



CD38 AND CALCIUM SIGNALLING

Raj Gurung^{1*} and Sushil Bhandari²

¹Medical school of Yangtze University, Jingzhou, Hubei, P.R. China

²The first affiliated Hospital of Yangtze University, Jingzhou, P.R. China

ABSTRACT

Human CD38 is a non-lineage-restricted type II transmembrane glycoprotein that has emerged as a multifunctional protein in recent years. It can serve as an ectoenzyme that catalyzes the synthesis and hydrolysis of cyclic ADP-ribose, Ca²⁺ mobilizing agent that acts independently of inositol triphosphate. The enzymatic functions of CD38 probably contribute to an array of its immune-regulatory functions. The release of soluble CD38 and the ability of membrane-bound CD38 to become internalized in response to appropriate stimuli suggest that extracellular and intracellular roles for this protein are equally plausible. Ligation of CD38 with agonistic antibodies induces diverse effects in hematopoietic cells that range from growth stimulation to induction and prevention from apoptosis, induction of cytokines, activation of kinases, and phosphorylation of certain proteins. Other molecules that share significant structural and functional homology to CD38 have been identified in humans and mice, suggesting that these molecules may represent a new family of proteins. Understanding the role of CD38 in certain pathological conditions such as myeloma, X-linked agammaglobulinemia, and HIV infection may provide insight into its physiological functions.

Keywords: Calcium Signaling, CD38 Protein, Smooth Muscle Cell, T-cell, B-cell.

INTRODUCTION

CD38 (cluster of differentiation 38), also known as cyclic ADP ribose hydrolase is a glycoprotein. [1] found on the surface of many immune cells (white blood cells), including CD4⁺, CD8⁺, B lymphocytes and natural killer cells. CD38 also functions in cell adhesion, signal transduction and calcium signaling. [2]

In humans, the CD38 protein is encoded by the *CD38* gene which is located on chromosome 4. [3,4]

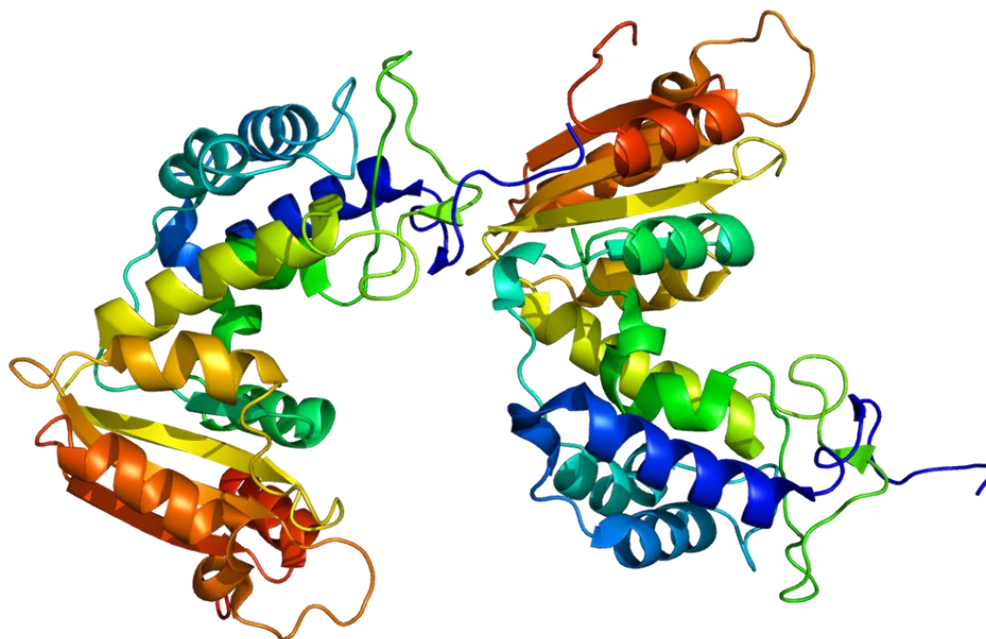
CD38 is a multifunctional ectoenzyme that catalyzes the synthesis and hydrolysis of cyclic ADP-ribose (cADPR) from NAD⁺ to ADP-ribose. These reaction products are essential for the regulation of intracellular Ca²⁺. [5]

