Cyclic ADP-Ribose and NAADP. Structures, Metabolism and Functions. Lee HC (ed), pp. 409-425, Dordrecht: Kluwer.

Retinoid-Mediated Signaling and CD38 Expression

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INTRODUCTION

CD38 was initially identified more than two decades back when murine monoclonal antibodies (mAbs) were being employed to define newer surface molecules expressed by the lymphocytes during various developmental stages. One such mAb (T10) was successfully employed in the analysis of distribution [1] and biochemical characterization of CD38 antigen [2]. The conclusions derived from the biochemical analysis of the molecule were independently confirmed by cloning of a cDNA for human CD38 in 1990 [3]. On the basis of its expression and allocation among immune cells, CD38 was referred to as an "activation marker" and till recently was used for phenotyping the differentiation state of lymphocytes and classifying leukemias.

In late 1992 a report described significant molecular and functional homology of CD38 to an enzyme purified from the mollusk, *Aplysia californica* [4], which rejuvenated the interest of the scientific community in this molecule. CD38 was then found to be capable of functioning as a bi-functional ectoenzyme, catalyzing the synthesis and hydrolysis of compounds involved in the regulation of intracellular calcium [5]. These findings were further extended to cells such as erythrocytes [6] and platelets [7] that were previously considered to be non-CD38 expressers, and suggested that the molecule may be ubiquitously present albeit at low levels in certain cell types or under certain conditions. The molecule was also found in mouse and in rat, where it maintained high level of homology among the species and with the *Aplysia* enzyme [5]. Because CD38 turned

out to be an ectoenzyme with multiple catalytic activities (NAD^+ glycohydrolase, ADP-ribosyl cyclase and cyclic ADP-ribose hydrolase), the biochemists working in the field raised the issue of topological paradox: how can an ectoenzyme operate in an environment where its substrate (NAD^+) is present only in trace amounts and whose product (cADPR) is used inside the cell? Immunologists, on the other hand, viewed CD38 as a cell-surface receptor with an intent that it may play a role in cell-to-cell communication by establishing contacts and communicating with neighboring cells.

The first evidence that CD38 antigen might play a role as a signaling molecule came from the observations that ligation of CD38 on peripheral blood mononuclear cells and T cell lines induced activation and proliferation signals [8]. Subsequent studies revealed that ligation of human CD38 with specific moAbs induces transcription of the cytokines IL-1, TNFα, interferon-gamma (IFNγ), and GM-CSF [9]. Studies with freshly isolated leukemia cells suggested that CD38 ligation could induce signals leading to the proliferation of leukemic cell clones [10]. These initial findings prompted the search for a natural ligand of CD38 and demonstration that CD31 (PE-CAM-1) can serve a counter-receptor for CD38 marked another important landmark in the field [11]. Proof for the receptorial nature of CD31 was revealed by its ability to reproduce the majority of the signaling events similar to those induced by agonistic anti-CD38 mAbs [12]. Thus, cross-talk between CD38 and CD31 induced increased cytosolic calcium levels, activation of the transcription factor NFκB, and release of cytokines [12].

The enzymatic and receptorial functions of CD38 are likely to influence certain biological processes by affecting cell growth, differentiation and apoptotic events. For example, the expression of CD38 during the life cycle of hematopoietic cells is considerably altered; immature precursor cells in the bone marrow have high expression, which declines as the cells differentiate to mature phenotype, and increases again on activated lymphocytes [13]. CD38 expression is also high on malignant hematopoietic cells. Moreover, the lymphocytes in neonates display much higher CD38 than do lymphocytes in adults. This distinct pattern of CD38 expression on hematopoietic cells suggests that this antigen may play a role in proliferation of immature cells and in the proliferation of certain leukemia clones [10, 14, 15]. Moreover, aberrant expression of CD38 has been linked to certain pathological conditions, such as diabetes [15-17], cancer [18-20], X-linked agammaglobulinemia, and human immunodeficiency virus infection [21]. In view of CD38's dual role in health and disease, it is imperative that we understand the molecular mechanisms that underlie the regulation of CD38 gene expression. The intent of this chapter is to inform the reader of our current understanding on transcriptional regulation of CD38. Special reference is made to retinoids (vitamin A and analogues), since they are the

most potent inducers of CD38 expression, and their mechanism of action is relatively well understood.

RETINOIDS AS MODULATORS OF TRANSCRIPTION

Table 1. tRA-induced expression of genes during granulocytic differentiation.

Target gene	RARE in the promoter	Function
CD38	Yes	Inflammation, cell growth
C/EBPα	?	Differentiation
CIP/WAF/p21	Yes	Cell cycle inhibitor
Transglutaminase	Yes	Apoptosis
CD11b	Yes	Adhesion
Myeloperoxidase	Yes	Pro-inflammatory
TRAIL	?	Apoptosis

Retinoids in general and all trans-retinoic acid (tRA) in particular, play important physiological functions by regulating organogenesis, organ homeostasis, and cell growth, differentiation and death [22]. The ability of retinoids to influence these complex biological processes resides in their ability to modulate the expression of multiple genes (Table 1). Some of these genes are expressed immediately following treatment with tRA while the others are delayed. The net effect of tRA-induced gene expression is the inhibition of cell growth and induction of differentiation or apoptosis. tRA-induced expression of the target genes is mediated through the nuclear receptors that belong to a superfamily of ligand-inducible transcription factors including steroid, vitamin D, thyroid hormone, peroxisome proliferator-activated receptor and orphan receptors, whose functions are still not known [23]. Specifically, retinoid ligands can bind and activate two major types of nuclear receptors, the retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Both, the RARs and RXRs consist of three subtyptypes (α-, β- and γ-) that are encoded by separate genes and have been discussed in great detail in a recent review [24].

