Additional Clues for a Protective Role ofVitamin D in Neurodegenerative Diseases: 1,25-Dihydroxyvitamin D3 Triggers anAnti-Inflammatory Response in BrainPericytes

Marie-France Nissou[a,b,d,1], Audrey Guttin[b,c,e,1], Cyril Zenga[a], François Berger[a,b,c,d], Jean-Paul Issartel[b,c,f,1]and Didier Wion[a,b,1,*]

[a]*INSERM U1167, CLINATEC, Centre de Recherche Edmond J Safra, MINATEC Campus CEA, Grenoble, France*

[b]*INSERM U836, Grenoble Institut des Neurosciences, Team Nanomedicine and Brain, Grenoble, France*

[c]*University of Grenoble Alpes, Grenoble, France*

[d]*CHU de Grenoble and Institut de Biologie et Pathologie, Grenoble, France*

[e]*CaCyS, EHPE, CNRS, UJF, Grenoble, France*

[f]*CNRS, Grenoble, France*

Abstract. Epidemiological and experimental studies suggest that 1,25-dihydroxyvitamin D3 (1,25D) plays a neuroprotectiverole in neurodegenerative diseases including Alzheimer's disease. Most of the experimental data regarding the genes regulatedby this hormone in brain cells have been obtained with neuron and glial cells. Pericytes play a critical role in brain function thatencompasses their classical function in blood-brain barrier control and maintenance. However, the gene response of brain pericyteto 1,25D remains to be investigated. Analyses of the transcriptomic response of human brain pericytes to 1,25D demonstrate thathuman brain pericytes in culture respond to 1,25D by regulating genes involved in the control of neuroinflammation. In addition,ericytes respond to the pro-inflammatory cytokines tumor necrosis factor $\alpha$and Interferon γ by inducing the expression of theCYP27B1 gene which is involved in 1,25D synthesis. Taken together, these results suggest that neuroinflammation could triggerthe synthesis of 1,25D by brain pericytes, which in turn respond to the hormone by a global anti-inflammatory response. Thesefindings identify brain pericytes as a novel 1,25D-responsive cell type and provide additional evidence for the potential value ofvitamin D in the prevention or therapy of Alzheimer's disease and other neurodegenerative/neuropsychiatric diseases associatedwith an inflammatory component.

