The role of APRIL in immunity and tumorigenicity

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CHAPTER 1

The expanding role of APRIL in cancer and immunity

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ABSTRACT

Proteins of the tumour necrosis factor (TNF) family are implicated in the regulation of essential cell processes such as proliferation, differentiation, survival and cell death. Altered expression of TNF family members is often associated with pathological conditions such as autoimmune disease and cancer. The TNF-like ligand APRIL (APRoliferation Inducing Ligand), first described in 1998, was named for its capacity to stimulate tumour cell proliferation in vitro. APRIL expression was initially reported in haematopoietic cells in physiological conditions, and it is overexpressed in certain tumour tissues.

APRIL is now known to be involved in activation and immune responses of B cells, as well as in B cell malignancies. This review focuses on recent advances in understanding APRIL and its receptors in physiology and tumour pathology, including the accumulating evidence that specific Toll-like receptor ligands can trigger APRIL-mediated responses, and the identification of new sources of APRIL such as epithelial cells and tumour-infiltrating neutrophils.
The TNF family of ligands and receptors is intimately connected to the regulation of various cellular pathways, such as activation, survival and cell death. A PRoliferation Inducing Ligand (APRIL) and B cell Activating Factor belonging to the TNF Family (BAFF) are two related members of the TNF ligand superfamily. The APRIL and BAFF system has been the subject of several reviews ([1-10]). However, recent discoveries on the functions of APRIL in immune responses and tumour control warrant a review on APRIL specifically. This review will therefore mainly deal with the emerging role of APRIL in both B cell immunity and malignancies.

**Cellular regulation of APRIL**

APRIL was first described in 1998 and named for its capacity to stimulate the proliferation of tumour cells in vitro and in vivo [11]. The gene encoding APRIL is located on chromosome 17 in humans and on chromosome 11 in mice, near another TNF family member, TNF-related weak inducer of apoptosis (TWEAK). APRIL and BAFF (frequently also called BLyS for B lymphocyte stimulator) share a higher amino acid sequence identity (~30%) with each other than with any member of the TNF family and are therefore often suggested to make up a subfamily. The crystal structure of APRIL consists of a compact trimeric ligand with a backbone fold similar to that of BAFF [12]. The human APRIL gene contains six exons, which can be transcribed as three alternatively spliced mRNAs respectively encoding the \( \beta \), \( \gamma \) and \( \delta \) forms of the protein. The biological relevance of this alternative splicing is not known and no similar sequences have been reported in ESTs of murine APRIL. APRIL consists of a 28-amino acid cytoplasmic domain, a 21-amino acid transmembrane domain and a 201-amino acid extracellular domain, consisting of a stalk and a TNF domain. The \( \beta \) form lacks exon 3 of the extracellular domain, reducing the protein to 234-amino acids. The \( \gamma \) form is generated by splicing of a cryptic intron in exon 6, resulting in a 257-amino acid protein. A rare splicing event in APRIL-\( \delta \) combines exon 1 of the cytoplasmic domain to exon 3 in the extracellular domain, generating a membrane-bound, uncleavable form of APRIL [1, 13]. Although TNF ligand family members are synthesised as type II transmembrane proteins, cleavage in the stalk region between the transmembrane and receptor-binding domains frequently occurs at the cell membrane. This allows conversion from a membrane-bound form to a soluble form of the ligands. However, in contrast to most TNF family members, APRIL is processed within the Golgi apparatus to a soluble form by a furin convertase and is thus normally secreted. This is not the case for the APRIL-\( \delta \) form, which lacks the cleavage site. Alternatively, APRIL can also be exposed on the cell surface due to a unique intergenic splicing between the TWEAK and APRIL locus, giving rise to a fusion protein called TWE-PRIL. TWE-PRIL is composed of the
cytoplasmic and transmembrane portions of TWEAK fused to the C-terminal TNF domain of APRIL [14]. Although BAFF and APRIL are known to form homotrimers, they can also associate with each other to form biologically active heterotrimers [15]. The exact composition, biological relevance and origin of these heterotrimers are however unknown.

**APRIL and its receptors in immunity**

APRIL is expressed by a variety of immune cell subsets that also produce BAFF: monocytes, macrophages, dendritic cells, neutrophils and T cells [11, 16-18]. A recent paper analysing expression of these ligands on B cell subsets in mice, reported APRIL and BAFF expression on resting B-1 B cells and developing B cells in the bone marrow as well as expression on activated B cells in vitro and in vivo [19]. One other report shows BAFF expression on human germinal center (GC) B cells [20]. The expression of APRIL by monocytes, macrophages and dendritic cells is dependent on the cytokine environment (e.g. interferon (IFN) gamma, IFN alpha) or the engagement of pattern recognition receptors (PRRs) and CD40 on the cell surface (see below). APRIL is also expressed in cells outside the immune system, such as epithelial cells, osteoclasts and in tumour tissues [11, 21, 22], while TWE-PRIL is expressed on resting and activated primary human T cells, monocytes and tumour cell lines [14].

![Figure 1. Putative binding partners within the APRIL/BAFF system. The ligands are depicted in the upper part of the picture, the receptors/binding partners in the lower part. The question mark indicates a possible interaction of HSPG and TACI on the surface of the same cell.](image)
APRIL binds to two known TNF receptors, Transmembrane Activator and CAML Interactor (TACI) and B Cell Maturation Antigen (BCMA). BAFF can also interact with TACI and BCMA, but additionally binds a third TNF receptor, called BAFF Receptor (BAFF-R or BR3). There is also evidence for the existence of a third receptor for APRIL, since cell lines that do not express TACI and BCMA (Jurkat T cells, NIH-3T3 fibroblasts, HT-29 colon carcinoma and A549 lung epithelial cells) have been shown to be susceptible to APRIL stimulation [11, 23]. Recently it was shown that APRIL, but not BAFF, can interact with heparan sulfate proteoglycans (HSPGs), but currently it is not clear whether these structures on membranes represent the unidentified third receptor (Fig. 1) [24, 25].

Conflicting values for the affinity of binding of APRIL and BAFF to their respective receptors are reported, mainly due to differences in the forms of the receptors used (bivalent receptor-Fc versus monovalent receptors) and to differences in assay methods. Most studies using bivalent receptor-Fc constructs report similar binding affinities for both APRIL and BAFF binding to BCMA and TACI [26, 27]. Reports employing monovalent forms of receptors however show that BAFF binds to BAFF-R and TACI with nanomolar affinity, but demonstrates two to three order of magnitude weaker binding to BCMA [28, 29]. Since BCMA lacks a pre-ligand-binding assembly domain (PLAD), which is needed for ligand independent assembly of receptor trimers, it is likely that BCMA only acts as a receptor for APRIL and not for BAFF in vivo. Similar studies performed with monovalent receptors show that APRIL binds TACI and BCMA with high affinity (nanomolar range), but not BAFF-R, while APRIL binding to HSPGs is weak (20–80 μM) [8]. As TWE-PRIL contains the complete APRIL extracellular domain, it is predicted to have similar binding characteristics as APRIL. APRIL/BAFF heterotrimers on the other hand are likely to only bind TACI, since TACI-Ig, but not BCMA-Ig and BAFF-R-Ig, can neutralise them [15].