Calcium ions are important for cellular signalling, as once they enter the cytosol of the cytoplasm they exert allosteric regulatory effects on many enzymes and proteins. Calcium act in signal transduction resulting from activation of ion channels or as a second messenger caused by indirect signal transduction pathways such as G protein-coupled receptors

The resting concentration of Ca²⁺ in the cytoplasm is normally maintained in the range of 10–100 nM. To maintain this low concentration, Ca²⁺ is actively pumped from the cytosol to the extracellular space and into the endoplasmic reticulum (ER), and sometimes in the mitochondria. Certain proteins of the cytoplasm and organelles act as buffers by binding Ca²⁺. Signaling occurs when the cell is stimulated to release calcium ions (Ca²⁺) from intracellular stores, and/or when calcium enters the cell through plasma membrane ion channels. [6]

Structure of CD38:

Human CD38 is a 45 kDa single-chain transmembrane glycoprotein with a short amino terminal cytoplasmic tail, a single membrane-spanning region, and a long extracellular carboxy-terminal domain. The cDNAs encoding human, mouse, and rat CD38 have been cloned [7,8], and the deduced amino acid sequences show significant homology among these CD38 proteins.



Based on its “amino terminus in and carboxy terminus out” topography, CD38 can be classified as a type II membrane protein. Four potential amino-terminal-linked glycosylation sites and two to four high-mannose amino terminal-linked oligosaccharide chains containing sialic acid residues contribute 25% of the molecular mass of the CD38 protein. A putative hyaluronate binding motif is also present in the extracellular domain of CD38 antigen. The gene that encodes human CD38 has been mapped to chromosome 4 by means of somatic cell genetics.[9] More recently, the subchromosomal localization of the human CD38 gene (4p15) has been achieved in the course of studies aimed at the genetic analysis of the molecule.[10] CD38 can be detected in vitro in culture supernatants from alloactivated T lymphocytes and CD38 tumor cell lines. It is also detectable in vivo in normal amniotic fluid and in serum and ascites from patients with multiple myeloma.[11] Shedding of membrane-bound CD38 can be induced in vitro by specific anti-CD38 antibodies and can be inhibited by N-ε-carboxymethyl-L-lysine chloromethyl ketone (a serine protease inhibitor). These results indicate that CD38 may act as a receptor for some unknown ligand, like many other receptors that are enzymatically cleaved from leukocyte membranes after their interaction with ligands or ligand-mimetic antibodies.[12] Recently, a high molecular mass form of CD38 (Mr 190 kDa) was identified in retinoic acid (RA) -induced human myeloid leukemia cells.[13] This high molecular weight form is a result of transglutaminase-catalyzed post translational cross-linking of mCD38. Of particular interest was the identification of a CD38-related gene, called BST-1 in the human [14] and BP3 in the mouse [15], that also maps to human chromosome 4 and to mouse chromosome 5 respectively. This may imply the existence of a CD38 gene cluster encoding a family of surface molecules that have similar structural and functional features.

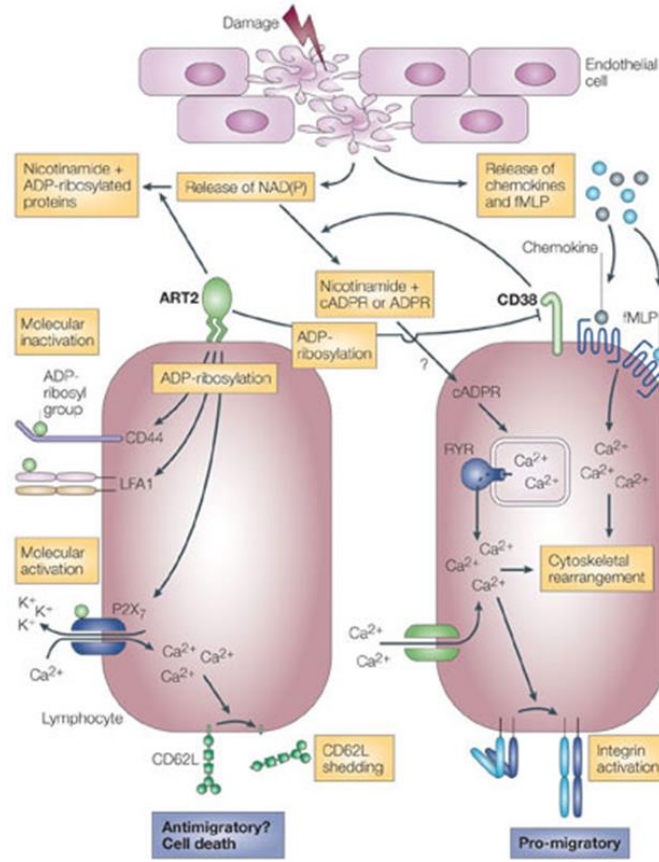
CD38 and transmembrane signalling:

CD38 involvement in transmembrane signaling is based on a substantial body of data obtained in diverse experimental systems. For the sake of simplicity, the results will be grouped according to the cell lineage in which the signaling effects have been observed. However, the results obtained in different cell lineages are too inconsistent to draw any definite conclusion. Most of the experiments involve the use of agonistic anti-CD38 mAbs, which should mimic the interaction between the CD38 receptor and its ligand (or ligands).

Effect on T cells:

The first hint that CD38 might play a role in signaling came from the observations that ligation of CD38 on peripheral blood mononuclear cells and T cell lines induced activation and proliferation signals.[16] Subsequent experiments revealed that ligation of human CD38 with specific mAbs induces the transcription of cytokines interleukin-1 (IL-1), tumor necrosis factor- α , and granulocyte-macrophage colony-stimulating factor at levels similar to those obtained after triggering T cell receptor CD3. However, the cytokines triggered in response to CD38 are qualitatively distinct from those induced via CD3.[11] For example, IL-6 mRNA and protein expression induced in response to CD38 ligation is greater than that induced via T cell receptor CD3. In contrast, interferon- γ , IL-2, and IL-1 β transcription is higher often activation by CD3 than after that by CD38. Another apparent difference in these two pathways is that CD38 mediated cytokine induction did not require either T cell proliferation or addition of antigen-presenting cells. The observation that signaling via CD38 in a T cell acute lymphoblastic leukemia (Jurkat) cell line led to Ca²⁺ mobilization with kinetics that were distinct from those induced via T cell receptor CD3 lend further support to this view.[12]

Another interesting observation was recently made after the analysis of protein phosphorylation induced in response to CD38 ligation. In Jurkat cells and a mutant cell line derived mutant cells, CD38 initiates a signaling pathway that leads to phosphorylation of a discrete set of proteins. The p120' proto-oncogene product is the major protein that is tyrosine phosphorylated in this signaling pathway. Furthermore, ligation of CD38 in a Jurkat variant lacking surface expression of the T cell receptor CD3 complex induces similar levels of tyrosine phosphorylation of p'20' as in the parental line. These results suggest that T cell receptor CD3 expression in T cells is not required to elicit some of the CD38-mediated activation event.



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Effects on B cells:

Its high levels of expression on immature lymphoid cells suggest that CD38 may play a role in the regulation of cell growth and differentiation, although direct evidence showing a functional activity in these cells is lacking. By using stroma-supported cultures of B progenitors and anti-CD38 mAbs, it has been shown that CD38 ligation inhibits the growth of immature B lymphoid cells in the bone marrow micro environment and that the interaction with a putative ligand represents a novel regulatory mechanism of B lymphopoiesis. The effects induced by CD38 ligation were further analyzed in mature B lymphocytes, where surprisingly it protected the cells from undergoing apoptosis. These effects were reminiscent of the effects seen in vivo by CD40 and its ligand (CD40L).

Effects on Smooth Muscle Cells:

Multiple lines of evidence support the second messenger role for CD38/cADPR in the regulation of $[Ca^{2+}]$ in a variety of smooth muscles. Kuemmerle and Makhlouf[16] demonstrated that addition of cADPR to

permeabilized longitudinal smooth muscle cells of the intestine results in a concentration-dependent increase in $[Ca^{2+}]_i$ and contraction. In airway smooth muscle, the role of cADPR in mediating calcium release has been investigated.[17] Addition of cADPR to permeabilized porcine airway smooth muscle cells resulted in a concentration-dependent increase in $[Ca^{2+}]_i$, which is inhibited by 8-amino-cADPR, a cADPR antagonist, and by ruthenium red and ryanodine, inhibitors of the RyRs. The intracellular calcium response to caffeine was also inhibited by ryanodine but not by 8-amino-cADPR. These observations suggested the possibility that caffeine and cADPR release SR calcium through RyR channels, but through different mechanisms. Caffeine most likely directly activates RyR channels, whereas cADPR acts through an indirect mechanism. The cADPR-induced elevation of $[Ca^{2+}]_i$ was not affected by heparin, an inhibitor of IP_3 Rs, suggesting that cADPR-mediated calcium release does not involve activation of IP_3 Rs. Furthermore, 8-amino-cADPR attenuated ACh-induced intracellular calcium oscillations, and addition of cADPR potentiated ACh-induced intracellular calcium oscillations. These studies together demonstrated the role of cADPR in the regulation of $[Ca^{2+}]_i$ in airway smooth muscle cells upon muscarinic receptor stimulation. Subsequent studies from our laboratory also confirmed that ADP-ribosyl cyclase and cADPR hydrolase enzyme activities are present in airway smooth muscle and are part of a single bifunctional protein CD38.[18] Recent studies performed using a membrane-permeant cADPR antagonist, 8-bromo-cADPR (8-Br-cADPR), reveal that several agonists utilize cADPR-mediated calcium release to mobilize calcium from intracellular stores in porcine and human airway smooth muscle cells (see recruitment of the cd38/cadpr signaling pathway below). In bovine tracheal smooth muscle cells, application of cADPR or 3-deaza-cADPR, a cADPR analog resistant to hydrolysis, potentiated ACh-induced elevation of $[Ca^{2+}]_i$ and contraction, which were inhibited by 8-amino-cADPR.[19] These studies provide the evidence for the role of cADPR in the regulation of $[Ca^{2+}]_i$ as well as contraction of smooth muscle isolated from bovine trachea.

In a study involving permeabilized smooth muscle cells obtained from porcine coronary vessels, it was demonstrated that addition of cADPR resulted in the release of calcium from intracellular stores.[20] Using reconstituted RyRs from coronary artery smooth muscle, Li et al.[21] have demonstrated that cADPR mobilizes calcium through RyR channels. In another recent study, Ge et al.[22] demonstrated the role of cADPR-mediated calcium release in agonist-induced calcium elevation and contractility of coronary arterial smooth muscle. In smooth muscle from seminiferous tubules, Barone and coworkers[23] demonstrated the role of cADPR in the calcium and contractile responses to endothelin (ET)-1. With freshly isolated cells and microsomes obtained from the myometrium, it has been shown that cADPR contributes to calcium and contractile responses elicited by oxytocin.[24]

The studies described above point to the role of CD38 and cADPR in the regulation of intracellular calcium and contraction of smooth muscle. However, the precise mechanisms by which agonist activation results in the recruitment of the CD38/cADPR signaling pathway are not clearly understood. Furthermore, how the extracellularly generated cADPR brings about intracellular calcium release is also not well understood.

Detailed investigations in this regard have been carried out by De Flora's group. In this regard, a recent study by Guida et al.[25] provides evidence for nucleoside transporters mediating influx of extracellular cADPR in 3T3 murine fibroblasts. Whether such transporters are expressed in smooth muscle cells and involved in cADPR influx remains to be determined.

Effects on Neurons:

Immunochemical studies performed by using polyclonal antibodies raised against selected peptide fragments of human CD38 indicate a specific immunoreactivity in the perikaryal and dendritic cytoplasm of neurons.[26] The neuronal localization of CD38 perfectly matches the predicted function of the molecule, which ultimately leads to the mobilization of intracellular Ca²⁺ and consequently may control certain brain functions such as neuronal plasticity. The CD38 antigen was also detected in the neurofibrillary tangles that occur in neuronal perikarya and proximal dendrites that are the pathological hallmark of Alzheimer's disease.[27]

