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ScienceDirect
Molecular Immunology 45 (2008) 1126–1135
Molecular Immunology
www.elsevier.com/locate/molimm

The inhibitory CD200R is differentially expressed on human and mouse T and B lymphocytes

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Received 4 May 2007; received in revised form 13 July 2007; accepted 18 July 2007
Available online 21 August 2007

Abstract

To ensure an adequate response against pathogens and prevent unwanted self-reactivity, immune cells need to functionally express both activating and inhibitory receptors. CD200R is an inhibitory receptor mainly expressed on myeloid cells that down-modulates cellular activation both *in vivo* and *in vitro*. Although previously mainly studied as a regulator of myeloid function, we now show that CD200R is differentially expressed on human and mouse T-cell subsets. In both species, $CD4^+$ T cells express higher amounts of CD200R than $CD8^+$ T cells, and memory cells express higher amounts of CD200R than naïve or effector cells. CD200R expression is up-regulated on both $CD4^+$ and $CD8^+$ T cells after stimulation *in vitro*. Furthermore, we show CD200R expression on human and mouse B cells. In human tonsils, CD200R is differentially expressed on B cells, with high expression on memory cells and plasmablasts. Mice lacking the ligand for CD200R, $CD200^{-/-}$ mice, do not show abnormal composition of the lymphocyte compartment and have normal B cell responses to antigenic challenge. Although the functional implications remain to be elucidated, the expression of CD200R on lymphocytes suggests a much broader role for CD200R-mediated immune regulation than previously anticipated.

Keywords: Immune regulation; CD200R; Lymphocytes; Inhibitory receptors

1. Introduction

The equilibrium between inhibition and activation of the immune system is delicate. Too much inhibition may result in immune deficiency, whereas too much activation could cause damage to the host. Therefore, every immune cell expresses inhibitory receptors to balance signals from activating receptors (Ravetch and Lanier, 2000).

Most immune cells express multiple inhibitory receptors. The function of these inhibitory receptors is not redundant, as has been shown by studies using mice deficient for several B-cell inhibitory receptors (Pritchard and Smith, 2003). Despite the fact that expression levels may be low, inhibitory immune receptors play an important role in maintaining immune homeostasis since absence of inhibitory immune receptors results in increased activation of immune cells, in some cases leading to spontaneous auto-immunity (Bolland and Ravetch, 2000; Takai et al., 1996).

CD200R is an inhibitory immune receptor that binds to CD200. Both molecules are members of the Ig superfamily and contain two Ig-like domains. CD200 is widely expressed and has a short intracellular tail which does not contain signalling motifs, whereas CD200R has a longer intracellular tail that contains three tyrosines, one of which is located in an NPXY motif (Wright et al., 2000). However, CD200R does not contain the common immunoreceptor tyrosine-based inhibition motifs (ITIMs) and has been described to regulate cellular activation via recruitment of the adaptor molecules Dok-1 and Dok-2 to the tyrosines in its intracellular tail (Zhang et al., 2004; Zhang and Phillips, 2005).

CD200R has been reported to be mainly expressed on myeloid cells such as monocytes, macrophages, granulocytes and dendritic cells and is an important signalling molecule in the inhibition of myeloid responses to challenge (Wright et al., 2000, 2003). *In vitro* studies have shown that ligation of CD200R results in decreased degranulation and cytokine secretion by

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doi:10.1016/j.molimm.2007.07.013

mast cells, monocytes and macrophages (Jenmalm et al., 2006; Cherwinski et al., 2005). In addition, deletion of the CD200 gene in mice results in enhanced susceptibility to auto-immune disease and increased myeloid response to inflammation (Hoek et al., 2000). Lack of CD200R signalling in CD200$^{-/-}$ mice was found to result in enhanced Th2 switching upon induction of tolerance, implicating a role for CD200R signalling in regulating Th2 cell function (Taylor et al., 2005). Moreover, expression of CD200R on lymphocyte subsets was shown, suggesting a broader role for CD200R in the regulation of immune cells (Wright et al., 2003; Rosenblum et al., 2005). Indeed, CD3-induced proliferation and cytokine secretion by dendritic epidermal T cells can be inhibited by crosslinking of CD200R on the surface of these cells (Rosenblum et al., 2005).

Here, we study the expression of the inhibitory CD200R on different lymphocyte subsets in human and mouse peripheral blood and lymphoid organs to elucidate the potential role of CD200R expression on these cells. Most surprisingly, we report clear CD200R expression on B cells in both species. Although the functional role of CD200R expression on lymphocyte subsets remains to be elucidated, its regulated expression pattern on lymphocytes suggests a broad role of CD200R in the regulation of immune function.