BCMA and BAFF-R are structurally different from other TNF receptor family members, since they consist of only one cysteine-rich domain (CRD) or a partial CRD respectively. The (partial) CRD of these receptors only contacts a single ligand monomer within the BAFF or APRIL trimer, while most TNF receptors contain several CRDs and establish more contact areas [30]. Although TACI contains two CRDs, only the second one is needed for high affinity binding to APRIL and BAFF. A short, functional form of TACI lacking this first CRD has been reported [31]. Structural and mutational analyses have revealed the molecular basis of the binding specificities of APRIL and BAFF to their receptors [1].

The signal transduction pathways used by BCMA and TACI have not yet been fully elucidated. BCMA has been reported to bind TNF receptor-associated factors (TRAFs) (1, 2 and 3; or 5 and 6, depending on the report) to activate the classical NF-kB pathway, p38 mitogen activating protein kinase (MAPK) and c-Jun NH2-terminal kinase (JNK)
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[32, 33]. Also, TACI interacts with TRAF 2, 5 and 6 and is a potent activator of the classical NF-κB pathway [34]. Furthermore, TACI has been reported to activate AP-1 and NF-AT [34].

In addition to binding to BCMA and TACI, APRIL interacts with HSPGs, which are structurally unrelated to TNF receptors [24, 25]. This involves interaction between acidic sulfated glycosaminoglycan (GAG) sidechains of proteoglycans with two distinct binding sites in APRIL: the basic NH2-terminal sequence of the secreted protein (amino acids 92-97 QKQKKQ) and basic amino acid residues on the surface of the molecule opposite of the receptor-binding pocket. These HSPG binding sites are present in APRIL, but not in BAFF, and are independent from the binding site for TACI and BCMA. The biological relevance for APRIL binding to HSPGs is not yet clear. Several different modes of involvement of HSPGs have been proposed and could be important for the biological activity of APRIL. HSPGs could play a role in concentrating the ligand and thereby increase receptor activation, as has been shown for hepatocyte growth factor (HGF) [35]. Apart from an effect on the local concentration of the ligand, HSPGs have been shown to serve a role in fibroblast growth factor-2 (FGF-2) function as cofactor. They are essential for the formation of an active signalling platform by connecting individual FGF-FGFR complexes. In the absence of HSPGs, FGF-2 signalling is transient, whereas simultaneous binding of FGF-2 to HSPGs and FGF-2 receptor results in sustained activation [36]. HSPGs could also play a role in the formation of gradients, as has been shown for the morphogen Wingless during wing formation in Drosophila [37, 38].

Thus HSPGs possibly concentrate APRIL at the receptor, might crosslink individual APRIL/TNF-receptor complexes and/or could play a role in chemotaxis. Whether HSPGs serve as the unidentified third receptor for APRIL and are in that function able to deliver signals through their intracellular tails or whether another, so far unidentified, APRIL receptor exists remains uncertain. Early evidence for a role of signalling through HSPGs in APRIL biology comes from experiments where co-ligation of TACI and HSPGs resulted in enhanced IgA production of B cells [39]. However, we have been unable to confirm a role for signalling through HSPGs in IgA production, but rather find a need for crosslinking via HSPGs (GH, unpublished observations). Additional complexity to the role of HSPGs in APRIL signalling comes from a recent study in which it was shown that the HSPGs syndecan-1, 2 and 4 can activate TACI [40]. A paper by Bossen and colleagues recently added more interest to this field by showing the need for higher order oligomerisation of both APRIL and BAFF in order to signal through TACI [41].

The receptors for APRIL are expressed on B cells at varying levels, depending on their maturation and activation state. In mice, TACI is expressed weakly in immature transi-
tional type 1 (T1) B cells, but is highly expressed by immature transitional type 2 (T2), marginal zone, B-1 and anti-B cell receptor (BCR) or Toll-like receptor (TLR) activated B cells [42-45]. BCMA is expressed in mouse plasma cells and BCMA expression increases upon activation of B cells [44, 46]. In humans, TACI is predominantly expressed on CD27+ memory B cells [47], whereas BCMA is expressed by plasma cells, plasmablasts and tonsillar GC B cells [42]. In contradiction with a report that BCMA resides intracellular in the Golgi apparatus, these studies measured extracellular BCMA expression [48].

The reports on receptor expression on T cells are controversial. TACI was first described to be expressed on activated human T cells using a polyclonal anti-TACI antibody for staining [49, 50]. Contrary to this finding, another group reported decreased TACI mRNA expression upon activation of human T cells [51]. In another report, several independent monoclonal anti-mouse and human TACI antibodies failed to detect TACI on both mouse and human activated T cells, respectively. In addition, these authors failed to detect TACI on activated human T cells using gene array experiments [42]. These contradictory findings may be due to the fact that only a subset of T cells expresses TACI. Indeed, expression of TACI was reported on a subset of synovial CD3+ T cells from patients with rheumatoid synovitis [49]. Similarly, we found substantial expression of TACI on mouse T cells derived from the peritoneal cavity, but only very limited expression in the spleen (GH, unpublished data). Together, these reports indicate that depending on their localisation, activation status and possibly the subset, mouse and human T cells express TACI.

**Effects of APRIL on T cells in vitro**

Several in vitro studies on the functions of APRIL and BAFF demonstrate that both factors can act as T cell co-stimulators. BAFF has been shown to enhance anti-CD3 activation of both mouse and human T cells [42, 52]. In addition, it was shown that blocking of endogenous BAFF from both the T cells themselves and dendritic cells in the presence of decoy Ig-fusion protein receptors such as BCMA-Ig, TACI-Ig (both blocking BAFF and APRIL) or BAFF-R-Ig (blocking only BAFF) prevents this effect [42, 53, 54]. However, some caution must be exercised in interpreting these data, since TACI-Ig showed more effect than BAFF-R-Ig [53] and BAFF-R-Ig failed to inhibit T cell proliferation in some individuals where TACI-Ig did block this effect [42], suggesting that APRIL can also account for part of this co-stimulatory signal. BAFF co-stimulation is dependent on BAFF-R as A/WySnJ-derived T cells, which express a defective BAFF-R, do not respond to BAFF. In contrast, T cells derived from TACI-deficient mice responded normally [42] or even showed increased responses [55]. The latter result could implicate TACI as a negative regulator of T cell activation, but this needs further
experimental support.

E.Coli derived soluble murine APRIL has been reported to co-stimulate anti-CD3-induced T cell activation [26]. The suggestion that anti-APRIL antibodies prevent T cell stimulation in vitro also supports a role for APRIL in primary T cell activation in vitro [56]. Another line of evidence for a role of APRIL in T cell biology comes from analysis of APRIL transgenic (Tg) mice, which display detectable transgene protein levels in their mature thymocytes and peripheral T lymphocytes. T cells derived from this mouse show increased proliferation after in vitro activation, especially after activation with a combination of anti-CD3 and anti-CD28 [57]. The APRIL-induced proliferation could be mediated by TACI, HSPGs and/or a yet unidentified additional receptor. Indeed, binding of APRIL to peritoneal T cells was largely prevented by heparin treatment, which blocks the HSPGs interaction. However, some residual binding remained, indicating that HSPGs are not the only binding partners of APRIL on T cells [24]. Furthermore, APRIL-induced co-stimulation of mouse CD4+ T cells could not be inhibited by heparin, thus HSPGs are not likely to play a role in APRIL induced co-stimulation [24]. Another study reported a lack of co-stimulatory activity on both human and mouse T cells using an engineered trimeric APRIL protein consisting of a trimeric coiled-coil domain fused to the APRIL TNF domain (ZZ APRIL) [8]. The reason for this inconsistency remains to be clarified.

