Evolution and Function of the ADP Ribosyl Cyclase/CD38 Gene Family in Physiology and Pathology

FABIO MALAVASI, SILVIA DEAGLIO, ADA FUNARO, ENZA FERRERO, ALBERTO L. HORENSTEIN, ERIKA ORTOLAN, TIZIANA VAISITTI, AND SEMRA AYDIN

Laboratory of Immunogenetics, Department of Genetics, Biology, and Biochemistry and Centro di Ricerca in Medicina Sperimentale, University of Torino Medical School, Torino, Italy

I. Introduction 842
II. Ontogeny and Tissue Distribution 844
   A. Thymocytes and T lymphocytes 845
   B. B lymphocytes 845
   C. Myeloid lineage 845
   D. Nonimmune tissues 845
   E. CD157 846
III. CD38/CD157 Structure 847
   A. CD38 847
   B. CD157 849
IV. Receptorial Functions 849
   A. Historical perspective 849
   B. Evidence of a cell-bound ligand 850
   C. Signaling in human models 850
   D. Signaling in murine models 854
   E. CD157 856
V. Enzymatic Activities 857
   A. Historical perspective 857
   B. Enzymatic activities controlled by CD38 857
   C. Enzymatic activities controlled by CD157 860
   D. Regulation of the enzymatic activities 860
   D. Functional models 861
VI. Animal Models 862
VII. Phylogeny and Gene Organization 863
   A. From one soluble gene to a family of transmembrane proteins 863
   B. Structure and organization of CD38 and CD157 genes 864
VIII. Human Disease Models 865
   A. Diabetes 866
   B. HIV infection 867
   C. Chronic lymphocytic leukemia 868
IX. Therapeutic Applications 869
   A. CD38 as a constitutive target 870
   B. De novo induced expression of CD38 for tumor targeting 871
   C. CD38 as a target for gene therapy 872
X. Concluding Remarks 872

Malavasi F, Deaglio S, Funaro A, Ferrero E, Horenstein AL, Ortolan E, Vaisitti T, Aydin S. Evolution and Function of the ADP Ribosyl Cyclase/CD38 Gene Family in Physiology and Pathology. Physiol Rev 88: 841–886, 2008; doi:10.1152/physrev.00035.2007.—The membrane proteins CD38 and CD157 belong to an evolutionarily conserved family of enzymes that play crucial roles in human physiology. Expressed in distinct patterns in most tissues, CD38 (and CD157) cleaves NAD⁺ and NADP⁺, generating cyclic ADP ribose (cADPR), NAADP, and ADPR. These reaction products are essential for the regulation of intracellular Ca²⁺, the most ancient and universal cell signaling system. The entire family of enzymes controls complex processes, including egg fertilization, cell activation and proliferation, muscle contraction, hormone secretion, and immune responses. Over the course of evolution, the molecules have developed the ability to interact laterally and frontally with other surface proteins and have acquired...
receptor-like features. As detailed in this review, the loss of CD38 function is associated with impaired immune responses, metabolic disturbances, and behavioral modifications in mice. CD38 is a powerful disease marker for human leukemias and myelomas, is directly involved in the pathogenesis and outcome of human immunodeficiency virus infection and chronic lymphocytic leukemia, and controls insulin release and the development of diabetes. Here, the data concerning diseases are examined in view of potential clinical applications in diagnosis, prognosis, and therapy. The concluding remarks try to frame all of the currently available information within a unified working model that takes into account both the enzymatic and receptorial functions of the molecules.

I. INTRODUCTION

The invitation from the Journal to write a review on the human CD38 gene family was extended after the CD38 Meeting held in 2006 in Torino, Italy. This meeting was the latest of what started as a series of mini-conventions initially attended by a limited number of devotees and that continues to attract a growing number of participating scientists as new publications and citations fuel interest in the molecule.

CD38 was identified by E. L. Reinherz and S. F. Schlossman (Boston, MA) while conducting detailed analysis of the cell surface by means of monoclonal antibodies, as part of their pioneer search for molecules acting as T-cell receptors and transducers of signals elicited by the encounter with specific antigens. This work resulted in the definition of a vast array of molecules, including some involved in the economy of discrete subsets of cells (e.g., CD4 and CD8), some playing a more general role as metabolic regulators (CD71/TFR-1), and several others, such as CD38, serving as markers for the study of thymocytes, activated T cells, and selected tissues.

Our interest in investigating human CD38 was piqued by its distribution, which was seen to range from discrete expression during lymphocyte differentiation to an extremely limited presence during the normal physiological life of both T and B cells. The molecule was reexpressed at high density by cells undergoing activation and in selected leukemias. Furthermore, in apparent contrast to the notion that CD38 acts merely as an activation marker, terminally differentiated plasma cells and their pathological counterparts expressed the highest surface density among human cells.

To study the function of the molecule, we took the approach of raising several monoclonal antibodies specific for discrete epitopes of CD38 so as to identify their contribution to the actual role played by the molecule.

Hon Cheung Lee, a biochemist working in unrelated fields, came across CD38 while studying new intracellular messengers in the sea mollusk Aplysia. During the course of his work, he made the surprising finding of the striking similarity between human CD38 and the enzyme ADP ribosyl cyclase, purified from a mollusk predating Homo sapiens by 700 million years.

This and other related observations confirmed that ectoenzymes could no longer be considered oddities in leukocyte biology; on the contrary, it was seen that ~3.5% of the molecules expressed on the surface of human and murine cells shared such enzymatic characteristics. This finding galvanized the attention and interest of basic and clinical scientists. Nucleotide-metabolizing ectoenzymes constitute a subgroup of the larger family of ectoenzymes, consisting in a set of molecules involved in the catabolism and scavenging of extracellular nucleotides. This process results in the synthesis of compounds that play critical roles in cell homeostasis and metabolism, suggesting that the physiological role of this complex family goes beyond that of nucleotide recycling (288). Initially, it was thought that one of the characteristics shared by several, though not all, of the nucleotide-metabolizing ectoenzymes was their ability to function in environments containing only trace amounts of the substrate and where the final product would be used prevalently inside the cell. This initial view of enzymes working in an antieconomical environment was later refined, thanks in part to more sophisticated experimental approaches confirming that substrates and final products are not so topologically confined.

The Torino Lab decided to approach the challenges derived from the intensely growing interest in this area by 1) prioritizing the human side, 2) deriving clues from the ontogeny and phylogeny of CD38, and 3) trying to infer information from disease models, which are some of nature’s best experiments. As an alternative to using CD38 knockout (KO) mice, we searched for naturally occurring CD38KO individuals by analyzing more than 5,000 blood samples from newborns. To do so, we tried to replicate work previously done by our Institute with individuals lacking the complement factors C2 and C4 (polymorphous genes located inside the HLA region), whose absence is occasionally detected in laboratory analysis. As the lack of these gene products has no apparent influence on normal life, the inference is that they are somehow redundant. Failing to identify any CD38KO individuals, we concluded that the absence of CD38 is incompatible with human life. This was one of the first inconsistencies noted between the human and the mouse model, where, although the CD38 KO is characterized by selected deficiencies in innate and adaptive immune responses, the animal is nonetheless able to live and reproduce.

Research in Japan has played an important role. H. Okamoto (Miyagi, Japan) focused on diabetes, designing
an original model of insulin secretion, while the H. Hirano group (Osaka, Japan) discovered that an old member of the Boston harvest (originally termed Mo-5) was identical to their anti-BST-1, providing the basis for the then-new CD157. Thus CD38 became the prototype member of a new family, whose molecular details were later analyzed by Enza Ferrero, who also derived CD157 and CD38 by gene duplication (91).

In the 1990s, this Lab became active in an international project overseen by the CD Workshop to use unknown antibodies for defining unknown molecules. While comparing locally and externally produced anti-CD38 antibodies, Ada Funaro observed that one of our reagents was able to induce cells to activate and proliferate (107). This meant that the monoclonal antibody was endowed with agonistic features, and raised the possibility that the antibody was standing in for a soluble or cell-bound natural ligand. The key to identifying this unknown molecule was found when U. Dianzani noticed selectin-type adhesion between CD38+ cells and endothelium (77). This meant that we could raise a huge number of monoclonal antibodies specific for the surface of endothelial cells and test each of them for its ability to block adhesion between cells expressing surface CD38 and the endothelium (63). The problem was ultimately solved as the result of the efforts of a young student, Silvia Deaglio. The finding of a key monoclonal antibody (christened Moon-1 for propitiatory reasons) initiated a year of intense characterization of the nonsubstrate ligand for CD38, which was later identified as CD31 (69).

One unintended consequence of increased interest in CD38 was that, by the 1990s, researchers were constructing such increasingly complex models and theories that scientific exchange between the different groups had objectively become extremely difficult. Admittedly, these problems in communication persist: researchers working in immunology risk finding it arduous to fully comprehend the biochemical theory and possibly vice versa. It may be, however, that these problems are related more to the unique nature of the molecule itself than to the inability of different specialists to understand one another. Unraveling the secrets of a molecule that has survived relatively unchanged over a 700 million year journey in phylogeny is bound to take more than a decade; it would seem naive to expect otherwise.

Thanks to continuing research efforts in the field, new information is constantly becoming available. Crystallization of the molecule by Q. Hao (Ithaca, NY) marked the long sought-after end of one quest on the one hand, while opening up entirely new frontiers for exploration on the other (216). Especially astonishing was a recent report by H. Higashida (Kanazawa, Japan), whose group showed a link between CD38 and short-term social memory in mice, offering novel vistas on human autism (172). Far from having coming to the end of our research, we are poised on the threshold of challenging new studies and experiences (Fig. 1).

The aim of this work is to provide an overview of what has been accomplished in the field to date, while keeping interpretation to a minimum. The Torino perspective is outlined in section x, but our own experience and background have inevitably colored the entire work. This will be obvious to any reader; however, it may also represent a strength of the presentation, and not necessarily a drawback.

![Fig. 1. Key dates and findings in defining the CD38 family.](http://physrev.physiology.org.org)
In setting out this review, we have attempted to combine and reconcile the results of studies rooted in different scientific backgrounds. We try to answer questions about where the molecules are expressed, what kind of structure they have, what they do in vivo, whether they are involved in disease, and whether they may find clinical applications. It is our desire to make the subject matter interesting and accessible to the widest possible audience, without sacrificing scientific detail or accuracy.

Section II focuses on tissue distribution and highlights changes induced by age and drug treatment. The section is split into subsections that report on the expression of the molecules in the cells and tissues where originally identified; the greater emphasis on CD38 than on CD157 is a result of the larger body of data of the former. Two tables provide easy reference to the data.

Section III focuses on analysis of the structure of CD38 and CD157; once again, the evidence concerning CD38 is more abundant than that on CD157. The section includes early data coming from immunoprecipitation of surface-labeled cells and more recent results coming from crystallography.

Section IV presents evidence of the molecule’s functional role as a receptor. Different subsections offer details concerning the existence of a ligand in humans and the ability to transduce signals in human and murine models. This area is the most controversial in terms of differences between humans and rodents. A separate subsection is dedicated to CD157, the Cinderella of the family.

Section V offers a brief summary of the vast literature on these molecules as enzymes and on the relevance of their products in physiology and pathology. This is a crucial component of the review 1) because of the very peculiarity of the enzymatic functions themselves, and 2) because it points to a clear link between the molecular structures, pleiotropic functions, and products of key relevance for the life of a cell. A table is dedicated to the regulation of the enzymatic activities by endogenous and exogenous agents, an extremely important consideration from the perspective of clinical transference. A separate subsection deals with the functional models described to date.

Section VI reviews the results obtained using murine models with inactivated CD38 and CD157 genes. The data obtained have led to distinct progress in defining the roles played by CD38 and, to a lesser extent, by CD157.

Section VII analyzes the phylogeny and gene organization of CD38 and CD157. Both the canonical and noncanonical aspects of the genes are presented, including the presence of a genetic polymorphism for CD38. Phylogeny is viewed as a means of tracing the history of the molecules and as a source of clues as to their functions during the distinct steps of evolution.

Section VIII summarizes the evidence on the role(s) played by CD38 in diseases. The choice of diabetes, human immunodeficiency virus (HIV) infection, and chronic lymphocytic leukemia (CLL) reflects again the historical perspective and the availability of data from independent groups. Admittedly, it is an arbitrary selection, but it was made because they represent models where CD38 is used for its ability to produce cyclic ADP ribose (cADPR) (i.e., diabetes), for a virus as a private surface receptor (i.e., HIV infection), and for tumor cells as a noninnocent bystander, leading to the acquisition of increased survival potential and hence to a poor clinical prognosis (i.e., CLL).

Section IX reviews the limited number of studies and unpublished experience on attempts to use CD38 in clinical applications. This includes conventional studies where CD38 is used as a constitutive or inducible target, to which antibodies are addressed as vectors for cytotoxic drugs or for purging protocols. An even more innovative approach is the use of CD38 to target tumors to be bound by modified oncolytic viruses.

Section X includes the concluding remarks, intended as a playground where to propose and discuss explanations for the several aspects that still need to be framed in a scientific and plausible model. A space is dedicated to the description of the CD38 gene family represents a bridge between the innate and adaptive immune systems. This is a way to provoke real scientific approaches, discussions, and contributions to answer the still open questions, to attract attention from a wider audience of scientists, and to convince the medical community that CD38 is not (only) a cell activation marker.

II. ONTOGENY AND TISSUE DISTRIBUTION

The expression of CD38 was first observed on thymocytes and T lymphocytes (23). As with many other leukocyte surface molecules, this early finding biased the direction of later research, which focused primarily on CD38 present in lymphoid tissues. Further studies led to a revised notion of the molecule’s distribution, and CD38 expression is now considered virtually ubiquitous, at least in the immune system. However, the underlying mechanism of action of the molecule’s expression in different cell lineages is still not entirely clear, and this is reflected in the sometimes contrasting reports in the literature.

While measuring the surface expression of CD38, several caveats apply. First, the expression of CD38 modifies significantly with age. The expression of CD38 measured in cord blood is extremely high in T cells (in 80–100% of both CD4+ and CD8+ T cell subsets) (24). The majority of these cells remain positive up until 2 years of age and decrease thereafter (237). Cord blood B cells also express CD38 at high levels, at variance with the very low numbers of CD38+ B cells seen in adults. Second, the range of CD38+ T cells varies greatly in healthy individu-
als. Pregnancy is also characterized by a peculiar expression of the molecule, with a significant increase in the percentage of CD38⁺CD8⁺HLA-class II⁺ lymphocytes. This population peaks during the third trimester and decreases to normal levels 1 mo after delivery (242).

What follows is a summary of currently available data on the distribution of CD38, arbitrarily split between immune and nonimmune cells.

A. Thymocytes and T Lymphocytes

CD38 is expressed by a significant fraction of human thymocytes, mainly at the double-positive stage. It is not found on subcapsular double-negative thymocytes and is only present on some medullary single-positive thymocytes (330). Within the circulating pool, CD4⁺/CD45RA⁺ naïve T cells express CD38 (77), as does a subset of regulatory CD4⁺/CD25⁺ T cells, at least in the mouse system (201). CD38 is not expressed or is present at very low levels in memory T cells (67). Among CD8⁺ T cells, CD38 is strongly expressed during chronic infection; the significance of CD38 expression in this subset is still controversial and will be discussed in the section on diseases. CD38 is also readily expressed at high levels by the majority of peripheral blood mononuclear cells upon in vitro and in vivo activation (226). Expression of CD38 on T cells residing in the tissues mainly depends on the degree of their activation (67).

B. B Lymphocytes

B lymphocytes were originally thought to be CD38⁻. However, this assumption has been revised, mainly in light of the fact that lymphomas, leukemias, and myelomas all express the molecule, and B cells are currently the focus of many detailed studies. CD38 expression is tightly regulated during B cell ontogenesis and is present at high levels in bone marrow (BM) precursors; it is downregulated in resting normal B cells and is then reexpressed in terminally differentiated plasma cells. This seesaw behavior suggests that CD38, though not a lineage marker, is expressed at certain points during B cell development, when cell-to-cell interactions are crucial (104, 227). CD38 has also proven extremely useful in classifying subsets of functional mature B lymphocytes. Simultaneous evaluation of CD38 and IgD surface expression levels allowed the identification of five discrete cell subsets corresponding to critical developmental stages of mature B lymphocytes (274). According to this model, CD38 expression is induced upon naive B lymphocyte activation, peaks when B cells enter the germinal center, decreases during centrocyte/centroblast differentiation, and completely disappears in memory B cells. Thus CD38 expression is one of the early markers of mature naive B cell activation and is upregulated before B cells undergo somatic mutations in the IgV genes within the germinal center (51, 328).

C. Myeloid Lineage

CD38 expression has been reported in circulating monocytes (256, 359) but not in resident macrophages. It is also a novel marker of the transition of monocytes to dendritic cells which is induced by inflammatory processes. The first step towards immature DC (iDC) is accompanied by progressive loss of surface CD38 as well as of CD14, and by the simultaneous acquisition of CD11c (88). Lipopolysaccharide (LPS) induces DC to shift to a mature state. LPS-induced maturation of iDC is paralleled by rapid reexpression of CD38 by the majority of the cells, a process highly reminiscent of lymphocyte differentiation. CD40 ligation (a late T-cell-derived signal of DC maturation operating in the secondary lymphoid organs) induces a similar, although weaker, upregulation of CD38 to that of CD83. CD38 may be considered an early maturation marker induced by microbial molecules and cytokines produced during the early steps of innate immune response. Late events (i.e., those driven by CD40 ligation) are less important to the induction of CD38 (98).

As members of the myeloid lineage, circulating osteoclast and osteoblast precursors express CD38 on the surface (310). Expression is maintained during tissue differentiation of these two populations (2).

