CD38 and CD157: Biological Observations to Clinical Therapeutic Targets

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The application of molecular knowledge for developing new medical technologies is the goal of molecular medicine. Success in this area is highly dependent on the interaction of investigators from fields as diverse as biochemistry, cell biology, immunology, physiology, epidemiology, and physics, with an eye toward applying their insights and discoveries to improving human health. Such interdisciplinary approaches rarely find the common ground and language necessary to achieve this goal. Recently, a meeting of researchers studying the ectoenzymes CD38 and CD157 brought together insights into the regulation of calcium signaling, the metabolism of pyridine nucleotides by CD38 and CD157, and subsequent effects on immune function. Together, these discoveries were being applied to the development of novel therapeutics and diagnostics for myeloma and chronic lymphocytic leukemia. This issue of *Molecular Medicine*, featuring several short reviews based on a conference held in Turin, Italy, 10–12 June 2006, showcases the current state of this field and highlights some recent progress in molecular medicine.
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CD38 AND CD157: NEW TARGETS IN MOLECULAR MEDICINE

The function of the mammalian immune system is to detect and destroy foreign antigens from viruses, bacteria, and other exogenous sources, as well as dysfunctional or aberrant endogenous antigens, such as cancer cells. In this capacity, cells of the immune system can have highly lethal effects on their targets, a characteristic that allows rapid and efficient clearance of undesired cells, microorganisms, and antigens. Left uncontrolled, however, these lethal activities can have adverse effects on normal cells and tissues, causing pathologies ranging from rheumatoid arthritis, inflammatory bowel disease, and septic shock to lupus, myeloma, and leukemia.

To maintain the proper balance between healthful and harmful immune responses, the cells of the immune system—including macrophages, dendritic cells, neutrophils, T cells, and B cells—rely on complex direct and indirect interactions that are mediated in part through cell-surface molecules, including some known as cellular differentiation (CD) markers. More than 500 leukocyte surface markers, many of them named with the CD convention managed by the Human Cell Differentiation Molecules (HCDM; www.hcdm.org) organization, have been characterized to date. These surface proteins serve as the interface between the immune cells and their environment and occupy a central role in transducing intracellular signals that regulate cellular activities. Among these cell-surface proteins are two members of a family of cyclases that metabolize nicotinic adenine dinucleotide (NAD^+) into nicotinic acid adenine dinucleotide phosphate (NAADP) and cyclic adenosine dinucleotide phosphate-ribose (cADPR) and are potent modulators of the immune system (1,2). These 2 ectoenzymes, known as CD38 and CD157, have been the focus of much research into the biology of the immune system. As presented at the Torino CD38 Meeting, the culmination of these efforts is the identification of these ectoenzymes as potential new therapeutic targets or diagnostic markers for myeloma and leukemia.

STRUCTURE AND FUNCTION OF CD38

CD38 and CD157 are highly conserved, arising from a gene duplication event, and have been found in organisms ranging from sea slugs to mammals (2,3). These NADase/cADPR cyclases act on the linear substrate NAD^+ to form the cyclic compounds $NAADP^+$ and cADPR, which play important roles in calcium signaling and immune function. In addition, crosslinking of CD38 molecules on the cell surface, via either the counter-receptor CD31 or agonist antibodies that bind CD38, activates intracellular signaling cascades independently of enzymatic function, leading to B cell proliferation

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and differentiation and affecting neutrophil trafficking and chemotaxis (3). CD38 is a type II glycosylated protein with a single transmembrane domain near its N-terminus (Figure 1). It is approximately 25% identical to CD157, which is a GPI-anchored protein. All three family members share ten cysteine residues, and thus are also highly conserved at the structural level (4). CD38 is one of the few enzymes known to convert a linear molecule, such as NAD^+, into a cyclic molecule such as cADPR. The primary enzymatic activity of CD38, however, is the hydrolysis of NAD^+ to ADP-ribose; in fact, it also hydrolyzes cADPR, its own product, into ADP-ribose, and is the only known enzyme to do so. The enzymatic activity of CD38 is highly dependent on pH, suggesting that the in vivo activity of this promiscuous enzyme may change according to its environment (4).

