All-*trans* Retinoic Acid Upregulates Reduced CD38 Transcription in Lymphoblastoid Cell Lines from Autism Spectrum Disorder

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Deficits in social behavior in mice lacking the *CD38* gene have been attributed to impaired secretion of oxytocin. In humans, similar deficits in social behavior are associated with autistic spectrum disorder (ASD), for which genetic variants of *CD38* have been pinpointed as provisional risk factors. We sought to explore, in an *in vitro* model, the feasibility of the theory that restoring the level of CD38 in ASD patients could be of potential clinical benefit. *CD38* transcription is highly sensitive to several cytokines and vitamins. One of these, all-*trans* retinoic acid (ATRA), a known inducer of *CD38*, was added during cell culture and tested on a large sample of N = 120 lymphoblastoid cell (LBC) lines from ASD patients and their parents. Analysis of *CD38* mRNA levels shows that ATRA has an upmodulatory potential on LBC derived from ASD patients as well as from their parents. The next crucial issue addressed in our study was the relationship between levels of *CD38* expression and psychological parameters. The results obtained indicate a positive correlation between *CD38* expression levels and patient scores on the Vineland Adaptive Behavior Scale. In addition, analysis of the role of genetic polymorphisms in the dynamics of the molecule revealed that the genotype of a single-nucleotide polymorphism (rs6449182; C>G variation) in the CpG island of intron 1, harboring the retinoic-acid response element, exerts differential roles in *CD38* expression in ASD and in parental LBC. In conclusion, our results provide an empirical basis for the development of a pharmacological ASD treatment strategy based on retinoids.

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INTRODUCTION

Autism is a complex neurodevelopmental disorder characterized by high heritability and considerable genetic and clinical heterogeneity (1). The behavioral phenotype is distinguished by impairment in social interaction and communication, as well as restricted and stereotyped behaviors and interests (2). Translational evidence based on the function of oxytocin (OT) in contributing to affiliative behavior in the vole has prompted investigations of peptidergic hormones in human social behavior, including autism (3–5). Some, but not all, genetic association studies have suggested that the oxytocin receptor (6–10) contributes vulnerability to autistic spectrum disorder (ASD). Interestingly, plasma measurements of OT showed that there are significantly lower plasma OT levels in ASD-affected individuals than in nonaffected subjects (11). In addition, OT can be administered intranasally and enhances affiliative behaviors such as the trust game (12) and parochial altruism (13) as well as improving social cognition (14,15), for example, reading of the Eyes in the Mind test. We have shown that intranasal OT enhances electroencephalogram (EEG) mu suppression, an index of mirror neuron activity in nonclinical subjects (16) that shows deficits in ASD (17). The results of all these studies suggest that social deficits characteristic of autism (7,18) may be related to dysfunctional OT peptidergic neurotransmission.

Recently, experiments with a mouse knockout of the *CD38* gene demonstrated the role of this transmembrane glycoprotein, with ADP-ribosyl cyclase activity, in the mediation of OT release in

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the brain (19). *CD38* knockout females are characterized by marked defects in maternal nurturing and social behavior that can be restored by OT administration. CD38 catalyses the formation of Ca^{2+} signaling molecules and is a crucial factor in the chain linking nucleotides, ectoenzymes and the endocrine system (20). Notably, *CD38* is highly expressed in the human hypothalamus (21), and is colocalized with oxytocinergic neuronal structures, the apparent source of OT in the brain (22). Moreover, two independent groups have demonstrated associations between *CD38* single-nucleotide polymorphisms (SNPs) and ASD (21,23). Our own first study also showed preliminary evidence for reduced expression of *CD38* in lymphoblasts from ASD subjects compared with parental lymphoblastoid cell (LBC) lines (23). Importantly, a CD38 deficit in ASD is hypothesized to be an upstream site of further dysfunction in OT peptidergic transmission in this disorder (4,24–27). Presumably, dysfunctional CD38 activity could partially explain the positive results recently obtained with intranasal OT administration in ASD (28–30).