CD38 is also expressed by cells of the innate immune system, including circulating and residential natural killer (NK) cells (229) and granulocytes (99, 100).

CD38 is also abundantly expressed in the BM, where it apparently stains CD34⁺ committed and proliferating precursors (34). However, the degree of stemness of CD38⁺ cells is still being investigated. This is the main reason caution must be exercised in the therapeutic use of anti-CD38 antibodies (27).

D. Nonimmune Tissues

Functionally active forms of human CD38 were also identified in the outer membrane of red blood cells (361) and on platelets (290). It later became clear that CD38 is widely expressed outside the immune system, often as a cytoplasmic and nuclear molecule (90). Among solid tissues, the molecule is expressed by normal prostatic epithelial cells (200) and pancreatic islet cells (194, 231). CD38 expression was also detected in perikarya and dendrites of many neurons, such as the cerebellar Purkinje cells (244), in rat astrocytes (341), and in perivascular autonomic nerve terminals (315) Other CD38⁺ cells include smooth and striated muscle cells (90), renal tubules (45), retinal ganglion cells (184), and cornea (314).
TABLE 1. Distribution of CD38

<table>
<thead>
<tr>
<th>Cell Population</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphoid tissues</strong></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td></td>
</tr>
<tr>
<td>T cells (precursors, activated)</td>
<td>292</td>
</tr>
<tr>
<td>B cells (precursors, activated)</td>
<td>225, 262, 317</td>
</tr>
<tr>
<td>Myeloid cells (monocytes, macrophages, dendritic cells)</td>
<td>256</td>
</tr>
<tr>
<td>NK cells</td>
<td>102, 225</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>361</td>
</tr>
<tr>
<td>Platelets</td>
<td>290</td>
</tr>
<tr>
<td>Cord blood</td>
<td></td>
</tr>
<tr>
<td>T and B lymphocytes,monocytes</td>
<td>115, 220, 287</td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
</tr>
<tr>
<td>Precursors</td>
<td>262</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>7, 90</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
</tr>
<tr>
<td>Cortical thymocytes</td>
<td>330</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td></td>
</tr>
<tr>
<td>Germinal center B cells</td>
<td>7</td>
</tr>
<tr>
<td><strong>Nonlymphoid tissues</strong></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td></td>
</tr>
<tr>
<td>Purkinje cells, neurofibrillary tangles</td>
<td>244</td>
</tr>
<tr>
<td>Cerebral cortex (rat)</td>
<td>351</td>
</tr>
<tr>
<td>Cultured astrocytes</td>
<td>341</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>59</td>
</tr>
<tr>
<td>Eye</td>
<td></td>
</tr>
<tr>
<td>Cornea</td>
<td>314</td>
</tr>
<tr>
<td>Retinal ganglion cells</td>
<td>184</td>
</tr>
<tr>
<td>Prostate</td>
<td></td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>200</td>
</tr>
<tr>
<td>Gut</td>
<td></td>
</tr>
<tr>
<td>Intraepithelial lymphocytes</td>
<td>90</td>
</tr>
<tr>
<td>Lamina propria lymphocytes</td>
<td>67</td>
</tr>
<tr>
<td>Small intestinal lymphatic vessels (rat)</td>
<td>87</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
</tr>
<tr>
<td>β-cells</td>
<td>174, 104</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
</tr>
<tr>
<td>Sarcolemma smooth and striated muscle</td>
<td>90</td>
</tr>
<tr>
<td>Myometrial smooth muscle cells (rat)</td>
<td>43, 80</td>
</tr>
<tr>
<td>Bone</td>
<td></td>
</tr>
<tr>
<td>Osteoclasts</td>
<td>322</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Glomeruli</td>
<td>45</td>
</tr>
</tbody>
</table>

Table 1 summarizes most of the reports available to date on the distribution of CD38. Table 1 is divided into lymphoid and nonlymphoid tissues.

E. CD157

After the first reports on CD38, it took over a decade for scientists to identify the second member of the family. Ironically, a specific monoclonal antibody for the molecule had been raised in the mid 1980s by the Harvard group, the same which first described T10/CD38 (336). Known as Mo-5, it was left in a sort of limbo until the Hirano group, using an entirely different approach, independently raised a specific reagent for the molecule, which they dubbed bone marrow stromal cell antigen-1 (BST-1) (175). Thanks to characterization of the target and molecular sequencing, researchers collaborating at the VI Workshop on Differentiation Antigens recognized the molecular homology between Mo-5 and BST-1, its sequence similarity with CD38, and also their analogy of function (167). This research effort culminated in attribution of an independent cluster. Because CD157 is a newcomer of the family, data on its distribution are still scant (Table 2).

Cytofluorographic analyses indicated that human CD157 is expressed by synovial cells, vascular endothelial cells, and follicular dendritic cells (140). Moreover, CD157 is also present on dermal fibroblasts; human mast cells from lung, uterus, and foreskin; and mesothelial cells from peritoneum (116, 295, 312, 346).

The earliest description of the distribution of murine CD157 was obtained using the BP-3 monoclonal antibody (239). The BP-3 antigen was originally described on early progenitors of murine B and T lymphocytes and on fetal liver B progenitors. The expression of CD157 during ontogenesis spans pre-B cells and circulating B cells with an immature phenotype (IgM<sup>high</sup>/IgD<sup>low</sup>) typical of cells recently migrated from BM (165). Analysis of CD157 expression in the thymus indicates its presence on pre-T cells in fetal thymus (122, 123).

In the murine myeloid lineage, CD157 is expressed at low levels by relatively mature myeloid cells in the BM and at high levels by polymorphonuclear cells and stromal cells from the BM (239). Immunohistochemical stain-

TABLE 2. Distribution of CD157

<table>
<thead>
<tr>
<th>Cell Population</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphoid tissues</strong></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>239</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>336, 346</td>
</tr>
<tr>
<td>Basophils</td>
<td>336</td>
</tr>
<tr>
<td>Monocytes</td>
<td>140, 265, 336</td>
</tr>
<tr>
<td>Macrophages</td>
<td>162, 265</td>
</tr>
<tr>
<td>Plasmocytoid dendritic cells</td>
<td>140</td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
</tr>
<tr>
<td>B cell precursors</td>
<td>165, 239</td>
</tr>
<tr>
<td>Myeloid precursors</td>
<td>220, 336</td>
</tr>
<tr>
<td>Stromal cells</td>
<td>145, 175, 282</td>
</tr>
<tr>
<td>Nurse-like cells</td>
<td>312</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
</tr>
<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt;/CD4&lt;sup&gt;+&lt;/sup&gt;/CD8&lt;sup&gt;+&lt;/sup&gt; thymocytes (mouse)</td>
<td>105, 166, 308, 342</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td></td>
</tr>
<tr>
<td>Follicular dendritic cells</td>
<td>A. Sapino, personal communication</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
</tr>
<tr>
<td>Reticular cells (white pulp)</td>
<td>238</td>
</tr>
<tr>
<td><strong>Nonlymphoid tissues</strong></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Fetal B cell progenitors</td>
<td>105</td>
</tr>
<tr>
<td>Vessels</td>
<td></td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>268</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td>116, 346</td>
</tr>
<tr>
<td>Uterus</td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td>346</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td>116</td>
</tr>
<tr>
<td>Gut</td>
<td></td>
</tr>
<tr>
<td>Brush border, epithelial cells of vili</td>
<td>238</td>
</tr>
<tr>
<td>Peyer's patches</td>
<td>238</td>
</tr>
<tr>
<td>Stromal cells in cryptopatches</td>
<td>329</td>
</tr>
<tr>
<td>Isolated lymphoid follicles</td>
<td>329</td>
</tr>
<tr>
<td>Peritoneum</td>
<td></td>
</tr>
<tr>
<td>Mesothelial cells</td>
<td>295</td>
</tr>
<tr>
<td>Macrophages/peritoneal exudates</td>
<td>239</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Collecting tubules</td>
<td>238</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
</tr>
<tr>
<td>α- and β-cells</td>
<td>176</td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>Gingival fibroblasts</td>
<td>200</td>
</tr>
</tbody>
</table>
ing of murine CD157 highlighted its expression within the collecting tubules of kidney, on the brush border of intestinal epithelial cells, and on a subset of reticular cells in lymph nodes, Peyer’s patches, and splenic white pulp (238). Finally, CD157 was identified in mouse pancreatic islet cells, including α and β cells (176).

III. CD38/CD157 STRUCTURE

A. CD38

CD38 was initially reported as a single chain of 45 kDa molecule which was occasionally associated with a 12-kDa molecule, making it reminiscent of a family of HLA class I-like molecules known at that time as T6 (now CD1a) (331). With the use of different technical approaches and multiple sources of antigens, it was later shown that purified CD38 is not associated with β2-microglobulin (β2m) (7). Two-dimensional isoelectrofocusing gel electrophoresis revealed 5 spots [isoelectric point (pI) range: 6.5 to 6.9]. Endoglycosidase-H treatment reduced the mass of CD38 by ~20%, revealing a broader band centered at 36 kDa. Treatment of the CD38 molecule with Staphylococcus aureus V8 protease yielded a single dominant band of 38 kDa, which contained the A10/IB4 epitope, the agonistic monoclonal antibodies (225). The CD38 molecule was trypsin-resistant in denatured and native conditions. It appeared as a glycoprotein comprising two to four N-linked oligosaccharide chains containing sialic acid residues. However, the various CD38+ cells or cell lines did not reveal apparent biochemical differences (7).

Direct cloning (169) ruled out the possibility of homology with murine Qa2. Furthermore, the use of somatic cell genetics indicated that the CD38 gene is located on chromosome 4 (4p15), and thus outside the canonical HLA locus in chromosome 6 (181).

The finding that CD38 is an ectoenzyme influenced later studies, which were mostly based on investigation of a link between structure and localization of the catalytic site. Initially, the A. De Flora group (Genoa, Italy) demonstrated that none of the cysteine residues is essential to the enzymatic activity, but also constitutes the epitopes exploited by the T. Katada group (Tokyo, Japan) mapped the catalytic domains by expressing COOH-terminal deletion mutants of CD38 in COS-7 cells (149). Mutants with fewer than 15 amino acids deleted at the COOH terminus of the 300-amino acid wild-type molecule maintained nicotinamide adenine dinucleotide (NAD) glycohydrolase (NADase) activity, whereas those with more than 27 amino acids deleted did not. The general inference was that the COOH-terminal 273–285 sequence bears the site of enzymatic activity. Introduction of site-directed mutation of Cys-275, a conserved cysteine residue located in the 273–285 sequence, completely abolished NADase activity. A panel of selected anti-CD38 monoclonal antibodies was tested using these mutants and various CD38 fragments as targets in immunoblot analyses to define an epitope of the agonistic anti-CD38 monoclonal antibodies. The epitopes recognized by monoclonal antibodies inducing protein tyrosine phosphorylation were mapped on an identical site containing the COOH-terminal sequence of 273–285. It was thus concluded that the discrete COOH-terminal sequence not only plays a key role in the ecto-NADase activity, but also constitutes the epitopes exploited by the agonistic anti-CD38 monoclonal antibodies for transmembrane signaling (149).

The same issue was taken up by the P. Deterre group (Paris, France), which showed that the epitopes recognized by distinct monoclonal antibodies and enzymatic
sites are both sensitive to proteases and reducing agents. Binding of the active site by specific monoclonal antibodies triggers conformational changes that shield critical backbone bonds and disulfide bridges, including protection from proteolytic cleavage or reduction. This trans-conformation takes place irreversibly after incubation with substrates (e.g., NAD) in the presence of dithiothreitol. The epitope is preserved, while enzymatic activity is lost. The resulting paracatalytic inactivation is likely secondary to a covalent trapping of the intermediate of the enzymatic reaction in the active site (21).

The group of H. C. Lee (Minneapolis, MN) used site-directed mutagenesis to identify the enzymatic active site of CD38. Replacement of Glu-226 (which corresponded to the catalytic residue of the cyclase) by Asp, Asn, Gln, Leu, or Gly results in complete loss of all enzymatic activities of CD38, meaning that Glu-226 is most likely a crucial catalytic residue. Homology modeling revealed that all of these critical residues are clustered in a pocket near the center of the CD38 molecule. The results also indicated a strong structural homology between the active sites of CD38 and the Aplysia cyclase (253). Later, the same group used a similar technique to study the role of the CD38 active site in ruling NAD cyclizing and hydrolyzing activities. CD38 mutants were produced in yeast, purified, and characterized by immunoblot. The results were consistent with Glu-146 being crucial in the specific and selective control of the cyclase and hydrolase activities of CD38 (127).

More recently, the crystal structure of the human CD38 enzymatic domain complexed with cADPR and with its analog cyclic GDP-ribose and NGD at different resolutions indicated that the binding of cADPR (or cGDPR) to the active site induces significant structural rearrangements in the dipeptide Glu146-Asp147 (217). This provides direct evidence of a conformational change at the active site during catalysis. Furthermore, the same paper confirmed that Glu-226 is critical for catalysis, but also plays an important role in driving cADPR to the catalytic site through strong hydrogen bonding interactions (215).

The structure of CD38 proved difficult to establish, but was finally determined by examining a product obtained from a construct with a missing transmembrane segment and mutated glycosylation sites. The resulting extramembrane domain is fully active in terms of enzymatic functions and is crystallized as head-to-tail dimers (216). The crystal structure of the extramembrane domain, solved to 1.9 Å (59), showed that the β-structures are found mainly in the C-domain, while the helices are in the N-domain. These secondary structures of CD38 and Aplysia cyclase are thus very similar. The impossibility of cocrystallizing CD38 with substrates (which would be transformed during crystallization) was overcome by using inactive mutants in the construct (Fig. 2).

The overall structure of the CD38 molecule is “L”-shaped and can be divided into two separate domains. The NH₂-terminal domain (residues 45–118 and 144–200) is formed by a bundle of α-helices (α1, α2, α3, α5, α6) and two short β-strands (β1, β3); and the COOH-terminal domain (residues 119–143 and 201–300) consists of a four-stranded parallel β-sheet (β2, β4, β5, and β6) surrounded by two long (α8 and α9) and two short α-helices (α4 and α7). These two distinct domains are connected by a hinge region composed of three peptide chains, including residues 118–119, 143–144, and 200–201. A disulfide bond (Cys119–Cys201), which is unique in CD38, in addition to the other five pairs of disulfide bonds (Cys64–Cys82, Cys99–Cys180, Cys160–Cys173, Cys254–Cys275, and Cys287–Cys296) conserved in ADPRC family members, further stabilizes the relative conformations of the two domains by linking peptides 118–119 with 200–201. The formation of the disulfide bond by residues Cys-119 and Cys-201 confirms the previous prediction (216, 284).

The membrane proximal domain is involved in binding the HIV coat protein gp120, resulting in a modulation of viral entry into the target cell (303). This region is also

![Fig. 2. Two views of a ribbon representation of soluble human CD38 structure related by 90° rotation around a vertical axis. (Figure kindly provided by Dr. Q. Hao, Cornell University, Ithaca, NY.)](image-url)
thought to be the one interacting with CD31 (U. Dianzani, personal communication).

In addition to the existence of a membrane form, a soluble form of CD38 (sCD38) was identified in cell culture supernatant of activated T lymphocytes, in several tumor cell lines, and in vivo in normal (fetal serum and amniotic fluid) and pathological [serum and ascites from patients with multiple myeloma, and serum from patients with acquired immune deficiency syndrome (AIDS)] biological fluids (103, 208).

### B. CD157

Human CD157 is a single chain of 42–45 kDa anchored to the membrane via a glycosylphosphatidylinositol (GPI) anchor (167). Deglycosylated CD157 is 31 kDa (142).

CD157 exhibits both monomeric and dimeric forms when heterogeneously expressed in MCA102 and CHO fibroblasts. The dimers can dissociate into monomers when SDS-PAGE is performed in the presence of β-mercaptoethanol, suggesting intermolecular disulfide bond(s) in the dimers. It seems plausible that the dimerization is a result of the high-density surface expression of CD157 on the recombinant cells.

When expressed and released into culture medium as a soluble form without the COOH-terminal GPI anchor, CD157 was found to further aggregate into oligomers, which could only be dissociated under reducing conditions (213).

The crystal structures of the extracellular region of human CD157 at atomic resolution was determined in the free form and also in complexes with five substrate analogs, namely, nicotinamide, nicotinamide mononucleotide, ATP, ethenoNAD phosphate (NADP+), and ethenoNAD. The CD157 subunit is divided into two domains, termed the N domain (residues 2–68, 98–150) and the C domain (residues 69–97, 151–251), which are connected by a hinge region of three peptide chains. All 10 cysteine residues, conserved among the cyclase family, form disulfide bonds, as observed in the structure of Aplysia cyclase (352). The internal architecture of each domain is essentially identical to that of the Aplysia cyclase. Each subunit of the CD157 dimer contains a substrate binding cleft, formed by the N and C domains. This large cleft, surrounded by a4, a5, a6, and a7 helices as well as β1 sheet, is covered mainly by hydrophobic and acidic residues, and its overall dimensions are essentially the same as those of the Aplysia cyclase.

Mutational analyses of CD38 and Aplysia cyclase suggested that the cyclase activity of CD157 requires Trp-77, His-81, Ser-98, Asp-99, Asp-107, Trp-140, and Glu-178, are similar in configuration, including the side chains. These two Trp residues and the Glu residue play key roles in substrate recognition and the catalytic reactions. In each catalytic cleft of the dimeric enzyme, substrates are recognized predominantly through van der Waals interactions with two Trp residues, leading to appropriate exposure of the N-glycosidic bond of NAD near a catalytic Glu residue. This conformation of the catalytic cleft also implies cyclization between the adenine base and ribose. The three key residues are invariant among the sequences of CD157, CD38, and Aplysia cyclase, which share a common substrate recognition mode and catalytic scheme (352).