Each of the RAR and RXR subtypes contains six distinct domains referred to as, A through F, and each of these domains has diverse functions (Fig. 1A). A and B domains are located at the amino terminal and contain isoform specific, ligand-independent transactivation functions, AF-1. The DNA-binding domain (domain C) is highly conserved among different retinoid receptor isoforms and binds to specific upstream nucleotide sequences (referred to as retinoic acid response element or RARE), located in the promoter region of the target genes. (Fig. 1). Ligands (retinoids) bind

to a ligand-binding domain (LBD) or E domain at the C-terminus of the receptors (Fig, 1C) that contain sequences involved in dimerization of the receptors, ligand dependent transactivation (AF-2), and translocation to the nucleus. The functions for D and F domains have not been clearly defined. RARs and RXRs mainly act as heterodimers to affect gene transcription after binding to RAREs in the promoter region of the target genes (Fig. 1B). The RARs can be transcriptionally activated by binding to either tRA or 9-cis-RA, however RXRs can be activated only by 9-cis-RA (FIG. 1C). Upon ligand binding, activated nuclear receptors that bind to RAREs found in the upstream sequences (promoters) of RA responsive genes lead to transcription of the target genes.

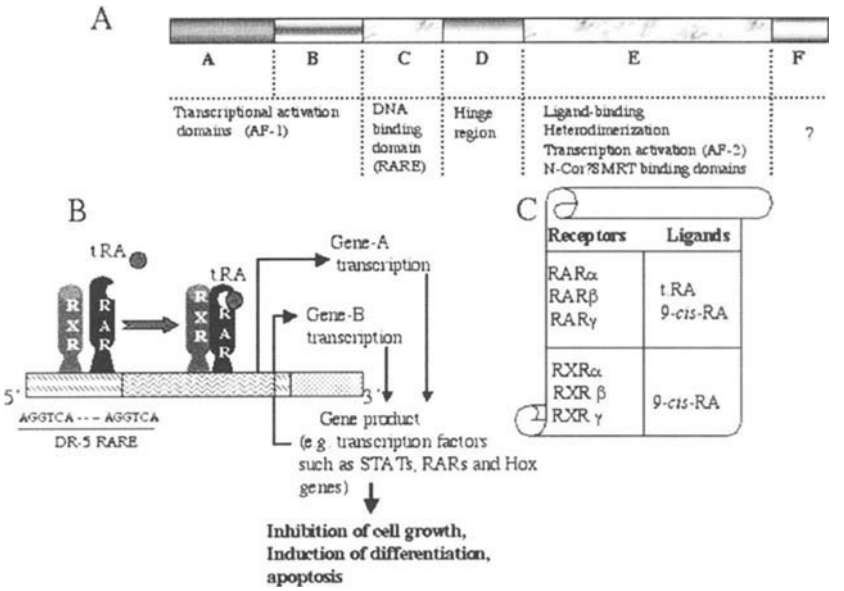

Figure 1. **Structure and functions of retinoid receptors. A**). Schematic representation of retinoid receptor depicting various functional domains. **B**). A molecular model for retinoid action. The ligated retinoid receptor forms heterodimer with RXR, binds to specific regulatory sequences (RARE) in the promoter region of a target gene A. Transactivation of gene A is a primary event of retinoid action. In an another scenario, the product of gene A can itself activate the transcription of a second gene B. Transactivation of gene B represents a secondary action of retinoid since its transcription requires the synthesis of protein A. This cascade of gene events leads to secondary and tertiary events that eventually produce a phenotype that is characteristic of retinoid action. **C**). Natural isoforms of retinoid receptors (RARs and RXRs) and their physiological ligands are listed.

The complex diversity and pleiotropic effects in tRA signaling pathway is provided not only due to existence of multiple isoforms of RA receptors but also as a result of combinations of RAR-RXR heterodimers and presence of

different ligands [24]. tRA treatment, for example, can induce the expression of RARα and RARβ genes, suggesting that tRA can modulate its own receptors, in addition to differentiation-related genes [25]. The availability of the retinoid ligands to its cognate receptors can be regulated by the level of certain non-receptor proteins, such as cytoplasmic retinoic acid binding proteins (CRABP) and heat shock proteins [26]. The formation of a RXR/RAR heterodimer is required for the high-affinity binding to RAREs that is critical for subsequent retinoid-induced transcription of target genes, such as CD38. Most RAREs have been identified in the regulatory regions of genes whose transcription is induced by retinoids. These cognate response elements consist of direct repeats (DR) of 2 core motifs in the sequence AGGTCA(X)nAGGTCA. The most common spacing observed in RAREs is 5 bp (DR5); however, RAREs containing these or similar motifs separated by 1 (DR1) or 2 bp (DR2) are also common.

As noted above, the RAR-RXR heterodimer constitutes a high affinity receptor that can bind to the specific regulatory elements of target genes. However, in the absence of ligands for RAR/RXR dimer, or in the presence of some antagonists, the receptor's target genes are repressed. This is due to the recruitment of histone deacetylase (HDAC) containing complexes that

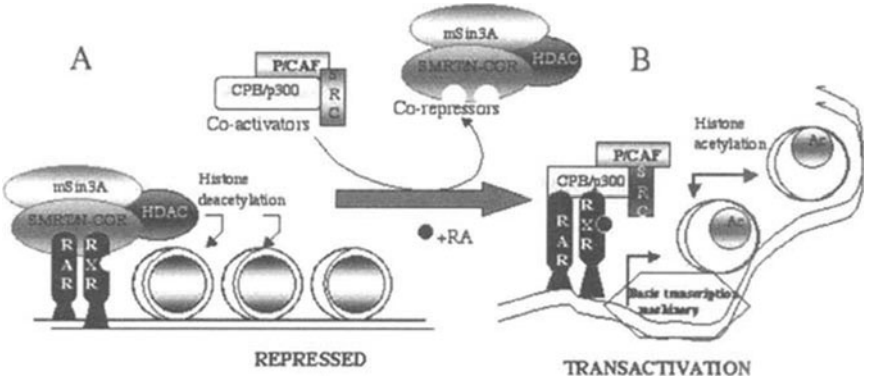

Figure 2. **Schematic representation of the basal repression and retinoid-induced transcriptional regulation of the target gene(s).** (**A**) The RAR/RXR heterodimer binds to a specific direct-repeat regulatory element that is separated by 5 bp (DR5-RARE) in the regulatory site of the target gene. In the absence of ligand (tRA), the RAR/RXR heterodimer recruits a transcriptional repressor complex that comprises N-CoR, mSin3a, SMRT, and a histone deacetylase (HDAC-1). The HDAC-1 activity helps suppress transcriptional activity. (**B**) Addition of tRA results in a conformational change in the RAR/RXR heterodimer that leads to release of the repressor complex and recruitment of a transcriptional-activator complex that has histone acetyltransferase (HAT) activity. That activity destabilizes nucleosomes and creates a permissive state for promoter activation.