In addition to the effect on proliferation, Tg APRIL-expressing T cells show increased survival in vitro if they are deprived of survival factors and receive no stimulation. This survival coincides with increased Bcl-2 expression in APRIL Tg T cells ex vivo. This effect on survival could not be blocked by addition of the soluble decoy receptors TACI-Fc or BCMA-Fc, suggesting that this is an indirect effect of APRIL expression in vivo or APRIL is able trigger a receptor inside the cell prior to secretion [57].

Modulation of T cell function in vivo

The role of APRIL in T cell function in vivo is not fully elucidated. Hints for an effect of APRIL on T cells in vivo are mainly derived from studies employing APRIL and receptor Tg or deficient mice. APRIL Tg mice show a reduction in absolute number of T cells in peripheral lymph nodes and an increase in the number of CD62L- T cells. The CD62L- T cells do not correspond to classically activated T cells, as they are CD44low, CD25+ and CD45RBhigh. The reduction in absolute numbers of T cells in the peripheral lymph nodes is likely connected to the increase in CD62L- T cells, as CD62L plays a role in lymphocyte homing from the blood to lymph nodes. To determine whether the increased T cell proliferation, observed in vitro, also plays a role in vivo, APRIL Tg versus non-Tg littermate mice received Staphylococcal enterotoxin B (SEB). A similar increase in numbers of both Vβ8+CD4+ and Vβ8+CD8+ T cells was observed in APRIL Tg mice and
corresponding non-Tg littermates, indicating that APRIL does not affect the expansion phase of this T cell response in vivo. In contrast, a delay in the deletion of Vβ8+CD4+ Tg T cells, but not Vβ8+CD8+ Tg T cells, was observed in comparison with SEB-reactive cells of littermates. The finding that Tg expression only affects the deletion of CD4+ Tg T cells can be explained by a different regulation of deletion of both subsets. Deletion of Vβ8+CD4+ Tg T cells is regulated by mitochondrial-dependent apoptosis and is thus inhibited by Bcl-2, which on its turn can be upregulated by APRIL. In contrast, Vβ8+CD8+ Tg T cells are dependent on perforin for their removal [58]. No significant differences were observed in the SEB-specific CD8+ T cell response [57]. In agreement with this, no effect of ectopic APRIL expression on the CD8+ T cell response to adenovirus was observed (GH, unpublished data). Furthermore, no differences were observed after adoptive transfer of in vitro activated CD8+ TCR Tg OT-1 APRIL Tg versus OT-1 cells into wildtype mice. Despite the effect of APRIL on SEB-triggered CD4+ T cells, no effect was observed on adoptively transferred and peptide-triggered CD4+ TCR Tg OT-2 T cells. In addition, Tg APRIL-expressing CD4+ or CD8+ T cells did not respond differently to other T cell immunisation protocols such as intranasal OVA or influenza A virus infection (GH, unpublished data). Thus, despite the differences observed after SEB immunisation, other, more physiological, immunisations did not reveal a role for APRIL in T cell responses in vivo.

Studies performed on two different APRIL-deficient mice strains confirm this conclusion. The strain by the group of A.Ashkenazi (subsequently referred to as APRIL-deficient mice 1) revealed no abnormalities in T cell numbers and distribution. In addition, no differences were observed during in vitro proliferation or in the T cell response after KLH immunisation in this strain [59]. The second strain of APRIL-deficient mice by the group of Geha (subsequently referred to as APRIL-deficient mice 2) showed significantly increased percentages of CD44hiCD62Llow effector/memory T cells, but no abnormality in in vitro proliferation. No detailed analysis of T cell responses in this strain is reported [60].

Since TACI expression could play a role in the effect of APRIL on T cells, studies in both the TACI-deficient and TACI Tg mice could shed light on the role of APRIL. TACI deficient mice do not show gross differences in number of T cells in the lymphoid organs compared with wildtype littermates. Only the Peyer’s patches showed a subtle but discernable increase in CD4+ T cells in these mice [61, 62]. A detailed analysis of T cell responses in this strain is lacking, except for one study showing no differences in cardiac allograft rejection [63]. Studies on different strains of TACI Tg mice do also not report an apparent T cell phenotype [64, 65].
Effects of APRIL on B cells in vitro

APRIL has been reported to stimulate proliferation of both murine and human naïve B cells in vitro [26, 66]. BAFF-BR3 ligation results in similar co-stimulatory effects, but this is thought to be the result of increased survival of B cells rather than a direct effect on proliferation [42, 67-71]. Besides this, APRIL has been shown to enhance antigen presentation of B cells by upregulation of important co-stimulatory molecules (such as CD40, MHC-II, B7.1 and B7.2). Additional stimulation with interleukin (IL)-4 and IL-6 was needed to observe this effect, presumably to upregulate BCMA expression on these cells. An agonistic anti-BCMA antibody also enhanced antigen presentation and BCMA is therefore implicated in the observed effect [72]. Both APRIL and BAFF have been shown to enhance the survival of plasmablasts from activated human memory B cells in vitro through their interaction with BCMA [73].

Apart from an effect on survival, APRIL and BAFF have been shown to promote immunoglobulin (Ig) class-switch recombination (CSR) to IgG and IgA independent of T cell help [17, 74]. This effect is solely dependent on TACI in the case of APRIL and both TACI and BR3 in the case of BAFF [75].

APRIL in B cell maturation and survival

BAFF-deficient mice show impaired numbers of transitional T2 B cells and subsequent stages in B cell development, such as mature B cells and marginal zone (MZ) B cells, but have normal numbers of B-1 B cells. BAFF-deficient mice share this phenotype with A/WySnJ mice, which express a defective BAFF-R, and BAFF-R-deficient animals. Although the regulation of CD21/35 and CD23 expression by BAFF has complicated these analyses, these findings clearly indicate that the BAFF-BAFF-R axis is important for the survival of the B-2/MZ B cell compartment [64, 76-78]. APRIL-deficient (1 and 2) and BCMA-deficient mice do not show impaired B cell survival, and TACI-deficient mice show quite the opposite, as they have elevated numbers of B-2/MZ B cells, suggesting a negative regulatory role for TACI in B cell homeostasis [46, 59-62] (see below).