INTRODUCTION

Recent data suggest a role for 1,25dihydroxyvitaminD3 (1,25D) in the prevention or in the therapy of neurodegenerative and neuropsychiatric diseasesincluding multiple sclerosis and Alzheimer's disease(AD) (for recent reviews, see [1–10]). 1,25D is a secosteroidhormone produced from vitamin D througha two-step hydroxylation process successively producing25-hydroxyvitamin D3 (25D) and vitaminD's most active metabolite 1,25D. This last stepis catalyzed by the 25-Hydroxyvitamin D3 1alpha-Hydroxylase named CYP27B1. Inactivation of 1,25Doccurs through another hydroxylation step catalyzedby the vitamin D 24-Hydroxylase named CYP24A1, and generating 1,24,25trihydroxyvitamin D3 [11, 12].1,25D regulates the transcription of its cell specifictarget genes by interacting with a protein named vitaminD receptor (VDR). The presence of VDR in brain[13–16], the capacity of 1,25D to regulate the expressionof neurotrophic factors (for reviews, see [17]),and its anti-inflammatory potential [2, 18] are strongarguments in favor of a role for 1,25D in brain function.Regarding brain diseases, many recent studiesreport an association between vitamin D insufficiencyand neurodegenerative/neuropsychiatric disorders [5,7, 10, 19, 20]. Experimental evidence also demonstratea preventive or therapeutic potential for 1,25Din brain disorders including multiple sclerosis, Parkinson'sdisease, AD, and traumatic brain injury (forrecent reviews, see [21–24]). Investigating the generesponse to vitamin D in brain cells is critical forunderstanding the molecular basis of its neuroprotectiveeffect. Most of the experimental data regarding thepotential of 1,25D to regulate the expression of genesable to prevent or delay the progression of neurodegenerativediseases have been obtained *in vitro* usingbrain-derived cells. These include neurons and macroor microglial cells (see [25–32]). However, little attentionhas been given to another abundant brain cell type,the brain pericyte.Pericytes are microvessel cells that wrap aroundendothelial cells [33–35]. The pericyte-to-endotheliaratio is 1 : 3, and the total length of capillaries inhuman brain is around 400 miles [36, 37]. The edge-toedgeinter-microvessels distance in rat brain is around20μm in gray matter and 30μm in white matter [38].This means that pericytes are very close to everyneuronal cell. Because of their perivascular localization,pericytes are components of the neurovascularunit (NVU), an anatomical and functional entity associatingneurons, glial and immune cells, endothelialcells, pericytes, and the extracellular matrix [33–35,39, 40]. How brain pericytes communicate with glialand neuronal cells in NVU is still largely unknown,but they are, however, essential for proper functionsof neurons [36, 41]. In addition to their role in angiogenesis,vessel maintenance, and blood-brain barrierformation and functions [33, 35], brain pericytes alsocontribute to immune and inflammatory responsesby synthesizing pro-inflammatory cytokines includingCXCL8/IL-8, CXCL11/ITAC, CCL5/Rantes, tumornecrosis factor-alpha (TNF-α), interleukin-1β(IL-1β),and interleukin-6 (IL-6) [42]. They participate to theadaptive immune response [43] and also have animmunosuppressive function by decreasing T lymphocyte proliferation [44]. The importance of pericytesin brain function is assessed by the observation thatage-dependent vascular damage in pericyte-deficientmice precedes neuronal degenerative changes, learningimpairment, memory deficiency, and the neuroinflammatoryresponse [41]. Pericyte loss or dysfunctioninfluences multiple steps of AD in several experimentalmodels [36, 41, 45]. Hence, targeting brain pericyteis now considered as a novel therapeutic option forAD [39, 41, 45, 46]. The emerging evidence for arole of vitamin D in brain function and its potentialpreventive and therapeutic interest in the managementof neurodegenerative disease prompted us to analyzethe transcriptomic response of human brain pericyte to1,25D. Our results provide evidence for a functionalrelationship between vitamin D and pericytes in thecontrol of inflammation that could explain some ofthe anti-inflammatory and neuroprotective effects ofvitamin D.

MATERIAL AND METHODS

Cell culture

Human brain pericytes and human brain endothelialcells were obtained from ScienCell Research Laboratories(San Diego, CA, USA). Cells were culturedon poly-lysine coated culture dishes according tothe provider instructions. Briefly, the growth mediumfor pericytes was made with Pericyte Basal CultureMedium supplemented with 2% fetal bovineserum, with Pericyte Cell Growth Supplement andwith penicillin and streptomycin, all from Science-Cell Research Laboratories (San Diego, CA, USA).

Brain endothelial cells were cultured in EndothelialBasal Culture Medium supplemented with EndothelialGrowth Supplement and with penicillin and streptomycin,all from ScienCell Research Laboratories. Inthe absence of a definitive pan-marker for pericytes, immunophenotyping of pericytes relies on the use of apanel of different markers [34]. Positive markers caninclude PDGFRβ, nestin, and CD13/aminopeptidaseN, whereas Ve-Cad can be used as a negative marker. Immunophenotyping of our cell cultures demonstrateda homogeneous positive labelling forPDGFR_, nestin,CD13/aminopeptidase N and no detectable synthesisof the endothelial marker VE-Cadherin (SupplementaryFig. 1A-D). On the contrary, brain endothelialcells stained positively for the endothelial markerVE-Cadherin (Supplementary Fig. 1E). TNF-α andInterferon-γ (Peprotech, Neuilly-sur-Seine, France)were used at 50 ng/ml.

Western blot analysis

For western blot analysis, cell pellets were resuspendedand lysed in RIPA buffer (Cell Signaling Technology, Danvers, USA) supplemented with completeProtease Inhibitor Cocktail (Cell Signaling Technology, Danvers, USA). The proteins (40 µg)were resolved with 8% polyacrylamide gel and transferredonto the Hybond N+ membrane (Amersham,Velizy-Villacoublay, France) according to standardprotocols. Blots were then probed with the VDRantibody N20 (Santa Cruz, sc-1009) or D6 (SantaCruz, sc-13133), followed by incubation with thecorresponding horseradish peroxidase conjugated secondaryantibodies (Santa Cruz, sc-2004 or sc-2005).

