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Research report

A novel bioassay for B-cell activating factor (BAFF) based on expression of a BAFF-receptor ectodomain-tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor-2 endodomain fusion receptor in human rhabdomyosarcoma cells

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ABSTRACT

B-cell activating factor (BAFF) is a type II transmembrane glycoprotein belonging to the tumour necrosis factor ligand superfamily. Active soluble forms of BAFF are generated either by cleavage of the extracellular domain or by recombinant DNA technology. The current bioassay for measuring the activity of soluble BAFF involves stimulation of the proliferation of mouse splenic B-cells in the presence of goat anti-mouse IgMμ chain which is rather cumbersome and lengthy and yields variable results. We have therefore developed an alternative functional assay which relies on the ability of BAFF to induce an apoptotic response in human rhabdomyosarcoma cells. For this, we constructed a chimeric receptor containing the ectodomain of the MuBAFF-R – the major cell receptor for BAFF – and the endodomain of the HuTRAIL-R2 – one of the two functional receptors for TRAIL – which is known to contain a death domain and trigger apoptosis. When the chimeric receptor was expressed in the TRAIL-sensitive human rhabdomyosarcoma cell line KD4 clone 21, recombinant BAFF of either human or mouse sequence stimulated apoptosis, similar to TRAIL, in a dose-dependent manner. The transfected cell population, called FL17, expressing the MuBAFF-R/ HuTRAIL-R2 thus provided the basis of a novel functional bioassay for BAFF that is simple and relatively fast to perform. The construction of the chimeric receptor, development of the transfected cells expressing this receptor and the development of sensitive and reproducible bioassays for BAFF and anti-BAFF neutralising antibodies are described.

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Abbreviations: AMD, actinomycin D; BAFF, B-cell activating factor; BAFF-R, B-cell activating factor receptor; BCMA, B-cell maturation factor; BLyS, B-lymphocyte stimulator; cpm, counts per minute; DD, death domain; *E. coli, Escherichia coli*; ED_{50}, effective dose 50; EPO, erythropoietin; HIFBS, heat-inactivated foetal bovine serum; mAb, monoclonal antibody; IgM, immunoglobulin M; IFN, interferon; IL-, interleukin; MTS, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfonyl)-2H-tetrazolium, inner salt]; PBL, peripheral blood lymphocytes; PBMC, peripheral blood mononuclear cells; RT-PCR, reverse transcriptase-polymerase chain reaction; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; TACI, transmembrane activator and calcium modulator and cyclophilin ligand interactor; TALL-1, tumour necrosis factor (TNF) and apoptosis ligand-related leukocyte-expressed ligand-1; THANK, TNF homologue that activates apoptosis, nuclear factor-κB, and c-Jun NH_2-terminal kinase; TM, transmembrane region; TNF-α, tumour necrosis factor alpha; TNFSF, tumour necrosis factor superfamily; TNFRSF, tumour necrosis factor receptor superfamily; TNFRSF, TRAIL, tumour necrosis factor-related apoptosis-inducing ligand; TRAIL-R, tumour necrosis factor-related apoptosis-inducing ligand receptor.

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1. Introduction

BAFF also known as BLyS, TALL-1 or THANK is a member of the tumour necrosis factor superfamily (TNFSF) which stimulates B-cell proliferation and immunoglobulin synthesis in concert with other immunological factors (Moore et al., 1999; Mukhopadhyay et al., 1999; Schneider et al., 1999; Mackay et al., 2003). Human (285 amino acids) and murine BAFF (309 amino acids) are type II transmembrane glycoproteins with relatively large extracellular domains, 21 amino acid transmembrane regions, and small 46 (Hu)-/ 45 (Mu)-amino acid cytoplasmic tails and share 86% sequence identity. As with other TNFSF members, the extracellular domain of BAFF can be cleaved at the cell membrane to yield biologically active soluble homotrimeric BAFF. The BAFF protein binds to three distinct receptors, BAFF-R, TACI and BCMA. BAFF-R, a 184 amino acid type III transmembrane glycoprotein of the TNF receptor superfamily (TNFRSF), which is expressed mainly on B-cells, appears to be the principal receptor and is probably responsible for inducing proliferation and immunoglobulin synthesis (Thompson et al., 2001; Schiemann et al., 2001; Mackay et al., 2003; Day et al., 2005). Unlike most other TNFRSF members which have multiple cysteine-rich domains in their extracellular or ectodomain, BAFF-R contains only one. While human and mouse BAFF-R share only 56% sequence identity, human BAFF binds to mouse BAFF-R and stimulates, with addition of appropriate co-factors, the proliferation of mouse primary B-cells, forming the basis of a bioassay for either human or mouse BAFF (Moore et al., 1999).