CD157 and Aplysia cyclase show a better match with each other than with CD38 (216), confirming at the structural level the results of evolutionary studies.

### IV. RECEPTORIAL FUNCTIONS

#### A. Historical Perspective

The finding that CD38 is a receptor was largely serendipitous. While performing a series of tests as part of the CD Workshop, an international collaborative study of cell surface molecules of human leukocytes, our laboratory noticed that binding of the A10/IB4 monoclonal antibody was followed by proliferation effects on preparations of human peripheral blood mononuclear cells (PBMC). This phenomenon proved to be accessory cell dependent and interleukin (IL)-2 dependent and additive with both the CD2 and CD3 activation pathways (107). Further experiments showed that the CD38 molecule is also involved in the transduction of activation and proliferation signals, which are line unrestricted (226). Analogous results were observed in a murine model by another group following a similar approach: the NIM-R5 monoclonal antibody was found to increase intracellular Ca2+ and to induce the expression of HLA class II molecules on resting B lymphocytes. Moreover, monoclonal antibody treatment proved weakly mitogenic on its own, but was strongly comitogenic when used with IL-4 (302).

The most plausible explanation for these results was that CD38 is a cell surface molecule with receptorial functions, most likely working in synergy with other receptors. This notion was substantiated by the identification of a specific ligand for CD38, expressed mainly by endothelial cells (63). Fifteen years have passed since those first attempts to shed light on the role of CD38, and, in the meantime, scores of papers have been published on the signaling properties of the molecule. The emerging picture is multi-faceted and complex. For the sake of clarity, this text has been organized as follows: 1) human and murine CD38 are treated in separate sections because of their marked differences in terms of tissue distribution
and modalities of signal transduction; and 2) each section has been subdivided by cell lineage, to make the vast amount of data now available on the signaling properties of CD38 easier to navigate.

B. Evidence of a Cell-Bound Ligand

The first clues to the existence of a nonsubstrate ligand for human CD38 emerged from experiments showing that anti-CD38 monoclonal antibodies blocked CD4+ CD45RA+ T cell adhesion to endothelial cells. Inhibition mediated by anti-CD38 monoclonal antibodies was apparent in binding assays that minimized integrin functions. This suggested that CD38 mediates weak cell binding to endothelium, effective even in dynamic conditions. Such behavior was reminiscent of that of selectins, which are adhesion molecules involved in leukocyte rolling on vascular endothelial cells and lymphocyte homing (77). We thus surmised that the endothelial cell membrane also harbors a ligand for CD38 and set out to test this hypothesis by producing a panel of murine monoclonal antibodies specific for human umbilical vein endothelial cells (HUVEC). Only a few reagents proved to consistently inhibit CD38-mediated adhesion of several cell lines to HUVEC (63). One of them, Moon-1, was shown to recognize CD31/PECAM-1, as evinced by (1) cross-inhibition assays between Moon-1 and reference anti-CD31 monoclonal antibodies; 2) sequential immunoprecipitation experiments with known anti-CD31 monoclonal antibodies, and 3) reactivity of Moon-1 with CD31 transfectedants. Furthermore, CD31 and CD38 cognate interactions were found to modulate heterotypic adhesion as well as to initiate cytoplasmic Ca2+ fluxes identical to those obtained by means of agonistic anti-CD31 monoclonal antibodies. The interplay between human CD38 and its ligand CD31 is thus crucial to the regulation of cell life and is fundamental in the migration of leukocytes (as well as of CD31 is thus crucial to the regulation of cell life and is fundamental in the migration of leukocytes (as well as of CD38 ligation by monoclonal antibodies was shown to increase [3H]thymidine incorporation (107). Subsequent studies explored the nature and (in)dependence of the signals mediated by CD38 by comparing them with what was known at the time about TCR/CD3 and CD2. As with these two molecules, ligation of CD38 induced multiple cytokine mRNA expression in cultured PBMC. What was surprising was the significant overlap between the types of mRNA molecules induced by CD38 and by TCR/CD3 activation. However, CD38 and CD3 activation differed in two main respects. IL-2 mRNA levels remained low upon CD38 ligation, while IL-1β and IL-6 mRNA steadily accumulated (14). Analogous studies were then performed on purified normal lymphocyte populations. CD38 ligation in purified peripheral blood T cells was followed by induction of discrete cytokines. Of these, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, and IL-10 mRNA expression were consistently found. Low levels of IL-2, IL-4, and IL-5 mRNA were also detected in the majority of CD38-activated T lymphocytes from all the individuals studied (13).

These anecdotal observations found support and explanation in experience gleaned at the bench. Researchers reported rapid and specific comodulation of the TCR/CD3 complex following CD38 ligation. The model hypothesized at the time was that CD38 was an accessory receptor, working in association with lineage-specific receptors. This prompted studies of lateral associations between CD38 and TCR/CD3, BCR/CD19/CR2, and CD16 in T, B, and NK cells, revealing physical proximity and functional interactions (102). It was generally assumed that TCR/CD3, CR2, and CD16 were ligand-binding structures within their respective lineages, whereas CD38 was thought to be involved in the intracellular transduction of the signals. Although this model has been partly revised, CD38 is still believed to be physically and functionally linked to supramolecular signaling complexes in the T, B, NK, and myeloid lineages (Fig 3).

C. Signaling in Human Models

1. PBMC and T lymphocytes

CD38 ligation by monoclonal antibodies was shown to increase [3H]thymidine incorporation (107). Subsequent studies explored the nature and (in)dependence of the signals mediated by CD38 by comparing them with what was known at the time about TCR/CD3 and CD2. As with these two molecules, ligation of CD38 induced multiple cytokine mRNA expression in cultured PBMC. What was surprising was the significant overlap between the types of mRNA molecules induced by CD38 and by TCR/CD3 activation. However, CD38 and CD3 activation differed in two main respects. IL-2 mRNA levels remained low upon CD38 ligation, while IL-1β and IL-6 mRNA steadily accumulated (14). Analogous studies were then performed on purified normal lymphocyte populations. CD38 ligation in purified peripheral blood T cells was followed by induction of discrete cytokines. Of these, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, and IL-10 mRNA expression were consistently found. Low levels of IL-2, IL-4, and IL-5 mRNA were also detected in the majority of CD38-activated T lymphocytes from all the individuals studied (13).

These anecdotal observations found support and explanation in experience gleaned at the bench. Researchers reported rapid and specific comodulation of the TCR/CD3 complex following CD38 ligation. The model hypothesized at the time was that CD38 was an accessory receptor, working in association with lineage-specific receptors. This prompted studies of lateral associations between CD38 and TCR/CD3, BCR/CD19/CR2, and CD16 in T, B, and NK cells, revealing physical proximity and functional interactions (102). It was generally assumed that TCR/CD3, CR2, and CD16 were ligand-binding structures within their respective lineages, whereas CD38 was thought to be involved in the intracellular transduction of the signals. Although this model has been partly revised, CD38 is still believed to be physically and functionally linked to supramolecular signaling complexes in the T, B, NK, and myeloid lineages (Fig 3).

The earliest evidence indicating a functional interrelationship between TCR/CD3 and CD38 emerged from studies using Jurkat T cell mutants. The mutants lacked a full complement of CD3 structures and the ability to signal via CD38. Reconstitution of a functional TCR/CD3 complex was accompanied by recovery of CD38 signaling. Moreover, like TCR/CD3, CD38 was able to induce apoptotic cell death of Jurkat cells, an event which was paralleled by specific upregulation of the Fas molecule and
which could be inhibited by cyclosporin A (251). A molecular explanation for these observations was found: CD38 ligation led to complete tyrosine phosphorylation of both CD3-ζ and CD3-ε polypeptide chains (365). As it turns out, however, the CD3-ζ immunoreceptor tyrosine-based activation motifs are not necessarily required for CD38 signaling in T cells. Indeed, cross-linking of CD38 or CD3 in TCR T cells that express a CD3-ζ mutant lacking the cytoplasmic domain still induces tyrosine phosphorylation of CD3-ε, ZAP-70, LAT, and Shc (364). Detailed analysis of the cytoplasmic events implemented immediately after binding of the agonistic monoclonal antibody revealed tyrosine phosphorylation of phospholipase C (PLC)-γ1, c-Cbl, ZAP-70, and Shc. CD38 signaling also induced CD69 expression and promoted Ras-dependent events, e.g., Erk2 mobility shift and increased kinase activity. CD38 ligation in JCam-1 (a Jurkat mutant lacking Lck) failed to induce substrate tyrosine phosphorylation or activation of Erk2 (366).

Our laboratory explored the interrelationship between CD38 and TCR/CD3 through comparative analysis of the events implemented by CD38 ligation in circulating versus resident T lymphocytes. The resident T cells adopted, in this case those colonizing the intestinal lamina propria (LP), proved to be relatively refractory to TCR/CD3-mediated signaling (281). Also, the results showed that, unlike peripheral blood T lymphocytes, LP T cells do not mobilize Ca<sup>2+</sup> upon CD38 ligation and that the impaired response is due to a failure to activate PLC-γ. Another difference in the two cell populations was the ratio of cytokines secreted upon CD38 engagement. These findings suggested that CD38 is active in an environment where the TCR/CD3 complex is apparently refractory to signals (67). From a teleological perspective, it seems logical that CD38 recruits the receptor most conducive to its signaling activities in each lineage and environment.

More recently, lipid rafts have been attributed key roles in the initiation of CD38-mediated signals. Indeed, lipid rafts are constitutively highly enriched in CD38, while cholesterol depletion substantially reduces CD38-mediated signaling. CD38/raft association may thus improve the signaling capabilities of the molecule through the formation of protein/lipid domains where signaling-competent molecules are recruited (364). This issue was investigated in detail in a follow-up paper by the same group which showed that most CD38 is preassembled in a subset of Brij98-resistant raft vesicles, containing high levels of Lck and of the CD3-ζ subunit, the latter directly associated with CD38. CD38 engagement induces LAT and Lck to be tyrosine phosphorylated exclusively in Brij98-resistant rafts (252). The relevance of Lck for CD38 signaling was confirmed in experiments showing a physical association between the cytoplasmic tail of CD38 and the Src homology 2 domain of Lck. These findings strongly suggested that CD38 ligation transduces activatory signals for T cells by means of the associated Lck (46).

Although CD38 was initially studied in the thymus (23), later attention shifted mainly to its presence in circulating leukocytes and leukemias. However, a recent
report addressed the signaling events mediated by CD38 in immature T cells. It showed that CD38 enhances apoptosis in thymocytes when it is cross-linked with a goat anti-mouse antiserum. It has the same effect when cross-linked with the CD31 ligand expressed by thymic epithelial cells or transfected into murine fibroblasts. Thymocyte death was also enhanced when CD38 interacted with CD31 expressed by accessory cells (330). These results confirmed the observations obtained using the Jurkat model (251).

2. B lymphocytes

Analysis of CD38 functions in human B lymphocytes originated from studies of the lymph nodes, where the molecule is expressed at high density. The M. Ferrarini group (Genoa, Italy) reported that CD38 ligation prevents apoptosis of human tonsillar germinal center (GC) B cells. This effect was specific for the IB4 monoclonal antibody and was not observed with other reagents, even when tested over a wide range of concentrations (367). The results in B lymphocytes were thus consistent with those in PBMC and T lymphocytes.

Attention was then turned to analyzing CD38 functions in the BM. By this time, the notion that CD38 was an ectoenzyme was generally well established. In contrast to results obtained using mature B lymphocytes, CD38 ligation in stroma-supported cultures of human B cell progenitors strongly inhibited B lymphopoiesis. This block in differentiation was due both to inhibition of DNA synthesis and to induction of apoptosis. Enzymatic activities were not influenced (positively or negatively) by monoclonal antibody ligation. Indeed, no changes in NAD⁺ hydrolysis or cADPR and ADPR production could be detected after CD38 ligation. Likewise, the addition of NAD⁺, ADPR, or cADPR to cultures of lymphoid progenitors failed to offset the inhibitory effects mediated by anti-CD38 monoclonal antibodies (204). This was the first of many studies to indicate that the agonistic monoclonal antibody binding has no influence on, nor is influenced by, enzymatic activities. It was thus hypothesized that the receptor and enzyme activities are two independent functions of the same molecule.

The same studies also revealed that CD19 is a major component of the CD38 signaling cascade in B cell precursors, serving as a cell surface membrane docking site for cytoplasmic kinases. Although they showed no physical link, CD38 and CD19 activated a similar set of kinases in human immature B cells (189) (Fig. 3).

CD38 ligation by the agonistic monoclonal antibody was followed by dimerization and tyrosine phosphorylation of the protein kinase syk and by increased syk kinase activity. CD38 dimerization also induced tyrosine phosphorylation of PLC-γ and of the p85 subunit of phosphatidylinositol 3-kinase (PI3K). These results are consistent with those obtained using T cell models (313). Btk was not tyrosine phosphorylated upon CD38 ligation, a peculiarity of human B cell precursors. However, btk is essential for CD38-mediated signals in murine B cell models (see below). A subsequent work of the same group specifically addressed this point and identified tyrosine phosphorylation of Tec, but not of btk, in RS4;11 cells following CD38 ligation (191). PI3K activity was shown to be essential to the process of CD38-mediated inhibition of lymphopoiesis. Furthermore, chl and PI3K were shown to be regulatory molecules whose activation suppresses cell proliferation and apoptosis in immature lymphoid cells (190).

There is strong evidence that CD38 channels an activation/proliferation signal in human mature B cells. Moreover, the CD38 pathway displays several features of autonomy from the known surface receptors. CD38 ligation in circulating human B cells was shown to induce expression of 1) CD25, 2) HLA class II, and 3) mRNA for selected cytokines. The final outcome was proliferation. Similar effects were observed on B blasts. In no instance did cross-linking of CD38 induce immunoglobulin production (105).

CD38 signaling in B lymphocytes was recently taken up again by our group, almost a decade after the seminal work of D. Campana (Memphis, TN). Interest in CD38 signaling in human B cells was sparked by reports of the molecule’s direct involvement in CLL both as a marker and as a pathogenetic agent (see relevant section). We have broached the issue once again, equipped this time with evidence from the study of T and myeloid cells that CD38 is a component of supramolecular complexes that reside mostly within lipid rafts. The results of our analysis, performed on tonsil B lymphocytes and on B cell lines, indicate that CD38-mediated signals are tightly regulated at three distinct levels. The first concerns the structural organization of CD38, which is clearly divided into monomeric and dimeric forms. The second is based on the dynamic localization of CD38 molecules in lipid microdomains within the plasma membrane. Lateral associations with other proteins, namely, with the CD19/CD81 complex, determine the third level of control. Raft localization and association with the CD19 complex are prerequisites for CD38-mediated signals in tonsillar B cells and in continuous lines, as shown by loss of CD38-mediated signals upon lipid raft disruption and/or silencing of CD19 (72).

3. Myeloid cells

Interest in the behavior of CD38 in myeloid cells stemmed from findings that the molecule is readily and consistently upregulated upon retinoic acid (RA) exposure in immature myeloid cells and acute promyelocytic leukemia blasts (83). The T. Katada group (Tokyo, Japan)
next investigated the intracellular signaling mediated by CD38 in RA-differentiated myelomonocytic HL-60 cells. The initial results revealed that c-cbl is one of the most prominently tyrosine phosphorylated substrates (197). These results suggest that c-cbl is a common substrate in the CD38 signaling pathway, regardless of cell lineage or differentiation stage. Later, it was shown that the p85 subunit of PI3K was immunoprecipitated with anti-cbl antibody only when c-cbl was tyrosine phosphorylated. PI3K activity was also observed in the immunoprecipitated fractions containing tyrosine-phosphorylated cbl (235), confirming results using T and B cell models.

The final effect of CD38 ligation in myeloid cell lines was to intensify the superoxide generation induced by chemotactic formyl-Met-Leu-Phe (fMLP) receptors. CD38 signaling alone did not trigger superoxide generation, suggesting that the CD38-induced tyrosine phosphorylation is involved in cross-talk with the chemotactic receptor/G_{beta}gamma-mediated signal transduction pathway. This cross-talk results in the enhancement of superoxide generation, probably through the activation of PI3K (339). Further investigation by the same group revealed that the profile of tyrosine phosphorylated proteins by HB-7 monoclonal antibody was identical to that induced by cross-linking of Fc_{y} receptors II (Fc_{y}RII/CD32). Furthermore, Fc_{y}RII itself was tyrosine phosphorylated in the treated cells. These results indicate that anti-CD38 monoclonal antibody-induced tyrosine phosphorylation and its associated cell response might be mediated through the Fc_{y}RII-induced signaling pathway, possibly resulting from stimulation of the cell surface human Fc_{y}RII with the murine IgG Fc domain (IgG1 subclass) of CD38-ligated monomodal antibodies (161). The possibility that the effects observed with the IB4 agonistic monoclonal antibody were mediated via FcR was ruled out by using F(ab')_{2} preparations. The divalent monospecific antibody fragment maintained all the effects mediated by the intact IgG_{2a} molecule, which is generally poorly bound by human FcRs (107). However, the observation by Inoue et al. (161) may also be read in a different light, highlighting interaction between CD38 and FcRs. Similar results have also been reported in NK cells and are discussed in the relevant section.

In addition to superoxide generation, the CD38 signaling pathway may be linked to proliferation, as observed in different cell lines. CD38 ligation caused a dose-dependent increase in the number of colony forming units (CFU) and increased cell division in BM. Furthermore, the anti-CD38 monoclonal antibody controlled proliferation of AML colony-forming cells in five out of six AML patients tested (196).