These results implicate that APRIL has no apparent function in the survival of B cell subsets. However, analysis of APRIL Tg animals did reveal an increase in the number of peritoneal B-1 B cells due to increased survival of this cell population upon ageing [79]. Interestingly, one strain of TACI Ig Tg mice shows, next to impaired survival of B-2/MZ B cells, decreased numbers of peritoneal B-1 B cells [64]. It is not clear why this was not seen in a second strain of TACI Ig mice described, although the B-2/MZ B cell were also decreased in this strain [65]. In addition, one study using both BAFF-deficient mice [76] and CD68 promoter driven BAFF Tg mice reported decreased and increased numbers of peritoneal B-1 B cells respectively. Together these reports indicate that B-1 B cell survival is controlled by both APRIL and BAFF [80]. In addition, it was recently
shown that BAFF promotes in vitro survival of B-1 B cells and synergises with TLR ligands to promote activation, proliferation and cytokine production of these cells [45].

**APRIL in humoral responses**

Although Tg APRIL expression did not alter B cell numbers in 6-12 weeks old mice, a twofold increase in natural serum IgM antibodies was observed in these mice. In addition, these mice show increased antigen-specific IgM in response to thymus-dependent (TD) vaccinia virus and increased IgM and IgG in response to the thymus independent (TI) NP-Ficoll antigen [57]. Natural serum IgM and IgA antibody titers increased upon aging, presumably as a result of increased B-1 B cell activity [79]. Despite an increased number of B-2/MZ B cells, TACI deficient mice were shown in one report to have the opposite phenotype, that is, reduced natural serum IgM and IgA antibodies, normal antibody responses to the TD antigen NP-KLH and deficient IgM, IgG and IgA antibody responses to immunisation with the TI type 2 antigen TNP-Ficoll, but not to the TI type 1 antigen NP-LPS [62]. This suggests that APRIL-TACI interactions may have a role in TI B cell responses.

TACI is reported to serve both as a positive and negative regulator for B cell responses depending on the conditions. In TACI-deficient mice TACI serves as a negative regulator of B cell homeostasis and its deficiency leads to B cell expansion, autoimmunity and lymphoproliferation [55, 61]. This inhibitory effect of TACI could also be shown in murine B cells in vitro where TACI agonistic antibodies inhibit B cell proliferation and induce apoptosis [55]. In contrast, TACI acts as a positive regulator during TI type 2 antibody production in TACI-deficient mice [62] and in TI CSR of B cells [17, 75]. Further evidence that TACI acts as a positive regulator of terminal B cell differentiation comes from the finding that two mutations in TACI (C104R and A181E) are associated with common variable immunodeficiency (CVID) in humans. B cells from patients with CVID who are heterozygous for these mutations fail to produce Igs in response to APRIL and/or BAFF. These patients have elevated serum TACI levels and suffer from humoral immunodeficiency [81-85]. The apparent paradox of both positive and negative regulatory functions of TACI seems to be caused by a dual effect on B cells: inhibiting prolonged B cell proliferation and stimulating plasma cell differentiation [86-88].

As mentioned before, two groups have generated viable APRIL-deficient mice (1 and 2) with different phenotypes [59, 60]. Both APRIL-deficient mice (1 and 2) show normal B cell development, but the levels of natural antibody IgA were shown to be decreased only in the second strain [60]. This disparity could be due to the difficulty of detecting very low levels of IgA antibody present in unimmunised mice. No differences in the in vivo humoral responses to the TD antigen NP-chicken γ globulin (NP-CGG) and TNP-keyhole limpet hemocyanin (KLH), a TI antigen according to the authors, were reported.
in APRIL-deficient mice 1 [59], whereas APRIL-deficient mice 2 showed increased IgG and decreased IgA responses to the TD antigen NP-CGG. Additionally, APRIL-deficient mice 2 showed decreased serum IgA responses to the TI type 1 antigen NP-LPS, but not to the TI type 2 antigen NP-Ficoll [60]. Consistent with this, we found the opposite phenotype in APRIL Tg mice, i.e. increased IgA responses to NP-LPS and NP-Ficoll (GH, Immunology and Cell Biology 2008, in press). Together, these results clearly implicate APRIL in CSR of B cell responses in vivo and are in line with the earlier finding that APRIL promotes Ig CSR in vitro [17, 75].

BCMA-deficient mice were initially shown to lack an apparent phenotype, as they had a normal B cell compartment and showed a normal primary response against the TI 2 antigen NP-Ficoll and a normal primary and secondary response against the TD antigen NP-chicken globulin (NP-CG) [89]. In a subsequent study, the survival of long-lived plasma cells (LLPCs) was shown to be impaired at 7 weeks after NP-CGG immunisation and BCMA was clearly shown to affect plasma cell survival in TD B cell responses [46]. This observation was in agreement with the expression of BCMA on mouse and human plasma cells, human plasmablasts and human GC B cells and implicates the relevancy of BCMA in later stages of B cell differentiation [42, 44, 46]. It is however not clear whether BCMA also plays a role in plasma cell survival after TI B cell responses.

**APRIL-producing cells in B cell physiology**

Next to the increased understanding of the role of the APRIL/BAFF system in both B and T cell biology, a lot of progress has been made on knowledge on the source and regulation of both ligands under physiological conditions. Monocytes, macrophages and dendritic cells are thought to represent the main immune cells responsible for APRIL and BAFF production for B cell function in vivo. In vitro, the ability of these myeloid cells to produce these ligands upon activation with different cytokines, PRR-ligands and CD40 ligand (CD40L) has been studied extensively. However, the findings differ somewhat between the different studies. Craxton and colleagues show that human macrophages and monocyte derived dendritic cells (moDC) enhance BCR-stimulated B cell proliferation and that this can be blocked with TACI-Fc, but not with prior immunodepletion of APRIL with an anti-APRIL antibody, showing the importance of BAFF in this effect. Furthermore they show increased secretion of BAFF by macrophages after IL-10, prostaglandin, IFN gamma and lipopolysaccharide (LPS) stimulation and increased production of APRIL production by moDC after stimulation with the latter two stimuli [66]. A study by Litinskiy et al. showed that both APRIL and BAFF derived from human monocytes and moDC can induce CSR to IgG and IgA in human B cells. This effect was inhibited by BCMA-Ig and, to a lesser extent, by TACI-Ig or a neutralising antibody to BAFF, indicating that both APRIL and BAFF play a role
MoDCs incubated with IFN alpha, IFN gamma or CD40L were shown to upregulate APRIL and BAFF, whereas monocytes upregulated BAFF after stimulation with IFN alpha, IFN gamma and LPS [17]. We studied the expression of both ligands on moDCs and found that of a whole panel of TLR ligands only CpG oligonucleotides and synthetic double-stranded RNA polyriboinosinic polyribocytidyllic acid (poly IC) could induce APRIL via a protein kinase receptor (PKR) dependent and transcription independent mechanism. Since poly IC, CpG and PKR are implicated in antiviral responses, this hints at a role for APRIL in the immune response against viruses [90]. In summary, these studies show that expression of APRIL and BAFF by monocytes, macrophages and dendritic cells is dependent on the cytokine environment and the engagement of PRRs and CD40 on the cell surface and plays an important role in the regulation of B cell responses.