CONCLUSION

In the past, CD38 has been viewed predominantly as a phenotypic marker of different subpopulations of T and B lymphocytes. Recent findings on its roles as multifunctional ectoenzyme and as a modulator of signal transduction pathways have renewed interests in this molecule. CD38 may mediate these effects either directly by interacting with a counter-receptor (on the surface of endothelial cells and BM stroma) or indirectly by virtue of its catalytic nature. cADPR, which is synthesized from NAD as a result of CD38 cyclase activity, is a potent inducer of Ca²⁺ release from intracellular stores. Intracellular calcium is a key factor in such important cell signaling pathways as cell growth, apoptosis, and differentiation. Similarly, the ability of CD38 to function as ADP-ribosyl transferase may account for some of its cell regulatory functions. The soluble form of CD38, released as a result of cell activation or interaction with cytokines, may compete for the counter-receptor with the cell-bound CD38 and play a role in regulating its activity. An understanding of the role of CD38 in hematopoiesis and in selected pathological conditions such as myeloma, HIV infection, and XLA may provide important information on the physiological functions of CD38.

REFERENCES

1. Orciani M, Trubiani O, Guarnieri S, Ferrero E, Di Primio R (2008). "CD38 is constitutively expressed in the nucleus of human hematopoietic cells". *J. Cell. Biochem.* 105 (3): 905-12. [doi:10.1002/jcb.21887](https://doi.org/10.1002/jcb.21887). PMID 18759251.
2. "Entrez Gene: CD38 CD38 molecule".
3. Jackson DG, Bell JI (1990). "Isolation of a cDNA encoding the human CD38 (T10) molecule, a cell surface glycoprotein with an unusual discontinuous pattern of expression during lymphocyte differentiation". *J. Immunol.* 144 (7): 2811-5. PMID 2319135.

4. Nata K, Takamura T, Karasawa T, Kumagai T, Hashioka W, Tohgo A, Yonekura H, Takasawa S, Nakamura S, Okamoto H (1997). "Human gene encoding CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase): organization, nucleotide sequence and alternative splicing". *Gene* 186 (2): 285–92. doi:10.1016/S0378-1119(96)00723-8. PMID 9074508.
5. Malavasi F, Deaglio S, Funaro A, Ferrero E, Horenstein AL, Ortolan E, Vaisitti T, Aydin S (2008). "Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology". *Physiol. Rev.* 88 (3): 841–86. doi:10.1152/physrev.00035.2007. PMID 18626062.
6. Clapham, D.E. (2007). "Calcium Signaling". *Cell* 131 (6): 1047–1058. doi:10.1016/j.cell.2007.11.028. PMID 18083096
7. Jackson, D.C., and Bell, J. I. (1990) Isolation of a cDNA encoding the human CD38 (T10) molecule, a cell surface glycoprotein with an unusual discontinuous pattern of expression during lymphocyte differentiation. *J. Immunol.* 144,2811-2817
8. Harada, N., Santos-Argumedo, L., Chang, R., Grimaldi, C. J., Lund, F. E., Brannan, C. I., Copeland, N. G., Jenkins, N. A., Heath, A. W., Parkhouse, R. M., and Howard, M. (1993) Expression cloning of a cDNA encoding a novel murine B cell activation marker: homology to human CD38. *J. immunol.* 151, 3111-3118.
9. I, Furuya, Y., Yonekura, H., and Okamoto, H. (1994) Cloning and characterization of cDNA encoding rat ADP-ribosyl cyclase/cyclic ADP ribose hydrolase from islets of Langerhans. *Biochim. Biophys. Acta* 1223, 160-162
10. Funaro, A., Spagnoli, C. C., Ausiello, C. M., Allesio, M., Roggero, S., Delia, D., Zaccolo, M., and Malavasi, F. (1990) Involvement of the multilineage CD38 molecule in a unique pathway of cell activation and proliferation. *J. Immunol.* 145,2390-239
11. Ausiello, C. M., 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
12. Deaglio, S., Dianzani, U., Horenstein, A. L, Fernandez, i.E., Van Kooten, C., Bragardo, M., Garbanino, C., Funaro, A., DiVirgilio, F., Banchereau, I., and Malavasi, F. (1996) Human CD38 ligand. A 120-kDa protein predominantly expressed by endothelial cells. *J. Immunol.* 156, 727-734
13. Kumagai, M., Coustan-Smith, E., Murry, D. J., Silvennoinen, O., Murti, C. P., Evans, W. E., Malavasi, F., and Campana, D. (1995) Ligation of CD38 suppresses human B lymphopoiesis. *J. Exp. Med.* 181, 1101-1110
14. Zupo, S., Rugari, E., Dono, M., Tamborelli, C., Malavasi, F., and Ferrarini, M. (1994) CD38 signalling by agonistic monoclonal antibody prevents apoptosis of human germinal center B cells. *Eur. J. Immunol.* 24,1218-1222
15. M.,Ito, C., Malavasi, F., Lin, Q., Conley, M. E., and Campana, D. (1996) CD38 signal transduction in human B cell precursors. *J. Immunol.* 156, 100-107
16. Kuemmerle JF and Makhlof GM. Agonist-stimulated cyclic ADP ribose. Endogenous modulator of Ca²⁺-induced Ca²⁺ release in intestinal longitudinal muscle. *J Biol Chem* 270: 25488–25494, 1995.