are tethered through corepressors, such as the silencing mediator for retinoid and thyroid hormone receptors (SMRT) or the nuclear receptor co-repressor (NCoR) to a non-liganded RAR-RXR dimmer (Fig. 2). This process results in histone deacetylation, chromatin compaction and silencing of target-gene promoter regions. Current understanding of RAR-mediated gene transactivation is illustrated in Figure 2. The binding of RAR ligand (e.g. tRA) destabilizes the corepressor binding and induces allosteric changes in the ligand binding domain. This results in the release of the repressor-protein complex and the subsequent recruitment of transcriptional coactivator complexes (containing CREB-binding protein [CBP]/p300 or other co-activators) that contain or recruit histone acetyltransferase (HAT) [22, 27-29]. Thus, the binding of ligand to the RAR/RXR dimer converts it from a transcriptional repressor (Fig. 2A) to a transcriptional activator (Fig. 2B). Evidently, modulation of transcriptional activity involves close links between acetylation/deacetylation and stabilization/destabilization of repressive chromosomes.

Retinoid-Mediated Regulation of CD38 Expression

The expression of the CD38 gene is physiologically regulated during hematopoiesis. A significant proportion of $CD34^+$ normal bone marrow precursor cells express CD38 antigen, whereas the expression is downmodulated or even lost once these cells differentiate into mature polymorphonuclear cells [30]. The human myeloblast cell line HL-60 offers a good model for studying the regulatory mechanisms of myeloid cell differentiation. This cell line can be terminally differentiated into mature neutrophils following their treatment with tRA. tRA-differentiated HL-60 cells, like normal neutrophils, have a limited in vitro life span and can be further induced to undergo apoptosis in response to certain stimuli [31]. tRA-induced differentiation of HL-60 cells is associated with a large increase in CD38 expression. In addition to HL-60 cells, other myeloid leukemia cell lines exhibit a similar increase in CD38 expression in response to tRA treatment [32]. As early as 1982, Hemmi and Brietman [33] observed that tRA-induced differentiation of HL-60 cells was associated with an increased ability of these cells to hydrolyze NAD^+, which was later shown to result from an increase in membrane enzyme that could prevent ADP-ribosylation reactions. More recent work on these lines suggested that tRA-induced increase in NAD-hydrolase activity was due to the induction of CD38 [34-37]. tRA is quite unique and selective in its ability to induce the expression of CD38, in that at picomolar concentrations an appreciable accumulation of mRNA and protein can be observed [32]. Moreover, t-RA-induced increase in CD38 expression is rapid and was not observed in response to other differentiation-inducing agents such as dimethylsulfoxide

(DMSO), G-CSF, GM-CSF, IFN-γ, and 12-O-tetradecanoylphorbol-13-acetate (TPA) [35]. Also, tRA could modify the catalytic functions of CD38 as a result of posttranslational cross-linking of CD38 into a high-molecular-weight form, p190 [36], which was determined to be a transglutaminase-catalyzed cross-linked product of native membrane-bound CD38. The expression of transglutaminase in myeloid cells has also been suggested to be under the control of retinoids [38, 39]. These results imply that retinoids can modulate CD38 antigen expression by functioning at the transcriptional as well as posttranslational level.

Since retinoids can intercede CD38 gene transactivation via 6 different subtypes of nuclear receptors (RARα, -β, -γ or RXRα, -β, -γ), next we delineated the nature of retinoid receptor(s) involved in CD38 regulation. We used an HL-60 cell line variant (HL-60R) in which the retinoid receptor function has been abrogated as a result of trans-dominant negative mutation. HL-60R cells were resistant to tRA-induced CD38 expression. However, retrovirally mediated transduction of cDNA coding for full-length functional RARα in HL-60R cells, fully restored this ability [35]. In contrast, transduction with cDNA coding for RARβ, RARγ, or RXRα failed to restore their responsiveness to tRA. These observations suggested that RARα may play a critical role in tRA-induced expression of CD38 in HL-60 cells. To further confirm the involvement of RARα, we used several synthetic

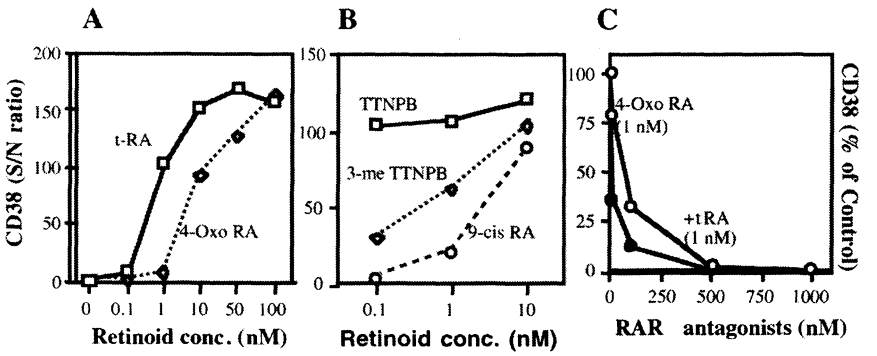

Figure 3. **RARα-selective driven expression of CD38 in myeloid leukemia HL-60 cells.** The ability of a particular retinoid to induce the expression of CD38 is dependent on its affinity for RARα. For example, tRA is about 10 times more active than 4-oxo RA (A). Similarly, the synthetic retinoid TTNB that has much higher affinity for RARs than for RXRs is more potent inducer of CD38 expression (B). Conversely, 3-methyl-TTNB has higher affinity towards RXRs than RARs and accordingly is less active in inducing CD38 expression (B). Similarly, 9-cis RA, a panagonist that can bind and activate all the RARs and RXRs, was least active (B). Interestingly, tRA and 4-oxo-RA that bind all three isoforms of RARs (α, β, and γ), were completely inactive when co-cultured in the presence of RARα selective antagonist (C), suggesting that retinoid-mediated expression of CD38 is completely dependent on RARα nuclear receptors.

