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Peptides of CD200 Modulate LPS-Induced TNF-α Induction and Mortality *In Vivo*

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Interaction of the ubiquitously expressed molecule CD200 with its receptor(s) CD200R, expressed on cells of myeloid and lymphoid origin, delivers immunoregulatory signals that modulate inflammation in a number of diseases, including transplant rejection and arthritis. A number of isoforms of CD200R have been described in mice with distinct function. We have synthesized small (9–13 mer) peptides defining distinct regions of CD200, and asked whether different peptides would function as agonists and/or antagonists of different CD200R isoforms. The assays used to characterize these functions include a model of inflammation and tumor necrosis factor-alpha production induced following lipopolysaccharide administration *in vivo*, and mixed leukocyte cultures *in vitro*. Discrete agonist and antagonist peptides were defined for different CD200Rs, which suggests this approach may have some clinical utility.

Key Words: **tolerance; immunosuppression; inflammation; immunoglobulin superfamily.**

INTRODUCTION

Activation of T lymphocytes by MHC-peptide complexes presented on antigen-presenting cells (APCs) is modulated by ligand binding to costimulatory and regulatory counter-ligands. Among the well-defined costimulatory molecules are CD28, CD154, CTLA4, 4-1BBL, and CD134 [1–5], while molecules contributing to negative signaling to T cells include PD-1 and B7-H3 [6–8]. Further regulation of activation depends upon receptor molecules encoded by other gene families, e.g., the triggering receptors expressed by myeloid cells (TREM) family [9]. We have characterized a member of the TREM family, CD200R, and shown that five members, R1-5, exist in mice, sharing structural homology with the immunoglobulin and lectin-like superfamilies, including MHC Class I molecules [10] and sialic acids [11]. The isoforms of CD200R for both mouse and human show tissue-restricted expression [12, 13] and heterogeneity of function [13, 14]. Thus, CD200R1 is expressed on macrophages/DCs and a subpopulation of T cells, and ligation by CD200 leads to association with *Dok* [15–18], phosphorylation of ITIM-like motifs in the cytoplasmic tail, and suppression of inflammation and T cell function [9, 19]. Alternate CD200R isoforms are expressed on cells of the myeloid lineage and triggering of CD200R2/3 results in signaling via DAP-10/-12 signaling molecules [12, 15, 17, 20], producing altered myeloid cell differentiation [9, 21].

CD200 regulates inflammation in a number of tissues and model systems [18, 22–27]. A viral homologue of CD200 has also been shown to modulate both macrophage and mast cell activation following interaction with CD200R1, as well as viral immunity to human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) [20, 28]. The onset of a generalized, often lethal, inflammatory state associated with surgical sepsis now believed to follow in part from activation of pattern recognition receptors of the innate immune system, has focused attention on mechanisms which might be brought to bear to suppress non-specific inflammation per se [29]. To date, strategies designed to suppress levels of the major inflammatory cytokine, tumor necrosis factor-alpha (TNF-α), have had little impact in clinical disease, but in studies of inflammation and TNF-α production in a mouse model of arthritis we have shown that CD200 is a potent immunomodulator [22].

The N-terminal regions of both CD200 and CD200R are important in their mutual interaction [12, 30–33]. Since the functional activity of different CD200R iso-

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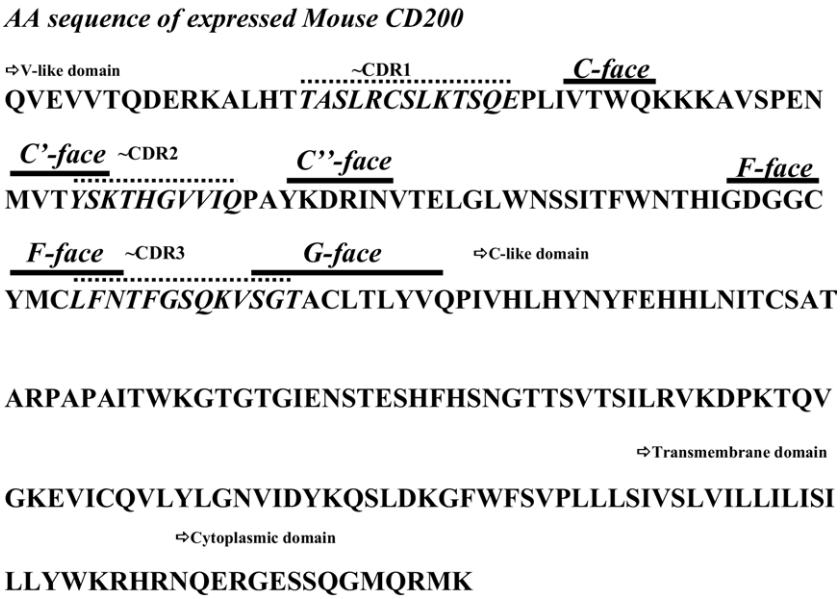

