The role of APRIL in immunity and tumorigenicity
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INTRODUCTION

A PRoliferation Inducing Ligand (APRIL) is a relatively new member of the TNF family and is implicated in various physiological and pathophysiological processes. Due to its relative novelty, the number of implications is still increasing. Likewise, the possibilities to exploit APRIL for therapeutic advantage are expanding. However, for safe and effective use of this therapeutic potential, a better understanding of APRIL’s biological action is indispensable. The present thesis focused on the role of APRIL in immunity and tumorigenicity. We demonstrated major implications of APRIL in thymus independent (TI) B cell responses (chapter 2), which in a physiological setting presumably work via dendritic cell-B cell interactions (chapter 3) and which involve crosslinking via heparan sulfate proteoglycans (HSPGs) (chapter 4). In contrast, the involvement of APRIL in influenza A infection and T cell responses was rather limited. Furthermore, we showed a clear effect of APRIL on erythroleukaemia formation in vivo (chapter 5-6) and on top of this we provided evidence for an important role of APRIL in murine B1 B cell malignancies and human B cell chronic lymphocytic leukaemia (B-CLL) (chapter 7).

APRIL IN TI B CELL RESPONSES

Recent advances clearly point to a physiological role for APRIL as potent inducer as well as survival factor for class switched plasma B cells. Ig class switch recombination (CSR) occurs in the classical germinal center reaction by cognate interaction between major histocompatibility complex (MHC) class II on B cells and the T cell receptor (TCR) on CD4+ T cells and subsequent CD40L-CD40 interaction between these two cell types [1]. Ig CSR can however also occur in a CD4+ T cell independent manner. This is for instance the case for IgA production in response to commensal bacteria in the gut, but also during certain infections [2-4]. IgA is mainly involved in preventing bacterial/viral attachment and neutralising bacteria and viruses at mucosal epithelia [5]. Initially, the mechanism via which TI CSR occurs was found to be dependent on the production of APRIL and BAFF by dendritic cells (DC) in vitro [6]. Not much later, these findings were extended with in vivo observations on the role of DC derived APRIL/BAFF in TI marginal zone B cell responses [7]. Subsequently, the role of APRIL in TI CSR was confirmed in APRIL deficient mice, which exhibited impaired IgA responses towards mucosal TI antigens [8] and TACI on the surface of B cells was shown to be the receptor involved [9]. Additional in vivo support for the role of APRIL in TI B cell responses came from the observation that tumor necrosis factor/inducible nitric oxide synthase (TNF/iNOS) producing DC in the mucosa-associated lymphoid tissue (MALT) are an important source of APRIL/BAFF in TI IgA production [10]. We could confirm the role of APRIL in TI CSR.
and plasma cell survival in APRIL transgenic mice (chapter 2) and showed that APRIL production by human monocyte derived DC (moDC) is tightly regulated by specific viral Toll-like receptor (TLR) ligands and dependent on protein kinase receptor (PKR) [11] (chapter 3). As previously shown by Litinskiy et al., interferon alpha induced the expression of APRIL by moDC. However, a more rapid and extensive production of APRIL was seen when moDC were stimulated with synthetic double stranded RNA (polyriboinosinic polyribocytidylic acid or poly IC) and CpG-oligonucleotides. Other TLR stimuli, such as lipopolysaccharide (LPS), failed to induce APRIL. The induction by poly IC and CpG was independent of transcription and, in the case of CpG, TLR engagement. Instead, we observed that 2-aminopurine, an inhibitor of PKR, could block the induction. We therefore propose that PKR activation during TI viral infections enhances translation of APRIL, which on its turn induces efficient (class switched) antiviral B cell responses. Besides PKR, other viral sensors are known to exist (e.g. RIG-I and MDA-5) and it would be interesting to determine their role in APRIL induction in DC. Besides DC, intestinal epithelial cells (IEC) were recently shown to produce APRIL and BAFF as well. However, these cells mainly react to bacterial products [12]. IEC and DC therefore seem to complement each other in conferring IgA-mediated protection against TI bacteria and viruses. Similar mechanisms are likely to play a role in the mucosal defense system of the airways. In agreement, primary bronchial epithelial cells were shown to produce APRIL and BAFF upon poly IC triggering, suggesting a role in antiviral responses [13]. However in chapter 5 we showed that APRIL does not regulate the IgA-mediated defense against influenza A. This is likely due to the fact that this IgA response is largely dependent on CD4+ T cell help (chapter 5) and thus APRIL-independent. We cannot exclude, though, that APRIL is involved in the antiviral defense against viruses that trigger a CD4+ T cell independent response.