retinoid analogues that selectively bind to and activate one of the six retinoid receptors. Indeed, the RARα-selective retinoids were highly potent inducers of CD38 expression (Figs. 3A and B; [40]); RARβ, -γ or RXRs-selective retinoids were relatively inactive in this regard. Moreover, RARα-specific antagonists, which bind but do not transcriptionally activate the receptor, completely blocked tRA-induced expression of CD38 in HL-60 cells (Fig. 3C). The significance of RARα in regulating CD38 gene expression was further confirmed in a study of RARα-antisense transgenic mice [37]. In these mice, tissues that expressed an antisense construct of RARα produced little or no CD38 and expressed 50-80% less RARα protein than did the same tissues from normal control mice. These results suggested that regulation of CD38 antigen, both in vitro and in vivo, is under the direct control of RARα subtype of nuclear receptors.

The low basal expression of CD38 antigen on APL blasts provides further evidence for the involvement of RARα in regulation of CD38 expression. In the t(15;17) variant of APL, the RARα locus is fused to that of the PML gene [25, 27], which results in the production of polymorphonuclear cells with abnormal granulation and CD38 expression. The basal expression of CD38 antigen on blast cells from patients with APL is very low (signal to noise ratio, 2.8 + 0.4) compared to the blasts cells from non-APL patients (S/N ratio, 58.6 + 8.2), which harbor full-length functional RARα. Treatment of APL cells with tRA, in vitro or in vivo, however, strongly induces CD38 expression [32, 35], suggesting that defective RARα-signaling pathways may attenuate basal CD38 expression in APL cells.

Retinoid-induced expression of CD38 protein depends on transcriptional regulation of the gene. Thus, accumulation of CD38 mRNA transcript in tRA-treated HL-60 cells could be blocked by actinomycin D but not by cyclohexamide. In an attempt to locate the RARE, Katada's group in Japan isolated and sequenced the genomic DNA ranging from the 5'-flanking region of CD38 gene to exon 1 and partial intron 1. Transient transfection experiments revealed that the responsiveness to tRA was conferred through a DR5 element consisting of 2 direct-repeat TGACCT-like hexamer motifs located at base pairs 437 to 453 of the first intron rather than in the 5'-upstream region of the CD38 gene [41]. On gel shift assays, the DR5 element interacted with a heterodimer composed of RAR and RXR. These results provided direct evidence that tRA-induced expression of CD38 is mediated by direct transcriptional regulation via activation of a RAR/RXR heterodimer through a RARE located in the first intron of the CD38 gene.

As discussed earlier in this chapter, retinoid receptors like other nuclear receptors, can regulate the basal and ligand-induced gene expression by interacting with a select group of proteins called corepressors and coactivators (Fig. 3). The 2 transcriptional corepressors that interact with the

RAR/RXR dimer include the 270-kDa N-CoR protein and the 168-kDa SMRT protein. Both of these corepressors can interact directly with retinoid receptors as well as with another corepressor, Sin3A. Furthermore, histone deacetylase (HDAC1) has been found to interact with Sin3A and SMRT to form a multisubunit repressor complex at the regulatory site of the target gene, which suppress basal transcriptional activity (Fig. 2A). Binding with the tRA ligand produces conformational changes in the RAR/RXR complex that result in the release of the repressor complex and the recruitment of another set of auxiliary proteins called transcriptional coactivators (Fig. 2B). Coactivators such as CBP (CREB-binding protein) or its homologue p300 either have intrinsic HAT activity or can associate with other HATs such as p/CAF. Recruitment of HATs to the DNA thus locally destabilizes nucleosomes, creating a permissive state for promoter activation. To gain insight into the possible role of HDAC in retinoid-dependent transcription of the CD38 gene, we studied the effect of trichostatin A (TSA), a specific inhibitor of HDAC, on HL-60 cells. TSA induced the expression of CD38 transcripts in these cells [40], suggesting that the CD38 gene in its basal state can stay silent due to compaction of the DNA caused by HDAC-catalyzed deacetylation.

Moreover, the 5' flanking region of the CD38 gene contains GC-rich sequences without classic TATA or CCAAT boxes [42], a feature that is usually shared by noninducible housekeeping genes [43]. Furthermore, in the upstream region (promoter) of the CD38 gene, potential binding sites for some common transcription factors, such as PEA-3, CP-2, and PuF have been identified [42]. Sites for T-cell transcription factor 1-alpha (TCF-1α), nuclear factor interleukin-6 (NFIL-6), and interferon responsive factor 1 (IRF-1) have also been identified [42]. The presence of an estrogen response half-palindromic element and a glucocorticoid response element suggests that the CD38 gene may also be hormonally regulated. Similarly, the 5' end of the human CD38 gene consists of a CpG-rich island. This island is approximately 900 bp long and encompasses exon 1 and the 5' end of intron 1. Within the intronic portion of the island, there are two binding sites for the SP1 transcription factor [42]. The functional significance of CpG island is that hypermethylation of this island may silence the expression of the gene. CD38 is likely to depend on the methylation or demethylation status of these regulatory sequences. Methylation of the CpG island generally represses gene expression, whereas demethylation induces it [34]. All these observations suggest the complex nature of CD38 gene regulation that may affect its expression during various physiological and pathological conditions.