RNA extraction and RT-qPCR

2µg of total RNA were transcribed intocDNA using iScriptTM Reverse TranscriptionSupermix for RT.qPCR (BioRad Laboratories, Marnes-la-Coquette, France). PCR primers(Eurogentec, Angers, France) for each gene weredesigned using the Universal ProbeLibrary AssayDesign Center (https://www.roche-applied-science.com/sis/rtpcr/upl/ezhome.html) and sequences of theprimers used are given as Supplementary Table 1.Then real-time PCRs were performed accordingto the SYBR Green methodology using theSsoAdvancedTM SYBRR Green Supermix (BioRadLaboratories, Marnes-la-Coquette, France), on aCFX96 TouchTMReal-Time PCR Detection System (BioRadLaboratories, Marnes-la-Coquette, France).Reference genes are _ actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), whoseexpressions were not affected by 1,25D according to our Affymetrix data (data not shown). Analyses were performed as previously described [25] with CFX ManagerTM software (BioRad Laboratories, Marnes-la-Coquette, France) using __Ct method. Each qPCR was performed in triplicate for PCR yieldvalidation and all reactions were performed on threedifferent biological samples. Data were quantifiedrelative to gene expressions of pericyte cells without1,25D treatment, which was standardized to 100.The statistical validation was given by the CFXManager software and $p < 0.005$ was considered significant.

Gene expression profiling

Total RNA (300 ng) extracted as described abovewas reverse transcribed and labelled with 3 prime IVTExpress kit (Affymetrix, High Wycombe, UK) followingthe manufacturer's instructions. Labelled sampleswere hybridized on GeneChipHumanGenomeU133Plus2.0 (Affymetrix, High Wycombe, UK). This microarray contains over 47,000 unique transcriptswhich correspond approximately to 39,000human genes. Subsequent wash, stain, and scan wereperformed according to the standard Affymetrix protocols.Raw expression data were normalized usingthe Robust Multi-array Average method. Experimentswere repeated twice using different RNA sampleson GeneChip (Affymetrix, High Wycombe, UK) andcorroborated by RT-qPCR for genes of interest. Thetranscriptomic data have been deposited in NCBI'sGene Expression Omnibus and are accessible throughthe Gene Expression Omnibus accession numberGSE54765.

RESULTS

Pericytes express VDR and respond to 1,25D byinducing CYP24A1 expression

1,25D regulates gene expression of its target genesby interacting with the nuclear receptor VDR.We firstinvestigated the presence of VDR in human brain pericyte.Western blot analysis of pericyte cell lysateswith the VDR polyclonal antibody N20 detected thepresence of a 53 kDa band consistent with the presenceof VDR in these cells (Fig. 1A). A similar resultwas obtained with the VDR monoclonal antibody D6(Supplementary Fig. 2).We next determined the functionality of the 1,25D/VDR transduction pathway in human brain pericytesby analyzing CYP24A1 regulation by 1,25D(CYP24A1 is a well-known 1,25D-responsive gene[47]). Results presented in Fig. 1B demonstrate thestrong induction of CYP24A1 gene expression in pericytestreated for 24 hours with 10^{-8}M 1,25D.