Using the same approach adopted with B cell precursors, the Campana group used stroma-supported cultures to assess the effects of CD38 ligation on myeloid differentiation. The addition of anti-CD38 T16 monoclonal antibody to the cultures induced a profound reduction of the most mature CD34^{+}/MPO^{+} cell population, which includes pro-myelocytes, myelocytes, and meta-myelocytes. The effects were strong enough to be maintained across species: CD38 ligation of 32D cells (a murine myeloid cell line transfected with human CD38) powerfully suppressed cell growth and survival (337).

Ligation of CD38 expressed by circulating monocytes inhibited superantigen-induced T lymphocyte proliferation. This effect was likely due to the implementation of an active signaling pathway, inducing tyrosine phosphorylation of several intracellular proteins (including c-cbl and the fgr and hck kinases). This would indicate that CD38 plays a role in the transduction of signal(s) involved in superantigen-induced activation of monocytes, operating in synergy with HLA class II (359). The same group recently built on these results by showing that CD38 and HLA class II are physically and functionally associated within lipid rafts of human monocytes. Furthermore, the integrity of these domains is required for HLA class II and CD38 signaling events. It was demonstrated that tetraspanin CD9 is a partner of the CD38/HLA class II complex and that HLA class II, CD38, and CD9 share a common pathway of tyrosine kinase activation in human monocytes (360) (Fig. 3).

CD38 expression on human monocytes is finely tuned in response to the proinflammatory cytokine IFN-γ. IFN-γ is a strong upmodulator of CD38, while lipopolysaccharide (LPS), tumor necrosis factor (TNF)-α, and granulocyte-macrophage colony stimulating factor (GM-CSF) had no detectable effects. CD38 upregulation is paralleled by increased ADPRC/cADPR hydrolase activities (256). The final outcome of CD38 signaling in circulating monocytes includes secretion of IL-1β, IL-6, and IL-10 cytokines (206).

More recently, attention has been turned to dendritic cells. CD38 was seen to be downmodulated during differentiation to immature monocyte-derived dendritic cells (MDDC) and expressed again upon maturation. The extent of CD38 expression is dependent on the stimulus adopted (LPS > IFN-γ > CD40 cross-linking). De novo-synthesized CD38 is enzymatically active, and its expression in mature (m) MDDC is dependent on NF-κB activity. However, CD38 is not merely a maturation marker but also mediates signaling in mMDDC, where it maintains its functions as a receptor. Activation via agonistic anti-CD38 monoclonal antibodies induces upregulation of CD83 and secretion of IL-12, whereas disruption of CD38/CD31 interactions inhibits CD38 expression, IL-12 secretion, and MDDC-induced allogeneic T cell proliferation (88).

The functional role of CD38 in MDDCs was analyzed in a follow-up paper by the Clara M. Ausiello group (Rome, Italy). They showed that CD38 signaling ensures efficient chemotaxis and transendothelial migration driven by CC chemokine ligand 21 (CCL21). Additionally,
CD38 signaling contributes to the longevity of LPS-matured MDDCs after growth factor withdrawal. A further effect is the production of IFN-γ by cocultured T lymphocytes, thus affecting Th1 polarization. From a molecular standpoint, CD38 was found to be laterally associated with the CCL21-specific CC chemokine receptor 7 and with CD83 and CD11b. It was also found to localize in membrane lipid domains, as was also observed in monocytes and T and B lymphocytes (98) (Fig. 3).

4. Lymphokine activated killer and NK cells

The role of CD38 in lymphokine activated killer (LAK) cells first came under scrutiny when it was observed that ligation with the monoclonal antibody was followed by a dramatic surge in the release of cytokines (GM-CSF, IFN-γ, TNF-α, and TNF-β). These effects were observed in both TALL-104 and LAK cells (37).

Further study by the U. Kim group (Seoul, Korea) led to the discovery of a functional loop involving IL-8 and CD38. According to this model, CD38 is stimulated by sequential activation of the IL-8 receptor, inositol trisphosphate (IP₃)-mediated Ca²⁺ rise, and cGMP/protein kinase G. It also plays an essential role in IL-8-induced migration of LAK cells (288). These observations have resulted in the finding that the nonmuscle myosin heavy chain IIA protein (MHCIIA) associates with CD38 upon IL-8 stimulation and that their association depends on PKG-mediated phosphorylation of MHCIIA. Binding studies using purified proteins revealed that the association of MHCIIA with CD38 occurred through the tyrosine kinase Lck. Moreover, all three molecules coimmunoprecipitated upon IL-8 stimulation of LAK cells. IL-8 treatment of LAK cells resulted in internalization of CD38, which colocalized with CD83 and CD11b. It was also found to localize in membrane lipid domains, as was also observed in monocytes and T and B lymphocytes (98) (Fig. 3).

5. Neutrophils

Investigation of the role and expression of CD38 in neutrophils was prompted by studies on CD38 KO mice, where CD38 was demonstrated to play a nonredundant role in neutrophil chemotaxis (271). Information concerning human cells is limited and mostly focused on CD157. However, the T. E. VanDyke group (Boston, MA) showed that IL-8 or fMLP exposure readily removed CD38 from the neutrophil surface, while mRNA levels remained stable. This effect is dependent on p38 mitogen-activated protein (MAP) kinase signaling (100).

The role of CD38 in neutrophil chemotaxis relates to the enzymatic activities leading up to cADPR production. The Frances E. Lund group (Saranac Lake, NY) found that a cADPR antagonist and a CD38 substrate analog inhibited chemotaxis of human phagocytic cells towards a number of fMLP-like-specific ligands. Moreover, the cADPR antagonist blocks chemotaxis of human monocytes to CXCR4, CCR1, and CCR5 ligands. According to this model, cADPR is responsible for increasing intracellular free Ca²⁺ levels following chemokine binding. Emerging evidence also attributes a crucial role to ADPR (273).

D. Signaling in Murine Models

The signaling properties of murine CD38 have been studied mostly in B cell models. This is a natural reflection of the mouse system environment, where B cells score the highest levels of CD38, and T and NK cells show only marginal expression. More recently, the focus has shifted to neutrophils and dendritic cells, as a consequence of the phenotype of CD38 KO mice.

1. B lymphocytes

The initial observation was that the NIM-R5 monoclonal antibody, a rat anti-murine CD38, increased intracellular Ca²⁺ due to influx from the extracellular milieu via Ca²⁺ channels. It also induced resting B lymphocytes to increase expression of HLA class II molecules and the cells for “spreading” in the presence of immobilized anti-HLA class II antibody. Furthermore, NIM-R5 monoclonal antibody induced B cells activated in vitro to proliferate and be rescued from apoptosis (302).

Another independent study on the role of CD38 in resting B lymphocytes found that CD38 ligation resulted in multiple protein tyrosine kinase activities, thus attributing to CD38 a potentially central role in the transduction of signals leading to B cell activation (187).
However, B lymphocytes from unstimulated X-linked immunodeficient (xid) mice proved unresponsive to CD38 ligation, both in terms of proliferation and surface antigen modulation. This was the first clue that btk is either an integral component or an indirect regulator of the CD38-induced signal transduction pathway (301). CD38 ligation was later found to induce tyrosine phosphorylation of btk, at variance with what was reported for the human counterpart (313), and also to enhance expression of the IL-5 receptor α-chain, suggesting a functional synergy between the CD38 and IL-5 signaling pathways (185).

Other studies showed that the proliferative response and IL-5 receptor α-chain expression of B cells from fyn-deficient (Fyn−/−) and lyn-deficient (Lyn−/−) mice were impaired by anti-CD38 monoclonal antibody CS/2. B cells from fyn/lyn double-deficient (Fyn/Lyn−/−) mice showed no response to CS/2 monoclonal antibody. These results indicate that the CD38 signaling pathway requires the presence of both Fyn and Lyn, and that their signals are synergistic (355).

In the search for partners for CD38 or for associated molecules or signaling pathways, a striking relationship was observed between CD38-mediated mitogenesis and the ability of surface IgM to promote B cell proliferation. A typical example is that of B-1 cells isolated from the peritoneal cavity of normal mice and splenic B cells isolated from newborn mice. Both populations are known to be unresponsive to IgM cross-linking. The same proved true upon CD38 ligation. However, signaling through CD38 and IgM does not always have identical effects on B cells, since anti-CD38 cannot deliver inhibitory growth or differentiation signals to normal B cells or immature B cell lines (223).

The relationship between CD38- and BCR-mediated signaling was examined in detail in a follow-up paper by the Maureen Howard group (Palo Alto, CA). Cross-linking of CD38 and the BCR gave rise to a synergistic response; moreover, expression of CD38 lowered the threshold for BCR-induced responses. As a model, the authors then proposed was that CD38 signaling and coreceptor activity are regulated in vitro by conformational changes induced in the extracellular domain upon ligand/substrate binding, rather than on the actual turnover or generation of products.

The alleged independence of receptor and enzyme activities was recently confirmed in a report by the same group. The authors showed that anti-CD38-induced apoptosis of Ba/F3 cells, a murine pro-B line, is affected neither by blocking the Ca2+-mobilizing activity of cADPR nor by inhibiting intracellular or extracellular Ca2+ mobilization. Furthermore, 1) apoptosis is also unaffected by blocking CD38 enzyme activity with 2'-deoxy-2'fluoronicotinamide arabinoside adenine dinucleotide and 2) Ba/F3 cells expressing catalytically inactive mutant forms of CD38 still undergo apoptosis upon CD38 cross-linking. Anti-CD38-induced apoptosis was found to depend on tyrosine kinase and caspase activation. In addition, this process appears to be intensified by the presence of membrane microdomains. Thus the receptor-mediated functions of CD38 can operate separately from its enzyme activity, thus reinforcing the view that CD38 plays multiple, but independent, biologic roles (221).

The signaling pathway implemented upon CD38 ligation in murine B cells was first studied in mature, resting B cells, where CD38 induced proliferation is in turn enhanced by cosignals such as IL-4 and LPS. Somewhat in agreement with what has been described for human monocytes, CD38-induced proliferation is abrogated by FcγRII engagement. This inhibition can be brought about by adding anti-FcγRII Ab during culture, suggesting that it delivers a potent negative signal to CD38-activated B cells. These findings indicate that FcγRII can act as a regulatory molecule that modulates CD38 signals in vivo (266).

The CD38 pathway was then tested in a cell line lacking the cytoplasmic domain of CD38. Results indicated that CD38 ligation induced tyrosine phosphorylation with an intensity and kinetics similar to those seen with the entire protein. It also induced cell aggregation and decreased cell recovery. This suggests that CD38 triggers remarkably similar signaling pathways in human and murine immature B cells and supports the existence of accessory transmembrane molecules associated with CD38 (192).

Looking further downstream, CD38 ligation of murine splenic B cells activated members of the NF-κB/Rel family of proteins, including c-Rel, p65, and p50. Activation of NF-κB-like proteins by CD38 is not observed in splenic B cells from btk−/− mice, or in the presence of inhibitors of protein kinase C (PKC) and P3K, also suppressing NF-κB activation in CD38-activated B cells. This would seem to imply that activation of btk, P3K, and PKC plays, at least in part, important roles in the induction of NF-κB in CD38-stimulated murine B cells. It thus appears that NF-κB proteins play an essential role in the induction of germline γ1 transcripts by CD38-ligated murine B cells, giving rise to the production of IL-5-induced IgG1 (177).
It has also been shown that loss of PKC activity blocks CD38-dependent, B cell proliferation, NF-κB activation, and subsequent expression of cyclin-D2. Because CD38 cross-linking does not result in the functional phosphorylation of PLC-γ2 nor does it cause an increase in IP₃ production, an alternate diacylglycerol-producing phospholipase must participate in CD38 signaling. Consistent with this idea, CD38 increased the enzymatic activity of the phosphatidyldicholine (PC)-metabolizing enzymes, PC-PLC and phospholipase D. The PC-PLC inhibitor D609 completely blocked CD38-dependent B cell proliferation, IκB degradation, and cyclin-D2 expression. Analysis of btk mutant B cells demonstrated a partial requirement for btk in the activation of both enzymes. Taken together, these data demonstrate that CD38 initiates a novel signaling cascade leading to btk-, PC-PLC-, and phospholipase D-dependent, PLC-γ2-independent, B lymphocyte activation (248).

2. Neutrophils

The finding that the loss of CD38 renders mice susceptible to bacterial infections touched off a highly fruitful investigation on the role of CD38 in innate immunity. It was shown that CD38 KO neutrophils are incapable of directionally migrating to the site of infection. The molecular basis of this defect lies in that neutrophil chemotaxis to iMLP is dependent on Ca²⁺ mobilization mediated by cADPR. Thus CD38 controls neutrophil chemotaxis and acts as a critical regulator of inflammation and innate immune responses (271).

3. Dendritic cells

Impaired migration towards sites of immune responses is not unique to CD38 KO neutrophils; it is also a feature of dendritic cells (DC). Indeed, DC precursors from CD38 KO mice were unable to migrate from the blood to peripheral sites, and mature DCs could not migrate from sites of inflammation to the lymph nodes. Thus T cells are inefficiently primed in CD38 KO mice, leading to poor humoral immune responses. It was also shown that CD38 and cADPR modulate Ca²⁺ mobilization in chemokine-stimulated DCs and that they are required for the chemotaxis of immature and mature DCs to CCL2, CCL19, CCL21, and CXCL12. Therefore, CD38 regulates adaptive immunity by controlling chemokine receptor signaling in DCs (272).

E. CD157

Like CD38, CD157 acts as a receptor able to generate signals. Early evidence of its receptorial nature derived from analysis of the BM microenvironment, where the molecule is expressed by stromal cells and supports the growth of a murine pre-B-cell line (175).

The signal-transduction ability of CD157 was analyzed using specific antibodies mimicking the natural ligand, which is yet to be identified. CD157 cross-linking by a polyclonal antibody induced tyrosine phosphorylation of a 130-kDa protein in the human myeloid cell lines U937 and THP-1. Instead, cross-linking of CD157 as overexpressed by a mouse transfectant induced tyrosine phosphorylation of p130, dephosphorylation of the 100-kDa protein, and cell growth inhibition (265). The identification of p130 with the focal adhesion kinase (FAK) is controversial; indeed, the correspondence has only been demonstrated in a limited number of cell lines (155, 213).

CD157 regulates calcium homeostasis in human myeloid cells and mediates superoxide production in the U937 line (164). Recent experimental evidence suggests that critical functions of human neutrophils are orchestrated by CD157. Indeed, CD157-mediated signals promote cell polarization, regulate chemotaxis induced through the high-affinity fMLP receptor, and control diapedesis (106, 268). Real-time microscopy revealed that CD157 engagement leads to a sort of disorientation of neutrophils, which meander towards the interendothelial junctions, where they eventually stop without transmigrating. These findings are supported by the observation that neutrophils obtained from patients with paroxysmal nocturnal hemoglobinuria (marked by defective expression of GPI-anchored molecules, including CD157) are characterized by severe defects in neutrophil migration (268).

Because it lacks a cytoplasmic domain, CD157 must associate with some professional receptor(s) to compensate for its structural ineptitude to transduce signals. Recent results suggest that CD157 is functionally and structurally associated with the CD11b/CD18 complex on human neutrophils (207).

The relationship between the molecule’s enzymatic activities and its receptorial functions is an intriguing issue. CD157-catalyzed generation of extracellular cADPR is followed by the concentrative uptake of the cyclic nucleotide by hemopoietic progenitors and may be a potentially relevant step in normal hemopoiesis (282). However, if this is due to the effects mediated by CD157 cyclase, it remains a controversial issue as to whether CD157 is enzymatically active in physiological situations or not, as reported by independent groups (154, 361). However, both CD157 and CD38 catalyze the production of other metabolites, including nicotinic acid adenine dinucleotide phosphate and adenine homodinucleotides (18), whose functional effects are largely unknown in physiology.

Ample evidence indicated that CD157 is an immunoregulatory molecule in the murine system; indeed, CD157 cross-linking enhanced the proliferative response of
sorted pre-T lymphocytes to anti-CD3 stimulation and accelerates the development of fetal thymic organ cultures (342). Moreover, the expression of CD157 by murine B cell progenitors parallels DJ rearrangement of the immunoglobulin heavy chain genes (165). This suggests that CD157 plays a role at critical stages of lymphopoiesis, being implicated in both early T and B cell lymphocyte growth and development. However, this working hypothesis was not confirmed by the murine KO model, which demonstrates that CD157 plays a central role in the regulation of humoral T-independent immune responses and the mucosal thymus-dependent response (164). Indeed, KO mice showed impairment of thymus-independent antigen-induced IgG3. In addition, oral immunization with CD157 plays a role at critical stages of lymphopoiesis, being implicated in both early T and B cell lymphocyte growth and development. However, this working hypothesis was not confirmed by the murine KO model, which demonstrates that CD157 plays a central role in the regulation of humoral T-independent immune responses and the mucosal thymus-dependent response (164). Indeed, KO mice showed impairment of thymus-independent antigen-induced IgG3. In addition, oral immunization with thymus-dependent antigens led to low production of specific IgA and IgG in the fecal extract, due to a reduced number of antigen-specific antibody-producing cells in the intestinal lamina propria (166).