The suggestion that CD38 plays a role in the etiology of ASD via modulation of central availability of OT should be represented by phenotypic differences in behavior, which correlate to basal expression in ASD probands. To test for this notion, we investigated the correlation between social skills and IQ in the ASD subjects and basal gene expression. Next, we aimed to demonstrate *in vitro* that the reduced *CD38* expression observed in ASD might be correctible by treatment with all-*trans* retinoic acid (ATRA), a potent inducer of this gene (31). Demonstration of this effect of ATRA would lay out a novel therapeutic approach in ASD and provide proof of principle for future clinical trials of ATRA in ASD therapy. We examined *CD38* expression in 42 LBC lines from ASD patients as well as their parental cell lines and treated all cell cultures with ATRA for 48 h. In addition, we examined the relationship between a reportedly functional *CD38* SNP variant (32) and expression in these cells.

MATERIALS AND METHODS

Participants

Forty-two probands with diagnosed autistic disorder (30 males, 12 females) and their parents were recruited through treatment centers, special schools, the Israeli National Organization for Children with Autism, and by acquaintance with other families who participated. Probands were between the age of 2 years and 1 month to 33 years and 8 months. None of the subjects were known (according to the parents' reports) to have chromosomal aberrations, tuberous sclerosis, or other medical complications that could be causally related to autism. The ethics committee of the Israeli Health Ministry approved this study, and written informed consent was obtained from participating subjects or with parental consent.

Many of the probands had a previous diagnosis within the autism spectrum made by independent clinicians. However, for the purpose of this study, two trained clinicians confirmed the probands' diagnosis of autistic disorder or pervasive developmental disorder–not otherwise specified (PDD-NOS). All probands were diagnosed by use of the Autism Diagnostic Observation Scale–Generic (33) and the Autism Diagnostic Interview–Revised (ADI-R) (34). The ADI-R is a standardized, semistructured interview based on the International Classification of Diseases–10 definition of autism (World Health Organization, 1993). Three areas of child functioning are assessed by this parent interview: communication and language; reciprocal social interaction; and repetitive, restrictive, and stereotyped behavior. To receive a diagnosis of autistic disorder, the child must meet the cut-off criteria for autism on each of the three aforementioned areas and show evidence for developmental abnormality before the age of 36 months. To receive a diagnosis of PDD-NOS a child must meet the cut-off criteria for autism for social interaction and for only 1 of the 2 other areas (35).

The ADOS-G is a semistructured, standardized assessment used for diagnosing children with autism spectrum disorders. It is an assessment of the child's social interaction, communication, play and imaginative use of materials, through an interaction with a trained professional. Diagnostic algorithms are provided for autism and for PDD-NOS. In case of a discrepancy between the two diagnostic measures, one yielding a diagnosis of autism and the other of PDD-NOS, the final diagnosis was that of PDD-NOS, as proposed by Risi *et al.* (35).

In addition to the ADI-R, parents were also interviewed by use of the Vineland Adaptive Behavior Scales–Interview edition (VABS) (36). The VABS is a structured interview administered to caregiver/s to assess the child's daily living skills in three domains: communication (receptive, expressive and written), daily living skills (personal, domestic and community) and socialization (interpersonal relationships, play and leisure time and coping skills). In addition to the three domain scores, a total score reflecting overall functioning was also computed. The VABS has excellent levels of split-half, interrater and test–retest reliability for each domain used in the current work (36).

The subjects' level of functioning was assessed by using standard intelligence measures selected according to the probands' age and abilities, that is, the Wechsler Intelligence Scale for Children (37) and the Kaufman Assessment Battery for Children (38–44). Because some tests yield mental ages rather than IQ, mental age was transformed to an IQ estimate by using the basic IQ formula: IQ = (mental age/chronological age) × 100 mental age.