Balázs and coworkers investigated an in vivo model and showed that blood-derived CD11c<sup>low</sup> immature dendritic cells efficiently capture and transport heat-killed and pepsin treated S.pneumoniae bacteria to the spleen marginal zone, where plasma cell differentiation takes place. These CD11c<sup>low</sup> DCs provide critical survival signals, which can be inhibited by TACI-Fc, to antigen-specific MZ B cells and promote their differentiation into IgM-secreting plasmablasts. After intraperitoneal immunisation with similar bacteria, macrophages were shown to provide similar support to B1 B cell derived plasmablasts. This study clearly shows that both DCs and macrophages are important players in TI humoral responses by inducing plasmablast survival and differentiation via APRIL and/or BAFF [91].

The recent findings on the role of APRIL in IgA CSR combined with the importance of IgA in the mucosal lining of the body, led the APRIL research into mucosal immunity. It was found that human intestinal epithelial cells (IECs) trigger IgA<sub>2</sub> class switching in B cells, through a TI pathway involving APRIL. IECs released APRIL after engagement of their PRRs by bacterial products (LPS and flagellin) and further increased APRIL production by activating dendritic cells via thymic stromal lymphopoietin (TSLP) [22]. Similar mechanisms could also play a role in the airway mucosa, as it has been shown recently that airway epithelial cells can also produce APRIL and BAFF. Interestingly, poly IC was the only TLR ligand that could induce APRIL and BAFF expression in primary bronchial epithelial cells [92]. In another intriguing study by Tezuka et al., it was shown that inducible nitric oxide synthase (iNOS)-deficient mice have impaired IgA levels, presumably because iNOS-deficient B cells show impaired CSR to IgA and these mice have reduced B-1 B cell numbers. Nitric oxide mediated these effects, by controlling B cell expression of TGF-b receptor II in TD IgA CSR and influencing APRIL and BAFF secretion by dendritic cells in TI IgA CSR. In addition, the authors found that iNOS is preferentially expressed in so called TNF-α/iNOS producing dendritic cells in the mucosal associated lymphoid tissue in response to the recognition of commensal
bacteria by Toll-like receptors. Adoptive transfer of iNOS proficient dendritic cells was shown to rescue IgA production in iNOS-deficient mice [93]. Together, these current studies clearly show the importance of APRIL in the immune regulation of B cell responses. This appears specifically evident for the provision of protective IgA in the mucosa (Fig. 2).

**Figure 2. APRIL in the intestinal immune defense system**

Upon recognition of pathogens (e.g. bacteria and viruses) by pathogen recognition receptors of dendritic cells and epithelial cells, APRIL is rapidly synthesised and released. Dendritic cells present bacteria and viruses in their native form to B cells and APRIL will engage TACI on the surface of these cells. Next, the B cells will become activated, start to produce pathogen specific plasma cells and class switch recombination to IgG and IgA will occur. IgG/IgA antibodies released by the plasma cells are able to opsonise bacteria or viruses for their subsequent removal. Secretory IgA is mainly produced upon recognition of commensal bacteria and plays an important role in the prevention of pathogen attachment and neutralisation in the intestinal lumen.
APRIL/BAFF in cancer

The first association of APRIL and cancer appeared with the initial description of this protein in 1998 [11]. In this study, it was shown that a panel of tumour cell lines and human primary tumours of diverse origins expressed APRIL mRNA, and that APRIL stimulates cell proliferation in vitro and in vivo. Also BAFF appeared to be associated with lymphoid expansion as mice overexpressing the BAFF transgene develop Sjögren’s syndrome and show a high incidence of B cell lymphomas [94]. Reports showing an association between APRIL, but also BAFF, and cancer have since multiplied. These two factors appear to be implicated in a broad spectrum of cancers, ranging from B cell malignancies to solid tumours, suggesting a broad and important function for APRIL and BAFF [4-9, 95] (Table 1).

B cell malignancies

APRIL in Chronic Lymphocytic Leukemia

Chronic Lymphocytic Leukemia (CLL), the most common leukaemia in Western countries, is characterised by the gradual accumulation of mature monoclonal B cells expressing surface CD5, CD23 and low levels of Igs. In CLL most systemic B cells are in a quiescent state characterised by prolonged survival [96]. Soon after their discovery, the cytokines APRIL and BAFF, which were already known B cell modulators in physiological conditions (as described in previous sections), were suspected to be involved in sustaining CLL survival.

Expression pattern of APRIL/BAFF and their receptors in CLL

Several laboratories have studied CLL B cell expression of BCMA, TACI and BAFF-R [97-99]. In the earliest report, Novak et al. detected TACI protein in the plasma membrane and BAFF-R mRNA in all primary CLL tumour samples tested (23 and 7, respectively); in the same analysis, BCMA mRNA was found in only one of the seven CLL cell samples tested [99]. In a second study, Kern et al. used RT-PCR to confirm CLL B cell expression of TACI and BAFF-R mRNA [98]. The recent availability of commercial antibodies to these receptors allowed Endo et al. to verify the presence of BCMA, TACI and BAFF-R on the CLL B cell surface [97]. A variety of approaches have been used to analyse APRIL and BAFF expression by
the CLL B cell as well as by other cells in the tumour proximity [79, 95, 97-100]. From a total of 23 patient samples tested by the Jelinek group, only 2 showed APRIL mRNA while BAFF transcripts or surface protein were detected in 11 CLL samples [99]. Using RT-PCR, we found that 18 of 40 CLL tumour cells expressed APRIL, whereas APRIL mRNA was not detectable in naive B cells [79]. Another study by Kern et al. described RNA expression of APRIL and BLyS on all 13 CLL B cell samples tested. In addition, by using flow cytometry they found APRIL and BAFF proteins on the cell membrane [98]; this observation nonetheless does not concur with the maturation pathway described for APRIL, in which there is no intermediate APRIL expression on the plasma membrane [101]. These authors suggested a possible alternative transport mechanism for APRIL in CLL B cells, although this hypothesis remains to be verified. A second possible explanation would be that the APRIL form detected by Kern et al. corresponds to TWE-PRIL [14].

An interesting study by Nishio et al. showed that not only CLL B cells, but also nurse-like cells (NLC), secrete large amounts of BAFF and APRIL when they are cultured in the presence of CLL B cells [100]. NLC are large, round, adherent cells that differentiate in vitro from CD14+ mononuclear cells derived from CLL patient blood samples; these NLC are thought to attract leukaemia cells and protect them from apoptosis since CLL B cell survival is rapidly reduced when NLC are removed from the culture [102]. Cells with a morphology and phenotype similar to those of NLC are abundant in secondary lymphoid tissues from CLL patients, which suggests that APRIL and BAFF expression by these NLC-like cells is also occurring in vivo. The data showing autocrine and paracrine expression of APRIL and BAFF by leukaemic cells and NLC imply a complex mode of action for these cytokines, as will be discussed in the next section.