Brain pericyte transcriptomic response to 1,25D

Having demonstrated the presence of VDR inhuman brain pericytes and the responsiveness of these cells to 1,25D, we analyzed the transcriptomicresponse of brain pericytes to 1,25D. RNA frompericytes cultured for 24 hours either with10^{-8}M 1,25D or with vehicle only (ethanol) wereextracted and processed for transcriptomic analyses. Experiments were performed on two different RNApreparations for each condition. Twenty genes werefound upregulated by a factor of at least 1.7, and 5down-regulated genes were also detected (expressionratios lower than 0.7) (Table 1). Eight of these geneswere already described as 1,25D targets in cells otherthan pericytes. These genes are cytochrome P450A1(CYP24A1) [47], elongation factor Tu GTP bindingdomain (EFTUD1) [48], thrombomodulin (THBD)[49], odd-skipped related 2 (OSR2) [50], Kruppel-likefactor 4 (KLF4) [51], insulin-like growth factorbinding protein 5 (IGFBP5) [52], fatty acid binding protein 4 (FABP4) [53], and tumor necrosis factor(ligand) superfamily member 4 (TNFS4/OX40L)[54]. An analysis of the 25 genes found regulated by1,25D was carried out with the GeneDeck analysis tool (http://www.genecards.org/). This allowed theidentification of 14 disorders associated with this setof genes with a p value lower than 10^{-5}. Six of thesedisorders were inflammatory diseases: endotheliitis($p = 2.2\times10^{-10}$), asthma ($p = 1.4\times10^{-6}$), rheumatoidarthritis ($p=2\times10^{-6}$), arthritis ($p = 5.1\times10-6$),inflammation ($p = 6.4\times10^{-6}$), and pancreatitis($p = 1.1\times10^{-5}$). The other disorders include variouscancers and hypertension. To confirm the relationshipexisting between the transcriptomic response of brainpericytes to 1,25D and inflammation, a bibliographicsearch in PubMed associating each of these 25 genes with the keyword "inflammation" was done. Relevant results were obtained for 8 genes. These genes arevascular cell adhesion molecule 1 (VCAM1), THBD,KLF4, tumor necrosis factor alpha-induced protein6 (TNFAIP6), chordin-like 1 (CHRDL1),

chemokine(C-C motif) ligand 2 (CCL2), FABP4, tumor necrosisfactor (ligand) superfamily member 4 (TNFSF4).THBD, KLF4, FABP4, and TNFSF4 have alreadybeen described as 1,25D-responsive genes in cellsother than brain cells or pericytes [49, 51, 53, 54].Confirmatory RT-qPCR analyses were performed thatvalidated the transcriptomic data for the 4 remaininggenes which were not previously described as 1,25Dtargets (Fig. 2). These are CCL2, CHDRL1, TNFPAIP6,and VCAM-1. KLF4 and TNFSF4 were alsoincluded as positive controls in these confirmatory experiments. Importantly the regulations observed areconsistent with a global anti-inflammatory response(see discussion below).

Induction of CYP27B1 expression by thepro-inflammatory cytokines TNF-α andInterferon-γ

25D is the most abundant metabolite of vitamin Din the circulation, but the most active metabolite is1,25D [11, 12]. Hydroxylation of 25D to active 1,25Dis required for the induction of the 1,25D-dependentsignaling pathway [11, 12]. This reaction is carriedout by 25-Hydroxyvitamin D3 1alpha-HydroxylaseCYP27B1, the rate limiting enzyme in 1,25D synthesis.Analysis of our transcriptomic data for CYP27B1failed to detect any expression for this gene in our pericytecultures (data not shown). Considering the globalanti-inflammatory gene response of pericytes to 1,25D, we hypothesized that inflammatory stimuli could upregulateCYP27B1 gene expression in these cells. Thiswould allow pericytes to metabolize 25D into 1,25Dand then limit the inflammatory response locally. Toinvestigate this point we treated brain pericytes withTNF-αand Interferon-γ which were recently foundto upregulate CYP27B1 in astrocytes [55]. Figure 3showed that a treatment of pericytes for 24 hourswith these two inflammatory cytokines stimulated theexpression of CYP27B1.

DISCUSSION

Epidemiological studies reveal a relationshipbetween several neurodegenerative/psychiatric diseasesand low vitamin D levels [5, 7, 10, 19–21,57, 58]. Although association does not mean causation,the possible role of vitamin D as a protectiveco-factor against brain disorders is further supportedby data demonstrating the neuroprotective effect ofvitamin D or of its active metabolite 1,25D in severalexperimental models of brain diseases (for recent reviews, see [1–10]). Understanding the molecularpathways involved in these effects is critical for optimizingclinical trials. At the transcriptomic level,1,25D is already known to upregulate several genes relevant to neuroprotection in brain cells. Several ofthese genes encode neurotrophins including NGF andGDNF [59–61]. 1,25D is a potent immunoregulatoryagent [2–4, 62, 63]. In neurons or glial cells1,25D regulates the expression of genes involved in thecontrol of neuroinflammation, such as cystathioninebeta-synthase, which codes for the enzyme producing the neuroprotective agent hydrogene sulfide [25], andgamma-glutamyltranspeptidase (gamma-GT) whichis the enzyme that synthesizes the antioxidant glutathione[64]. 1,25D also reduces the expression of theinflammatory cytokines M-CSF and TNF-_ in astrocyteschallenged with lipopolysaccharides [65], andprotects brain cells from nitric oxide excessive productionby down-regulating iNOS [30]. However, allthese data have been obtained with cells others thanpericytes, and the global gene response of pericytes to1,25D remained to be investigated. This point is criticalregarding the importance of pericytes in brain function[33–46]. The present study reveals that eight out of thetwenty five genes we found regulated by 1,25D in brainpericytes are related to inflammation. With exceptionof VCAM1, all of these genes are involved in criticalanti-inflammatory processes. This regulation is consistentwith a global anti-inflammatory process sincepro-inflammatory genes are found down-regulated,while anti-inflammatory genes are upregulated.