Genotyping

DNA was extracted by use of a Master Pure kit (Epicentre, Madison, WI, USA). The SNP rs6449182 was identified by searching through the dbSNP public database (http://www.ncbi.nlm.gov/SNP) and genotyped by using the

SNaPshot Method™ (Applied Biosystems, Foster City, CA, USA), as described in a previous report (7). The forward and reverse primers for the first PCR were 5′-CCG GGT GGT GCT GAG TAG GGA GTC-3′ and 5′-CCG TCC CTG AAG CGG TGA AG-3′, respectively, and the specific primer extension oligo was 5′-TTC GCT CGG TGC CAA GGC CA-3′. The SNP was in Hardy-Weinberg equilibrium for the genotyped samples. Since there were only n = 3 probands with the GG genotype, these samples were excluded from the analyses of that SNP.

Cell Cultures and Retinoic Acid Treatment

Epstein-Barr virus (EBV)-transformed LBC lines were established from all participants and grown for at least 20 passages in 25-cm^2 flasks (Nunclone™; Thermo Fisher Scientific, Waltham, MA, USA) containing 10 mL RPMI 1640 medium supplemented with 16% heat-inactivated fetal calf serum, 2 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Biological Industries, Kibbutz Bet Ha'emek, Israel) at 37°C in 5% CO_2 humidified air. For cryopreservation, LBC lines were grown to a density of 5×10^5 cells/mL and the pellet was redissolved to a density of 1 million cells/mL in freezing medium (RPMI 1640 medium with 10% DMSO), aliquoted, kept on ice for 1 h, at –20°C for 1 h, then at –70°C for 24 h and transferred to liquid nitrogen. For the experiments, cell lines were thawed and grown for several passages under conditions as described above. Six parental cell lines (three fathers, three mothers) did not grow even after all available frozen aliquots were used.

The growing cell lines were seeded into two 25-cm^2 flasks at an average density of 2.5×10^5 cells/mL ($\pm 6.6 \times 10^4$) each and grown for 48 h. On day 0, one flask per proband was incubated with 0.1 μmol/L ATRA (all-*trans* retinoic acid; Sigma, St Louis, MO, USA). For that purpose, ATRA was dissolved in pure ethanol in dimmed light to a 200-μmol/L working solution, and 5 μL of this solution was added to 10 mL cell culture. The equal volume of pure ethanol was added to the remaining flask and represented the vehicle-treated control. At 48 h after incubation, the cells were washed in cold DPBS (Sigma) and the cell pellet was shock-frosted in liquid nitrogen and transferred to –70°C until extraction of total RNA by use of TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. RNA concentration was measured photospectrometrically and samples were stored at –70°C.

QUANTITATIVE PCR

cDNA was synthesized by reverse transcription of 2 μg RNA by use of the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) following the manufacturer's protocol. β-Actin was used as a reference gene. Quantitative PCR was performed using 10 μL of 2 × SYBR green ROX mix (Thermo Fisher Scientific), 40 ng cDNA and a primer concentration of 150 nmol/L each in 20-μL reaction volume. For CD38, the forward primer was 5′-TTG GGA ACT CAG ACC GTA CCT TG-3′ and the reverse primer was 5′-CCA CAC CAT GTG AGG TCA TC-3′, resulting in a 149-bp amplicon comprised of a part of exon 2 and 3. The forward primer for β-actin was 5′-ACA GAG CCT CGC CTT TGC CG-3′ and the reverse primer was 5′-ACA TGC CGG AGC CGT TGT CG-3′, resulting in a 104-bp amplicon comprised of a part of exon 1 and 2. The reactions were performed on a RotorGene 3000 (Corbett Life Science, Quiagen, Hilden, Germany) with the following conditions. For CD38: a heat-activation step for 15 min at 95°C, 40 cycles at 95°C for 5 s, 55°C for 30 s and 72°C for 15 s. For β-actin: heat-activation step for 15 min at 95°C, 35 cycles at 95°C for 5 s, 62°C for 30 s and 72°C for 15 s. All samples were run in duplicate and with a nontemplate control. CD38 expression was normalized to β-actin expression for the corresponding sample.