17. Prakash YS, Kannan MS, Walseth TF, and Sieck GC(1998). Role of cyclic ADP-ribose in the regulation of $[Ca^{2+}]_i$ in porcine tracheal smooth muscle. *Am J Physiol Cell Physiol* 274: C1653–C1660.
18. White TA, Johnson S, Walseth TF, Lee HC, Graeff RM, Munshi CB, Prakash YS, Sieck GC, and Kannan MS(2000). Subcellular localization of cyclic ADP-ribosyl cyclase and cyclic ADP-ribose hydrolase activities in porcine airway smooth muscle. *Biochim Biophys Acta* 1498: 64–71.
19. Franco L, Bruzzone S, Song P, Guida L, Zocchi E, Walseth TF, Crimi E, Usai C, De Flora A, and Brusasco V(2001). Extracellular cyclic ADP-ribose potentiates ACh-induced contraction in bovine tracheal smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 280: L98–L106.
20. Kannan MS, Fenton AM, Prakash YS, and Sieck GC(1996). Cyclic ADP-ribose stimulates sarcoplasmic reticulum calcium release in porcine coronary artery smooth muscle. *Am J Physiol Heart Circ Physiol* 270: H801–H806.
21. Li PL, Tang WX, Valdivia HH, Zou AP, and Campbell WB(2001). cADP-ribose activates reconstituted ryanodine receptors from coronary arterial smooth muscle. *Am J Physiol Heart Circ Physiol* 280: H208–H215.
22. Ge ZD, Zhang DX, Chen YF, Yi FX, Zou AP, Campbell WB, and Li PL(2003). Cyclic ADP-ribose contributes to contraction and Ca^{2+} release by M1 muscarinic receptor activation in coronary arterial smooth muscle. *J Vasc Res* 40: 28–36.
23. Barone F, Genazzani AA, Conti A, Churchill GC, Palombi F, Ziparo E, Sorrentino V, Galione A, and Filippini A(2002). A pivotal role for cADPR-mediated Ca^{2+} signaling: regulation of endothelin-induced contraction in peritubular smooth muscle cells. *FASEB J* 16: 697–705.
24. Barata H, Thompson M, Zielinska W, Han YS, Mantilla CB, Prakash YS, Feitoza S, Sieck G, and Chini EN(2004). The role of cyclic-ADP-ribose-signaling pathway in oxytocin-induced Ca^{2+} transients in human myometrium cells. *Endocrinology* 145: 881–889.
25. Barata H, Thompson M, Zielinska W, Han YS, Mantilla CB, Prakash YS, Feitoza S, Sieck G, and Chini EN(2004). The role of cyclic-ADP-ribose-signaling pathway in oxytocin-induced Ca^{2+} transients in human myometrium cells. *Endocrinology* 145: 881–889.
26. Mizuguchi, M., Otsuka, N., Sato, M., Ishii, Y., Kon, S., Yamada, M., Nishina, H., Katada, I., and Ikeda K. (1995) Neuronal localization of CD38 antigen in the human brain. *Brain Res.* 697, 235-240
27. Otsuka, K., Mizuguchi, M., Aizawa, I., Haga, S., Sato, M., Inoya, H., Namba, Y., Machinami, R., and Ikeda, K. (1994) Immunoreactivity in Alzheimer's neurofibrillary tangles. *Brain Pathol.* 4, 558 (abstr.)