Pro-inflammatory genes down-regulated by 1,25Din pericytes

CCL2/MCP-1 is an inflammatory chemokine thatis also able to modulate blood-brain barrier permeability.It is upregulated in AD patients and notablyin brain microvessels [66]. High plasma concentrationof CCL2 are found in mild AD [67] and is associatedwith a faster rate of cognitive decline during the earlystages of AD [68]. CCL2 is also associated with multiplesclerosis patients and experimental autoimmuneencephalomyelitis (EAE). Moreover deficiency in theCCL2 receptor CCR2 confers resistance to EAE [69].TNFSF4/OX40L is the ligand of the OX40 receptor.The OX40-OX40L interaction is critical for theregulation of T cell tolerance and T-cell mediatedinflammatory diseases [70]. Consequently, OX40 signalingis involved in allergic inflammation [71, 72]and in multiple sclerosis, and is also involved inthe development of EAE [73]. Down-regulation ofTNFSF4/OX40L is suggested to provide a novelapproach for treating inflammatory disease [72, 74–76].The fatty acid binding protein FABP4 acts at theinterface of metabolic and inflammatory pathways[77]. It is involved in the development of a chronicmetabolic inflammatory state recently referred asmetaflammation that connects obesity with inflammation[77]. FABP4 is associated with inflammatoryfactors relatedto obesity [78], and is also implicated in asthma [79] and rheumatoid arthritis [80]. Toour knowledge, the involvement of FABP4 and the potential of inhibitors of FABP4 have not yet beeninvestigated in inflammatory neurodegenerative disorders.

Anti-inflammatory genes upregulated by 1,25D inpericytes

Thrombomodulin (THBD) is a well characterized1,25D responsive gene proposed as a biomarker for evaluating the effect of vitamin D supplementation[49]. The anti-inflammatory properties of thrombomodulinhave been recently reviewed [81, 82]. THBDsequesters thrombin which has pro-inflammatoryactivities, in addition to its role in hemostasis andthrombosis. Note that thrombin is highly expressed inAD brain vessels and is a mediator of cerebral inflammation in AD. Thrombomodulin also promotes theactivation of protein C, which, in addition to its anticoagulant activity, also has anti-inflammatory effectsby suppressing the production of the pro-inflammatory cytokine TNF-α [83].Tumor necrosis factor, alpha-induced protein 6(TNFAIP6/TSG6) is a secreted protein whose expressionis upregulated in response to pro-inflammatorycytokines such as TNF and IL-1. It has anti-inflammatory effects and acts as a negative feedbackmodulator by down-regulating the inflammatoryresponse [84].The identification of KLF4 as a 1,25D upregulatedgene is in agreement with a previous findingobtained with keratinocytes [51] and is in line witha general role of 1,25D and KFL4 in the controlof inflammatory responses. KLF4 inhibits endothelialinflammation [85] and represses the expression ofhistidine carboxylase, which is the enzyme that convertshistidine to histamine, a major actor of allergyand inflammation [86]. In macrophage KLF4 promotesthe anti-inflammatory macrophage/microglia M2 phenotype at the expanse of the M1 pro inflammatoryphenotype [87]. In this regard, it is worth mentioningthat M2 microglia is reported to drive oligodendrocytedifferentiation during CNS remyelination. Althoughsuch effects are observed with macrophages, they maybe relevant in the case of pericytes. Indeed these cellsare a source of macrophage activity, macrophage markers,phagocytosis, and antigen presentation [88]. Note,however, that KLF4 also increases the synthesis ofseveral pro-inflammatory cytokines in M1 microglialcells [89]. This suggests that the effects of 1,25Don KLF4 expression could participate to the control of abalanced inflammatory response, thus preventing the development of chronic inflammation. Chordin-like protein 1 (CHRDL1), also known asVentroptin, is a bone morphogenetic protein-4 antagonist[90, 91]. It antagonizes the function of BMP4 bybinding to it and preventing its interaction with receptors.Increased expression of BMP4 mRNA withinthe hippocampus dentate

