Developmental and Comparative Immunology (2008) **32**, 1076–1087

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/dci

The BAFF-Interacting receptors of chickens

Shalini K. Reddy[a], Tuanjun Hu[a], Rao Gudivada[a], Karen A. Staines[a], Kathryn E. Wright[a], Lucy Vickerstaff[a], Sonja Kothlow[b], Lawrence G. Hunt[a], Colin Butter[a], Bernd Kaspers[b], John R. Young[a,*]

[a]*Institute for Animal Health, Compton, Newbury, Berkshire RG20 7NN, UK*
[b]*Institut für Tierphysiologie, Veterinärstr. 13, 80539 München, Germany*

Received 17 December 2007; received in revised form 14 February 2008; accepted 16 February 2008
Available online 11 March 2008

KEYWORDS
Bursa;
B cells;
TACI;
BAFFR;
BCMA

Summary
The TNF superfamily cytokine BAFF has crucial roles in homoeostatic regulation of B cell populations in mammals. Similar effects on peripheral B cells have been reported for chicken as for mammalian BAFF. Unlike mammalian BAFF, chicken BAFF is produced by B cells, implying an autocrine loop and consequent differences in regulation of B cell homoeostasis. Understanding of these mechanisms requires investigation of BAFF-binding receptors in chickens. We identified and characterised chicken receptors BAFFR and TACI, but found that the gene encoding the third BAFF-binding receptor, BCMA, was disrupted, implying differences in mechanisms for maintenance of long-lived antibody responses. A BAFFR-Ig fusion protein expressed *in vivo* lowered B cell numbers, showing that it was functional under physiological conditions. We found changes in the ratio of BAFFR and TACI mRNAs in the bursa after hatch that may account for the altered requirements for B cell survival at this stage of development.

Introduction

Signals mediated by structurally related members of the tumour necrosis factor superfamily (TNFSF), have important roles in the homoeostasis and effector differentiation of mammalian B cells. The TNFSF ligands BAFF and APRIL share an untypical ligand–receptor-binding geometry. They and their receptors, BAFFR, TACI and BCMA, have important roles in B cell homoeostasis. The availability of BAFF may have a decisive role in regulating the numbers of mature, antigen-responsive B cells in the periphery by modulating pro- and/or anti-apoptotic pathways [1]. Both BAFF and the related molecule APRIL have been shown to be capable of inducing Ig class switching and influencing its direction [2]. The effects of BAFF are considered to be predominantly mediated through its receptor, BAFFR, a member of the tumour necrosis factor receptor superfamily (TNFRSF), which binds only to BAFF. BAFF and APRIL both bind two other TNFRSFs called TACI and BCMA [3]. Signalling through TACI may generally be inhibitory, but may also be involved in class switching to IgA [2]. The role of BCMA is less clear, but it may be important in receiving signals required for the maintenance of long-lived plasma cells [4].

*Corresponding author. Tel.: +44 1635 578411; fax: +44 1635 577263.
E-mail address: john.young@bbsrc.ac.uk (J.R. Young).

0145-305X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dci.2008.02.004

In man and mouse, the mature antigen-responsive B cell population is continuously replenished by cells that have recently undergone diversifying Ig gene rearrangements in the bone marrow. However, the majority of other studied species depend on an intense burst of diversity generation in gut-associated lymphoid tissue in early life. This process was first described, and has been most thoroughly studied, in chickens, where diversity is generated after gene rearrangement in a specialised organ, the bursa of Fabricius [5]. The bursal follicles are colonised by pre-bursal stem cells that have undergone Ig gene rearrangements [6] that produce only very limited diversity. In the bursa their Ig genes are diversified by gene conversion [7,8], and cells selected for intact Ig expression emigrate to form the mature antigen-responsive peripheral population [9]. Thus, the bursal B cell constitutes a new stage interposed between the BAFF-independent immature B cells of mammals and the BAFF-dependent mature B cells that develop from them. Chicken BAFF was identified as a major transcript in the bursa [10,11] implying an important role in this stage of development, which was confirmed by more recent studies [12]. Administration of BAFF, or blocking its action with fusion proteins increased or decreased, respectively, both bursal and peripheral B cell populations, consistent with a homoeostatic role for BAFF similar to that in mammals. Mammalian B cells generally do not produce BAFF, although expression can be induced [13–15]. However, the majority of chicken B cells not only produce BAFF, but probably produce most of the BAFF in this species, in the periphery as well as the bursa [12]. Thus in the chicken, BAFF appears to be an autocrine B cell survival factor, at least for the class of cells if not for individual cells. How this can effect homoeostatic regulation is not entirely clear, since autocrine circuits are inherently unstable as the result of positive feedback. It is likely that other regulatory factors are involved, among which might be the balanced expression of the positive and negative BAFF receptors BAFFR and TACI. Thus, given the demonstrated importance of anti-apoptotic signalling by BAFF in development and homoeostasis, regulation of expression of these receptors may provide a mechanism for regulation of B cell numbers, in the bursa and at later stages of differentiation. Because of the difference in the B cell life cycle in this species, and the potential involvement of autocrine signalling, the roles of the receptors may differ substantially from those in mice. We therefore set out to characterise the BAFF receptors in chickens. We identified transcripts from both BAFFR and TACI orthologues and showed that both encoded molecules that bound chicken BAFF. BAFFR fusion proteins were shown to be capable of blocking BAFF signalling *in vivo*. Where a BCMA gene would be expected, the chicken genome sequence carries a disrupted BCMA-related sequence, probably a pseudogene, implying the absence of a functional BCMA orthologue. BAFFR and TACI mRNA expression followed different developmental programs in the developing bursa.

