Immunobiology 217 (2012) 147–157

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.de/imbio

Mice expressing human CR1/CD35 have an enhanced humoral immune response to T-dependent antigens but fail to correct the effect of premature human CR2 expression

Isabel Y. Pappworth[a], Christine Hayes[a], Jason Dimmick[b], B. Paul Morgan[b], V. Michael Holers[c], Kevin J. Marchbank[a,*]

[a] Applied Immunobiology and Transplantation, Institute of Cellular Medicine, Newcastle University, Framlington Place, Newcastle-upon-Tyne NE2 4HH, UK
[b] Department of Medical Biochemistry and Immunology, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XX, UK
[c] Departments of Medicine and Immunology, University of Colorado, SOM, Denver, CO, USA

ARTICLE INFO

Article history:
Received 29 March 2011
Received in revised form 6 June 2011
Accepted 13 June 2011

Keywords:
Complement receptor
B cell
Humoral immune response
Anergy
Marginal zone

ABSTRACT

We have previously demonstrated that mice expressing human complement receptor type 2 (CR2/CD21) during the CD43$^+$/CD25$^-$ late pro-B cell stage of B cell development have marked changes in their subsequent B cell ontogeny. Here, we show that the humoral immune response to the T cell dependent antigen, sheep red blood cells (SRBCs) can be moderately enhanced with the addition of human CR1 (driven by the lambda promoter/enhancer transgene) to endogenous mCR1/CR2 expression on the B cell surface but that hCR1 expression alone (on the mouse CR1/2 deficient background) has no effect on the humoral immune response or general B cell development. Furthermore, expression of hCR1 had no recuperative effect on the markedly altered B cell phenotype noted with premature expression of hCR2 (either in the presence or absence of endogenous mCR1/2). We conclude that hCR1 alone cannot replace the role of CR2 in mice and that the effects of premature hCR2 expression during BCR development are not significantly altered by the addition of hCR1 at that developmental stage or beyond; thus hCR2 signaling in the mouse remains dominant over subsequent input from either hCR1 or endogenous receptors.

Introduction

Complement receptor type 1 (CR1, CD35, C3b/C4b receptor) is a highly polymorphic multifunctional glycoprotein expressed primarily on erythrocytes and leukocytes. It contains both common blood group antigens and alleles associated with increased susceptibility to malaria or protection from development of SLE (Krych-Goldberg and Atkinson 2001). CR1 binds C3b and C4b (also with lower affinity iC3b and C3dg) and can function as an immune adherence receptor as well as act as powerful inhibitor of both the classical and the alternative pathway C3- and C5-convertases. It achieves this through both decay-accelerating capacity and co-factor activity and is important in the breakdown of C3b to C3dg, which is a major ligand for complement receptor type 2 (CR2, CD21) (Holers et al. 1986). CR1 has been demonstrated to have both inhibitory effect and enhancing effects on B cell proliferation/differentiation (Hivroz et al. 1991; Jozsi et al. 2002; Tsokos et al. 1984). Further, simultaneous ligation of BCR and CR1 has been shown to be associated with reduced autoantibody levels (Iking-Konert et al. 2004; Voynova et al. 2008) and CR1 stimulation on phagocytes can lead to production of proinflammatory cytokines such as Il-1β (Bacle et al. 1990; Thieblemont et al. 1993). Thus, CR1 plays multiple roles in immune cell function and modulates the effects of complement activation at several levels.

In man (and other primates) CR1 and CR2 are two distinct genes but in mouse, CR1 and CR2 are products of alternative splicing of a single *Cr2* gene (Kurtz et al. 1990; Molina et al. 1990). Despite this fundamental difference, there remain significant similarities in function across the species. For instance, expression of CR2 is tightly regulated (Takahashi et al. 1997) both in cell type (majority expressed on B cells and follicular dendritic cells) and particularly with respect to stage in B cell development (expression restricted to transitional and mature B cells). CR2 in both mouse and man associates primarily with CD19 to form an important B cell signaling complex. Simultaneous cross-linking of BCR and CR2/CD19 by iC3d and C3dg coated antigens dramatically lowers the threshold for B

Abbreviations: CR, complement receptor; hCR1, human complement receptor 1; mCR1/2, mouse complement receptor 1 and complement receptor 2; SCR, short consensus repeat; HRPO, horse-radish peroxidase; SRBC, sheep red blood cells; BM, bone marrow; GC, germinal center; MZ, marginal zone; FO, follicular; SLE, systemic lupus erythematosus; Ig, immunoglobulin; B6 or WT, C57BL/6 mice; RT-PCR, reverse transcriptase polymerase chain reaction; IC, immune complex; FDC, follicular dendritic cells.