STATISTICAL ANALYSIS

SPSS 17 (Windows) or the STATA software suite was used for all statistical analyses. All relative CD38 mRNA values normalized to β-actin were $\log_{10}$ transformed. To test whether there is significant difference of log CD38 mRNA between the parent group and ASD group, the log CD38 mRNA is regressed on the group dummy variable (parents = 0 and ASD = 1) and the constant term, with family ID as cluster in robust SE using STATA 10. All statistics were two-tailed.

RESULTS

Sample

The starting point of this work was our initial observation that *CD38* expression is lower in EBV lines derived from ASD patients than in cell lines from the patients' parents (23). We then investigated whether this difference was maintained after modulation of *CD38* expression by ATRA, a vitamin reported to powerfully induce this molecule (45). We therefore reanalyzed the EBV lines described in the first report (42 from ASD patients and 40 from the parental lines), enriching the sample with 38 additional cell lines so that in the current investigation for each of the probands both of their parents were included in the analysis.

Cells in culture, or frozen lines, were first thawed and then cultured, and their *CD38* mRNA levels were measured. It was important to determine whether expression of this gene is stable and can resist the stress of multiple cycles of freezing and thawing. The results obtained in the context of the new sample confirm that *CD38* expression in ASD patient lines is substantially lower than in those derived from the patients' parents (Figure 1).

Influence of Familiality on *CD38* Expression

Because the comparative analysis involved cell lines from children and their parents, we needed to confirm the validity of the differences observed between ASD and parental lymphoblast lines. We therefore adopted a robust estimator of the standard error based on a STATA linear regression. The results clearly indi-

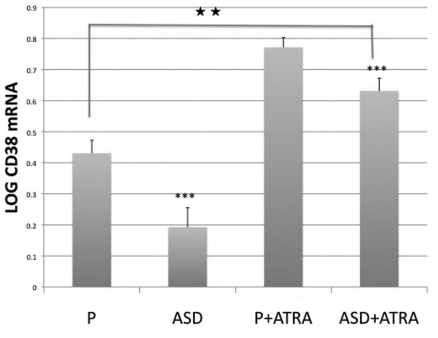

Figure 1. The effect of 48 h 0.1μm ATRA treatment on *CD38* mRNA levels in LBC lines. **, independent samples *t* test, *t* = –3.199; *P* = 0.002; prolonged ATRA treatment elevates reduced *CD38* mRNA levels in LBC lines from ASD patients (n = 42) above parental (P) basal expression (n = 78). Also, basal and induced *CD38* mRNA levels are significantly reduced in ASD cell lines compared with parental (P) cell lines (***, independent *t* test; *P* < 0.001).

cate that the difference under analysis was maintained in the sample (Table 1).

Influence of Age on CD38 Espression in LBC Lines

The surface expression of CD38 on blood cells varies significantly throughout the life course. Normally, expression is high on cord blood cells and diminishes in cells obtained from adults (46). However, no age-related differences indexed by mRNA levels were observed in LBC lines obtained from the ASD patients (Pearson correlation = –0.104; *P* = 0.51; age range: 3–17 years; n = 42) or from their parents (Pearson correlation = 0.057; *P* = 0.62; age range: 31–64 years; n = 78), at least in the disease model currently adopted and with the use of immortalized cell lines.

In Vitro Effects Induced by ATRA on *CD38* mRNA

We wanted to determine whether the diminished expression of *CD38* in ASD could be reversed through simple treatment with ATRA. Following 48 h of ATRA treatment, the results indicated that the *CD38* gene in the EBV lines obtained from the ASD probands conserved its ability to respond with a significant induction of *CD38* mRNA (Figure 1). The parental lines display the same ability, although to a lesser extent (paired *t* test *t* = –13.26; *P* < 0.001 ± ATRA).