Materials and methods

Materials

Two cDNA libraries were used for PCR-based cloning of chicken cDNAs. These were derived from 17-day embryonic bursa of Light Sussex chickens in pCDM8 [16] and from Line 0 chicken spleen in pcInX [17]. Fertile eggs of RPRL Line 0, Compton sub line, were obtained from the Poultry Production Unit at the Institute for Animal Health. All procedures were conducted in accordance with the UK Animals Scientific Procedures Act 1986 and local ethical review procedures. Raw chicken genome sequence (http://genome.wustl.edu/pub/organism/Other_Vertebrates/Gallus_gallus/assembly/Gallus_gallus-1.0/, [18]) and annotated assembled sequence browsers (http://www.genome.ucsc.edu, [19]; http://www.ensembl.org/Gallus_gallus/index.html, [20]) were used extensively.

Analysis of conserved genes in human and chicken genomes

The UCSC browser was used for inspection of the ordering of potentially orthologous genes in relevant regions of the chicken and human genomes. Tracks identifying known human genes and those showing similarities with genes in other species were inspected. The presence of potential orthologues in the two species was inferred wherever both genomes had identified similarities with the same sequence. Regions were considered to have conserved gene order if both genomes carried a series of potential orthologues in the same relative orientations and positions, without intervening non-orthologous genes.

Cell cultures and binding assays

CHO-K1 cells [21] were grown in Ham's F12 medium with 10% FBS, using puromycin HCl (BioMol, Exeter, UK) at 20 μg/ml for selection and at 15 μg/ml for maintenance of transfected cells. COS-7 cells were maintained as described previously [22]. Chicken embryo fibroblasts (CEF) from Line 0 embryos were kindly provided by Dr. Venugopal Nair (Institute for Animal Health, Compton, UK), and were grown in Iscove's modified Dulbecco's medium (IMDM), 5% heat inactivated FCS, 2% chicken serum (InVitrogen, Paisley, UK). All cell cultures were maintained at 37 °C in humidified 5% CO_2.

FLAG-tagged chicken BAFF (FlagBaff) was produced in the supernatant of COS cells transfected with the vector pcIpac [17] into which had been inserted cDNA sequences encoding the mouse CD8 α-signal peptide [23] followed by an isoleucine zipper trimerisation motif [24], the FLAG peptide epitope [25] and finally residues 137–288 of chicken BAFF peptide [10] (AF506010), downstream of the furin cleavage site, obtained by PCR amplification of cDNA from the bursal cDNA library. Standard DNA manipulation procedures were used and all final constructs were sequenced to confirm the intended sequences. COS cells were transfected with Lipofectamine 2000 (InVitrogen) or Effectene (Stratagene, La Jolla, CA) according to manufacturers' instructions. Fresh medium, DMEM+10% FCS, was added at 8 h and collected 2 days later (FlagBaff SN).

For detection of binding of FlagBaff to COS cells, 40 h after transfection with either pcBaffR or pcTACI, in chamber slides (Fisher Scientific, Loughborough, UK), the cells were washed once with PBS and then incubated with the FlagBaff SN for 30 min at 4 °C. The cells were then washed three times with cold PBS, 0.05% sodium azide, fixed in ice-cold