NAD METABOLISM AND SIGNAL TRANSDUCTION

CD38 utilizes NAD(P) as a substrate to form a range of biologically active compounds, including cADPR. Because CD38 is expressed on the surface of many cell types, this ectoenzyme is positioned as a potential regulator of extracellular NAD levels, a hypothesis that is supported by CD38 knockout studies. NAD levels are critical for ADP-ribosylation of proteins, a reversible posttranslational modification that regulates protein function. T cells derived from CD38 knockout animals have significantly elevated levels of ADP-ribosylated proteins on their surface, and CD38 deficiency accelerates autoimmune diabetes in NOD mice. NAD^+ also can activate granulocytes via CD38, and thus can induce pro-inflammatory responses. Extracellular NADP(H) induces contractions in mouse aorta, which can be blocked by pharmacological inhibitors of P2X receptors, suggesting that NADP(H) can interact directly with receptors (5).

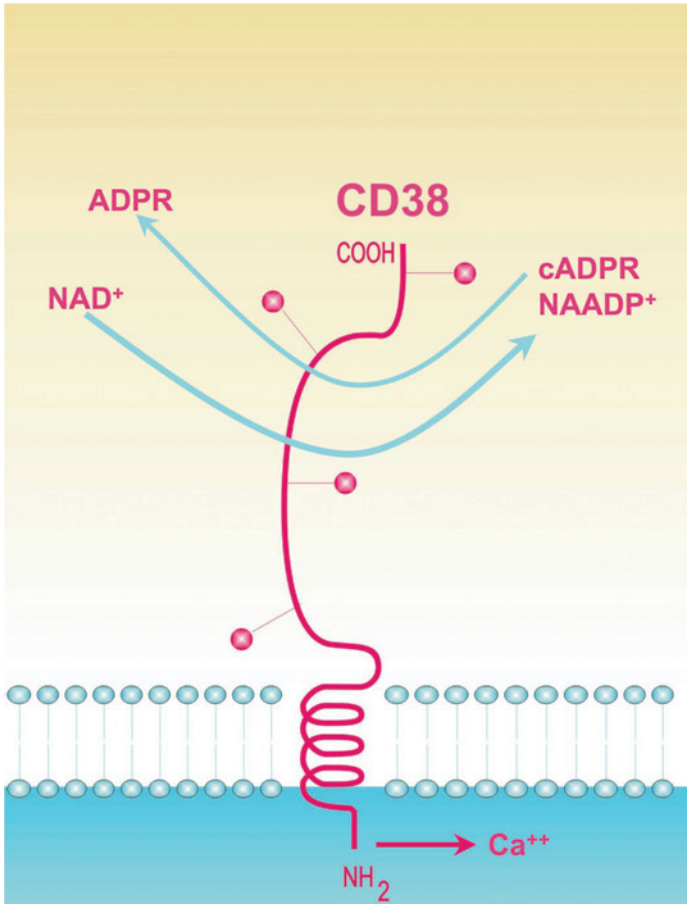

Figure 1. Schematic representation of the structure and enzymatic activity of CD38. CD38 converts the substrate NAD^+ into cyclic ADP ribose and $NAADP^+$; it can also act on its own product, cADPR, to form the linear molecule ADP-ribose. Independently of its enzymatic activity, CD38 can trigger intracellular signaling cascades that mobilize intracellular calcium stores.

NAD, CADPR, AND NAADP IN CALCIUM SIGNALING

cADPR, a product of the enzymatic activity of CD38, induces calcium mobilization from calcium stores in the endoplasmic reticulum and regulates many calcium signaling pathways in plants, invertebrates, and vertebrates. $NAADP^+$ and adenosine diphosphoribose (ADPR), other metabolites of CD38 activity, also play important roles in calcium signaling. Evidence from CD38 knockout mice indicates that CD38 is required for glucose-induced increases of cADPR, intracellular calcium, and insulin secretion in pancreatic islet cells. Enzymatic generation of cADPR by CD38 regulates intracellular calcium release and calcium influx during neutrophil chemotaxis and is required for bacterial clearance in vivo. CD38 can mediate the entry of cADPR and NAADP into cells; thus, NAD, cADPR, NAADP, and other substrates or products of CD38 can play autocrine/paracrine functions and potentially act in a hormone-like manner on cells distant from the site enzymatic activity (4,5).