gyrus is correlated with adecrease in cell proliferation inA_PPswe/PS1DeltaE9transgenic mice [92]. BMP4 is upregulated duringEAE [93], and this cytokine is also reported to mediateinflammation in endothelial cells [94]. Hence, thesynthesis of CHRDL1 by pericytes would protect cells from inflammation.Vascular adhesion molecule-1 (VCAM1) is a cellsurface protein with adhesion properties. It is alymphocyte adhesion molecule induced on humanendothelium by inflammatory stimuli [95] and hasbeen initially considered as an inflammatory marker.However, it is not clear whether its function is onlydirected toward the aggravation of the original insultor if, depending of the context, is also involved inthe restoration of homeostasis. VCAM1 plays a keyrole in the brain in maintaining subventricular zoneadult stem cell niche structure and function [96]. Thissuggests that VCAM1 could have similar functions inthe maintenance of the structure and functions of theNVUby regulating cell-cell junctions. Upregulation ofVCAM1 has been suggested to play a protective role inmaintaining the integrity of the ependymal zone duringneuroinflammation [96]. VCAM-1 depletion leadsto the rapid decrease of the subventricular zone neuralstem cell population, suggesting that chronic VCAM-1insufficiency/deficiency would lead to neural stem celldepletion [96].
Collectively, our gene expression data providestrong evidence that the gene response of human brainpericytes to 1,25D is directed toward the control ofneuroinflammation.

Expression of CYP27B1 in pericytes can beinduced by an inflammatory stimulus
1,25D results from the metabolism of 25D by 25-Hydroxyvitamin D3 1alpha-Hydroxylase (CYP27B1). Thus, the availability of 1,25D at the cellular leveldepends on both circulating levels of 25D, and CYP27B1 expression level. 25D is detected in humancerebrospinal fluid [58]. Therefore, the characterizationof stimuli able to induce CYP27B1 is critical.Here we report that TNF-α and Interferon-γ, two inflammatory cytokines recently described to regulateCYP27B1 expression in astrocytes and microglial cells [55], also induce the expression of CYP27B1 in pericytes.
Note that pericytes are reported to secrete TNF-α[42]. Our data strongly suggest the existence of a regulatoryintracrine/autocrine feedback loop in pericytesbalancing the inflammatory potential of TNF-α andInterferon-γ. A paracrine role for the 1,25D producedby pericytes in the NVU during inflammation can alsobe considered.In conclusion, our results point to brain pericytes and 1,25D as two active players in the regulation of neuroinflammation.They provide additional evidence fora role of vitamin D metabolites in the prevention and the therapy of neurodegenerative/neuropsychiatric diseasesthrough the modulation of neuroinflammation.They also strengthened the interest of targeting brainpericytes for controlling brain disorders such as AD [41, 45, 46, 97, 98].

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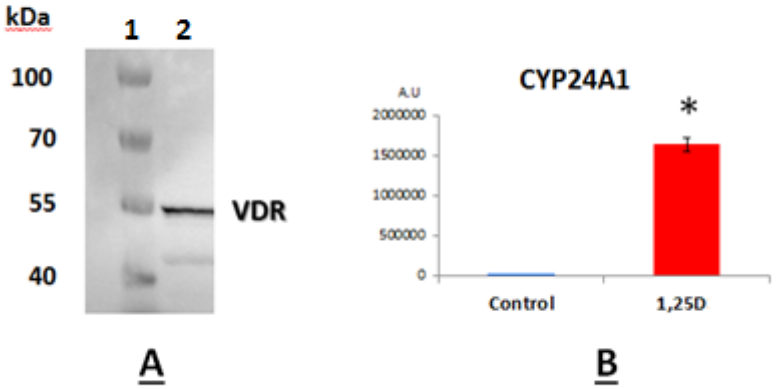


Fig. 1. Human pericyte cells express VDR and respond to 1,25D3. A) Immunoblot showing the presence of VDR in human pericyte brain cellcultures. Lane 1, molecular weight markers; lane 2: pericyte cell lysate. The blot was incubated with the VDR antibody N20. B) RT-qPCR assay of the mRNA of the 1,25D inducible gene vitamin D 24-Hydroxylase (CYP24A1) demonstrates a significant increase in CYP24A1 mRNAamount when pericytes are cultured for 24 hours with 10−8 M 1,25D3. (∗$p < 0.005$); A.U: arbitrary units.