Table 1. Log *CD38* mRNA comparing parental lines to ASD-derived cell lines.[a]

Dependent variable	Independent variable	Coefficient	SE	*t*	*P*	95% CI	
CD38 mRNA	Parents/ASD children	–0.238	0.057	–4.160	0.001	–0.353	–0.122
Basal	Constant	0.430	0.053	8.060	0.001	0.323	0.538
CD38 mRNA	Parents/ASD children	–0.140	0.038	–3.680	0.001	–0.217	–0.063
0.1 μmol/L ATRA	Constant	0.771	0.040	19.460	0.001	0.691	0.851

[a]The log *CD38* mRNA values were regressed by using STATA 10 on group dummy variables (parents = 0 and ASD = 1) and constant terms with family ID as clusters in robust standard error. The first column contains the dependent variable of log *CD38* mRNA. The second column contains the independent variables of the group dummy variable (parents = 0 and ASD = 1) and constant term in each regression analysis. The subsequent columns contain regression coefficients, robust standard error (SE), *t* value, *P* value and 95% confidence interval (CI), respectively.

CD38 Genotype and ATRA Response

The next question we addressed was whether the *CD38* genotype might differentially influence ATRA sensitivity in the ASD and parental line samples. The cell lines were genotyped for the rs6449182 SNP, which leads to a C→G variation. This SNP is located in intron 1 of the regulatory region of human *CD38*, proximal to the retinoic acid response elements (RARE). The presence of the allele G is reported as being paralleled by increased binding of the transcription factor E2A (47). Furthermore, the G allele marks an increased risk in chronic lymphocytic leukemia (CLL) patients of transformation into Richter's syndrome (32).

The current results (Figure 2) indicate that the presence of the G allele is paralleled by reduced transcriptional levels of *CD38* mRNA, a characteristic ASD lines share with parental lines, although in LBC lines obtained from ASD probands the difference does not attain statistical significance. Furthermore, the G allele is accompanied by reduced sensitivity to ATRA treatment (+ ATRA treatment in parental lines CC = 0.82 ± 0.04; CG = 0.69 ± 0.04; *P* = 0.04). Both are original findings.

Link between *CD38* Expression and Clinical Characteristics in the ASD Sample

The final issue we addressed was the potential for correlation between *CD38* expression and the clinical characteristics of ASD, for which we used social functioning and communication skills as a proxy. Deficits in social functioning are a core characteristic of autism and to our knowledge no robust biomarker for this variable has yet been reported. The results obtained clearly show a significant correlation between transcriptional levels of *CD38* mRNA and IQ and VABS scores,

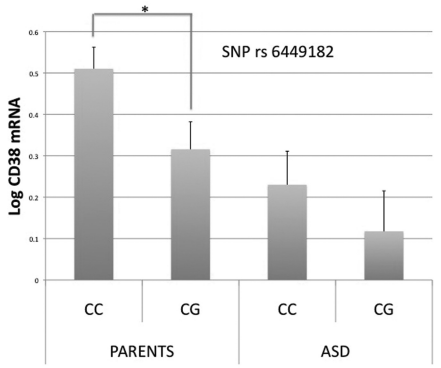

Figure 2. Log *CD38* mRNA levels stratified by rs6449182 alleles in both parental and proband LBC cell lines. The analysis compared CG versus GG in cell lines derived from probands diagnosed with autism (n = 42) and both of their parents (n = 78). A significant difference between CC and CG genotypes was observed only in the parental cell lines.