**APRIL AND HSPGs**

Understanding the biology of the APRIL/BAFF system is complicated by the multiple ligands and receptors that make up the system (chapter 1). Additional complexity comes from the recent discovery of HSPGs as binding partners for APRIL [14, 15]. It is however unclear whether these structures represent the long assumed third receptor and much remains to be learnt about their function. In analogy to HSPG functioning in other biological processes [16-19], they could either play a role in concentrating APRIL at the receptor or in crosslinking individual APRIL/BCMA or APRIL/TACI complexes, or in signaling. By comparing flag-tagged APRIL and flag-tagged HSPG-mutant APRIL, which is devoid of HSPG binding sites, we found that the HSPG interaction is essential for both proliferation and IgA production of murine B cells. Importantly, crosslinking with
anti-flag antibodies could compensate for the absence of HSPG interaction, indicating that HSPG could serve as crosslinker for APRIL (chapter 4). These findings are in line with a paper showing the need for higher order oligomerisation of both APRIL and BAFF in order to signal through TACI [20], but now provide support for the idea that the HSPGs can provide a platform for crosslinking. Previously, HSPGs were also suggested to signal, as co-ligation of TACI and HSPGs with specific antibodies resulted in enhanced IgA production of B cells [21]. However, our current data do not support this notion and rather suggest that crosslinking is the sole effect of HSPGs. Whether this is mediated by HSPGs present on the surface of target cells or whether APRIL circulates in complex with HSPGs is currently unknown. It is, however, clear that increased understanding of the role of HSPGs and thereby more information on a potential third receptor will drive the field forward.

APRIL AND T CELLS

Based on in vitro findings showing APRIL expression by T cells [22] and co-stimulation of T cell activation, it has been envisaged that APRIL also plays a role in T cell biology. However, analysis of APRIL deficient or transgenic (Tg) mouse strains did not reveal a significant role so far [8, 22-26]. In agreement with this, we could not detect an effect of APRIL on T cell responses against influenza A (chapter 5), adenovirus and OVA-protein (chapter 6). Although these data suggest that APRIL does not play a significant role in T cell biology, we cannot exclude a role for APRIL in T cell responses. Closer examination of specific T cell responses in APRIL deficient animals could potentially still reveal an important function. Our observation that TACI is expressed on a subset of T cells, which mainly resides in the peritoneal cavity, hints at such a role (chapter 6). Moreover, TACI expression was found to partly coincide with expression of the transcription factor foxp3 in CD4+ T cells (GH, unpublished observation), thereby possibly implicating APRIL in regulatory T cell function. In analogy to the proposed negative regulatory function of TACI in B cells [24, 27], TACI-APRIL interactions could dampen regulatory T cell responses. In agreement, we found decreased numbers of regulatory T cells in APRIL Tg mice (GH, unpublished observations). Future experiments should thus be directed towards immune responses that are influenced by T regulatory cells. Surprisingly, we also did not observe a role for APRIL in Moloney murine leukaemia virus induced T cell lymphomagenesis, despite a suggested association between APRIL and cutaneous T cell lymphoma. In contrast, we detected an unexpected correlation between APRIL and erythroleukaemia formation induced by both Molony and Friend virus (chapter 6). Since the role of APRIL in human erythroleukaemia has not been studied to date, it would be interesting to determine the role of APRIL signaling in
preleukaemic erythroblastosis and to look for a possible association between APRIL expression in the serum of erythroleukaemia patients and disease severity.

**APRIL AND B1 B CELLS**

Our study in the APRIL Tg mouse model [28] (chapter 7) has revealed an important role of APRIL in the biology of B1 B cells, which primarily reside in the peritoneal cavity in mice. These cells accumulate in the peritoneal cavity of APRIL Tg mice due to a survival advantage and give rise to malignancies upon ageing. This process is reminiscent of human B cell chronic lymphocytic leukaemia (B-CLL) and in agreement, APRIL has been shown to act as a survival factor for B-CLL [29-32]. B1 B cells express high levels of TACI and BCMA (chapter 6) and excessive signaling through these receptors is the likely reason for their enhanced survival. Despite the fact that all APRIL Tg animals show accumulation of B1 B cells, not all of them develop malignancies. Therefore we propose that a “second hit”, e.g. in the form of an intestinal pathogen, might be necessary to give rise to full-blown disease. Manually introducing a “second hit” in the form of an infection or by knocking out important pro-apoptotic molecules could worsen the disease. In addition to APRIL’s role in B1 B cell survival in mice, we could show that human B-CLL patients have significantly increased levels of APRIL in their serum (chapter 7). Our group recently extended this study to a 95 B-CLL patients cohort in which elevated serum APRIL levels were shown to inversely correlate with survival, while BAFF was rather decreased in the sera of these patients [33]. APRIL is thought to act on cell surface BCMA and/or TACI on B-CLL after secretion by the B-CLL themselves or by so-called nurselike cells (NLC) [29, 30, 32]. Neutralising APRIL therefore represents an opportunity to treat B-CLL and currently, trials are underway using TACI-Fc, which blocks both APRIL and BAFF, to treat B-CLL patients. Importantly, similar observations have been made for other B cell malignancies [34-38]. Blocking agents may thus provide a therapeutic tool. We are therefore developing antagonistic antibodies, which specifically target APRIL in diseases in which APRIL is negatively implicated.

**CONCLUDING REMARKS**

The present thesis deals with the expanding role of APRIL in immunity and tumorigenesis. With respect to its role in immunity, we showed that DC regulate TI IgA B cell responses via virally induced APRIL production and subsequent binding of HSPGs and TACI on B-cells. Furthermore, we obtained more insight in its elusive role in T cell biology. Concerning the role of APRIL in tumorigenicity, we found that APRIL increases
virally induced erythroleukaemia formation and is involved in B cell malignancies in mice and humans. Additional roles for APRIL are likely to be uncovered in the future and will certainly help to improve and extent the therapeutic use of APRIL antagonistic approaches.
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