### Serum APRIL and BAFF levels in CLL

The clinical relevance of APRIL and BAFF in CLL has been partly investigated by measuring the concentration of these factors in the patient serum. Our initial study, which included 22 CLL samples, showed increased serum APRIL levels in CLL patients compared to healthy donors [79]. Later on, we confirmed this result using a larger 95 patient cohort and found a significant correlation between serum APRIL levels and overall patient survival, with a poorer prognosis for patients with the highest APRIL levels [103]. Kolb's group has also analysed serum APRIL levels in CLL patients and found that some of them have elevated levels of circulating APRIL, although the differences were not significant compared to those in healthy donors [95]. New studies are needed to reinforce and broaden these findings and to further clarify the clinical significance of APRIL serum levels in CLL. In addition, it would be of interest to evaluate whether the measurement of APRIL, alone or combined with other clinical, biological, or genetic
markers, could be a useful indicator of CLL progression. Three reports describe the analysis of circulating BAFF levels in CLL patients. In a first study, Kern et al. used SELDI-TOF (surfaced-enhanced laser desorption/ionization time-of-flight) mass spectrometry and detected elevated levels of a soluble, 28 kDa BAFF species in the sera from five CLL patients compared to control donors [98]. The same group subsequently reported significantly lower soluble BAFF (sBAFF) levels in CLL patient sera (n = 88; detected using a commercial ELISA kit) compared to healthy donors [95]. To explain these apparently contradictory data, the authors suggested that the presence of sBAFF-containing complexes in CLL patient sera might account for the distinct results obtained using ELISA and mass spectrometry. Another hypothesis put forward by the authors is that the initially detected 28 kDa BAFF species, which differs from the 17 kDa sBAFF form, corresponds to a recently described BAFF isoform that is generated by alternate splicing [80]. Compared to sBAFF, this isoform lacks 57 nucleotides, is inefficiently released by proteolysis, and may heterodimerise with sBAFF [80].

We have also studied our 95 CLL patient cohort and found a slight decrease in serum BAFF levels [103], using the same commercial ELISA kit employed by the group of Kern. A possible explanation for lower BAFF serum levels, which contrasts the described BAFF overexpression in CLL cell lines, could be a poor cleavage efficiency of the membrane-bound protein. Further studies are needed to determine whether or not a decrease in circulating BAFF levels is associated with CLL development.

Finally, Novak et al. showed that, whereas serum BAFF levels were unaltered in the overall CLL patient cohort (n = 46) compared to healthy donors, sBAFF was increased in a restricted subset of CLL patients with a family history of B cell lymphoproliferative malignancies (n = 24) [104]. Variations in the ELISA system used might explain why no significant differences were detected in patient BAFF serum levels compared to controls [99], in contrast to the moderate differences observed in the two reports mentioned previously [98, 103].

These observations indicate that BAFF overexpression by CLL tumour cells (or any other cell type) does not significantly increase systemic BAFF concentrations; its potential CLL tumour-supporting effect could thus be due to the membrane-anchored form of BAFF. Alternatively, increased local concentrations of sBAFF by NLC in the tumour cell proximity could mediate a stimulatory effect on CLL cells, as suggested previously [100].

The role of APRIL/BAFF in CLL maintenance

Several in vitro studies clearly showed that APRIL and BAFF can protect tumour cells from spontaneous as well as drug-induced apoptosis; in addition, the use of specific agents to block either BAFF or APRIL was found to reduce tumour cell survival [97-
Addition of exogenous BAFF to primary CLL cell samples resulted in a 55% reduction in the activity of caspase-3, a hallmark of apoptosis, suggesting that BAFF reduces spontaneous apoptosis levels [99]. To confirm the specificity of the effect of soluble APRIL and BAFF on CLL cell survival, the authors used BCMA-Fc, which binds with high affinity to both cytokines, and measured caspase-3 activity. Four of the five patient cell samples treated with BCMA-Fc showed an increased apoptosis rate, suggesting an autocrine role for APRIL and/or BAFF in enhancing cell survival and decreasing apoptosis in CLL B cells. Experiments by Kern et al. support these observations. In their study, exogenous BAFF or APRIL also protected leukaemic cells from spontaneous and flavopiridol-induced apoptosis [98].

In addition, in vitro survival of CLL B cells can be increased by addition of recombinant APRIL or BAFF or when the cells are cultured in the presence of NLC, which express high levels of APRIL and BAFF [100]. Moreover, treatment of co-cultures of CLL cells and NLC with BCMA-Fc, greatly reduced cell viability. Unexpectedly, such a reduction was not observed when a decoy BAFF-R:Fc protein, which binds BAFF exclusively, was used. This observation suggests a pivotal role for APRIL in the protective effect of NLC on CLL survival. Indeed, Haiat et al. also mentioned that in some CLL patients, APRIL protected leukaemic cells from apoptosis better than BAFF [95]. The observation by Nishio et al. that BCMA-Fc treatment had no effect on cell survival when CLL were cultured in the absence of NLC contrasts previous data on the autocrine effect of BAFF and APRIL on CLL survival. The reason for this discrepancy could be that in this case the cells were already prone to undergo apoptosis due to the absence of NLC.

All of these in vitro-based results strongly suggest that the BAFF/APRIL system uses both autocrine and paracrine activation loops to promote survival and apoptosis resistance in CLL tumour cells [97-100]. It is however clear that the individual participation of BAFF, APRIL as well as TACI, BCMA and BAFF-R in sustaining CLL requires further examination.

There is little information available about the in vivo function of the APRIL/BAFF system in CLL, in part due to the limited number of appropriate experimental CLL models. Our analysis of APRIL-Tg mice showed the first in vivo evidence of APRIL participation in B cell malignancies [79]. 9-12 months old APRIL-Tg mice develop lymphoid malignancies that originate from a peritoneal B-1 B cell expansion [79]. The hyperplasia we observed in lymphoid organs and Peyer’s patches was accompanied by tissue disorganisation and mucosal and capsular infiltration. In addition, we found CD5+ B cell infiltration into nonlymphoid organs such as kidney and liver at later stages [79]. The B-1 B cell associated neoplasms observed in APRIL-Tg mice are highly reminiscent of human CLL, and the B cell accumulation in APRIL-Tg mice is due to a cell survival advantage, concurring with the in vitro studies in human CLL described above. BAFF overexpression also
leads to development of B cell neoplasia in mice [105].
There are nevertheless substantial differences between the respective murine and human leukaemic B cell populations, and caution should therefore be employed when extrapolating conclusions from experimental animal models. It thus remains to be determined whether APRIL is involved in CLL leukaemogenesis.

**Effects of APRIL/BAFF on signalling pathways**
The APRIL and/or BAFF-mediated survival effect in CLL B cells involves activation of the transcription factor NFκB [97, 98]. This was not a surprising finding, since in normal B cells the NFκB2 pathway is activated in response to BAFF [106, 107]; in addition, the NFκB transduction pathway appears to be critical for cancer cell growth and survival [108]. Addition of recombinant BAFF and APRIL to leukaemia cells stimulated a moderate increase in NFκB activation, which is already high in these cells [98]. The increment observed in NFκB activation was dose- and time-dependent, as measured by the translocation of the NFκB p65 subunit in ELISA. In addition, blockade of the NFκB pathway inhibited the capacity of APRIL and/or BAFF to promote CLL cell survival. Using other approaches, the groups of Nishio [100] and Haiat [95] confirmed these observations and Endo et al. recently reported a more complete characterisation of the NFκB signalling pathways activated in response to APRIL and BAFF in CLL [97]. In an in vitro system, these authors detected IkBα phosphorylation and degradation, as well as translocation of the NFκB p65 subunit to the nucleus, showing that BAFF and APRIL activate the classical NFκB pathway in CLL cells. Nevertheless, they found degradation of the NFκB p100 subunit to p52 and translocation of p52 to the nucleus only when BAFF bound to BAFF-R, but not to BCMA or TACI, indicating that signalling through BAFF-R is able to induce the alternative NFκB pathway. Using specific inhibitors of the alternative and canonical NFκB pathways, the authors conclude that the canonical NFκB pathway appears to have a dominant role in promoting APRIL- or BAFF-induced survival in CLL cells and postulate that its blockade could have therapeutic potential.