CD38/CD157 AND IMMUNOREGULATION

CD38 plays important immunomodulatory roles through both enzyme-dependent and -independent mechanisms. CD38 regulates inflammatory responses by modulating the activity of

both leukocytes and nonhematopoietic cells in the inflamed tissue. Crosslinking of CD38, with either agonist antibodies or the counter-receptor CD31, induces proliferation and/or differentiation of B cells (depending on the cytokine milieu and the presence of bacterial products such as endotoxin) and induces transcription of cytokine genes, apoptosis, and phosphorylation of intracellular proteins. The intracellular signaling events triggered by CD38 appear to depend, at least in part, on machinery it shares in common with the B cell receptor pathway. In vivo, CD38 independently regulates allergen-induced airway hyperresponsiveness and inflammatory responses to allergen. Disruption of the CD38 gene appears to predispose mice to autoimmune disease, because combination deficiencies in CD38 and Fas exacerbate the development of a lupus-like disease, and CD38 disruption in the NOD/Lt mouse leads to a more rapid development of diabetes (2,3). CD38 knockout mice display other phenotypes as well, including impaired osteoclast formation and function, impaired dendritic cell trafficking, and reduced humoral responses (4).

CD38 AND B-CLL

CD38 expression levels are a negative prognostic marker in B cell lymphocytic leukemia, and its proliferative effects on B cells suggest that CD38 may be a valuable therapeutic target in several lymphoid tumors (6). Mature B cells express CD38; as they mature within germinal centers and undergo somatic hypermutation within variable regions of immunoglobulin genes, CD38 expression is lost in cells that differentiate into B memory cells, but is increased further in terminally differentiated plasma B cells. B cell chronic lymphocytic leukemia (B-CLL) patients with >30% of B cells expressing CD38 have poorer prognoses than patients with fewer B cells expressing CD38, suggesting that understanding the differentiation state of B cells in B-CLL may lead to increased understanding of the mechanisms of this disease. Recent studies demonstrate that CLL B cells express higher levels of Ki-67, indicating that, compared with nondiseased cells, more B-CLL cells have passed the G_0/G_1 phase of the cell cycle and that they have shorter chromosomal telomeres, indicating more extensive replicative history than cells from disease-free donors. CD38 may contribute to the pathogenesis of B-CLL, because CD38 is a cell-surface receptor that induces proliferation and increases survival of B cells by acting in part through intracellular signaling pathways that include ERK1/2 and ζ chain-associated protein (ZAP)-70; the latter is also a prognostic factor in B-CLL. CD38 has been established as a negative prognostic marker in B-CLL and may be an important therapeutic target (6).

CD38 AND THERAPY

The CD38 molecule is well represented on cell surfaces in many cases of a variety of lymphoid tumors, notably multiple myeloma, AIDS-associated lymphomas, and posttransplant lymphoproliferations, making it a promising target for antibody therapy. Three groups have recently described anti-CD38 antibodies with strong cytolytic potential. First, a human monoclonal anti-CD38 IgG, raised in mice transgenic for human immunoglobulin, induces potent complement and cellular cytotoxicities against both myeloma cell lines and primary myeloma and leukemic cells. This antibody also exhibits the unique property of inhibiting the cyclase activity of CD38. Second, a series of CD38-specific human antibodies, with high affinities and activities against cell lines and primary cultures of myeloma, has been selected from a unique phage-display library. Third, to enhance specificity for myeloma cells, bispecific domain antibodies targeting both CD38 and CD138 have been developed, which lack the Fc domain, and thus rely on a tumor cell toxin for cytotoxicity. The list of candidate CD38-bearing neoplasms as targets for these antibody constructs can now be expanded to include acute promyelocytic leukemia, and possibly other myeloid leukemias (7).

OVERVIEW

The Torino CD38 Meeting brought together many of the active members of the CD38 ectoenzyme family research community, who all have a common interest in understanding the biology of these novel cell-surface proteins in health and disease. Research being conducted by molecular biologists, protein chemists, immunologists, and clinicians has generated a better understanding of the biological functions of the CD38 family of ectoenzymes and their roles in health and disease. Discoveries in basic biology, combined with clinical observations and groundbreaking translational research, are setting the stage for targeted therapeutics and fulfilling the mission of molecular medicine.

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