Other Modulators of CD38 Expression

Table 2 lists some physiological and pharmacological agents that have been shown to regulate the expression of CD38. For example, cytokines, such as interferon-γ (IFN-γ) and IFN-α are known to induce CD38 expression in resting mature B cells and myeloma cells. In one study, IFN-α could upregulate CD38 expression on activated B cells, monocytes and the monocytic leukemia cell line THP-1 [44], but other cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, granulocyte-macrophage colony stimulating factor (GM-CSF), and tumor necrosis factor alpha [TNF-α]) were completely ineffective. Similarly, up-regulation of CD38 was observed in monocytes and monocytic cell lines in response to IFN-α treatment whereas TNF-α, GM-CSG, and lipopolysaccharide were completely ineffective [45]. In support to these findings is the observation that the regulatory region of CD38 gene contains an interferon-responsive element [42].

Table 2. Agents known to induce CD38 antigen expression

Retinoids
HL-60 cell line
KG-1 cell line
$CD34^+$ progenitors
Myeloid leukemia cells (APL, AML)
Myeloma cell line
Lymphoma cell line
T- and B-cells
Vitamin D3
T- and B-cells
HL-60 cell line
Interferons
Activated T- and B-cells
Myeloma cell lines
$CD34^+$ progenitors
Monocytic cell lines and monocytes
Cyclic-AMP
T-cells
Promonocytic cell lines
Rotenone and arsenic tetraoxide
HL-60 cell line

A metabolite of vitamin D, vitamin D3 (1-25 α-dihydroxyvitamin D3) is known to induce monocytic differentiation in the human myeloblastic leukemia cell line HL-60. Like retinoids, vitamin D also exerts its biological effects by interacting and activating a distinct class of nuclear receptor, called vitamin D receptor (VDR). In one study, vitamin D3 induced CD38 expression in HL-60 cells as well as in mature tonsillar B cells and peripheral T cells [44]. Similarly, rotenone, a mitochondrial NADH dehydrogenase inhibitor, can also induce expression of CD38 in HL-60 cells [46]. β-estradiol, an archetypal steroid hormone, has been shown to induce ADP ribosyl-cyclase activity in its target organ, the uterus, and thus to enhance the synthesis of cADPR from NAD^+ [47]. However, it is not known whether the β-estradiol-induced increase in cyclase activity is due to an increase in CD38 expression. Nevertheless, the genomic action of estrogens could be augmented by the release of calcium triggered by cADPR, which would enhance calcium-dependent contractile responses of myometrial smooth muscle cells as well as activate the calcium- dependent phosphorylation of nuclear transcription factors. Other ligands that act on nuclear receptors, namely tRA and triiodothyronine, could also induce ADPR-cyclase activity in renal cell carcinoma cell lines without an increase in CD38 protein expression.

CLINICAL IMPLICATIONS OF RETINOID-INDUCED CD38 EXPRESSION

Retinoid-Induced CD38 Expression and Retinoic Acid Syndrome

The clinical outcome of acute promyelocytic leukemia (APL) has been dramatically altered by the discovery of the striking differentiating potential of tRA. tRA treatment induces complete remission in >90% of APL patients. Although tRA therapy is well tolerated by patients, two problems frequently occur. The first problem is leukocytosis, reflecting an increase in peripheral leukocytes to >20,000 cells/mm3 that occurs in about half of the APL patients treated with tRA. The second, more serious problem is a condition called, retinoic acid syndrome (RA syndrome), which develops in about 20 - 30% APL patients treated with tRA. Early signs of this problem are characterized by fluid retention, weight gain, hectic fever and musculoskeletal pain. Later signs include progressive respiratory distress, pulmonary infiltrates, pleural effusions, renal insufficiency, skin infiltrates, hypotension and death [25]. It is postulated that RA syndrome is secondary to the aberrant interactions taking place between differentiating APL cells and host tissues. Indeed, the autopsies of patients who died of the syndrome have revealed extensive organ infiltration by differentiating APL cells.

Because the clinical signs of RA syndrome closely resemble those of endotoxin shock and acute respiratory distress syndrome (ARDS), it has been suggested that tRA treatment may affect in some way the cytokine expression by differentiating granulocytes. Although the pathobiology of ARDS is poorly understood, several observations suggest an important role for interaction between polymorphonuclear neutrophils (PMN) and cytokines in this process. High levels of IL-1, TNF-α, IL-8, IL-6, and macrophage inflammatory protein-2 (MIP-2) have been found in plasma and in bronchoalveolar lavage supernatant of patients with ARDS [48]. Induction of IL-1 and granulocyte-colony stimulating factor (G-CSF) like molecules under tRA influence may contribute towards hyperleukocytosis. Whereas, secretion of IL-6, IL-8, and TNF-α like molecules, all of which can activate leukocytes and are implicated in the development of ARDS, can induce lung injury observed in RA syndrome-related pathogenesis. Since RA syndrome occurs only in patients with APL who have been treated with tRA, this syndrome is considered to reflect an aberrant interaction between maturing granulocytes and host tissues.