V. ENZYMATIC ACTIVITIES

A. Historical Perspective

The identification of a sequence similarity between the human lymphocyte antigen CD38 and the Aplysia ADPRC (318) marked the beginning of long-term investigations into the enzymatic properties of CD38 and into their role in human physiology and pathology. Fifteen years of continuous research have revealed that CD38 is a multifunctional (ecto)-enzyme that is involved in the catabolism of NAD⁺ and NADP⁺, the two main substrates of the molecule thus far identified. This reaction leads to the generation of potent intracellular Ca²⁺-mobilizing compounds (cADPR, NADAP, and ADPR); its main product, ADPR, can be covalently attached to proteins whereby it modifies the protein functions. The importance of these enzymatic pathways has been demonstrated not only in the immune system, but also in tissues and organs, including uterus, bronchi, pancreas, and kidney (130).

Several issues continue to baffle researchers. Perhaps the most intriguing concerns the relation(s) between the molecule’s enzymatic and receptorial functions. It is clear from evolutionary studies that the enzymatic function precedes the receptorial one and that the dual behavior of the molecule is likely the long-term outcome of selective pressure. If evolution has indeed played such a role on CD38, particular caution must be exercised when translating the results obtained in animal models to the human system. Moreover, all the evidence collected to date indicates that the receptorial functions are limited to surface CD38, which is in turn prevalently found in immune cells.

This section provides an overview of the enzymatic activities mediated by CD38 and CD157 and outlines their regulation and the main functional models in which they have been assessed.

B. Enzymatic Activities Controlled by CD38

The enzymatic properties of CD38 were formally demonstrated after analyzing a purified recombinant soluble form of the molecule. When added to NAD⁺, sCD38 catalyzed the formation and hydrolysis of cADPR (150). Similar results were obtained using solubilized human erythrocyte membranes: the three ectoenzyme activities, i.e., NADase, ADPRC, and cADPR hydrolase, could be purified to homogeneity using an anti-CD38 monoclonal antibody (361).

These results were confirmed in human T cell models, where the NAD-hydrolyzing enzymatic activity proved to correlate with the amount of CD38 on the cell surface. Moreover, CD38 immunoprecipitated from thymocytes behaved as an authentic NADase enzyme, by transforming NAD stoichiometrically into nicotinamide and ADPR (111) (Fig. 4).

Furthermore, transient transfection of CD38 cDNA in COS1 cells resulted in the expression of CD38 molecules capable of converting NAD to cADPR in the extracellular medium, as assessed by Ca²⁺ release from sea urchin egg microsomes (321). Further results indicated that cADPR is a reaction product rather than an obligatory intermediate reaction during glycohydrolase activity (22).

Initially, most research focused on the production of cADPR, given its central role in cell physiology. Only later did attention turn to investigation of the various other enzymatic activities. The soluble extracellular domain of CD38 was shown to mediate ADP ribosylation of several proteins, including CD38 itself. This process occurs at cysteine residues and can be reversed by the addition of HgCl₂, which specifically cleaves thiol-glycosidic bonds (128). It was suggested that during exposure of activated T cells to NAD⁺, CD38 is modified by cysteine-ADP-ribosylation, whereas cysteine-ADP-ribosylation leads only to inhibition of the ADPRase activity. Arginine-ADP-ribosylation results in inactivation of both the cyclase and hydrolase activities of CD38, whereas arginine-ADP-ribosylation leads only to inhibition of the hydrolase activity. Arginine-ADP-ribosylation causes a drop in intracellular cADPR and a subsequent decrease in Ca²⁺ influx, causing the death of the activated T cells (134). This is additional evidence that nucleotide-metabolizing (ecto)-enzymes are not just solo players, but likely work in a tightly orchestrated and interchained fashion (64) (Fig. 5).

The role of ADP ribosylation in controlling T cell homeostasis was subsequently addressed by studying NAD-induced cell death (NIDCD). It was shown that extracellular accumulation of NAD induces ADP ribosylation of the cytolytic P2X7 purinergic receptor. This phenome-
non induced ATP-independent activation and initiated the apoptotic process (309). The model thus proposed is that ARTs can sense and translate the local concentration of ecto-NAD$^+$ into corresponding levels of ADP-ribosylated cell surface proteins, while CD38 controls the level of cell surface protein ADP-ribosylation by limiting the substrate availability for ARTs (201). This process is thought to culminate in selective expansion of primed T cells at the expense of resting lymphocytes, which are uniquely sensitive to NICD. NICD therefore contributes to the dynamic regulation of T cell homeostasis (4).

The role of CD38 in controlling NAD$^+$ levels was further examined by the E. N. Chini group (Rochester, MN). Shifting the focus from the cell surface to the intracellular compartment, these authors postulated that CD38 is the major NADase in mammalian cells and that it regulates intracellular NAD$^+$ levels (6). In a subsequent study, the same authors proposed that CD38 could modulate the activity of sirtuins, NAD-dependent deacetylases implicated in ageing, cell protection, and energy metabolism in mammalian cells. This regulation occurs inside the nucleus and is mediated by CD38 expressed by the inner nuclear membrane (5). Recently, the CD38/sirtuin axis was reported as playing a major role in the regulation of body weight in mice (16).

The complex relationships between CD38 and other NAD-dependent enzymes are illustrated in Figure 6.

A separate enzymatic activity attributed to CD38 involves the catalytic exchange of the nicotinamide group of NAD$^+$ with nicotinic acid (NA). The product is nicotinic acid adenine dinucleotide phosphate (NAADP), a potent Ca$^{2+}$-mobilizing metabolite (1, 300). This activity occurs selectively at an acidic pH; the acidic dependence of NAADP metabolism, coupled with its biological function in targeting the acidic Ca$^{2+}$ stores in cells, suggests that NAADP serves as a specific Ca$^{2+}$ messenger for the acidic organelles of the endocytic pathway in cells (210). There is recent support for NAADP-elicited Ca$^{2+}$ release from lysosomes and endosomes (241). However, it was also shown that NAADP can release Ca$^{2+}$ from the endoplasmic reticulum (113, 114).

Thus, in addition to IP$_3$, internal Ca$^{2+}$ stores can be mobilized by at least two other molecules, cADPR and NAADP, which target separate Ca$^{2+}$ stores and are bound by distinct receptors (48, 353). However, these two new Ca$^{2+}$ agonists are intimately related, since the same enzymes can, under appropriate conditions, synthesize either one. This suggests that a unified mechanism may regulate both pathways (209) (Fig. 4). The importance of NAADP as a second messenger was further confirmed in pancreatic acinar cells, which have properties that make them particularly attractive for studies of Ca$^{2+}$ signaling.
events initiated by Ca\textsuperscript{2+} release from intracellular stores (280). In these cells, the hormone cholecystokinin elicits a mixture of local and global cytosolic Ca\textsuperscript{2+} signals (354). These signals may be abolished by blocking NAADP receptors. The effect is selective, since inactivation of the same receptors does not inhibit acetylcholine-induced Ca\textsuperscript{2+} (35). The conclusions from the studies in the model are that irrespective of whether the primary stimulus is acetylcholine, cholecystokinin, or one of the internal messengers, Ca\textsuperscript{2+} spiking can be abolished by either IP\textsubscript{3} receptor or ryanodine receptor antagonists. Further studies revealed a functionally important interplay between IP\textsubscript{3}, cADPR, and NAADP in the transformation of local cytosolic Ca\textsuperscript{2+} spikes (via IP\textsubscript{3}) to global Ca\textsuperscript{2+} transients (via cADPR and NAADP) (36).

Aplysia ADPRC and CD38 share another enzymatic activity. They both have the ability to exchange the base group of NAD\textsuperscript{+} with various nucleophiles, leading to the formation of a dimeric ADPR (ADPR\textsubscript{2}). Although ADPR\textsubscript{2} did not release Ca\textsuperscript{2+} from sea urchin egg microsomal vesicles, it specifically enhanced the Ca\textsuperscript{2+}-releasing activity of subthreshold concentrations of cADPR (58).

Recently, the cyclases from lower and higher Metazoa were also found to synthesize diadenosine diphosphate and two of its isomers, all of which are adenylc dinucleotides from cADPR and adenine. These dinucleotides are present and metabolized in mammalian cells and influence intracellular Ca\textsuperscript{2+} and cell proliferation (18). The Ap2A isomer P24 was shown to affect mitochondrial function, while the other products of ADPRC activity, namely, Ap2A and cA cDPR, antagonize P24-induced proton gradient dissipation and cytotoxicity. This indicates that the relative concentration of P24, cADPR, and Ap2A may rule the balance between cell life and death (30).

Specific attention has recently turned to ADPR. Although it is the main product of the enzymatic activities, ADPR initially lacked a clear role as an intracellular signaling molecule in vertebrate systems (279). It was later shown that ADPR activates the melastatin-related transient receptor potential cation channel TRPM2 after bind-
ing to the Nudix domain. These data revealed that ADPR and NAD$^+$ act as intracellular messengers and may play an important role in Ca$^{2+}$ influx by activating TRPM2 in immunocytes (299). Closer investigation showed that cADPR and NAADP can facilitate ADPR-mediated activation of TRPM2 by lowering its threshold. The mechanism of action is thought to involve mobilization of ADPR via metabolic conversion (19, 94, 195). This novel activation pathway has been studied in Jurkat T cells activated by high concentrations of concanavalin A, which induced an increase in ADPR concentration, TRPM2 activation, and, eventually, cell death (110).

C. Enzymatic Activities Controlled by CD157

Similarly to the Aplysia enzyme and to CD38, soluble CD157 incubated with NAD$^+$ produces cADPR and subsequently ADPR, indicating that this molecule is endowed with both ADPRC and cADPR hydrolase activities (142, 176). However, the catalytic efficiency of CD157 is several hundredfold lower than that of CD38 (154).

The enzymatic activities are pH dependent and require metal ions: the addition of Zn$^{2+}$ and Mn$^{2+}$ remarkably increases both the cyclase and hydrolase activities of CD157, as is the case for CD38. In contrast, Cu$^{2+}$ shows inhibitory effects on both catalytic activities of CD157, whereas it increases the cyclase activity of CD38 on the erythrocyte membrane (142, 362). These discrepancies may be useful in discriminating between CD157 and CD38 activities and suggest that the enzymatic activity of each molecule can play distinct roles in different environments.

D. Regulation of the Enzymatic Activities

The study of the regulation of the enzymatic activities of CD38 has been hampered by the lack of specific and effective inhibitors and by the unexpected finding that none of the monoclonal antibodies available in mouse and rat influences the enzymatic activity, either negatively or positively. A recent report shows that a human monoclonal antibody is endowed with inhibitory potential (320).

Table 3 is a list of compounds and their corresponding regulatory activities on CD38, as known to date and as

![Diagram of NAD$^+$-CONSUMING REACTIONS](image-url)

D. Functional Models

The finding that the enzymatic site of CD38 is located outside the plasma membrane raised a number of questions about 1) the accessibility of the NAD\(^+\) substrate, which is present only in minute amounts outside the cells, and 2) the transfer of cADPR to the cytoplasm (57). A possible solution was proposed based on the finding that connexin 43 (Cx43) works as a NAD\(^+\) channel located on the cell surface (96). Furthermore, it was proposed that CD38 is an active cADPR pump (97). Subsequent studies showed that cADPR may be transferred inside the cell also by nucleoside transporters (NT) (one equilibrative and three concentrative) (275). The deriving model provides evidence of an extensive trafficking of nucleotides and their metabolites across the plasma membrane and likely the membranes of the cytoplasmic vesicles. The NAD\(^+\) channels function in a paracrine and in an autocrine way, pointing to the fact that the enzyme activity of CD38 could be regulated simply by modifying the availability of the substrate.

Thus Cx43\(^+\) and CD38\(^+\) cells can provide cADPR to neighboring RyR\(^+\) parenchymal cells and enhance their intracellular Ca\(^{2+}\) levels and Ca\(^{2+}\)-dependent functions (97). Examples of cADPR-responsive cells via paracrine processes include 1) bovine smooth myocytes (95), 2) murine fibroblasts (31), 3) rat hippocampal neurons (341), and 4) human hemopoietic stem cells (283).

The role of cADPR as a signaling molecule in T lymphocytes was investigated by the A. Guse group (Hamburg, Germany). These authors showed causal relations between the increased concentrations of cADPR, sustained Ca\(^{2+}\) signaling, and activation of T cells (131). These chained effects are directly mediated through cADPR binding to RyRs (205).

Other groups investigated the role of CD38 outside lymphocytes. ADPRC activity has been detected in most cells and tissues, and cADPR is a second messenger involved in most eukaryotic cell functions (209). The best studied models include smooth muscle cells of different origins (vascular, bronchial, and uterine), epithelial cells, and secretory cells (including pancreas, kidney, and hypophysis). The basic mechanism of action is dependent on the production of cADPR, whereas the final outcome is dependent on the specific role of cADPR in the tissue.

1. **Myometrium**

The CD38/cADPR pathway of intracellular Ca\(^{2+}\) mobilization is upregulated in the rat myometrium by estrogens. This process is inhibited by the presence of progesterone. The effects of estrogens on CD38 and on the differential regulation of its enzyme activities are also detectable in the myometrium at term. In preterm rat myometrium, the level of CD38 expression and its enzyme activities are comparable to those seen in myometrium obtained from ovariectomized rats or from ovariectomized rats treated with progesterone along with estrogens (78). The effects on CD38 expression and the differential regulation of its enzyme activities seem to favor cADPR production, cADPR-induced Ca\(^{2+}\) release, and increased contractility of the myometrium. It has recently been demonstrated that the cADPR system is important in oxytocin-induced Ca\(^{2+}\) transients in human myometrial cells (15). This suggests that cADPR may be an endogenous regulator of the Ca\(^{2+}\)-induced Ca\(^{2+}\) release mediated through RyR in human myometrial cells and may play an important role in successful delivery (44, 79).

2. **Smooth muscle cells**

CD38 expression in airway smooth muscle cells contributes to the contractile response in the bronchi and is tightly regulated at a genetic level. This finding is particularly relevant for pathological conditions such as asthma, where proinflammatory cytokines induce CD38 expression and glucocorticoids attenuate it. This may prove clinically relevant in the treatment of asthma (76).

The expression and function of CD38 has been evaluated in several other smooth muscle cell models, including peritubular (17) and vascular (61) muscle cells.
3. Bone metabolism

The M. Zaidi group (New York, NY) conducted extensive studies on the expression and function of CD38 in osteoblasts and osteoclasts of different species. They found that the molecule is expressed by both cell types in different cellular compartments, including the nucleus. In addition, they showed that CD38 activation in the osteoclast triggers Ca\textsuperscript{2+} release, stimulates the production of IL-6, and inhibits bone resorption (2, 322). Consistent with these findings, CD38 KO mice displayed strikingly reduced bone mineral density at 5 mo, with full normalization at 5 mo. Hematopoietic stem cells isolated ex vivo from CD38 KO mice showed a dramatic, approximately fourfold increase in osteoclast formation (323). Furthermore, cADPR or exogenous addition of ADPRC stimulated osteoclast formation. Conversely, blocking cADPR action with the analog 8-bromo-cADPR strongly inhibited osteoclast formation. These effects seem to be predominantly related to the NADase activity of CD38 (162).

VI. ANIMAL MODELS

Animal models deficient or hyperexpressing a single molecule have in many instances been fundamental tools for understanding its biological role in the murine and likely the human system. The underlying assumption is that if a protein exerts a nonredundant function, then its complete lack will result in the complete loss of that function. This has been the first problem with the CD38 KO mice. Indeed, these animals show an almost complete loss of tissue-associated NADase activity, with a significant reduction of cADPR values (43, 49, 357). Accordingly, NAD\textsuperscript{+} levels are altered in multiple tissues, even though the physiological consequences are still to be determined.

Moreover, cADPR loss however is not complete, suggesting that CD157 and/or yet unidentified members of the family may compensate for the absence of CD38. A double CD38/CD157 KO has not yet been published, somewhat preventing general conclusions.

The CD38 KO mice are viable and appear to breed normally, leading to the conclusion that CD38 and its enzymatic activities are not necessary for life. Further examination of these mice confirmed that the development of the hematopoietic lineages is not influenced by the absence of CD38. On the contrary, the molecule is required for optimal T cell-dependent humoral immune responses (49). In later reports, the F. Lund group (Saranac Lake, NY) extended the analysis of the immune system of these animals under stressful conditions, including bacterial infections. Loss of CD38 renders mice susceptible to bacterial infections due to an inability of CD38 KO neutrophils to migrate to the site of infection (271). When infected with the gram\textsuperscript{+} organism S. pneumoniae, CD38 KO mice upregulated normally the expression of inflammatory cytokines systemically as well as in the lung. However, the inflammatory cell infiltrate was significantly reduced in the lungs of these mice, and the bacteria rapidly disseminated from the lungs to the blood, causing death within 36 h in the majority of the animals (271). The defect can be attributed to the lack of cADPR, which directly induces intracellular Ca\textsuperscript{2+} release in wild-type neutrophils and is required for sustained extracellular Ca\textsuperscript{2+} influx in neutrophils that have been stimulated by fMLP, a bacterial chemoattractant (271). Likewise, CD38 KO neutrophils are unable to migrate in response to several endogenous chemoattractants and chemokines, including serum amyloid A and MIP-1\textalpha (273).

The first conclusion is that CD38 controls neutrophil chemotaxis to bacterial chemoattractants through the production of cADPR. This concept was further strengthened by successive studies on DC. Indeed, CD38 regulates migration of DC precursors from the blood to peripheral sites and controls the migration of mature DCs from sites of inflammation to lymph nodes. The consequence for the immune response is that T cells are inefficiently primed in CD38 KO mice, leading to poor humoral immune responses. The molecular mechanism relies on the lack of cADPR, which is required for the chemotaxis of immature and mature DCs to CCL2, CCL19, CCL21, and CXCL12 (272).