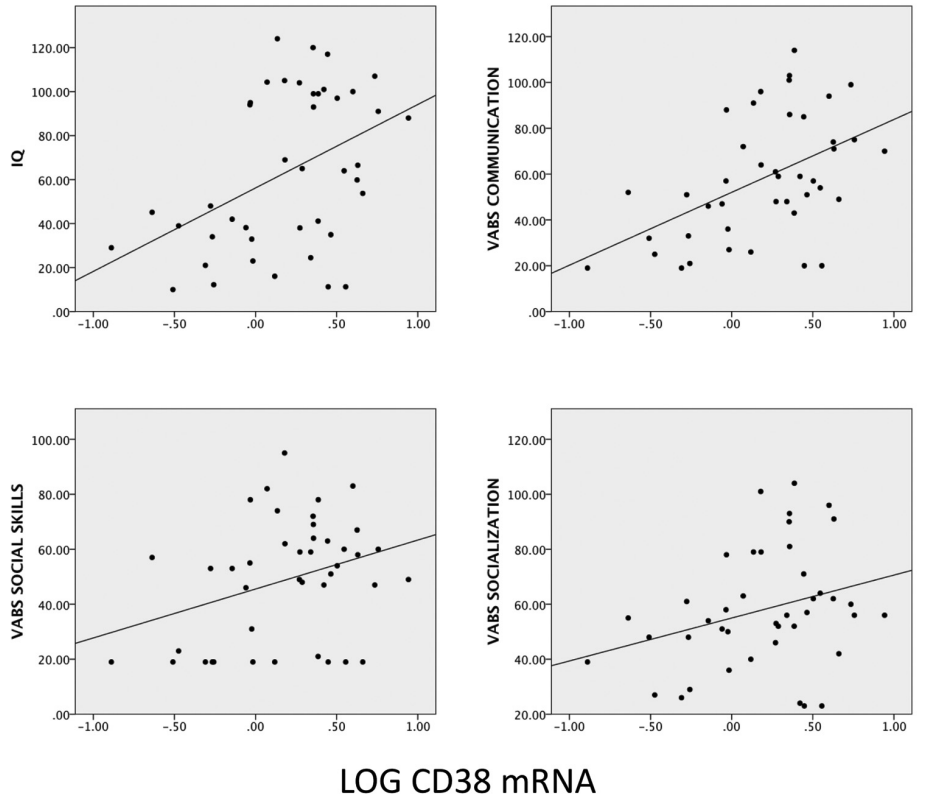

Figure 3. Correlation between CD38 mRNA expression and IQ and VABS subscores in lymphoblast lines derived from ASD subjects. Pearson correlations (IQ $r = 0.431$; $P = 0.004$; n = 42); VABS communication ($r = 0.487$; $P = 0.001$; n = 42); VABS social skills ($r = 0.329$; $P = 0.034$; n = 42); VABS socialization ($r = 0.294$; $P = 0.059$; n = 42).

except for VABS socialization (Figure 3). Nonetheless, the correlation with the VABS total scores did prove significant ($r = 0.431$; $P = 0.008$; n = 42).

DISCUSSION

A role for the CD38/cADPR system in the regulation of hormone secretions was recently proposed by Higashida and his collaborators (19). Observation of adult male and female *CD38* KO mice led to the discovery of marked defects in maternal nurturing and social behavior. Detailed examination of the mice revealed that although OT continued to be synthesized and detectable in the neurohypophysis, it was not released into the circulation. Surprisingly, the production and secretion of arginine vasopressin was unaffected. The impaired release of OT was reversed upon restoration of CD38 expression in the neurohypophysis (19), providing convincing evidence that CD38 is linked to OT secretion. Notably, OT levels and clinical phenotype could be rescued by correcting the genetic defect of the mice. The work concluded that *CD38* KO mice are characterized by a deficit in short-term social memory, which can be restored by either OT or CD38.

These and related observations on affiliative behavior in vole models (48) led us and other groups to investigate whether CD38 and OT might exert roles in human pathologies characterized by impaired social cognition. One of these is ASD, which has high heritability (49) and demonstrates marked genetic and clinical heterogeneities. Individuals with this disorder typically exhibit significant deficits in social interaction and communication and engage in restricted and stereotyped behaviors.