In contrast, neither the mitogen-activated protein-kinases (MAPK) ERK1/2 nor the serine/threonine kinase PKB/AKT seem to be activated in CLL cells in response to BAFF and APRIL [100]. When CLL cells were treated with either BAFF or APRIL, no phosphorylation of the respective kinases was detected [100].

In an attempt to identify other mechanisms responsible for the APRIL- and BAFF-mediated effects in CLL, Nishio et al. analysed the expression levels of various pro- and anti-apoptotic proteins. Mcl-1 was found to be upregulated in CLL B cells co-cultured with NLC, or with recombinant APRIL or BAFF [100]. It is, however, difficult to draw firm conclusions from these experiments, since CLL cells also express APRIL and BAFF endogenously.
The expanding role of APRIL in cancer and immunity

Table 1. Summary of APRIL/BAFF role in human B cell malignancies

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<tr>
<th>Expression pattern of APRIL/BAFF and receptors</th>
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Function of APRIL/BAFF

- **APRIL&BAFF**
  - Increase survival: CLL [97, 100] NHL [20, 113, 114], HL [115], MM [47, 122]
  - Reduce apoptosis: CLL [95, 97-99] NHL [20, 114], HL [115], MM [121, 122]
  - Enhance proliferation: NHL [20], HL [115], MM [47, 122]

Signal transduction pathways involved

- **APRIL&BAFF**
  - Activate NFκB: CLL [95, 97, 98, 100], NHL [20, 114], HL [115], MM [122]
  - Activate PI3K, AKT, MAPK: MM [122]
  - Up-regulate Mcl1: CLL [100], MM [122]
  - Up-regulate Bcl-2 and Bcl-xL: NHL [20], HL [115], MM [122]
  - Down-regulate Bax2: NHL [20], HL [115]
  - Up-regulate c-maf, cyclin D2, integrin [7]: MM [120]

Suggested associations of APRIL/BAFF with outcome disease

- **APRIL**
  - High serum APRIL levels correlate with poor survival: CLL [103]
  - High tissue APRIL expression correlates with poor survival: NHL [111]

- **BAFF**
  - High serum BAFF levels correlate with tumor aggressiveness and poor survival: NHL [113]

Abbreviations: CLL (Chronic lymphocytic leukemia), NHL (Non-Hodgkin’s lymphoma), HL (Hodgkin’s lymphoma), MM (multiple myeloma), nd (not determined). [ ] See the references in the text.
Non-Hodgkin’s lymphoma

Non-Hodgkin’s lymphomas (NHL) encompass a large number of cancers that can be divided into aggressive (fast-growing) and indolent (slow-growing) types, and can be classified as either B cell or T cell NHL. B cell NHL include chronic lymphocytic leukaemia (CLL), Burkitt’s lymphoma, diffuse large B cell lymphoma (DLBCL), follicular lymphoma, immunoblastic large cell lymphoma, precursor B lymphoblastic lymphoma, and mantle cell lymphoma [109]. Prognosis and treatment of NHL depends on the disease stage and type [110]. Schwaller et al. recently analysed in situ APRIL expression in a panel of NHL samples, and detected elevated levels of secreted APRIL in cancer lesions from two types of aggressive NHL, DLBCL (26 of 56 patient samples) and a small proportion of Burkitt’s lymphomas (2 of 10 samples) [111]. APRIL protein levels in DLBCL patient sera (n = 5) appeared to be higher than those of controls. Analysis of a larger number of patient samples is however required to ascertain such a connection. In addition, it has been shown that circulating BAFF levels were also increased in NHL patients (n = 17 and n = 56) [112, 113] and that elevated serum BAFF levels correlated with more aggressive NHL and poorer disease outcome [112]. In the cancer lesions, DLBCL cells themselves express low APRIL levels [20, 111] and the main APRIL producers are the infiltrating neutrophils; APRIL expression by the neutrophils is constitutive and contributes to cancer aggressiveness [111]. A retrospective analysis of 39 DLBCL patients showed that high APRIL expression in cancer lesions correlated with a poor survival rate [111]. BAFF expression has also been reported in aggressive NHL cell lines derived from DLBCL and mantle cell lymphoma patients [114]. DLBCL cell lines and primary cancer cells show variable expression of BCMA and TACI receptors [20, 111], and also express HSPGs [111]. In NHL, HSPGs are suggested to retain APRIL in the lesion and provide binding to malignant cells [111]. APRIL binding to HSPGs has been shown to induce proliferation in some cell lines [24, 25], suggesting that HSPGs might have a role in APRIL-mediated effects on NHL. Exogenous APRIL and BAFF enhance cancer cell survival in NHL [20, 114]. A recent study by Fu et al. showed that BAFF expression in NHL is regulated by the transcription factors NFκB and NFAT, although the individual role of each factor was not clarified [114]. These authors also identified binding sites for NFκB and NFAT in the BAFF promoter critical for BAFF gene transcription [114]. NFκB activation in NHL induces APRIL and BAFF expression, which in turn participate in maintaining NFκB activity. All of these observations on APRIL/BAFF expression, function and regulation in NHL provide further support for the positive activation loop described previously for APRIL and BAFF in CLL.
Hodgkin’s lymphoma
Classical Hodgkin’s lymphoma (HL) is recognised as B cell lymphoma, since cases of T cell origin are exceptional. HL is characterised by the clonal expansion of malignant mononuclear Hodgkin and Reed-Sternberg cells (HRS). Two reports published in 2007 showed that APRIL and/or BAFF are involved in HL [115, 116]. The Cerutti group analysed the expression pattern of APRIL/BAFF and their receptors in HL, as well as the cell survival and proliferation signals activated in response to these factors [115]. They report that HL cell lines and primary HRS cells express BCMA and TACI, whereas the BAFF-R receptor is notably absent. In addition, they observed that all HL cell lines tested expressed BAFF protein at the cell membrane, whereas APRIL protein was detected inside the cells. Analysis of primary HL (n = 15) showed that CD30+ HRS cells as well as inflammatory cells that form part of the lesion infiltrates express both cytokines at variable levels. Concurring with these observations is a study by the Huard laboratory, which reported high APRIL levels in 64% (182 of 285) of the HL samples analysed [116]. Nevertheless, these authors concluded that infiltrating neutrophils, but not HRS cells are the main source of APRIL production in these cancer lesions. Both reports describe that paracrine APRIL, secreted by the infiltrating inflammatory cells, is retained on or near the HRS cells via binding to HSPGs [115, 116]. Using siRNA approaches the Cerutti group described that in HRS cell lines the inhibition of TACI reduced APRIL/BAFF-mediated proliferation in vitro, whereas BCMA inhibition diminished APRIL/BAFF-induced survival. In addition, they showed that APRIL, as well as BAFF, induces NFκB activation in HRS cells, as well as an altered expression of anti- and pro-apoptotic proteins (Bcl2, Bcl-xL and Bax) thus enhancing cell viability and proliferation [115]. Further studies are nonetheless needed to examine these effects not only in cell lines but also in primary HL cells [115].