The first interest in generating animals overexpressing or lacking CD38 was linked to the proposed model of NAD\textsuperscript{+} as a central agent in the pathogenesis of pancreatic \( \beta \)-cell damage and consequent onset of diabetes. The Okamoto group designed a model of insulin release and pancreatic \( \beta \)-cell damage based on a complex interplay between NAD\textsuperscript{+}, PARPs, CD38, and cADPR. To confirm this model, the authors initially prepared mice overexpressing CD38 and showed that the transgenic serum insulin level was higher than that of control in glucose-tolerance tests, suggesting enhanced release (179). The general inference is that Ca\textsuperscript{2+} release from intracellular cADPR-sensitive Ca\textsuperscript{2+} stores and the Ca\textsuperscript{2+} influx from extracellular sources plays important roles in insulin secretion (179). The experiments conducted later by generating CD38 KO mice highlighted impaired glucose tolerance, with serum insulin levels lower than controls. The pathological phenotype was rescued by \( \beta \) cell-specific expression of CD38 cDNA (180).

The interest on diabetes was recently continued by showing that CD38 KO islets are significantly more susceptible to apoptosis compared with islets isolated from littermate controls. The conclusion of these experiments is that CD38 plays a role in novel antiapoptotic signaling pathways, but does not directly control glucose signaling in pancreatic \( \beta \)-cells (173).

The observations brought about in the genetically modified animals were assessed in spontaneous disease
models of autoimmune type 1 diabetes, typically of the NOD strain. The onset of diabetes is significantly anticipated in the CD38 KO NOD/Lt mice, due to an impairment of both the regulatory T cell compartment and of the invariant NKT (iNKT) cells. The molecular mechanisms remain partially unknown, even though an interplay between CD38 and ART2 is hypothesized (38, 40).

A role for the CD38/cADPR system in regulating hormone secretions has very recently been proposed by the Higashida group. By observing adult CD38 KO female and male mice, the authors were able to highlight marked defects in maternal nurturing and social behavior, respectively. This correlated with higher locomotor activity, while plasma levels of oxytocin (OT) were strongly decreased in CD38 KO mice. Surprisingly, vasopressin was unaffected in the model. The group went on to show impaired OT release in the CD38 KO mice, which could be reverted by genetic reconstitution of CD38 expression in the neurohypophysis. These results reveal that CD38 has a key role in neuropeptide release, thereby critically regulating maternal and social behaviors, and may be an element in neurodevelopmental disorders (141, 172).

The other area of interest concerns the role of CD38 in smooth muscle contraction in distinct organs and tissues. In the lung, the CD38/cADPR system contributes to airway smooth muscle tone and responsiveness through its effects on agonist-induced elevation of intracellular Ca$^{2+}$ (76). This model was further expanded by examining the effects on lungs exposed to IL-13, a cytokine involved in the pathogenesis of asthma. Under these conditions, CD38 contributes to airway hyperresponsiveness by increasing airway smooth muscle reactivity to contractile agonists (129). Other districts concern smooth muscle in heart (324) and vessels (243).

There is still an enormous amount of data derived from the use of CD38 KO mice waiting to be organized for publication. A number of papers report on data inferred by using KO mice: other ones concern the role of CD38 in bone (323) and fat control (16). Table 4 tries to comprehensively summarize the majority of published reports.

The experience using CD157 KO mice is more limited. The phenotype of the animals is characterized by a partial impairment of thymus-independent and thymus-dependent antigen-specific immune responses. Oral immunization of CD157 KO mice with cholera toxin, a potent thymus-dependent antigen for the induction of IgA response, resulted in the poor production of specific antibodies at the intestinal mucosa accompanied by reduced numbers of IgA-producing cells in the LP. These results suggest that CD157 has roles in B cell development and antibody production in vivo (166) (Table 4).

### VII. PHYLOGENY AND GENE ORGANIZATION

#### A. From One Soluble Gene to a Family of Transmembrane Proteins

NADase/ADPRC enzymatic activities are found in bacteria (29, 186), plants (347), and metazoans (297). The eukaryotic NADase/ADPRCs form a unique gene family of very small size, which makes it convenient when cataloguing new members as orthologs (same gene, different species) or paralogs (genes related by duplication). From

### TABLE 4. Phenotypes of the CD38 and CD157 knockout mice

<table>
<thead>
<tr>
<th>System/Organ</th>
<th>Strain</th>
<th>Defect</th>
<th>Outcome</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune system</td>
<td>C57BL/6</td>
<td>↓ Antibody responses to T cell-dependent antigens</td>
<td>↓ IgG titers</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>C57BL/6</td>
<td>Impaired neutrophil chemotaxis</td>
<td>↑ Death by infection</td>
<td>271</td>
</tr>
<tr>
<td></td>
<td>C57BL/6</td>
<td>Impaired dendritic cell chemotaxis</td>
<td>↑ Death by infection</td>
<td>272</td>
</tr>
<tr>
<td>Pancreas (β-cells)</td>
<td>ICR</td>
<td>↑ Apoptosis, ↓ insulin secretion</td>
<td>Impaired glucose tolerance</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>NOD/Lt</td>
<td>↑ CD8$^+$ T cells, impaired Treg development, ↑ apoptosis of iNKT cells</td>
<td>↑ Autoimmune diabetes</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>C57BL/6</td>
<td>Normal glucose receptor signaling, but increased apoptosis</td>
<td>No impairment in glucose tolerance, no diabetes</td>
<td>40</td>
</tr>
<tr>
<td>Bone</td>
<td>C57BL/6</td>
<td>↑ Osteoclast formation</td>
<td>↓ Mineral density</td>
<td>323</td>
</tr>
<tr>
<td>Lung</td>
<td>C57BL/6</td>
<td>↓ Smooth muscle contractility</td>
<td>Constitutively impaired airway responsiveness and impaired airway responsiveness in response to IL-13 challenge</td>
<td>76</td>
</tr>
<tr>
<td>Heart</td>
<td>ICR</td>
<td>Altered myocyte contractility</td>
<td>Cardiac hypertrophy in male</td>
<td>324</td>
</tr>
<tr>
<td>Aorta</td>
<td>ICR</td>
<td>↓ Contractility</td>
<td>↓ α-Adrenoreceptor-induced contraction</td>
<td>243</td>
</tr>
<tr>
<td>Fat</td>
<td>C57BL/6</td>
<td>Regulation of mitochondrial biogenesis and energy homeostasis</td>
<td>↓ High-fat-diet-induced obesity</td>
<td>16</td>
</tr>
<tr>
<td>Brain</td>
<td>ICR</td>
<td>↓ Oxytocin release</td>
<td>Altered short-term social memory</td>
<td>172</td>
</tr>
<tr>
<td>CD157</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune system</td>
<td>C57BL/6</td>
<td>Delayed development of B-1 cells, altered Ig responses</td>
<td>Altered B cell homeostasis</td>
<td>166</td>
</tr>
</tbody>
</table>
Mammals have two NADase/ADPRCs identified as CD38 and CD157. Their genes are very similar in intron-exon structure and reflect their common origins with the Aplysia ADPRC gene. Arranged head-to-tail in tandem on Homo sapiens (HSA) chromosome 4 (telomere → CD157 → CD38 → centromere) and Mus musculus (MMU) chromosome 5, CD38 and CD157 are clearly derived by gene duplication (92). Analysis of the chicken genome databases and our unpublished data confirm that orthologs of CD38 and CD157 lie in tandem on Gallus gallus (GGA) chromosome 4 (E. Ferrero, unpublished data). The presence of both CD38 and CD157 in the chicken genome indicates that gene duplication predated the reptile/mammal separation over 300 mya.

The products of the NADase/ADPRC genes are either soluble (e.g., Aplysia and sea urchin) or membrane-bound enzymes [schistosome (NACE) and CD38 + CD157 in mammals]. These proteins range in size from the soluble 29-kDa Aplysia ADPRC to the 45-kDa membrane glycoproteins CD38 and CD157. Their stringent conservation of the three-dimensional structures owes much to the 10 cysteine residues as well as to the inter-cysteine spacing (216, 284).

Aplysia cyclase shares 25–30% of its amino acid sequence with the CD38 and CD157 polypeptides, but it is the modifications of the NH2 and COOH terminals which determine protein topology and localization. The short NH2-terminal hydrophobic tract found in Aplysia cyclase is longer in CD38, the result of a process simply requiring the addition of a few amino acid residues to produce a type II membrane protein with an NH2-terminal transmembrane domain. The fate of most type II proteins is associated with the plasma membrane, Golgi apparatus, and endoplasmic reticulum (ER) (11). This is also the case with CD38, which thus became an ectoenzyme during its long course through evolution.

CD157 devised a more innovative mechanism for acquiring membrane attachment. With an NH2-terminal ER-targeting signal peptide in place, membrane binding was acquired through the addition of a ninth exon whose sole raison d’être is to encode a COOH-terminal hydrophobic stretch, which is subsequently removed upon addition of the GPI anchor. The process generated a membrane-bound, GPI-anchored NADase/ADPRC.

The microevolutionary processes that designed the modifications of the vertebrate NADase/ADPRC genes and their effects on protein dynamics provide an explanation for how the vertebrate enzymes reached the cell surface. On the contrary, the sectorial distribution of the metabolic effects to within cellular organelles may help to explain why.

A working hypothesis is that early CD38/CD157 precursors are part of the innate immunity, and their passage to the surface of immune cells may have gone in parallel with the transition to adaptive immunity.

It would be nice to find evidence that the first lymphocytes of the vertebrate protoimmune system to emerge in some primitive jawed fish over 500 mya expressed a NADase/ADPRC on their surface. Indeed, the first in silico evidence of a tandem duplication of NADase/ADPRC genes comes from Tetraodon nigroviridis.

In conclusion, the CD38 and CD157 genes are duplicates, and as such, at least one copy could have undergone pseudogenization and disappeared without trace. They have survived with modification to join the ranks of genes pertaining to chemosensation, immunity, and reproduction, those most frequently duplicated during mammalian evolution (Fig. 7).

B. Structure and Organization of CD38 and CD157 Genes

The CD38 gene is localized on the short arm of chromosome 4 (4q15) (169, 259). The CD38 polypeptide is encoded by a >80 kb gene, more than 98% of which is represented by intronic sequences. The gene has eight exons, and exon 1, the largest coding exon, determines the intracytoplasmic and transmembrane regions and the membrane proximal 33 amino acids of the extracellular region (91, 93).

Control of human CD38 expression appears to be multilayered. The first level of control lies in the 5′-flanking promoter region of the gene, which, with no TATA box and the presence of a CpG island, resembles many non-inducible housekeeping genes. The island is ~900 bp long, encompasses exon 1 and the 5′-end of intron 1, and contains a binding site for the transcription factor Sp1, suggesting that methylation may intervene in the process of CD38 gene regulation (92).

A second level of control involves trans-interactions with sequences that lie further upstream from the transcription start site. Potential binding sites have been identified for the T cell transcription factor-1 (TCF-1α), nuclear factor for interleukin-6 (NF-IL-6), interferon-responsive element-1 (IRF-1), and binding sites for glucocorticoid hormones. A third level of control is located at the 5′-end of intron 1 and is involved in the induction of CD38 expression by retinoids in myeloid cells (188) via a retinoic acid-responsive element (RARE).

CD38 has a well-characterized single-nucleotide polymorphism (SNP) located at the 5′-end of this intron (184).
C→G), which leads to the presence (or absence) of a Pvu II restriction site (92). The frequency of the three genotypes has been established in healthy Italian-born adults as 70% CC, 26% CG, and 4% GG. Similar frequencies have been reported in Spanish (125) and Irish (84) populations. The SNP is located in an intronic hot spot, containing part of the CpG island /H11001 RARE mentioned above. In addition, evidence based on a novel truncated CD38 mRNA transcript suggests that a further mechanism for controlling CD38 gene expression involves transcriptional elongation, where a stop-or-go decision is taken within the 5'-end of intron 1 (E. Ferrero, unpublished data).

CD157 extends over 35 kb and consists of nine exons (255). CD38 and CD157 are highly conserved as demonstrated by the fact that exons 1–8 are similar in length and maintain the same phase of intron insertion. As described above, exon 9 of CD157 encodes the GPI anchor signal (Fig. 8).

VIII. HUMAN DISEASE MODELS

The study of disease models has been fruitful in revealing vital immune mechanisms. As a direct consequence, an increasing number of CD family members have been implicated in the pathogenesis of diseases or as markers of their prognosis and progression. The CD38 family is no exception. Analysis of CD38 expression is currently used for the diagnosis of leukemia and myeloma, and the molecule is also used as a powerful independent prognostic marker for CLL patients. Moreover, CD38 expressed by CD8+ and CD4+ cells constitutes a very reliable, economic, and easy-to-use prognostic tool for the progression to AIDS in HIV-infected patients. Additionally, functional impairment of the CD38/cADPR system has been associated with the disordered insulin metabolism typical of type II non-insulin-dependent diabetes mellitus (NIDDM).
Here, we focus on the involvement of CD38 in NIDDM, HIV infection, and CLL, both as a marker and as a pathogenetic agent. Not analyzed are the recent results reported by the H. Higashida group, which promise to open an exciting new field concerning the link between CD38 and behavior. Extensive analysis in CD38 KO mice led the authors to conclude that CD38 plays an indirect role in influencing short-term social memory, mediated through an impaired release of OT (172). The impact on human medicine is still to be explored.

### A. Diabetes

The earliest report of involvement of the cADPR axis in insulin metabolism came from experiments using a cell-free system of islet microsomes. In this model, cADPR induced Ca\textsuperscript{2+} release, whereas IP\textsubscript{3} did not. Moreover, cADPR and Ca\textsuperscript{2+} both induced insulin secretion in digitonin-permeabilized islets, but IP\textsubscript{3} did not. These results suggested not only that cADPR helps mediate Ca\textsuperscript{2+} release from islet microsomes, but that cADPR might be generated in islets by glucose stimulation, thus serving as a second messenger for Ca\textsuperscript{2+} mobilization in the endoplasmic reticulum (326).

An independent paper reported on the cloning of a rat CD38-homologous protein, expressed in pancreatic islets. Rat CD38 is highly homologous to the human molecule, exhibiting 58% overall identity and 76% similarity and is expressed at comparable levels in Wistar rats and GK rats (a genetic model of NIDDM). The presence of rat CD38 in the pancreatic islets suggested that it is involved in insulin secretion by synthesizing cADPR (212).

Further studies on GK rats provided the first accepted link between CD38 and diabetes. It was observed that CD38 mRNA levels are reduced by ~50% in islets (236); in fact, the cADPR signal system for insulin secretion is replaced by IP\textsubscript{3} in these islets. It was later seen that the microsomes in the diabetic β-cells release Ca\textsuperscript{2+} in response to IP\textsubscript{3} but not to cADPR. In addition, they overexpress IP\textsubscript{3} receptor mRNAs (325).

Later studies exploring the role of the CD38/cADPR axis exploited animals genetically modified to express either high or low amounts of CD38. More specific information concerning the effects of the overexpression or deficiency of CD38 is reported in the relevant sections. Generally speaking, however, it can be said that mice overexpressing CD38 exhibit enhanced glucose-induced insulin release, while CD38 KO mice display severe impairment of β cell function.

A report from the Okamoto group on human diabetes revealed that 13.8% of Japanese NIDDM patients have autoantibodies against CD38; moreover, sera containing such autoantibodies inhibited the ADPRC activity of CD38. The addition of NIDDM sera containing anti-CD38 antibodies to cultured rat pancreatic islets significantly inhibited insulin secretion induced by glucose, whereas recombinant soluble CD38 abolished this effect. These results strongly suggested that the presence of anti-CD38 autoantibodies in NIDDM patients may be one of the major causes of impairment in the secretion of glucose-induced insulin (159).

The presence of anti-CD38 autoantibodies was confirmed in Caucasian diabetic patients. High anti-CD38 autoantibody titers were found in 9.7% of type 2 diabetic patients (231). Another study by the same authors also revealed anti-CD38 autoantibodies in 13.1% of type 1 diabetes (DM1) patients (286). Anti-CD38 autoimmunity defines a clinical phenotype similar to nonautoimmune type 2 diabetes, with relatively preserved β-cell function and low genetic influence (10). A more recent study reported...
B. HIV Infection

The expansion of the CD8<sup>+</sup>/CD38<sup>+</sup> lymphocyte subset was an early alteration frequently observed prior to the detection of antibodies against HIV and diminished levels of CD4<sup>+</sup> cells in subjects at risk of developing AIDS. An increase in the number of HLA class II<sup>+</sup>, CD38<sup>+</sup>, and Leu-8<sup>-</sup> CD8<sup>+</sup> lymphocytes was associated with a decline in the number of CD4<sup>+</sup> cells and development of the disease (119). Subsequent studies by independent groups confirmed this observation (349). These pioneer works disclosed a stage-associated pattern of HLA class II and CD38 expression on CD8<sup>+</sup> T lymphocytes during HIV infection (182). However, it remained unclear as to whether specific phenotype patterns could have a resulting functional correlation in the host response to the virus. Indeed, it later turned out that CD8<sup>+</sup>/HLA class II<sup>+</sup>/CD38<sup>+</sup> cells exhibited higher HIV-specific CTL activity than other CD8<sup>+</sup> cells, indicating that CD38 is expressed in vivo on virus-specific CD8<sup>+</sup> CTL (143).

