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Characterisation of monoclonal antibodies to the TNF and TNF receptor families

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Abstract

Tumour necrosis factor (TNF) family ligands and their corresponding receptors play important roles in the immune system and are involved in immune regulation such as lymphoid development, cell proliferation, differentiation, activation and death. Antibodies against these ligands and receptors together with Fc-fusion proteins, have been particularly useful as immunological tools in addressing the underlying involvement of these proteins in these contexts and furthermore, have given us hope in using them as potential therapeutic agents. Over last few years, there have been many additions to these ever-growing TNF family ligands and their receptors. Here, we have generated and characterised a set of monoclonal antibodies, together with mAbs from the HLDA workshop, against DcR1, DcR2, DR4, DR5, TRAIL, APRIL, BAFF, BAFF-R, BCMA, and TACI, which may be useful in phenotypic and functional studies of the role of TNF and TNF receptor family in immune function and regulation in relation to health and disease.

Keywords: DcR, Decoy receptor; DR, death receptor; TRAIL, TNF related apoptosis inducing ligand; APRIL, a proliferation inducing ligand; BAFF, B cell activating factor of the TNF family; BAFF-R, BAFF receptor; BCMA, B cell maturation antigen; TACI, transmembrane activator and CAML integrator

1. Introduction

Programmed cell death and cell proliferation are important immunological processes whereby the involvement of many members of the tumour necrosis factor (TNF) and TNF receptor (TNFR) family play crucial roles in determining these physiological outcomes. The balance of these two processes is a scientific paradox in that one TNF family member may induce cell death (also known as apoptosis) while others, and at times even the same protein, may in fact lead to proliferation, maturation or even cell survival. This fine balance of apoptosis versus proliferation/maturation/survival in the latter case is especially evident for TNFR [1] and Fas [2–4], as they can not only signal for apoptosis but also help cell survival by upregulating anti-apoptotic proteins and survival factors under certain conditions, thus having the potential to act as a molecular switch for cell death or cellular activation [5]. Both processes are tightly regulated and required for immune homeostasis, yet when the balance is tipped, this may result in the breakdown of immune tolerance and the onset of various diseases ranging from autoimmunity to cancer.

Several TNF family members, including TNF and CD40L [6–9], have already been valuable therapeutic targets for the treatment of immune-related diseases.

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Sequence-homology database searches and bioinformatics have led to the identification of several members of the TNF, of which there are now at least 19 members. For example, TNF related apoptosis inducing ligand (TRAIL) and its receptors has been of much interest with its ability to induce apoptosis in a wide range of cancer cells without causing any harm to normal cells in vitro [10,11] or in experimental animals [12,13] although its true physiological role is still unknown. Like Fas, the TRAIL receptors death receptor 4 (DR4) [14] and death receptor 5 (DR5) [15] are type I transmembrane proteins that have cytoplasmic death domains [16,17], which trimerise [18] upon ligand binding. This in turn leads to a cascade of signalling events, activation of apoptotic genes and ultimately, programmed cell death. Interestingly, the decoy receptors 1 (DcR1) [19] and decoy receptor 2 (DcR2) [20,21] that also are able to bind to TRAIL are not able to induce apoptosis as DcR1 lacks a death domain while DcR2 has a non-functional death domain being two-thirds truncated compared to a typical death domain. Their activity within a physiological setting has yet to be proven [22] but it is thought that these decoy receptors may determine the sensitivity to apoptosis by acting as decoys to sequester any soluble TRAIL [23] which may induce apoptosis, thereby protecting the cells. In contrast, a new subfamily of TNF-related ligands/receptors whose signalling pathways are similar to that of CD40L-CD40, have been recently discovered by sequence-homology database searches, which appears to be particularly important in B cell immunity. These include two ligands known as B cell activating factor of the TNF family (BAFF) [24] (also known as BlyS [25]/TALL-1 [26]/THANK [27]/zTNF-4 [28]) and a proliferation-inducing ligand (APRIL) [29] (also known as TALL-2/TRDNL-1/TNFSF-13) and at least three receptors: B cell maturation protein (BCMA) [30–32] and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) [33,34], which bind both ligands, and BAFF receptor (BAFF-R) [35], which binds only BAFF, and furthermore, a possible APRIL specific receptor. These receptors, lacking in death domains, can signal through the association of TRAF molecules [36] when their cytoplasmic domains upon trimerisisation, lead to downstream signalling and promote the survival of B cells and regulate their proliferation and differentiation. Overproduction of BAFF has been implicated in many autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis and Sjögren syndrome [37–41] together with B cell malignancies [42–46], while APRIL may act as an autocrine growth factor for certain tumour cells in light of its high expression on tumour cells compared to normal cells and its function as a proliferation inducing ligand [29,47].

Here we have characterised a panel of monoclonal antibodies against DcR1, DcR2, DR4, DR5, TRAIL, APRIL, BAFF, BAFF-R, BCMA, and TACI using flow cytometry and western blotting to submit to the HLDA workshop.

2. Material and methods

2.1. Isolation of TRAIL, DR4, DR5, DcR1, DcR2, APRIL, BAFF, BAFF-R, BCMA, TACI, and TWE-PRIL cDNA clones

Full length human APRIL, BAFF, BCMA, and TACI cDNA were isolated by PCR using reversed transcribed poly(A)+ RNA from EBV-transformed B-cells, PBMC, PHA activated PBMC, U937 cDNA as their templates and the appropriate primers (TRAIL and TRAIL-R YFP constructs were a generous gift from Dr. J. Mongkolsapaya and TWE-PRIL was a generous gift from Dr. M. Hahne). These were cloned into pcDNA3-Hydgro vector (Clonetech) and the YFP fusion constructs into eYFPN1 and eYFPC1 (Clonetech) for mammalian cell expression.

2.2. Production of hybridoma

Female BALB/c mice (8 weeks old) were immunized with 20 μg of recombinant human TRAIL, DR4, DR5, DcR1, DcR2, APRIL, BAFF, BAFF-R, BCMA or TACI fusion proteins in complete Freund's adjuvant by subcutaneous (s.c) injection. Subsequently, immunizations were carried out twice with 20 μg of the proteins in incomplete Freund's adjuvant by s.c and intra-peritoneal (i.p) injection respectively, at 3-week-intervals. Ten days after the third immunization, mice were bled from caudal vein and the anti-serum titres were determined by indirect ELISA. The immunized mice were boosted with 20 μg of antigen by i.p. injection. Three days later, splenocytes from immunized mice and SP2/0 myeloma cells which were cultured in RPMI 1640 containing 10% fetal calf serum (FCS) were fused in the presence of PEG (MW4000, Merk, Germany). The positive hybrids were selected by ELISA and subcloned four times using limiting dilution. Monoclonal antibodies were produced either from supernatants of the hybridoma culture or from ascites fluid of BALB/c mice in which hybridoma had been injected intraperitoneally. The Ig isotypes were identified using an isotype kit (Pierce, Rockford, IL).

2.3. Flow cytometry

The binding of mAbs to cell surfaced expressed TRAIL, APRIL, and BAFF or their receptors, were determined by indirect fluorescent staining and flow cytyometric analysis. Stable cell lines for TRAIL, DcR2, DR4, DR5, BAFF, BAFF-R, and BCMA were generated using 293T fibroblast cells to ensure appropriate expression while transient DNA transfection using the $CaCl_2$–HeBS system were performed if necessary. Cells