As discussed in the previous section, tRA-treated APL cells express large amounts of CD38 antigen; it is therefore likely that maturing APL cells in

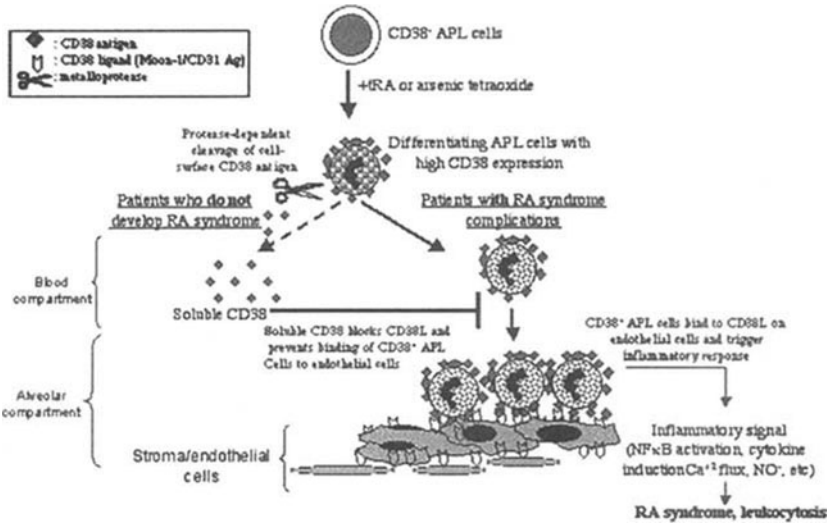

Figure 4: **Hypothetical model for RA-induced CD38 antigen's involvement in RA syndrome.**

tRA treated patients interact with CD31 antigen on the lung endothelial cells and trigger inflammatory signals. Human lung endothelial cells express high basal levels of CD31, a cell-surface protein that serves as a ligand for CD38

[11]. In support to this contention, our results suggested that tRA-treated leukemia cells accumulate large amounts of transcripts for inflammatory cytokines such as TNFα, IL-1β, IL-6, and IL-8 after co-culture with human lung endothelial cells. These cytokines and possibly other mediators produced in the alveolar interstitium may contribute to the pathogenesis of RAS. Possible CD38-mediated events that might lead to the development of RA syndrome are illustrated in Figure 4. Further delineation of these pathways may yield important benefits in designing effective strategies to control not only RA syndrome pathogenesis but also for evaluating the therapeutic superiority of tRA in combination with other chemotherapeutic agents, given tRA's ability to downregulate Bcl2 and perhaps other proteins that inhibit chemotherapy-induced apoptosis.

Retinoid-Induced CD38 as Target for Immunotherapy

The use of monoclonal antibodies for delivering drugs or toxins to molecular structures expressed on the surfaces of tumor cells represents an attractive and potentially useful strategy. Theoretically, such a targeted approach to cancer therapy could selectively eliminate tumor cells while reducing the toxicity of the treatment to normal, non-target tissues. Nevertheless, in practice many problems exist that need to be addressed before immunotoxin or antibody-drug therapies become practical. One limitation to the success of such targeted therapy is the heterogeneity of target antigen expression within a population of tumor cells. If a small number of cells within a tumor were negative for the target antigen or expressed the antigen only weakly, then these cells would escape destruction from the antibody-mediated delivery of an agent that is cytotoxic to those cells. One possible means of overcoming this problem would be to identify agents that induce high levels of cell surface target molecules, in the expectation that target tumor cells that were antigen-negative could be induced to express those target molecules in abundance. We have exploited the ability of tRA to induce high levels of CD38 antigen on leukemia cells and then used this antigen as a target for delivering the anti-CD38-conjugated plant toxin gelonin. Our preliminary results indicate that tRA treatment of leukemia cells, even at sub-nanomolar concentrations made those cells exquisitely sensitive to immunotoxin-induced killing. Co-culture of leukemia cells with as little as 0.1 nM tRA in the presence of picomolar concentrations of the immunotoxin killed more than 90% of the leukemia cells. In contrast, similar concentrations of the immunotoxin without tRA killed only 40-50% of the leukemia cells (Fig. 5). Since tRA is currently being used in the clinic for treating APL patients, its combination with anti-CD38-based therapies may offer new exciting opportunities for the treatment of certain leukemias.

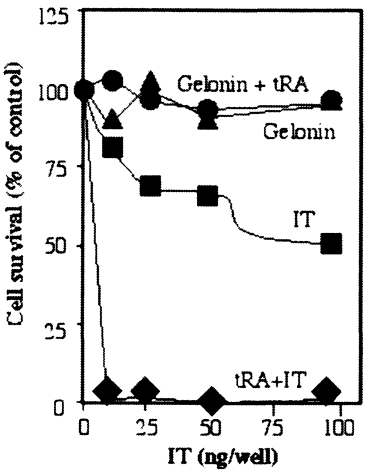

Figure 5. **tRA-induced CD38 expression augments anti-CD38-based delivery of the plant toxin (gelonin) to leukemia (HL-60) cells.** HL-60 cells were incubated with gelonin alone, gelonin plus tRA (5 nM), anti-CD38-conjugated gelonin (IT) or IT plus tRA for 48 h at 37^0 C. The cells surviving under various culture conditions were determined by MTT assay. As a control, the simultaneous presence of unconjugated anti-CD38 antibody (100-fold excess) completely abrogated IT-induced cytotoxicity in the presence of tRA (not shown).

CONCLUSION

CD38 has emerged out as a multifunctional protein from a simple cell-surface differentiation antigen title. Originally identified as a differentiation marker on hematopoietic cells, CD38 is now known to be present on nonhematopoietic tissues as well. The best-characterized feature of the CD38 antigen is its ability to catalyze the synthesis of cADPR, which serves as an important second messenger in regulating calcium release from intracellular pools. By virtue of its ability to synthesize cADPR as well as to serve as a signal transduction molecule, CD38 may participate in physiological processes as diverse as cell growth, apoptosis, differentiation, and inflammation. Equally interesting is the decline in CD38 expression on hematopoeitic cells as they mature and on normal prostate epithelial cells as they become cancerous. Little information is available on the factors that regulate the expression of CD38. In this chapter, I have attempted to summarize current knowledge with special reference on retinoic acid, which to my knowledge is the most well-characterized and potent positive regulators of CD38 expression. The retinoid-induced expression of CD38 is mediated via selective activation of RARα. The tRA-induced upregulation of CD38 in APL cells can be considered pathologic and may contribute to the development of RA syndrome and leukocytosis like conditions that frequently occur and are often fatal in patients treated with tRA. However, tRA-induced CD38 expression may offer new means for treating certain leukemias in combination with anti-CD38-based therapies.