These findings led to several multicenter studies aimed at determining whether the CD8<sup>+</sup>/CD38<sup>+</sup> cell subset had prognostic value for progression to AIDS. They concluded the following:

1. The percentage of CD8<sup>+</sup>/CD38<sup>+</sup> cells marks disease progression towards the development of AIDS, independently of CD4 count and β<sub>1m</sub> levels (219, 245). The Multicenter AIDS Cohort Study confirmed that the elevated expression of CD38 on CD8<sup>+</sup> T cells is a more reliable marker for the risk of chronic HIV disease progression to AIDS and death than CD4<sup>+</sup> cell count, soluble immune activation markers, or combinations of HLA class II and CD38 expression (218). The finding that the percentage of activated CD8<sup>+</sup> cells expressing CD38 can predict the rate of decline of CD4<sup>+</sup> T cells before it becomes clinically relevant is relevant for the disease. Furthermore, early identification of the CD4<sup>+</sup> T cell slope will allow clinicians to target treatment to those patients who are most likely to benefit (25). This assay was therefore deemed to be potentially useful, in conjunction with blood CD4 counts and serum β<sub>1m</sub> levels, in patient management and clinical trial design.

2. The test can be performed either on fresh or frozen cells with identical results (278).

3. The evaluation of CD38 expression on CD8 cells is a rapid, simple, and economical method of studying HIV infection and progression to AIDS. Not less important is the fact that such a low-cost test may result in wide application in developing countries, generally burdened by high rates of disease (267).

4. In children, too, expression of CD38 on CD8<sup>+</sup> T cell predicts maintenance of high viremia in highly active anti-retroviral treatment (HAART)-treated HIV-infected patients (311, 343). This is consistent with the view that increased CD38 expression on CD8<sup>+</sup> T cells has the same prognostic significance in pediatric as in adult HIV disease.

5. High numbers of CD38<sup>+</sup>/CD8<sup>+</sup> cells may indicate an underlying chronic but blunted activation, which is ultimately unable to control disease progression (121). Indeed, profound CD8<sup>+</sup> cell activation was seen in all subjects at seroconversion and at 6 and 12 mo later. The CD8<sup>+</sup>/HLA class II<sup>+</sup>/CD38<sup>+</sup> cell population, which has potent direct HIV cytotoxic T cell activity, was markedly elevated at seroconversion. In individuals that maintained high HLA class II<sup>+</sup>/CD38<sup>+</sup>/CD8<sup>+</sup> expression throughout the first year of infection, CD4<sup>+</sup> cell counts were stable during the next 5 yr. Long-term survivors also had elevated levels of this subset, despite the paucity of other activated CD8<sup>+</sup> cells. Thus selective elevation of HLA class II<sup>+</sup>/CD38<sup>+</sup>/CD8<sup>+</sup> cells proved to be a marker of subsequent stable HIV disease (120).

The issue has recently been taken up again, after a study revealed that the distribution of HIV-specific CD8<sup>+</sup> T cells was heavily skewed toward CD38<sup>+</sup>/CD8<sup>+</sup> T lymphocytes. However, a significant percentage of this subpopulation underwent spontaneous/Fas-mediated apopto-
sis, indicating that a substantial proportion of the HIV-specific CD8\(^+\) T cells arise from HIV-driven aberrant immune activation. The absence of effective cytolytic activity by these cells against infected targets could be related to their high susceptibility to apoptosis. This would explain why HIV is not successfully contained by CD8\(^+\) T cells in such patients (47). It would seem that an analogous situation occurs in the CD4 compartment, where a population of highly activated cytotoxic effector CD4\(^+\)/CCR5\(^+\)/CD38\(^+\) cells is expanded immediately after HIV infection. These cells are prone to both apoptosis and cytopathic infection by HIV, and rapidly decline (358).

Studies of CD4 cells expressing CD38 also showed that the number of cells expressing both markers dependably anticipates the clinical signs of progression to AIDS (183).

Researchers and clinicians also asked whether the number of CD38\(^+\)/CD8\(^+\) cells could be predictive of response to therapy. HAART was followed by a rapid decline in CD8\(^+\)/CD38\(^+\) lymphocytes in parallel with viral load, allowing for rapid normalization of CD8\(^+\)/CD38\(^+\) T cell numbers. This might indicate that CD38 is a marker of residual viral replication when the viral load falls below detectable levels following HAART intervention (335). Likewise, children with high CD38 levels in CD8\(^+\) Tlymphocytes had a higher incidence of and relative risk for virological failure than did those with low CD38 expression. This means that CD8\(^+\)/CD38\(^+\) T cell count is a dependable marker of therapeutic failure in HIV-infected children (20, 293).

Deciding on the best method for measuring CD38 expression has been the subject of lengthy debate. In general, CD38 can be quantified by using either Quantibrite beads or CD4 expression on CD4\(^+\) T lymphocytes as calibrators (152, 168, 211). The most recent evidence is that combining measurement of CD38 expression on peripheral blood monocytes with measurement of CD8\(^+\) and CD4\(^+\) T cells is more useful for monitoring HIV-infected patients under HAART than measurement of CD38 expression on CD8\(^+\) T lymphocytes alone (8).

The main issue still to be resolved concerns the biologic role of CD38 in CD8\(^+\) T lymphocytes in HIV-infected individuals. What is known is that CD38 is expressed in its fully active form when upregulated on T cells from HIV patients. The increased expression of CD38 on lymphocytes during the chronic phase of HIV infection potentially compensates for the impaired capacity of cells to synthesize ribonucleotides de novo (26).

Also known is the negative correlation between the levels of surface CD38 expression and the rate of acute cell death from HIV observed 96 h after infection. This implies that high CD38 expression correlates with resistance to HIV infection, leading to a lower rate of cell death. This assumption is further supported by the observation that nicotinamide, a reaction product of CD38, confers partial protection against acute cell death from HIV to CD38\(^{low}\) cell lines. Nicotinamide may thus be at least partially responsible for the correlation observed between high levels of CD38 and low rates of acute cell death from HIV (306). A follow-up paper reported that CD38 expression is negatively correlated to susceptibility to HIV infection, likely because of interference with gp120/CD4-dependent viral binding to target cells (304). This is due to strong lateral associations between CD38 and the HIV receptor CD4. The association is further accentuated by the HIV envelope gp120 glycoprotein. According to this model, CD38 prevents HIV infection by specifically inhibiting gp120/CD4 binding. The anti-HIV activity exerted by CD38 was mapped in the membrane-proximal region, which displays significant sequence similarity with the V3 loop of the HIV gp120 glycoprotein. Similar effects were obtained by using synthetic soluble peptides derived from this region, reproducing the anti-HIV effects of full-length CD38. Moreover, the same peptides inhibited primary HIV-1 and HIV-2 isolates from different subtypes and with a variety of coreceptors (303, 305).

Another independent study showed a link between the susceptibility of CD4\(^+\) T cells to different strains of HIV and their surface expression of CD38 (148). However, the Tropic HIV proliferated in vitro more efficiently in the CD4\(^+\)/CD38\(^+\)/CD62L\(^-\) subset than in the CD38\(^-\) counterparts. A likely explanation is that the CD4\(^+\)/CD38\(^+/\)CD62L\(^-\) subset secretes endogenous Th2 cytokines (e.g., IL-4), which promote efficient production of T-tropic HIV through upregulation at a certain stage of the viral life cycle, probably after the adsorption step (147).

C. Chronic Lymphocytic Leukemia

The original observation linking CD38 expression to prognosis in CLL patients was published in a seminal work by the N. Chiorazzi group (New York, NY) (55). The authors suggested that CLL cases could be divided into two groups with prognostic implications according to the mutational status of their IgV genes. Patients with unmutated IgV genes presented with a more aggressive disease from diagnosis, had a shorter time to therapy, and ultimately died sooner. The same subset also displayed higher percentages of CD38\(^+\) CLL cells, raising the possibility that CD38 could be used as a surrogate marker for the absence of IgV mutations. This observation was confirmed by many groups operating in different institutions and clinical centers. A general conclusion from all these studies is that CD38 expression is a reliable negative prognostic marker for CLL patients (52, 75, 81, 85, 158, 170, 246, 247). The second conclusion is that the association between IgV mutational status and CD38 expression is less stringent than originally believed. Both parameters
are now considered independent prognostic markers. This issue was initially discussed by the T. J. Hamblin group (Southampton, UK) (132) and later confirmed by others (171, 234, 334). CD38 was thus seen to be an independent risk factor that can be used together with IgV mutation and clinical staging to identify CLL patients with the poorest prognosis (133). Furthermore, CD38 expression positively correlates with all of the other negative prognostic markers examined, including cytogenetic abnormalities (202, 269), soluble CD23, soluble β₂m (138), p53 function (41, 214), and cell size (232). However, CD38 does not appear to be a prognostic marker in the context of familial CLL cases (163).

Notwithstanding the impressive number of patients studied, there is still no generally accepted standardized procedure for determining CD38 expression. As a consequence, there is no clear threshold above which CD38 is said to be positive (28, 112, 333). The F. Caligaris-Cappio group (Milan, Italy) has proposed that it is the presence of a distinct CD38⁺ population within the leukemic clone that correlates with IgV mutational status, rather than a fixed numerical cut-off. Notwithstanding its size, the CD38⁺ population identifies CLL patients who will have progressive disease (118).

Other issues to be resolved concern 1) the stability of CD38 expression over time, which some investigators think can change over time and in response to therapy (53, 117, 133, 137), and 2) the feasibility of using cryopreserved material (133, 151).

Our interest in CLL derives primarily from the exploitation of human diseases as strategic models for defining the in vivo biological role of CD38. The underlying working hypothesis is that CD38 is not a mere marker, but that its surface expression has pathogenetic potential. In line with this hypothesis, we have shown that CD38 ligation by means of agonistic monoclonal antibodies is followed by proliferation and blast transformation of a subset of CLL cells, demonstrating that the molecule may perform as an active signaling receptor (62). The in vitro signaling properties mediated by CD38 are significantly enhanced by the simultaneous presence of IL-2, which acts through strong upregulation of CD38 expression. To determine which mechanism(s) maintain CD38⁺ cell proliferation, we showed that CD38-mediated signals may be activated upon interaction with the cell surface CD31 ligand. CD38⁺ CLL cells juxtaposed to murine fibroblasts transfected with CD31 exhibit increased growth and survival. Furthermore, CD38/CD31 contacts upregulate the survival receptor CD100, a semaphorin family member involved in sustaining CLL growth and survival (71). The model is indirectly confirmed by evidence that nurselike cells (NLC) derived from CLL patients express high levels of functional CD31 and plexin-B1, the high-affinity ligand for CD100 (71) (Fig. 9).

More recently, the cytoplasmic kinase ZAP-70 has been identified as a further independent negative prognostic marker (50). Thus aggressive CLL can be identified by absence of mutations in the IgV genes and by the presence of both surface CD38 and intracellular ZAP-70. Preliminary results indicate that a combination of CD38 and ZAP-70 provides better identification of high risk CLL patients (54, 74, 153, 307). The CD38⁺/ZAP-70⁺ subset also appears to be genetically distinct (156, 277). The clinical indication that combined expression of CD38 and ZAP-70 more accurately identifies high-risk patients prompted us to evaluate the existence of a functional link between the two molecules in neoplastic B cells. CD38 engagement leads to a transient but significant tyrosine phosphorylation of ZAP-70 in CD38⁺/ZAP-70⁺ CLL cells. Furthermore, ZAP-70 expression seems to be a limiting factor for CD38-mediated functions. Extensive analysis of CD38 signals proved that the CD38 pathway is selectively active in CD38⁺/ZAP-70⁺ cells. This observation is clinically relevant because it provides a molecular rationale for simultaneous testing of CD38 and ZAP-70 for the risk stratification of patients (70). The question that remains to be answered is where the CD31/CD38/ZAP-70 axis leads. The first possibility is that CD38 and ZAP-70 somehow synergize with (or add to) the signals mediated by the B cell receptor (BCR). Independent studies have shown that BCR cross-linking results in ZAP-70 activation and, ultimately, in a stronger signal (39). According to this line of thinking, CD38 might exert a coreceptorial function, further sustaining the signal mediated by the BCR. The second possibility is that the presence and functions of ZAP-70 are linked to the signaling pathways controlled by CXCR4, CXCR3, and CCR7. Chemokines are reported to significantly contribute to the delivery of growth signals to CLL cells expressing functional receptors. Indeed, results obtained in T cell models indicate that the signaling pathway driven by CXCR4 involves ZAP-70 (270, 276). In support of this view is evidence that ZAP-70⁺ CLL cells migrate better in response to CXCL12 (294).

Other results obtained using mature dendritic cells indicate that CD38 engagement ensures efficient chemotaxis in response to CCL21. Additionally, the presence of lateral associations between CD38 and the CCR7 receptor may help in fine-tuning cell motility (98). Another report shows that cADPR antagonists block chemotaxis of human monocytes to CXCR4, CCR1, and CCR5 (273).

IX. THERAPEUTIC APPLICATIONS

The peculiar pattern of cell surface expression of CD38 made it attractive for the design of therapeutic protocols driven either by cells or by monoclonal antibodies. In both cases, de novo expression of the molecule is considered a useful asset for improving the efficacy and
specificity of these models. CD38 has also been used for gene therapy approaches relying on the selective killing of tumor cells by means of oncolytic viruses.

A. CD38 as a Constitutive Target

CD38 is expressed at high epitope density by a variety of lymphoid tumors, including most cases of myeloma (225), some cases of AIDS-associated lymphoma (144), and many cases of posttransplant lymphoproliferations (109). The marked quantitative differences in cell surface expression between normal cells and their leukemic counterparts made CD38 an attractive target for immunotherapy treatment.

In 1991, the G. T. Stevenson group (Tenovus, Southampton, UK) prepared a chimeric antibody (mouse Fab × human Fc) specific for human CD38. The recombinant monoclonal antibody efficiently mediated antibody-dependent cellular cytotoxicity (ADCC) against a CD38⁺ lymphoid cell line when using human blood mononuclear cells as effectors (319). The effector functions of T and NK cells (both CD38⁺) proved not to be impaired or influenced in any apparent way. More importantly, the growth potential of erythroid and myeloid progenitors was not inhibited, furnishing possible evidence that the earliest stem cells do not express CD38 (86).

Along with attempts at using monoclonal antibodies to redirect effector cells towards tumors, scientists explored another strategy in which monoclonal antibodies are used as carriers of toxins. Myeloma had been identified early on as an appealing target for therapeutic applications. Anti-CD38 antibodies were devised to be used alone or for enriching a therapeutic armamentarium which was quite poor at that time. In 1994, V. Goldmacher (ImmunoGen, Cambridge, MA) and K. C. Anderson (Harvard Medical School, Boston, MA) (124) developed a potent immunotoxin capable of killing human myeloma and lymphoma cells, but with only limited toxicity to normal BM progenitors. Another significant finding was that normal resting peripheral blood lymphocytes were not activated by the presence of the immunotoxin.

In spite of their promising results, these early investigations did not lead to clinical applications. The molecule’s widespread distribution in lymphoid, myeloid, and epithelial cells as well as in specialized tissues and organs including the eyes caused general reluctance to use CD38 as a target in human therapy (see Table 1).

A. Bolognesi et al. (Bologna, Italy) persisted in this line of research and recently developed an immunotoxin based on an anti-CD38 monoclonal antibody (IB4) coupled to saporin-S6, a type 1 ribosome-inactivating protein. This drug was designed for ex vivo or loco-regional therapeutic applications in human myelomas and lymphomas (27). The ability of this immunotoxin to eliminate cells expressing the target was studied in vitro on selected CD38⁺ human cells and cell lines. Concentrations as low

FIG. 9. CD38 as a pathogenetic agent in CLL. Left: CD38⁺ CLL lymphocytes (white arrows) interact with nurse-like cells. Red staining: Alexa-red phalloidin. Green staining: anti-CD31 followed by FITC-conjugated goat anti-mouse IgG. Images were acquired using an Olympus 1X71 confocal microscope at ×60 magnification. Full experimental details may be found in Ref. 71. Right: schematic representation of the molecular interactions taking place between CLL lymphocytes and closed microenvironments. The biological and clinical implications of these signals are listed in the bottom part of the image.
as 100 pM of the immunotoxin completely inhibited protein synthesis. CD38\(^{+}\) neoplastic cells from non-Hodgkin lymphoma patients were totally eliminated after treatment with the immunotoxin at a concentration of 10 nM. Once again, CFU-c in culture from BM was maintained after exposure to the immunotoxin. These results indicate that IB4/saporin-S6 has strong and specific cytotoxic effects on selected CD38\(^{+}\) tumor cell lineages. On the basis of these results, it is reasonable to propose clinical use of IB4/saporin-S6 for ex vivo purging of undesired cells (e.g., for the depletion of contaminating neoplastic cells in aphereses obtained from G-CSF-treated patients) or for loco-regional therapies of CD38\(^{+}\) tumors (27). The likelihood of developing clinical applications using the immunotoxins grew even stronger after their behavior was more systematically analyzed in vivo (see summary in Ref. 199).

Further impetus for designing clinical models based on anti-CD38 reagents came from significant structural modifications of the antibodies and variations in the species used. P. Parren (Genmab, Utrecht, The Netherlands) prepared a vast panel of human anti-CD38 monoclonal antibodies raised after immunizing mice transgenic for human Ig genes. These monoclonal antibodies stained both CD38\(^{+}\) cell lines and freshly isolated myeloma cells. The reagents revealed potent ADCCs against CD38\(^{+}\) cell lines of B derivation, against clinical samples of myeloma and plasma cell leukemias (both CD38\(^{-}\)/CD138\(^{+}\)). The antibodies also elicited a significant complement-dependent cytotoxicity (CDC) against primary myeloma cell cultures isolated from a panel of 13 patients (320).

The growth of a human B lymphoma xenografted in SCID mice was strongly inhibited in both preventive and therapeutic settings. Similar effects were observed using plasma cells from human rheumatoid synovium engrafted in SCID mice. Of particular relevance for biochemists, the human monoclonal antibody from Genmab had the unique ability to inhibit the enzymatic properties exerted by cell surface CD38, a result never achieved with mouse, rat, or rabbit antibodies (320).