Multiple myeloma
Multiple myeloma (MM) is a progressive incurable disease characterised by the accumulation of malignant plasma cells in the bone marrow (BM). Three stages of MM can be distinguished based on progression and localisation. In the inactive phase there is little or no cancer cell proliferation in the BM; in the active phase, plasma cells proliferate in the BM and can be detected in the blood, and the advanced phase is identified by an increase in plasmablastic cells, which proliferate inside and outside the BM [117-119]. Similar to cells of other haematological malignancies, MM cells resist apoptosis and show prolonged survival, leading to their accumulation. Several groups have studied the implication of the APRIL/BAFF system in MM [21, 47, 120-122], and reported that both MM cell lines and primary MM cells show variable levels of BCMA and TACI, with rare BAFF-R expression [21, 47, 120].
In the case of TACI, gene profiling analysis was recently used to show that TACI\textsuperscript{high} MM cells resemble normal BM plasma cells, which are highly dependent on the BM environment; TACI\textsuperscript{low} MM cells have a plasmablastic gene signature that reflects behaviour less dependent on the BM environment [120]. The authors showed that TACI expression in MM cell lines is associated with expression of the c-maf transcription factor. APRIL- or BAFF-induced activation of TACI can upregulate c-maf expression, which in turn controls other genes (such as cyclin D2 and integrin β7) that are critical in MM pathogenesis [120].

BAFF and APRIL are detected both in MM cell lines and in the BM of MM patients, and high levels of circulating APRIL and BAFF are also found in MM patient serum [121, 122]. In the BM of MM patients, MM cells express both ligands, as do CD14\textsuperscript{+} cells and osteoclasts, which appear to be the main APRIL/BAFF producers [121, 122]. 70 to 80\% of MM patients develop osteolytic bone disease, mainly due to enhanced activation of osteoclasts and to the interaction between MM cells and the BM microenvironment [123]. MM cells co-cultured with osteoclasts have greater viability due to cell-cell contact dependent factors [124]. It is therefore tempting to suggest that osteoclast-derived APRIL and BAFF are involved in MM cell growth and survival. This would again support a paracrine role for these two factors in B cell malignancies. Indeed, a preliminary analysis shows enhanced apoptosis when osteoclast/MM cell co-cultures are treated with TACI-Fc [122]. Exogenous BAFF and APRIL enhance survival of MM cell lines and primary MM cells, increase proliferation, and protect cells against deprivation-induced apoptosis [120-122]. In the presence of TACI-Fc these effects were abolished [21, 47]. Diverse molecular mechanisms are activated in MM cell lines by APRIL and BAFF, including the signalling pathways NF\textsuperscript{κ}B, PI3K/ AKT(PKB) and MAPK, as well as the anti-apoptotic proteins Mcl-1 and Bcl-2 [121].

**APRIL in solid tumours**

APRIL mRNA expression was reported initially in various solid tumours, including thymomas, colon carcinomas and glioblastomas [11, 13, 125]. At that time, APRIL was also found to stimulate in vitro proliferation of cell lines derived from solid tumours [11, 13]. These effects were reproduced in vivo, since APRIL-transfected NIH3T3 murine fibroblasts were shown to grow more rapidly in immunodeficient mice [11, 13]. In addition, in vivo growth of carcinomas expressing endogenous APRIL could be blocked by a soluble APRIL receptor [23].

Roth et al. found APRIL expression in several malignant glioblastoma cell lines, although APRIL appeared not to affect tumour cell proliferation [125]. A later study by Deshayes et al. reported expression of APRIL as well as BCMA and TACI in a panel
of eight malignant glioblastomas and one colon carcinoma cell line [126]. Their RT-PCR and northern blot analyses did not reveal BAFF expression and found BAFF-R transcript in only one of these cell lines. APRIL, but not BAFF, bound strongly to the cell surface and enhanced cell proliferation in these experiments. A similar effect was observed in astrocytes from healthy donors, and this effect could be abrogated by addition of BCMA-Fc.

Mhawech-Fauceglia and colleagues used in situ immunohistochemistry to examine APRIL expression in a set of solid tumour cells from cervix, colon, and hepatocellular carcinomas and melanomas, as well as 2159 tumour microarray tissue sections [127]. To characterise APRIL-producing cells, they used specific antibodies to distinguish the secreted form of APRIL from the intracellular domain of APRIL, which is retained in the Golgi after cleavage. APRIL was detected in the epithelium of melanomas and basal cell carcinomas and produced mainly by the tumour-infiltrating neutrophils and by normal epithelial cells proximal to the tumour. In contrast, APRIL-producing neutrophils were not detected in healthy donor samples, suggesting that the tumour recruits APRIL-secreting cells. The proteoglycan syndecan-1 on the plasma membrane of skin epithelial tumour cells was suggested to bind secreted APRIL. Analysis of APRIL expression in the 2159 tumour samples showed that approximately 66% produced APRIL, and that stromal cells were the major source of this cytokine [127].

The abundant expression of APRIL in human solid tumours and the preliminary functional studies in vitro raise the question as to whether APRIL promotes formation and/or maintenance of solid tumours. Studies are under way in a number of laboratories to address this issue.

**Therapeutic approaches**

APRIL- and BAFF-mediated effects on cancer progression have led several researchers to postulate that these factors could be targeted as a therapeutic strategy in cancer, combined with other treatments already in use. Several clinical trials are underway using neutralising APRIL and/or BAFF molecules in patients with autoimmune diseases (review in [8]). In cancer, a phase I/II clinical trial using a TACI-Fc fusion protein is currently ongoing in patients with refractory or relapsed MM [128]. TACI-Ig, co-developed by ZymoGenetics and Serono [64] is also in a phase I trial in MM, NHL and CLL patients. Results from these ongoing clinical trials will provide us precious information about the efficacy of targeting APRIL/BAFF in cancer.

Another approach to generate APRIL/BAFF-specific antagonists is based on computational analysis of the 3-D complex structure. Detailed crystal structural information as well as reliable homology models are now available for TNF-type ligands and their re-
ceptors [12, 31] and allow prediction of the crucial amino acids in the BCMA and TACI ligand-binding domains. This approach has been used to develop a BCMA-Ig mutant form that exclusively binds APRIL [29], as well as a BAFF-antagonist peptide [129]. The therapeutic potential of this antagonist is so far unknown.

Concluding remarks

From the data presented in this review it is clear that APRIL plays an important role in both immunity and cancer. Efforts are under way to determine the therapeutic potential of inhibiting APRIL in cancer patients. A better understanding of the biological role of APRIL is however necessary to improve the therapeutic use of APRIL and is likely to uncover new roles for this pleiotropic molecule.

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