ACKNOWLEDGMENTS

The work in author's laboratory was supported in part by grants from the U.S. Food and Drug Administration (FDR-000923) and Department of Defense (DAMD 17-1-1-0645). I wish to thank all the research colleagues working in my laboratory whose dedicated efforts made this publication possible. Also, I would like to acknowledge the invaluable help of several collaborators who made the reagents available for these studies.

REFRENCES

1. Reinherz EL, Kung PC, Goldstein G, Levey RH and Schlossman SF. 1980. Discrete stages of intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T lineage. *Proc. Natl. Acad. Sci. USA* 77: 1588-1592.
2. Terhorst C, van Agthoven A, Le Clair K, Snow P, Reinherz EL and Schlossman SF. 1981. Biochemical studies of the human thymocyte cell-surface antigens T6, T9 and T10. *Cell* 23: 771-778.
3. Jackson D and Bell JI. 1990. Isolation of a cDNA encoding the human CD38 (T10) molecule, a cell surface glycoprotein with unusual discontinuous pattern of expression during lymphocyte differentiation. *J. Immunol.* 144: 2811-2817.
4. States DJ, Walseth TF and Lee HC. 1992. Similarities in amino acid sequences of *Aplysia* ADP ribosyl cyclase and human lymphocyte antigen CD38. *Trends Biochem. Sci.* 17: 495.
5. Howard M, Grimaldi JC, Bazan JF, Lund FE, Santos-Argumedo L, Parkhouse RM, Walseth TF and Lee HC. 1993. Formation and hydrolysis of cyclic ADP ribose by lymphocyte antigen CD38. *Science* 262: 1056-1059.
6. Zocchi E, Franco L, Guida L, Benatti U, Bargellesi A, Malavasi F, Lee HC and De Flora F. A single protein immunologically identified as CD38 displays NAD^+-glycohydrolase, ADP ribosyl cyclase and cyclic ADP-ribose hydrolase activities in the auter surface of human erythrocytes. *Biochem. Biophys. Res. Commun.* 196: 1459-1465.
7. Ramaschi G, Torti M, Tolnai Festetics E, Sinigaglia F, Malavasi F and Balduini C. 1996. Expression of cyclic ADP-ribose-synthetizing CD38 molecule of human platelet membrane. *Blood* 87: 2308-2313.
8. Dianzani U, Funaro A, DiFranco D, Garbarino G, Bragardo M, Redoglia V, Buonfiglio D, DeMonte LB, Pileri A and Malavasi F. 1994. Interaction between endothelium and $CD4^+CD45RA^+$ lymphocytes. *J. Immunol.* 22: 952-959.
9. Ausiello CM, Urbani F, LaSala A, Funaro A and Malavasi F. 1995. CD38 ligation induces discrete cytokine mRNA expression in human cultured lymphocytes. *Eur. J. Immunol.* 25: 1477-1480.
10. Konopleva M, Estrov Z, Zhao S, Andreeff M and Mehta K. 1998. Ligation of cell surface CD38 protein with agonistic monoclonal antibody induces a cell growth signal in myeloid leukemia cells. *J. Immunol.* 161: 4702-4708.
11. Deaglio S, Morra M, Mallone R, Ausiello CM, Prager E, Garbarino G, Dianzani U, Stockinger H and Malavasi F. 1998. Human CD38 (ADP ribosyl cyclase) is a counter-receptor of CD31, an Ig superfamily member. *J. Immunol.* 160: 395-402.
12. Deaglio S, Mehta K and Malavasi F. 2001. Human CD38: a (r)evolutionary story of enzyme and receptor. *Leuk. Res.* 25: 1-12.
13. Funaro A, Ferrero E, Mehta K and Malavasi F. 2000. Schematic portrait of CD38 and related molecules. *Chem. Immunol.* 75: 256-273.
14. Podesta M, Zocchi E, Pitto A, Usai C, Franco L, Bruzzone S, Guida L, Bacigalupo A, Scadden DT, Walseth TF and De Flora A, Daga A. 2000. Extracellular cyclic ADP-ribose increases intracellular free calcium and stimulates proliferation of human hematopoietic progenitors. *FASEB Lett.* 14: 680-690.