Probe set ID	Gene Name		Exp 1	Exp 2	Average	Ref
206504_at	cytochrome P450, family 24, subfamily A,	CYP24A1	158,2	355,8	237,2	[24]
203868_s_at	vascular cell adhesion molecule 1	VCAM1	3,4	3,2	3,4	
218973_at	elongation factor Tu GTP binding domain	EFTUD1	3,0	3,8	3,4	[25]
203887_s_at	thrombomodulin	THBD	2,8	2,8	2,8	[26]
213568_at	odd-skipped related 2 (Drosophila)	OSR2	2,0	3,7	2,7	[27]
221841_s_at	Kruppel-like factor 4 (gut)	KLF4	2,3	3,1	2,7	[28]
224762_at	serine incorporator 2	SERINC2	2,2	3,4	2,7	
206025_s_at	tumor necrosis factor, alpha-induced prot.	TNFAIP6	2,4	2,8	2,6	
210002_at	GATA binding protein 6	GATA6	2,4	2,4	2,4	
206765_at	potassium inwardly-rectifying channel, s	KCNJ2	2,1	2,6	2,3	
225532_at	Cdk5 and Abl enzyme substrate 1	CABLES1	2,3	2,0	2,1	
204345_at	collagen, type XVI, alpha 1	COL16A1	1,5	2,4	1,9	
229581_at	extracellular leucine-rich repeat and fi	ELFN1	1,7	2,1	1,9	
209763_at	chordin-like 1	CHRDL1	1,6	2,3	1,9	
219025_at	CD248 molecule, endosialin	CD248	1,7	1,9	1,8	
212190_at	serpin peptidase inhibitor, clade E	SERPINE2	1,9	1,6	1,7	
211958_at	insulin-like growth factor binding protein	IGFBP5	1,7	1,8	1,7	[29]
221872_at	retinoic acid receptor responder (tazaro	RARRES1	2,0	1,5	1,7	
209840_s_at	leucine rich repeat neuronal 3	LRRN3	1,7	1,7	1,7	
200878_at	endothelial PAS domain protein 1	EPAS1	1,6	1,7	1,7	
216598_s_at	chemokine (C-C motif) ligand 2	CCL2	0,6	0,7	0,7	
203980_at	fatty acid binding protein 4, adipocyte	FABP4	0,6	0,7	0,6	[30]
207426_s_at	tumor necrosis factor (ligand) superfami	TNFSF4	0,6	0,6	0,6	[31]
222862_s_at	adenylate kinase 5	AK5	0,6	0,6	0,6	
239909_at	ADAMTS-like 1	ADAMTSL1	0,4	0,4	0,5	

Table 1: List of differentially expressed genes in human brain pericyte cultures in the presence of 10^{-8} M 1,25D (average induction fold $\geq$1.7 or $\leq$0.7compared to control cells). Fold changes are the average of two independent experiments. Published references for genes previously reported to be modulated by 1,25D are indicated.