2. Materials and methods

2.1. Mice

Wild type C57BL/6J and CD200$^{-/-}$ mice were bred at the Amsterdam Medical Centre animal facility. For some experiments, wild type C57BL/6J mice were obtained from Charles River (France). All animals were used as approved by the Utrecht and Amsterdam University animal ethics committees.

For antigen challenge, mice received a single dose of either 10 μg TNP–Ficoll (T-cell-independent (TI) antigen) or 25 μg TNP–KLH (T-cell-dependent (TD) antigen) in 100 μl PBS by intravenous (i.v.) injection in the lateral tail vein. Serum was collected weekly, until 4 weeks after immunization, from blood obtained via the tail vein. Anti-TNP antibody titres were assessed by an isotype-specific ELISA.

2.2. Cells

Mouse spleens and inguinal, axillary and brachial lymph nodes were strained through nylon sieves to obtain single cell suspensions, after which erythrocytes were lysed. Human peripheral blood leukocytes (PBL) were isolated from peripheral blood from healthy volunteers by density gradient centrifugation on Ficoll–Paque combined with Histopaque. Human peripheral blood mononuclear cells (PBMC) were isolated by centrifugation on Ficoll-Paque. Human tonsils were rest material from adenotomies and/or tonsillectomies. Tonsils were minced and leukocytes were washed out to obtain single cell suspensions. All cells were resuspended in RPMI 1640 medium (Gibco BRL, Life Technologies, Paisley, UK) supplemented with 10% heat-inactivated foetal calf serum (Integro, Dieren, The Netherlands), penicillin and streptomycin (Gibco).

2.3. T cell stimulation in vitro

A 24-well plate was coated with 1 μg/ml anti-CD3 (OKT3, Janssen Cilag, Tilburg, The Netherlands) and/or 5 μg/ml anti-CD28 (15E8, kindly provided by Dr. R. van Lier) in a final volume of 200 μl PBS for 2 h at 37 °C. 1.5×10^6 PBMC/ml were cultured for 4 days in the coated wells.

2.4. Flow cytometry

Antibodies against human CD3, CD4, CD8, CD25, CD27, CD45RO, CD19, CD38, IgD, CD11b, CD56 and mouse CD3, CD4, CD8, CD44, CD62L, CD25, CD69, DX5, NK1.1, B220, Gr-1, CD11b were obtained from Beckton Dickinson Biosciences (San Diego, United States). Antibodies against human IgG and IgM were obtained from DAKO (Glostrup, Denmark) and anti-human IgA antibodies were obtained from Jackson Bioscience (West Grove, United States). Antibodies against human and mouse CD200 and CD200R were obtained from Serotec (Kidlington, Oxford, UK), as was anti-mouse F4/80. Cells were incubated with antibodies on ice for 30 min. A FACSCalibur with BD CellQuest software (BD Pharmingen) was used for acquisition and analysis. For quantification of CD200R expression, MFI of isotype control stainings, although low in all cases, was subtracted from MFI values of CD200R stainings.

2.5. Mouse antibody ELISA

Flat-bottom 96-well MAXIsorp plates (Nunc, Roskilde, Denmark) were coated with 50 μl of 1 μg/ml isotype-specific anti-immunoglobulin antibodies in PBS and incubated overnight at 4 °C. Plates were washed with PBS and blocked with 3% BSA in PBS for 1 h at room temperature. Sera were added in three-fold dilutions starting at 1:150 in a total volume of 50 μl. After incubation for 2 h at room temperature, plates were washed and 50 μl biotinylated isotype-specific antibodies (0.1 μg/ml in PBS) were added. Plates were incubated for 1 h at room temperature and antibodies were detected by the addition of poly-streptavidin-HRP. Plates were developed with ABTS according to the manufacturer's protocol.

Anti-TNP antibody titres were assessed by an isotype-specific ELISA. MAXIsorp plates were coated with 50 μl of 1 μg/ml TNP–BSA in 0.1 M carbonate buffer (pH 9.7) overnight at 4 °C. Plates were blocked by incubating with 2% non-fat dry milk (NFDM) in PBS for 1 h, and washed with PBS-T. Sera were applied in a three-fold dilution series starting at 1:100 and ending at 1:24,300 and plates were incubated overnight at 4 °C. After washing plates were incubated with 1 μg/ml biotinylated Ig isotype-specific antibody in 2% NFDM, washed again and incubated with streptavidin-AP conjugate. Following a final wash, the plates were developed in 100 μl pNPP buffer (Sigma, St. Louis, United States) according to the manufacturer's protocol.

2.6. Statistical analyses

Nonparametric statistical tests were used since the data were not normally distributed. Differences between the groups were