Because the *CD38* gene is present and functional in ASD patients, the mouse model cannot be directly extrapolated to the disorder. The absence of an "all-or-nothing" effect in human pathology suggests the possibility of subtler causative or correlative links arising either from polymorphisms of the CD38/OT/OXTR axis or from the regulation of gene expression. Indeed, the regulatory region of the *CD38* gene is quite complex (46). Vulnerability to ASD may depend on one or more combinations of these genes and their products.

Despite these complexities, several interesting observations can be made. OT plasma levels are known to be significantly lower in ASD patients than in individuals without the disorder (11). In addition, intranasal administration of OT enhances affiliative behaviors such as the trust game (12), parochial altruism (13) and social cognition (14,15) as demonstrated by, for example, performance in the Reading of the Eyes in the Mind Test. We have also shown that intranasal OT enhances EEG mu suppression, which is an index of mirror neuron activity in nonclinical subjects (16) but is deficient in ASD (17). These reports implicate dysfunctional OT peptidergic neurotransmission in the poor performance of ASD patients. Likewise, evidence from genetic association studies suggests that the oxytocin receptor (OXTR) (6–10) also contributes vulnerability to ASD.

Our focus on CD38 was motivated by several factors. First, CD38 catalyses the formation of Ca^{2+} signaling molecules and is a crucial factor in the network that links nucleotides, ectoenzymes and the endocrine system (46). Moreover, CD38 is highly expressed by the human hypothalamus (21) and is colocalized with oxytocinergic neuronal structures, the apparent source of OT in the brain (22). Finally, two independent groups have demonstrated an association between *CD38* SNPs (21,50) and ASD.

Our initial goal was to transfer these observations to a clinical setting and verify the working hypothesis and, at the same time, to help design a model of

pharmacological intervention for ASD patients. After immortalizing B-lymphocytes from ASD patients and from their parents, we were able to confirm that CD38 is present in the EBV lines at lower levels than in their parents and that this characteristic is apparently independent of age and sex. The results of the present work support our initial findings(23), indicating that they are stable and resistant to the stress of multiple freezing and thawing of the human lines. The inference is that the reduction observed in the lines likely reflects a generalized defect in ASD.

Of the several physiological and pharmacological components controlling the expression of *CD38*, we selected retinoids as the focus of analysis. Our interest in retinoids was two-fold: first, ATRA has been reported since 1994 as the most potent inducer of surface *CD38* expression (45), and second, retinoids and vitamin A are already widely used in therapy because of their low toxicity even at high dosages (51). They were thus our candidates of choice for rescuing the reduced CD38 expression observed in cell lines derived from ASD patients.

The results obtained indicate that vitamin A has the ability to upmodulate *CD38* expression in ASD lines by 3 log units and by 2.5 log units in the control parental lines. The physiologic regulatory mechanism is maintained in ASD lines and may reach levels similar to those of parental lines not treated with ATRA.

As observed in our cohort, the differential expression of the *CD38* gene may be paralleled by variable functionality lying between the two extremes: probands with higher levels of CD38 should have better social and cognitive skills than those with lower levels of CD38. In fact, the results of the current study highlight a significant correlation between *CD38* expression and VABS scores and IQ. These findings are also reminiscent of those from results of a recent study, which suggested the occurrence of functional interactions between OXTR and CD38 in hypothalamic neurons upon OT signaling (24). Such a link would require sufficient availability of all partners involved in the pathway.

Our next step was to evaluate the hypothesis that *CD38* polymorphisms influence *CD38* expression, whether quantitatively and/or qualitatively. *CD38* is characterized by an SNP (rs6449182) located at the 5′-end of this intron SNP, 184C>G, which leads to the presence or absence of a Pvu II restriction site (32). The frequencies of the three genotypes established in healthy Italian-born adults (70% CC, 26% CG and 4% GG) are similar to those observed in the Israeli population. The SNP is located in an intronic hotspot containing part of the CpG island that spans the RARE transcription factor site. This SNP was analyzed as potentially regulating *CD38* expression in our sample. The results provide evidence that the presence of the G allele is associated with a significantly reduced expression of *CD38* by the LBC lines derived from the parents. There also appears to be a nonsignificant reduction in ASD lines in the presence of the same allele. Clearly, the role of this and other SNPs in ASD warrant further study.