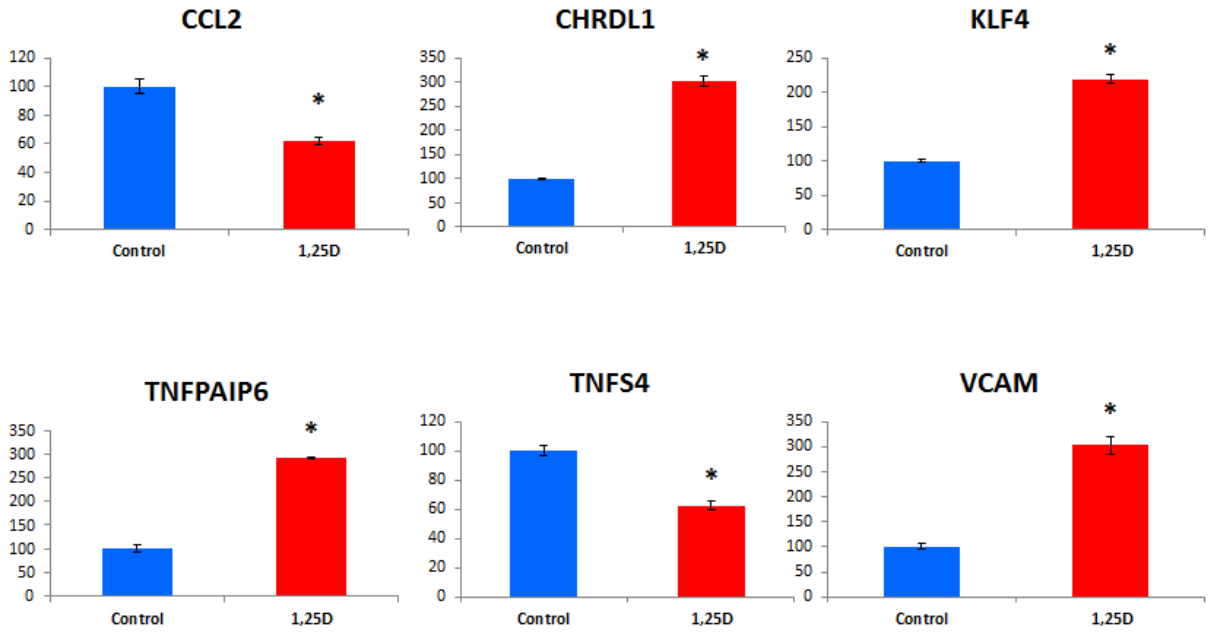


Fig. 2. Confirmatory RT-qPCR of the transcriptomic data for the genes newly identified as upregulated by 1,25D and involved in the regulationof inflammation. Results are depicted relative to control and normalized to actin and GAPDH mRNA. (∗$p < 0.005$ compared to control). KLF4and TNFSF4, two genes previously described as induced by 1,25D [51, 54], were included as positive control. CCL2, Chemokine (C-C motif)ligand 2; CHRDL1, chordin-like 1; KLF4, Kruppel-like factor 4; TNFAIP6, Tumor necrosis Factor, alpha-induced protein 6; TNFSF4, Tumornecrosis factor (ligand) superfamily member 4; VCAM, Vascular cell adhesion molecule 1.

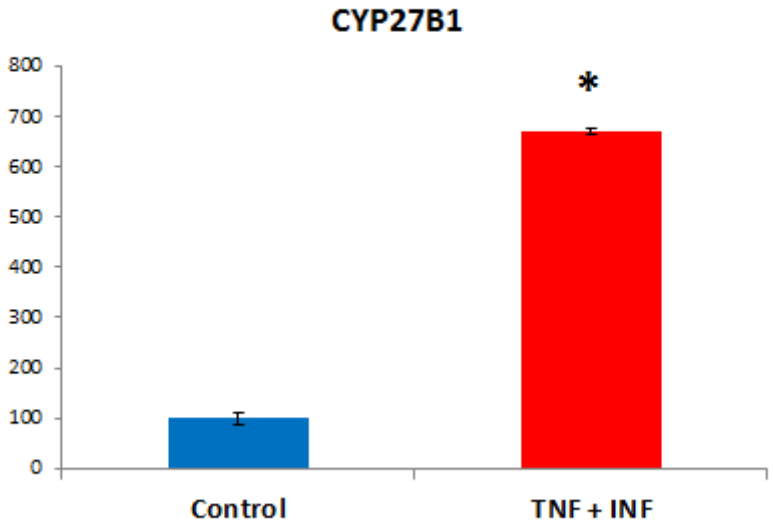


Fig. 3. TNF-α and Interferon-γinduce the expression of 25-Hydroxyvitamin D3 1alpha-Hydroxylase (CYP27B1) in human brain pericytes.Human brain pericytes were cultured in the presence or absence of TNF-α(50 ng/ml) and Interferon-γ (50 ng/ml) for 24 hours. Then RNA were extracted and CYP27B1 expression quantified by RT-qPCR. (*$p < 0.005$ compared to control).