* Corresponding author. Tel.: +44 191 222 5998; fax: +44 191 222 8988.
E-mail address: k.j.marchbank@ncl.ac.uk (K.J. Marchbank).

0171-2985/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2011.06.001

cell activation (Dempsey et al. 1996; Mongini et al. 1997). Association of human CR1 and CR2 in signaling complexes on B cells has also been shown and might be important in modulating signals (Tuveson et al. 1991). CR1 in both mouse and man is expressed by follicular-dendritic cells (and B cells), where its role in the retention of opsonised antigens in germinal centers is probably highly important in maintenance of immunological memory (Krych-Goldberg and Atkinson 2001). The similarity in CR1 intrinsic function in both mouse and man has come to light during the development of a soluble human recombinant CR1 (sCR1) as a C inactivating therapeutic (Mulligan et al. 1992; Ramaglia et al. 2008; Weisman et al. 1990a,b). hCR1 can bind to mouse C3b and has co-factor function (against hC4b) with mouse factor I (Alexander et al. 2010; Kai et al. 1980) and crucially, sCR1 was shown to be a viable C regulatory protein in the murine system (Pemberton et al. 1993). However, there are also distinct differences between CR1 function in mouse and man. For instance, CR1 in man has a broad expression pattern, including most peripheral blood cells except platelets, natural killer cells and the majority of T lymphocytes (Fearon 1980). Furthermore, CR1 on erythrocytes, in man, acts as an immune adherence receptor that binds C3b/C4b-opsonized immune complexes (IC) and ferries them to the liver and spleen for removal, a function carried out by factor H in the murine system (Alexander et al. 2001; Alexander and Quigg 2007).

Targeted deletion of the *Cr2* gene in mice has demonstrated that mCR1/2 protein expression is important for B cell development and function. The mCR1/2 deficient mice have an expanded marginal zone B cell population (Haas et al. 2002) and a poor germinal center/memory cell/adaptive immune response to T-dependent antigens (Ahearn et al. 1996; Haas et al. 2002; Molina et al. 1996). Furthermore, mCR1/2 deficient mice, when crossed to autoimmune susceptible stains develop signs of disease much more rapidly (Wu et al. 2002). We have previously created mice expressing hCR2 using a P1 genomic DNA construct (P1.hCR2) (Marchbank et al. 2000) or hCR2 cDNA under the control of a B cell specific lambda promoter/enhancer mini-gene (lambda CR2) (Marchbank et al. 2002) and took advantage of CR1/2 deficient mice (Molina et al. 1996) to begin to investigate the individual roles of CR2 and CR1 in B cell fate. The P1 hCR2 study yielded mice with low level expression of hCR2 at the correct developmental stage, giving essentially a wild type phenotype with expression of hCR2 giving a small recovery of immune response when compared with CR1/2 deficient mice (Marchbank et al. 2000). On the other hand, the lambda CR2 transgene mice displayed marked changes compared to wild type and CR1/2 deficient animals, particularly those that expressed the highest levels of human CR2 protein (henceforth $hCR2^{hi}$) (Marchbank et al. 2002). The $hCR2^{hi}$ mice displayed marked reduction in peripheral blood B cell numbers, a reduction in IgG isotype antibodies, loss of follicular and reciprocal expansion of 'innate' B cell populations in the spleen and as well as a marked reduction in the immune response to both T dependent and T independent antigens beyond that seen in CR1/2 deficient mice. Indeed, the $hCR2^{hi}$ mice were subsequently shown to be highly resistant to collagen induced arthritis and have a degree of protection from developing systemic autoimmune disease as a result of these changes (Kulik et al. 2007; Pappworth et al. 2009; Twohig et al. 2007, 2009). All these alterations in B cell function are almost certainly a result of the premature expression of CR2 on the B cell surface during B cell development in the bone marrow (Kulik et al. 2007), which irreversibly alter the signaling potential of these B cells whether in the presence or absence of endogenous mCR1/2 expression or signaling (Birrell et al. 2005; Kulik et al. 2007; Marchbank et al. 2002).

