but again not substantiated by molecular analyses.\(^{183}\) Mice with constitutively active IKK2, enhancing the canonical NF-\(\kappa\)B pathway, showed a mild B-cell hyperplasia due rather to prolonged survival than to proliferation. Cell proliferation was dramatically enhanced when the IKK2 B cells were stimulated via the BCR or TLR4/9.\(^{184}\) Mice with B-cell-specific expression of a chimeric CD40/laten membrane protein 1 (LMP1) protein showed an increased number of FO- and MZ- B cells in secondary lymphoid organs. The constitutive CD40-like signaling via the cytoplasmic LMP-1 tail in the B cells induced activation of the non-canonical NF-\(\kappa\)B pathway, but also of MAP kinases, Jnk and ERK.\(^{185}\) Interestingly, in mice of >12 months, oligo- and mono-clonal B-cell lymphomas developed at a high incidence. These B-cell lymphomas did not resemble human MZBCLs as they did not express CD21.

Some mouse models nicely underscore the role of chronic T-cell help in the development of B-cell lymphomas. For example, in IgA transgenic mice with B cells presenting IgA idotype (Id)-derived peptides on MHC class II, the transferral of Id-specific CD4 T cells resulted in B-cell lymphoma development after approximately 40 weeks. The lymphomas resembled MZBCLs as they expressed CD21, CD35, CD1d and IgM. Moreover, the lymphomas were shown to be mono-/bi-clonal, harbored some somatic IgV\(\gamma\) mutations and had major cytogenetic aberrations.\(^{186}\) Mice deficient for the autoimmune regulator (Aire) gene showed a high frequency of MZBCLs after 15-24 months. The B-cell lymphoproliferations were shown to be oligoclonal in the spleens of 4 out of 9 mice and displayed a MZ B-cell phenotype with low IgD and high CD1d.\(^{187}\) Interestingly, Aire is a recently discovered transcription factor that is expressed in thymic medullary epithelial cells and plays a key role in central tolerance induction.\(^{188}\) Thus, in these mice MZBCL development is most likely related to chronic help from autoactive T cells.

In conclusion, the assembled literature points towards a key role of constitutive NF-\(\kappa\)B signaling in MZBCL development. This requirement is fulfilled by the combination of persistent BCR triggering, chronic T-cell help and TLR stimulation elicited by chronic infection or autoimmunity. In the ectopically formed lymphoid tissue, these physiological stimuli can be overruled by genetic alterations which guarantee constitutive NF-\(\kappa\)B signaling, thus making the cells less dependent on the environmental stimuli.

**Authorship and Disclosures**

RJB designed, discussed, wrote and performed original work, FVM wrote, discussed and performed original work and CJVN wrote, approved and discussed original work.

The authors reported no potential conflicts of interest.

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Inflammation, lymphoid neogenesis and extranodal marginal zone B lymphoma

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