FIG. 1. Amino acid sequence for murine CD200, with the location of the important structural domains, including CDR regions and the important "faces" of the 3-D CD200 molecule, which interact with CD200R1 [32].

forms is not the same, characterization of CD200-derived N-terminal peptides, which may discriminate between CD200R1 or other CD200R isoforms in terms of agonist/antagonist activity, is of crucial importance. We have used responder cells from wild-type mice, or from mice lacking the gene encoding CD200R1 [34], as tools to characterize functional properties of CD200-derived peptides. We classified a peptide agonist (of CD200) as a molecule that produced the equivalent biological effect to full-length CD200Fc; an antagonist peptide in contrast is a competitive inhibitor of the biological effect produced by CD200Fc. We report use of *in vitro* models of allo-activated cells (induction of CTL/cytokines in mixed leukocyte cultures [MLCs]), and *in vivo* studies of mice receiving lethal intraperitoneal injections of the non-specific inflammatory stimulus, lipopolysaccharide (LPS), to define the sequence of CD200 peptides agonists/antagonists for CD200R1 and alternate CD200R isoforms.

MATERIALS AND METHODS

Mice

Female C3H/HEJ, C57BL/6, and BALB/c mice were purchased from the Jackson Laboratories, Bar Harbor, ME. A CD200R1 KO mouse (BL/6 background) in which the genetic region encoding the extracellular domains of CD200R1 were deleted was generated and characterized as described elsewhere [34]. All mice were housed 5/cage and allowed food and water ad libitum. Mice were used at 8 to 12 wk of age.

Purification of CD200Fc

The mouse fusion protein (CD200Fc) encoding the extracellular domains of CD200 linked genetically to a mouse IgG2aFC region was cloned into CHO cells using a PIRES neo vector (Clonetech, Palo Alto, CA) and an Fc construct from mouse IgG2a, obtained from Dr. T. Strom [33, 35]. The molecular mass of the recombinant product was within the range (~66 kDa) of that anticipated from the cell-surface expressed glycosylated molecule. A rat monoclonal antibody to mouse CD200Fc, 10A5, was prepared and characterized as described elsewhere [36].

Peptide Synthesis

Nine to 15-mer peptides corresponding to regions encompassing domains of CD200 believed important in interaction with CD200R, as well as the CDR1-3 regions of mouse CD200 [32], were synthesized by the Hospital for Sick Children's protein sequence facility (University of Toronto, ON). All peptides were stored at a concentration of 10 mg/mL. Figure 1 and Table 1 show a schematic illustrating the sequence and location of the CD200 peptides used.

Preparation of Cells, Cytotoxicity, and Cytokine Assays

Single cell suspensions from different tissues of C57BL/6 or CD200R1KO mice were prepared aseptically by incubation of teased

TABLE 1

Primary and Secondary Sequence Identification of CD200 Peptides

Peptide sequence	Identification no.	Secondary sequence region
TASLRCSLKTSQE	#4004	CDR1⇨ FR2
RCSLKTSQE	#6061	CDR1⇨ FR2
SPENMVTYSKT	#4005	FR2⇨ CDR2
ENMVTYSKT	#6060	FR2⇨ CDR2 and C'-face
TYSKTHGVVTQ	#4012	CDR2⇨ FR3
YKDRINVTE	#6062	C"-face
TELGLWNSSIT	#8000	Control peptide
NTIGDGGCY	#6059	F-face
LFNTFGSQKVSGT	#4013	CDR3
SQKVSGTACLTLY	#4006	CDR3⇨