TRAIL, another TNFSF member, like BAFF also occurs naturally as a membrane-bound ligand, but its ectodomain too may be cleaved to produce a soluble, active, homotrimeric molecule (Locksley et al., 2001; Bodmer et al., 2000). Soluble TRAIL induces programmed cell death or apoptosis in many tumour-derived cell lines *in vitro* but, interestingly, has no apoptotic effect in most non-transformed cells (Almasan and Ashkenazi, 2003). In humans, four TNFRSF members expressed at the cell surface bind TRAIL: TRAIL-R1 (*TNFRSF10A*), TRAIL-R2 (*TNFRSF10B*), TRAIL-R3 (*TNFRSF10C*) and TRAIL-R4 (*TNFRSF10D*) (Pan et al., 1997; Walczak et al., 1997; Ashkenazi and Dixit, 1999). TRAIL-R1 and -R2 possess an intracellular tail containing a conserved motif known as the “death domain or DD”, which allows interaction with downstream adaptor proteins to initiate apoptotic signals in tumour cells. In contrast, TRAIL-R3 and -R4 do not have DDs or any other recognisable signalling motifs and appear to act as “decoy” receptors that either inhibit or modulate TRAIL's apoptotic activity (Ashkenazi and Dixit, 1999; Ashkenazi, 2002).

Since BAFF-R does not naturally activate a ‘TRAIL-type’ apoptotic pathway, we constructed a functional chimeric receptor comprising the murine BAFF-R ectodomain and the TRAIL-R2 DD to stimulate a BAFF-induced apoptotic pathway in stably transfected cells to provide a simple, rapid and reproducible bioassay for BAFF.

2. Materials and methods

2.1. Cytokines and antibodies

Recombinant human and mouse BAFF and recombinant human TRAIL were obtained from R&D Systems Europe. The glycosylated rHuBAFF (Ala 134-Leu 285) and rMuBAFF (Ala 127-Leu 309) expressed in the NS0 mouse myeloma cell line contain the 151 and 182 amino acids respectively of the C-terminal ectodomain of BAFF. The mature proteins consist of homotrimers with monomeric subunits of approximately 22 kDa (HuBAFF) and 23.2 kDa (MuBAFF) as determined from migration in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. A non-glycosylated preparation of rHuBAFF derived from *Escherichia coli* and containing 153 amino acids of the extracellular domain was generously donated by Human Genome Sciences, Inc. (Rockville, MD, USA). The rHuTRAIL was derived from *E. coli*. It contained the extracellular domain - Val114-Gly281, 168 amino acids – of the membrane-bound TRAIL molecule, and formed biologically active homotrimers. Anti-HuBAFF mouse monoclonal antibody (MAb), anti-MuBAFF goat polyclonal antibody, anti-MuBAFF-R goat polyclonal antibody, and recombinant human BAFF-R/Fc chimera were obtained from R&D Systems Europe. Anti-HuTRAIL mouse MAb (2E5 clone) and goat anti-mouse immunoglobulin M (IgM; human absorbed) were obtained from Serotec (Oxford, UK).

2.2. Cell lines

The KYM-1D4 (KD4) human rhabdomysarcoma cell line (Meager, 1991) was cloned from the KYM-1 parental cell line (Sekiguchi et al., 1985). An adherent cloned cell line (cl21) was derived from KD4 (Meager, 1999) and used for TRAIL bioassays and for transfection with the chimeric receptor/blasticidin expression plasmid. KD4cl21 cells were cultured in DMEM plus 10% heat-inactivated foetal bovine serum (HIFBS), 2 mM L-glutamine and penicillin (50 U/ml) and streptomycin (50 μg/ml). Tissue culture flasks of cells were incubated at 37 °C in a 5% CO_2-gassed incubator and maintained by passaging every 5 days at a split ratio of 1:20. The human pre-B-cell line REH and the human B-lymphoblastoid cell line RPMI 1788 were cultured in RPMI1640 medium plus 7% HIFBS, 2 mM L-glutamine, 1 mM sodium pyruvate and penicillin (50 U/ml) and streptomycin (50 μg/ml).

2.3. Cloning strategy

In order to confer BAFF-induced biological responses into cell lines, a DNA sequence encoding a chimeric MuBAFF-R ectodomain-HuTRAIL-R2 endodomain was constructed, comprising the following elements: 5′-(ClaI)-MuBAFF-R ectodomain bases 42-251 (Gen-EMBL Acc No AF373847)-(KpnI)-HuTRAIL-R2 endomain bases 775-1467 (Gen-EmBL Acc No AF016849)-EcoRI-3′. The MuBAFF-R sequence was obtained by RT-PCR amplification from BALB/c mouse spleen mRNA using the following primer sequences: Forward—5′-GACATCGATGGCGACATGGGCGCCAGGAGACTCCGG-3′, reverse—5′-GACCCATGGGGGTCTCAGCGCGGAGCCCTCCTGA-3′. The TRAIL-R2 sequence was obtained by RT-PCR amplification from REH cell mRNA using the following primer sequences: Forward—5′-GACGGTACCCTCTCAGGCATCATCATAGGAGTCAC-3′, reverse-5′-GACCTTAAGTTAGGACATGGCAGAGTCTGCATTAC-3′. In all cases, the desired restriction sites were contained in the 5′ primer extensions. Intermediate amplified sequences were cloned using the pGEM-T Vector System

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