A team from MorphoSys (Martinsried, Germany) produced a number of fully-human antibodies selected by cell-panning strategies from a unique phage-display library (332). The affinity constants of the antibodies were in the low nanomolar range, and they efficiently stained myeloma cells by flow cytometry. In addition to the ability to kill CD38\(^{+}\) cell lines and clinical samples in ADCC tests, the antibodies did not exert negative effects on circulating leukocytes or BM progenitors. Indeed, the effects related to the exposure of anti-CD38 monoclonal antibodies in normal blood progenitors were negligible, and clonogenic potential appeared to be unaffected. The therapeutically favorable characteristics displayed in vitro were confirmed by the reduction of tumor growth in a SCID mouse xenograft model (320).

S. Holmes (Domantis, Cambridge, UK) designed a strategy of bypassing the intrinsic limitations of conventional monospecific murine monoclonal antibodies by exploiting antibody derivatives with dual specificity. The monomeric domain antibodies (dAbs) were isolated using phage display techniques by selecting independent specificities for CD38 and CD138, which are simultaneously expressed by myeloma cells. When combined, the two phage display ligands yielded a final product (dAbs) dual targeting with good avidity human myeloma cells (CD38\(^{-}\)/CD138\(^{+}\)). Cells expressing just one of the target molecules were only weakly stained or bound. The dual targeting derivative construct was also easily internalized, highlighting its potential for use as an immunotoxin (320).

B. De Novo Induced Expression of CD38 for Tumor Targeting

A growing body of data suggest that a positive therapeutic index may be generated for drugs or drug combinations by immunotherapeutic targeting of chemotherapy-induced antigens (296). The K. Mehta group (M. D. Anderson Cancer Center, Houston, TX) observed that the expression of CD38 is highly sensitive to exogenous and endogenous all-trans retinoic acid (ATRA) and derivatives and that the sensitivity is highly magnified in tumor and leukemic cells (82).

These initial observations were reevaluated from therapeutic perspectives in acute promyelocytic leukemia (APL), as well as in other myeloid leukemias. ATRA is an in vitro inducer at nanomolar concentrations of cell surface CD38 in myeloid leukemia blasts. The same reagent is a key component in clinical differentiation therapy adopted for APL cells, which are generally CD38\(^{-}\) before treatment.

The CD38 molecules expressed de novo at high epitope density may be used as therapeutic targets. Indeed, the combination of ATRA and an anti-CD38-gelonin immunotoxin induces synergistic killing of leukemia cell clones and blasts from patients (240).

Despite the good results of ATRA treatment, the drug unfortunately has clinically significant side effects in ~20% of patients. The most important is the Retinoic Acid Syndrome (RAS), a potentially fatal condition in APL patients, marked by acute respiratory distress and pulmonary edema. Autopsies of RAS patients demonstrated extensive infiltration of ATRA-differentiated myeloid cells into lungs, skin, kidney, liver, and lymph nodes. The only available treatment consists of high-dose dexamethasone, even though the mechanisms of action of the drug are still unknown.

From this, we may speculate that RAS is secondary to the aberrant interactions taking place between differentiated myeloid cells and host tissues. This may be at-
tubuted to the effects elicited by ATRA on the mechanism(s) regulating adhesion between myeloid cells and endothelium in the lungs. Indeed, ATRA treatment was shown to modulate the secretion of certain cytokines as well as the expression of different adhesion molecules, among which is CD38 (68, 108).

These effects are mediated by the retinoic acid receptor-α (RAR-α), which acts as a ligand-inducible transcription factor, while the RA response element is localized in the first intron of the CD38 gene (82, 92).

Intense cross-talk between the CD38 expressed by myeloid cells and the CD31 present on the surface of endothelial cells lining lung small vessels is likely to occur and to activate signal transduction pathways in both cell partners. This event could be the first step towards the cytokine storm which characterizes the late phases of the disease. Although it has not been described in individuals taking retinoids for other reasons, RAS cannot be attributed to retinoids alone; indeed, inorganic arsenic trioxide (As2O3 or ATO), which was recently proposed as an alternative treatment for APL patients, provokes the same syndrome in some instances. Preliminary data indicate that ATO is also a potent inducer of CD38 expression and that this compound may alter the tendency of myeloid cells to adhere to the vessel walls (S. Deaglio, unpublished observations). This may indicate that CD38 is a link between RAS induced by ATRA or by ATO.

C. CD38 as a Target for Gene Therapy

CD38 was also used for gene therapy applications using modified viruses. To guarantee therapeutic success, transfer vehicles for gene therapy must be capable of transducing target cells while avoiding bystander cells. In several instances, there is an imbalance between transduction efficiency of viral vectors and cell tropism not compatible with in vivo use. The lack of appropriate targeting has limited the potential of gene therapy for many years (reviewed in Ref. 344).

The strategy adopted by the S. J. Russell group (Mayo Clinic, Rochester, MN) was to use oncolytic viruses as anticancer drugs. These viruses propagate selectively in tumor tissue and destroy it without causing excessive damage to normal or surrounding noncancerous tissues. The group at Mayo Clinic provided an in vivo demonstration of antibody-directed tumor destruction by ad hoc retargeting of oncolytic viruses. They observed that live attenuated measles viruses of the Edmonston lineage have potent anti-tumor activity; however, they are not entirely tumor specific because of the wide distribution of their native receptors, namely, CD46 and SLAM. This limitation was overcome by retargeting the viruses by inserting single-chain antibody surface fragments; this consents full rescue and propagation of the virus and, at the same time, provides a receptor for specifically infecting the tumor cells. Viruses retargeted to tumor-selective CD38 and other surface molecules efficiently entered neoplastic cells through their respective targeted pseudoreceptors either in vitro or in vivo, but not through CD46 or SLAM. When administered intratumorally or intravenously to mice bearing human tumor xenografts expressing CD38, the targeted viruses demonstrated specific receptor-mediated anti-tumor activities (257).

Antiviral immunity remains a significant barrier to the clinical efficacy of oncolytic viruses. Nonetheless, it is reasonable to expect that this limit will eventually be overcome by new strategies for evading the immune system and the use of immunosuppressive drugs (157).

X. CONCLUDING REMARKS

The questions surrounding CD38 and CD157, and ectoenzymes in general, are fascinating and continue to prompt experiments and kindle debate. A review published more than 10 years ago by our group (227) ended with no firm conclusions as to how the two molecules actually function in vivo. Today, the situation has changed significantly: we can now start to piece together a general picture from the mosaic of information emerging from a number of different perspectives, with the ultimate aim of integrating these insights into a single, coherent view.

The first solid data are that CD38 is old and phylogenetically conserved. The human variant continues to share ~35% identity with that of a primitive sea mollusk. Of even greater note, the enzymatic activity of the molecule has been maintained intact over the entire known course of its evolution. The salient difference between mammalian CD38 and Aplysia cyclase lies in the acquisition by the former of an additional location on the cell surface. From a teleological perspective, this change may reflect increased biological complexity and the corresponding need for new functions in different locations. It would also seem to imply new role(s) for the reaction products outside of cells. Yet, whereas cADPR, ADPR, and NAADP play an essential and nonredundant role as Ca2+ mobilizing agents inside the cell (209), no clear physiological function has been identified for them in the extracellular fluids. Several models have been proposed as to how the extracellularly generated products may reach their final destination inside the cell, even though they appear quite complex (60).

Another way to look at the picture gives privilege to the substrate of the enzymatic activity. CD38 is the most important NADase in mammals, where it degrades NAD+, which is released in significant amounts during tissue inflammation and consequent cell death. The coordinated enzymatic activities of CD38 and CD157 and of a number of nucleotide-metabolizing enzymes would therefore per-
mit recovery of energetically costly products, which would otherwise go wasted. Indeed, CD38 and CD157 are part of a larger family of nucleotide-metabolizing (ecto-)enzymes sharing a common evolutionary history. The network includes CD26, CD39, CD73, and PC-1, along with P2X7 (formally not an ectoenzyme) and the related ADP ribosyl-transferases/ARTs. These molecules tend to cluster in specialized areas of the plasma membrane, creating a sort of hub for external activities, which may yield products eventually destined for internal cell use (Fig. 5). However, new roles as signaling molecules regulating life and death are emerging for each of these substrates, intermediates, final products and for the enzymes in charge of their metabolism (193). It is thus safe to conjecture that this network serves more important purposes than mere scavenging.

An alternative possibility is that the localization on the cell surface serves to inactivate CD38 and store it outside its true working environment in the cytosol. According to this view, the enzymatic functions would be superfluous when the molecule is expressed on the cell membrane, where its substrates are largely unavailable. They would become prominent, instead, when the molecule is expressed in the cytoplasmic or nuclear compartments of the cells (65).

Each of the models outlined has some merit, but they all fall short of providing a single and cohesive account of how and where the receptorial functions of CD38 integrate with its enzymatic activities.

It is the view of this Lab that the CD38 gene family represents a bridge between innate and adaptive immunity. According to this hypothesis, the enzymatic activi-

![Diagram](http://physrev.physiology.org/)

**Fig. 10.** Proposed model that integrates enzymatic and receptorial functions mediated by CD38. Details are outlined and discussed in section X.
ties would modulate crucial immune functions such as migration, defense from pathogens, cell activation, and immune synapse formation. At the same time, the enzymatic functions are regulated through lateral and frontal associations with surface molecules sharing an Ig-like domain (Fig. 10). Although still partially speculative, the hypothesis is supported by evidence from different fields.

To begin with, CD38 plays an important role in chemotaxis, which is crucial for both innate and adaptive immune responses. In fact, the most striking feature of the CD38 KO mouse is its inability to direct neutrophils to sites of infections, a typical context of innate immunity (271, 272). Moreover, data from human myeloid cells and human lymphocytes suggest that CD38 can also influence their migration into the lymph nodes where the antigen is taken up, processed, and presented, a typical context of adaptive immunity (98).

A second line of evidence derives from the link between CD38/CD157 signaling pathways and the release of defensins, which are among the most ancient and basic forms of defense against pathogens. Dating back as far as plants, defensins were later acquired by polymorphonucleates as a means of rapidly eliminating harmful external and internal agents (178). We have observed that CD38 signals in human peripheral blood leukocytes are followed by release of significant amounts of defensins (A. Funaro, E. Tibaldi, F. Saccucci and F. Malavasi, unpublished data). This may indicate that when CD38 acquired its surface expression, it connected with the most efficient defense/offense system of the cell. We are also collecting preliminary evidence along these lines from the association between CD38/CD157 and toll-like receptors.

Further substantiation for our view derives from the structural/functional relationship between CD38 and members of the Ig superfamily, first with the receptor for the Ig Fc region (FcR) and later with antigen receptors. FcRs are expressed by different cell types, the most relevant of which for our present purposes are NK cells. NK cells are the most important lineage controlling the elimination of tumor cells and exogenous pathogens and operate through an age-old system of recognition (56). In NK cells, CD38 developed a symbiosis with CD16, the low-affinity receptor for IgG. Later on in its evolution, CD38 contacts widened by acquiring lateral associations with TCR and BCR complexes. Support of the relevance of this type of cross-talk comes from recent findings that CD38 plays a prominent role in the formation of the immune synapse, the most sophisticated and well-developed sys-

---

FIG. 11. Proposed model of evolution of the CD38 gene family. The working hypothesis is that the CD38 gene family acts as a bridge connecting innate (blue) and adaptive (pink) immune systems. Mya, million years. NLR, nucleotide binding domain, leucine-rich repeat containing family; TLR, Toll-like receptors; RLH, retinoic acid inducible gene-I like RNA helicases.
tem of cell-cell interactions. Indeed, there is strong evidence that CD38 is recruited to the contact areas that are dynamically formed between T lymphocytes and antigen-presenting cells (251a). CD38 gained further complexity through the acquisition of frontal interactions with the nonsubstrate ligand CD31, another member of the Ig family (69).

Our view is also supported by evolutionary analysis of the family. It is accepted that CD38 and CD157 genes derive from duplication, even though the ancestor is unknown (91) (Fig. 11). Intuitively, one might suppose that the CD157 gene derives from CD38; however, the reverse could also be true, and CD157 could be the eldest of the family. If this were indeed the case, the soluble ancestral enzyme may have found its surface location on innate immune cells by acquiring a simplified anchor to the membrane through a lipid link. Later, CD157 could have duplicated as CD38, a modified form of the molecule with a more highly structured membrane organization and the ability to establish lateral and frontal interactions. This newer molecule may also have found expression in a wider variety of cells, including the effectors of adaptive immunity. This is a key step in our hypothetical account and would have led to significant enhancement of the overall performance of the enzyme.

Evolution is an ongoing process: reminders of this can be found in the marked differences between rodent and human CD38 in terms of tissue distribution, regulation of expression, and frontal associations. Furthermore, human (but not murine) CD38 is genetically polymorphic, while no information is currently available for CD157. Also, the stability in gene frequencies of the two CD38 alleles in the Caucasoid population, including Sardinians, may indicate that the alleles are the result of a process of natural selection that took place thousands of years ago and conferred an adaptive advantage. Indirect proof of this would come from the finding of enrichment of the rare allele in the context of selected diseases.

Finally, one must also bear in mind that the role of the CD38 family in immunological responses could be a fairly recent acquisition during evolution. The prototype enzyme is present in the ovotestis of Aplysia where it plays a role in fertilization. It is well established that cADPR from NAD$^+$ in the brain, where NAD$^+$ is as abundant as ATP is in neuronal cells (172).

The data and models presented and discussed in this review undoubtedly reflect our background, resulting in a strong immunological orientation. It is important to recognize, however, that immunity is a relative newcomer in the history of life, and the scientific community may have missed more informative clues to be found in older systems. Future research over the next few years should seek to fill this gap.

ACKNOWLEDGMENTS

Thanks are given to H. C. Lee (Minneapolis, MN and Hong Kong, China) and to Q. Hao (Ithaca, NY) for kindly providing figures about enzymatic activities and crystal structures, respectively. G. Magni (Ancona, Italy) contributed to the design of the ectoenzyme network figure. Thanks are also given to F. Land (Saranac Lake, NY) for friendly assistance.

Dr. L. McLean provided a passionate and inspired assistance in the stylistic assembly of the review.

Address for reprint requests and other correspondence: F. Malavasi, Laboratory of Immunogenetics, Dept. of Genetics, Biology, and Biochemistry, via Santena 19, 10126 Torino, Italy (e-mail: fabio.malavasi@unito.it).

GRANTS

This work was supported by grants from University of Torino (F. Malavasi, S. Deaglio, A. Funaro), Associazione Italiana Ricerca Cancro (AIRC; Milan, Italy) (F. Malavasi, S. Deaglio), Progetti di Ricerca di Interesse Nazionale (PRIN; Rome, Italy) (F. Malavasi, S. Deaglio, A. Funaro), Ricerca Sanitaria Finalizzata (Regione Piemonte, Turin, Italy) (A. Funaro), and the Chronic Lymphocytic Leukemia Global Research Foundation (CLL-GRF) (S. Deaglio) and Teleton (A. L. Horenstein).

Fondazione Internazionale Ricerca in Medicina Sperimentale (FIRMS), Compagnia di SanPaolo, Fondazione CRT, and Fondazione Cariverona provided valuable financial contributions.

T. Vaisitti is supported by a FIRC fellowship (Milan, Italy). S. Aydin is supported by Dr. Werner Jackstadt-Stiftung (Wuppertal, Germany).

S. Aydin is on a leave of absence from the Department of Hematology, University of Essen Medical School, Essen, Germany and is a member of the PhD Program "Tumor Localization," University of Torino.

REFERENCES

3. Adebanjo OA, Koval A, Moonga BS, Wu XB, Yao S, Bevis PJ, Kumegawa M, Zaidi M, Sun L. Molecular cloning, expression, and functional characterization of a novel member of the CD38...


21. Chorro J, Chen YG, Reifensnyder PC, Schott WI, Lee CH, Osborne M, Scheuplein HF, Haag F, Koch-Nolte F, Serreze DV, Leiter EH. Targeted disruption of CD38 accelerates autoimmune diabetes in NOD/Lt mice by enhancing autoimmunity in an ADP-


91. Ferrero E, Malavasi F. Human CD38, a leukocyte receptor and ectoenzyme, is a member of a novel eukaryotic gene family of nicking ribose adenine dinucleotide’-converting enzymes: extensive structural homology with the genes for murine bone marrow stromal cell antigen 1 and Aplysia ADP-ribosyl cyclase. J Immunol 159: 3858–3865, 1997.


100. Fujita T, Zawawi KH, Kurihara H, Van Dyke TE. CD38 cleavage in fMLP- and IL-8-induced chemotaxis is dependent on p38 MAP kinase but independent of p44/42 MAP kinase. Cell Signal 17: 167–175, 2005.


Huttmann A, Klein-Hitpass L, Thomale J, Deenen RJ, Carpin Hussein AM, Lee HC, Chang CF.


Kappa B220(+)CD19(+)IgM(+)CD5(+) resting B cells are required for the development of anti-inflammatory and immunosuppressive IL-10 producing regulatory T cells. Blood 95: 4191–4197, 2000.


Kikuchi Y, Yasue T, Miyake K, Kimoto M, Takatsu K. Kitanaka A, Ito C, Coustan-Smith E, Campana D.

Kitanaka A, Mano H, Conley ME, Campana D.


242. Moreno-Garcia ME, Partida-Sanchez S, Primack J, Sumoza-Toleda A, Muller-Stefferl H, Schuber F, Oppenheimer N, Lund FE, Santos-Argumedo L. CD38 is expressed as nonco-

882 MALAVASI ET AL.