15. Okamoto H, Takasawa S, Nata K, Tohgo A and Noguchi N. 2000. Physiological and pathological significance of the CD38-cyclic ADP-ribose signaling system. *Chem. Immunol.* 75: 122-145.
16. Antonelli A, Baj G, Marchetti P, Fallahi P, Surico N, Pupilli C, Malavasi F and Ferrannini E. 2001. Human anti-CD38 autoantibodies raise intracellular calcium and stimulate insulin release in human pancreatic islets. *Diabetes* 50: 985-995.
17. An NH, Han MK, Um C, Park BH, Park BJ, Kim HK and Kim UH. 2001. Significance of ecto-cyclase activity of CD38 in insulin secretion of mouse pancreatic islet cells. *Biochem. Biophys. Res. Commun.* 282: 781-786.
18. Ibrahim S, Keating M, Do KA, O'Brien S, Huh YO, Jilani I, Lerner S, Kantarjian HM and Albitar M. 2001. CD38 expression as an important prognostic factor in B-cell chronic lymphocytic leukemia. *Blood* 98: 181-186.
19. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, Buchbinder A, Budman D, Dittmar K, Kolitz J, Litchman SM, Schulman P, Vinciguerra VP, Rai KR, Ferrarini, M and Chiorazzi N. 1999. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood* 97: 1840-1847.
20. Kramer G, Steiner G, Fodinger D, Fiebiger E, Rappersberger C, Binder S, Hofbauer J and Marberger M. 1995. High expression of a CD38-like molecule in normal prostatic epithelium and its differential loss in benign and malignant disease. *J. Urol.* 154: 1636-1641.
21. Bofill M and Borthwick NJ. 2000. CD38 in health and disease. *Chem. Immunol.* 75: 218-234.
22. Altucci L and Gronemeyer H. 2001. The promise of retinoids to fight against cancer. *Nature Rev.* 1: 181-193.
23. Collinwood TN, Urnov FD and Wolffe AP. 1999. Nuclear receptors: coactivators, corepressors and chromatin remodeling in the control of transcription. *J. Mol. Edocrinol.* 23: 255-275.
24. Chambon P. 1996. A decade of molecular biology of retinoic acid receptors. *FASEB J.* 10: 940-954.
25. Melnick A and Licht JD. 1999. Deconstructing a disease. RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood* 93: 3167-3215.
26. Boylan JF and Gudas LJ. 1991. Overexpression of the CRABP-1 results in a reduction in differentiation specific gene expression in F9 teratocarcinoma cells. *J. Cell Biol.* 112:965-979.
27. Ozpolat B, Lopez-Berestein G and Mehta K. 2001. ATRA(ouble) in the treatment of acute promyelocytic leukemia cells. *J. Biol. Regul. Homeost. Agents* 15: 107-122.
28. Glass CK, Rose DW and Rosenfeld MG. 1997. Nucelar receptors coactivators. *Curr. Opin. Cell Biol.* 9: 222-232.
29. Collingwood TN, Urnov FD and Wolffe AP. 1999. Nucelar receptors: coactivators, corepressors and chromatin remodeling in the control of transcription. *J. Mol. Endorinol.* 23: 255-275.
30. Malavasi F, Funaro A, Rogerro S, Horenstein A, Calosso L and Mehta K. 1994. CD38: A glycoprotein in search of a function. *Immunol. Today* 15: 95-97.
31. Mehta K, McQueen T, Neamati N, Collins S and Andreeff M. 1996. Activation of retinoid receptors RAR alpha and RXR alpha induces differentiation and apoptosis, respectively, in HL-60 cells. *Cell Growth Diffren.* 7: 179-186.
32. Drach J, Zhao S, Malavasi F and Mehta K. 1993. Rapid induction of CD38 antigen on myeloid leukemia cells by all trans-retinoic acid. *Biochem. Biophys. Res. Commun.* 195: 545-550.
33. Hemmi H and Brietman TR. 1982. Induction by retinoic acid of NAD-glycohydrolase activity of myelomonocytic cell lines HL-60, THP-1 and U937, and fresh human acute promyelocytic leukemia cells in primary culture. *Biochem. Biophys. Res. Commun.* 109: 669-674.

34. Kontani K, Nishina H, Ohoka Y, Takahashi K and Katada T. 1993. NAD-glycohydrolase specifically induced by retinoic acid in human leukemic HL-60 cells. Identification of the NAD-glycohydrolase as leukocyte cell surface antigen CD38. *J. Biol. Chem.* 268: 16895-16898.
35. Drach J, McQueen T, Engel H, Andreeff M, Robertson K, Collins SJ, Malavasi F and Mehta K. 1994. Retinoic acid-induced expression of CD38 antigen in myeloid cells is mediated through retinoic acid receptor-alpha. *Cancer Res.* 54: 1746-1752.
36. Umar S, Malavasi F and Mehta K. 1996. Post-translational modification of CD38 protein into a high molecular weight form alters its catalytic properties. *J. Biol. Chem.* 271: 15922-15927.
37. Mehta K, McQueen T, Manshouri T, Andreeff M, Collins S and Albitar M. 1997. Involvement of retinoic acid receptor-alpha-mediated signaling pathway in induction of CD38 cell-surface antigen. *Blood* 89: 3607-3614.
38. Mehta K and Berestein GL. 1986. Expression of tissue transglutaminase in cultured monocytic leukemia (THP-1) cells during differentiation. *Cancer Res.* 46:1388-1394.
39. Davies PJA, Murtaugh MP, Moore WT, Johnson GS and Lucas D. 1985. Retinoic acid-induced expression of tissue transglutaminase in human promyelocytic leukemia (HL-60) cells. *J. Biol. Chem.* 260: 5166-5174.
40. Mehta K. 2000. Retinoid-mediated signaling in CD38 antigen expression. *Chem. Immunol.* 75:20-38.
41. Kishimoto H, Hoshino S, Ohori M, Kontani K, Nishina H, Suzawa M, Kato S and Katada T. 1997. Molecular mechanism of human CD38 gene expression by retinoic acid. *J. Biol. Chem.* 273:15429-14434.
42. Ferrero E, Sccucci F and Malavasi F. 2000. The making of leukocyte receptor: origin, genes and regulation of human CD38 and related molecules. *Chem. Immunol.* 75:1-19.
43. Ernst P and Smale ST. 1995. Combinatorila regulation of transcription: general aspects of transcriptional control. *Immunity* 2: 311-319.
44. Stoeckler JD, Stoeckler HA, Kouttab N and Maizel AL. 1996. Dihydroxyvitamin D3 modulates CD38 expression on human lymphocytes. *J. Immunol.* 157: 4908-4917.
45. Musso T, Deaglio S, Franco L, Calosso L, Badolato R, Garbarino G, Dianzani U and Malavasi F. 2001. CD38 expression and functional activities are upregulated by IFN-γ on human monocytes and monocytic cell lines. *Leuk. Biol.* 69: 605-612.
46. Matsunaga T, Kudo J, Takahashi K, Dohmen K, Hayashida K, Okamura S, Ishibashi H and Niho Y. 1996. Rotenone, a mitochondrial NADH dehydrogenase inhibitor, induces cell surface expression of CD13 and CD38 and apoptosis in HL-60 cells. *Leuk. Lympho.* 20: 487-494.
47. Chini EN, de Toledo FG, Thompson MA and Dousa TP. 1997. Effect of estrogen upon cyclic ADP ribose metabolism: β-estradiol stimulates ADP ribosyl cyclase in rat uterus. *Proc. Natl. Acad. Sci. USA* 94: 5872-5876.
48. Abraham E, Carmody A, Shenkar R, Arcaroli J. 2000. Neutrophils as early immunologic factors in hemorrhage-induced acute lung injury. *Am. J. Physiol.* 279: 1137-1145.

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