Hence, we have developed mice expressing human complement receptor 1 (CR1*1 allotype) with the aim of establishing whether the presence of hCR1 could alter mouse B cell development and function; whether the lambda transgene has an negative impact on B cell function and whether the combination of hCR1 and hCR2 in the murine system can rectify the negative effects on humoral immune response seen in the CR1/2 knockout and the $hCR2^{hi}$ mice.

Materials and methods

Cells

Peripheral blood lymphocytes (PBL) were isolated from blood collected into 20 μl of heparin following a tail vein nick and washed once in cold PBS. Bone marrow B cells were collected by flushing mouse femurs with cold PBS. Splenocytes were isolated from whole spleens by disrupting them between two frosted glass slides in PBS buffer and transfer to 15 ml conical tubes on ice. Once large debris had settled, the supernatant was transferred to a new tube. Cells were pelleted by centrifugation and washed once with staining buffer (PBS, 1% FCS, 0.02% sodium azide). Samples were incubated with 0.5–1 ml of red blood cell (RBC) lysis buffer (0.83% NH_4Cl, 0.1% KCO_3, 0.1 mM EDTA) and incubated at RT for 1–2 min. The cells were then washed with 1 ml staining buffer. Cells were then counted and $1–3 \times 10^6$ cells/ml were used per analysis. Cells were stained as described below.

Antibodies

Purified, biotin and allophycocyanin (APC) conjugated mAb 171 (anti-hCR2, IgG_1 isotype) (Guthridge et al. 2001) and mAb 543 (anti-hCR1, ECACC); biotin mAb 7e9 (anti murine CR2, IgG) (Kinoshita et al. 1988) and IgG_1 isotype control were produced in the laboratory following standard methods. 2.4G2 (anti-mCD16/mCD32, Fc Block), phycoerythrin (PE) conjugated B-Ly-4 (anti-hCR2), anti-CD35 (anti-hCR1); anti-CD43, anti-IgG_1 and anti-CD1d; APC, fluorescein isothiocyanate (FITC) or PerCP conjugated RA3-6B2 (anti-mCD45R, B220), FITC conjugated anti-$IgG_{2a/c}$, IgG_{2b}, IgG_3, Gl-7 and anti-CD23, biotin anti-CD138 (Syndecan – 1) and anti-CD24; Streptavidin (SA)-APC and Quantibrite beads were all obtained from Pharmingen (BD, Oxford, UK). Cy5.5- conjugated anti-IgM or IgG, SA-FITC and SA-PE were obtained from Jackson Immunoresearch Laboratories (Stratech Scientific, UK). Purified goat anti-mouse IgG/IgM, alkaline phosphatase (AP) conjugated goat anti-mouse Ig isotype secondaries were obtained from Caltag-Medsystems Ltd. (Buckingham, UK).

Transgenic mice

All mice in this study are on the C57BL/6 (B6) genetic background with or without the transgenes and/or genetic deletions described below.

In order to generate the hCR1 cDNA transgene, we introduced a short multiple cloning site including Xho I and Cla I restriction sites into the unique Pme I (cDNA cloning) site of the $v\lambda_{2-4}$ vector (a kind gift from Prof. J. Hagman, Denver, USA) using standard techniques. This vector was then double digested with Xho-I and Cla-I prior to ligation with a 6.5 kb Cla I to Xho I fragment isolated by sequential digest of a CDM8 vector containing the full cDNA sequence for human CR1 (F allotype; a kind gift from Prof. J. Atkinson, St. Louis, USA). Purified vλ hCR1 DNA was then cut with Sal I to release the transgene and purified from low melt point agar gel prior to injection into FVB/n mouse ova. Pups were screened using hCR1 specific PCR, briefly, 3 μl of DNA solution underwent standard PCR using the following primer set (vλ 5′-GTGAATTAAGGCCTGGACTTCACTT-3′ and hCR1-r 5′-TTATCCCAGGTTCCATCCAC-3′; at cycles of 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 1 min). PCR reactions were carried out for 35 cycles. Genotyping was also confirmed by flow cytometric analysis of PBL.