Another focus of potential interest is whether the *CD38* locus is regulated by methylation, which may partially account for its reduced expression in ASD and perhaps provide a target for environmental insults in conferring vulnerability to this disorder. A parallel may once again be made with OXTR, in which epigenetic modifications can regulate *OXTR* expression and have been associated with autism (52,53). DNA methylation status was independently reported as lower in the peripheral blood cells and in the temporal cortex in datasets of ASD individuals than in control samples (54,55). Associated with the increased methylation of these CpG dinucleotides is the observation that *OXTR* mRNA levels are reduced in the temporal cortex tissue of ASD cases matched to controls for age and sex (52). Taking these results into account, it may be reasonable to assume that ASD individuals are characterized by the simultaneously reduced expression of *CD38* and of *OXTR*.

Our hypothesis, that retinoids play a beneficial role in ASD, was tested by inducing CD38 in LBCs from ASD lines, and using parent-derived lines as controls. The idea was that *CD38* mRNA levels measured in LBC lines might parallel those of hypothalamic neurons in the same proband. Interestingly, Sullivan *et al.* (56) have observed that on a transcriptome level, whole blood shares significant gene expression similarities with multiple CNS tissues. The expected outcome is that the upregulation observed with retinoids *in vitro* could lead to enhanced expression in the brain of patients treated with retinoid. Our assumption is that the enhanced presence of CD38 in paraventricular and supraoptic nuclei following retinoid treatment may produce and release more OT. This, in turn, will fuel the brain areas that actively modulate human social behavior and cognition.

Jin *et al.* (19) provided a valid basis for this assumption by demonstrating that the impaired maternal behavior and social memory observed in mice deficient in ADP-ribosyl cyclase was rescued by site-directed reexpression of CD38 in mouse hypothalamus and that the subcutaneous administration of OT in knock-out mice had an identical effect.

The current observation that CD38 expression is reduced in lymphoblastoid cells derived from ASD subjects suggests the prospect that CD38 might be an early hallmark for this disorder. As noted by Yirmiya and Charman (60), "the primary motivation for identifying the earliest signs of emerging ASDs is the desire to develop and test early or even 'preventative' interventions to lessen morbidity by changing the course of early emerging developmental perturbation, thus preventing 'secondary' neurodevelopmental disturbances." Toward evaluating the potential of CD38 as a hallmark in ASD, reduced CD38 expression must first to be verified in circulating lymphocytes. Then it must be stressed that CD38 transcription is a marker for other diseases. It is a prognostic marker for HIV-infected subjects (57), in chronic lymphocytic leukemia (CLL) (58) and for diabetic patients with

nephropathy (59). Hence reduced *CD38* transcription cannot be pathognomonic for ASD but nevertheless might prove of salient clinical value in a disorder diagnosed solely using behavioral assessments only at the age of 3 years (60). Moreover, CD38 expression changes throughout the lifespan (46), and age-dependent CD38 expression in circulating lymphocytes is a potential confounder in its use as a diagnostic indicator in ASD. However, the critical need in ASD is for very early (prenatal or perinatal) diagnostic tools and hence, from this perspective, *CD38* mRNA levels in cord blood or amniotic fluid might be of substantial value notwithstanding subsequent age-related changes in lymphocyte CD38 expression.

The results of the current study offer evidence that retinoids may play a beneficial role in the treatment of ASD. At the same time, it provides proof of principle for using lymphocytes and LBC lines (which are both readily available and easily accessible) from human subjects in whom CD38 is robustly expressed. Because CD38 is sensitive to both pharmacological and genetic modulation, these cell lines may be useful in exploring the role of this ectoenzyme in behavioral phenotypes relevant to social cognition. CD38 thus stands to become a key element in the diagnosis and therapy of ASD.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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