Antibodies directed to Neisseria gonorrhoeae crossreact with human *post mortem* choroid plexus epithelial cells and HIBCPP choroid plexus papilloma cells *in vitro*

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Introduction

First trimester maternal infections with the Gram-negative bacterium Neisseria gonorrhoeae (NG), increase the risk for the offspring to suffer from psychotic symptoms in later life.* Both morphological and functional findings indicate that this may be a result of a dysfunction of the choroid plexus (Figs. 01 and 02). We hypothesize here an antibody mediated mechanism for this, and investigated therefore interactions of a rabbit antiserum directed to NG (α-NG) with postmortem samples of human choroid plexus and with HIBCPP cells, an in vitro model of human choroid plexus epithelium.

* Sørensen et al., 2009, Schizophrenia Bull. 35, 631-637.

Materials & Methods

Antibodies and proteins: Anti-Neisseria gonorrhoeae (**α-NG**): rabbit polyclonal, Antikoerper-online.de, Cat. Nr. ABIN285584. Anti-Neisseria meningitidis (**α-NM**): rabbit polyclonal, Antikoerper-online.de, Cat. Nr. ABIN285585. **Anti-rabbit IgG,** goat polyclonal, Peroxidase coupled, Sigma-Aldrich, Cat. Nr. A9169. **Anti-rabbit IgG**, goat polyclonal, Biotin coupled, Sigma-Aldrich, Cat. Nr. B8895. Recombinant human heat shock protein **Hsp60**, Antikoerper-online.de, Cat. Nr. ABIN621577. Recombinant human Retinal Dehydrogenase 1 **(ADH1)**, Antikoerper-online.de, Cat. Nr. ABIN666900. Recombinant human Aldehyde Dehydrogenase 2 **(ADH2)**, Antikoerper-online.de, Cat. Nr. ABIN1525864. ATP-Binding Protein **(ATPB)**, Antikoerper-online.de, Cat. Nr. ABIN1346052.

Tissue samples and cell culture: Human postmortem plexus choroideus was derived from paraformaldehyde fixed brains used for anatomical dissection, donors of which have given written consent to the use of their bodies for this purpose. HIBCPP cells were maintained as monolayers at 37°C and 5% CO_2, in high serum (HS) medium (DMEM-F12 supplemented with 15% FCS, 5µg/ml Insulin, and Penicillin/Streptomycin) with the medium being exchanged every other day. For differentiation cells were cultivated in low serum (LS) medium, containing 1% FCS.

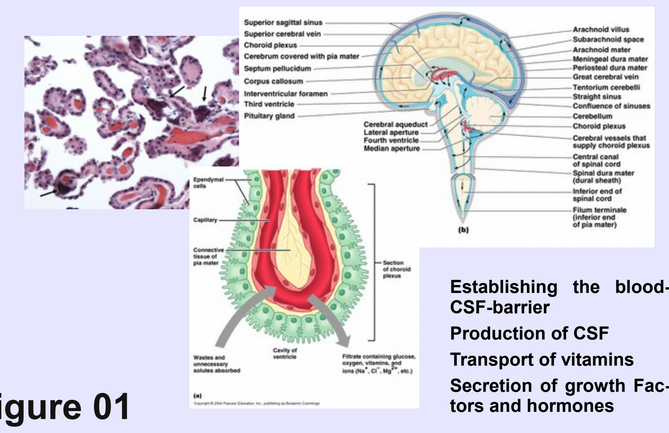
Immunocytochemistry: Tissue samples were postfixed in 4% PFA overnight. Cryofixed tissue blocks were cut at 10µm thick sections on a Cryomicrotome and mounted on glas slides. Also cultured cells were fixed for 10' with 4% paraformaldehyde. After treatment with Acetone/ Methanol (1:1), followed by three washes with PBS, sections/cells were blocked for 1h with goat serum (1:50) in PBS. Primary antibodies were applied over night at 4°C, followed by three washes with PBS and biotin-coupled secondary antibodies (1:400 at RT). After three washes with PBS, Peroxidase-coupled Streptavidin was used to label the antibody tagged proteins by DAB-staining. After three final washing steps and covering, microscopic imaging was performed using an Axiocam digital camera system mounted on an Axiophot microscope (Zeiss).

Western blot analysis: Cells were harvested in 5x Laemmli sample-buffer and protein concentration was densometrically determined. 5µg of total cellular protein was separated by polyacrylamide gel electrophoresis (8,5%). After tank-blot Western-transfer on PVDF-membranes, they were blocked with dry milk and incubated with primary antibodies (1:2000, 4°C) over night. After washing, rabbit specific peroxidase-coupled secondary antibodies (1:10.000) were applied for 90' at RT, and after washing, ECL-detection was performed. For some experiments, blots were stripped with NaOH (0,1mol/l) for 15' and after blocking, were re-incubated with another primary antibody.

2D-Gel electrophoresis: Cells were harvested in 2x ampholyte sample buffer, and protein concentration was determined densometrically. For the 1st dimension by pH-dependent isoelectric focusing, 30µg of total cellular protein was loaded on amphpholyte (pH3,5-10) containing polyacrylamide gel slices and separated electrophoretically. For the 2nd dimension, gel slices were equilibrated in Laemmli sample buffer and located on top of a standard SDS polyacrylamide gel, where proteins were separated according to their molecular weight. After tank-blot Western-transfer onto PVDF membrane, proteins were stained by immune incubation, and α-NG immunreactive protein spots were localized by ECL-detection and film autoradiography.

Digestion and LC-Q-TOF MS/MS analysis: Labeled protein spots were excised and digested as described previously*. Obtained peptides were reconstituted in an aqueous solution and introduced onto two consecutive nano-C18-reversed-phase chromatography columns using an auto CapLC sampler (Waters). The separated peptides were analyzed in a Q-TOF Ultima Global mass spectrometer (Waters) equipped with a nanoflow ESI Z-spray source in the positive ion mode. Data were acquired with the MassLynx (v4.0) software on a Windows NT PC and further processed using ProteinLynx Global Server (PLGS; v 2.2; Micromass). Obtained peak lists were searched online (http://www.matrixscience.com) by the MASCOT algorithm against the NCBI and SwissProt database.
*Asif et al., 2010, Electrophoresis 31, 1947-1958.

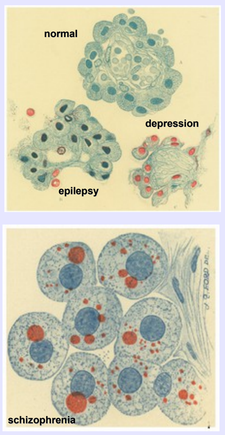
Functions and morphology of the human choroid plexus



- Establishing the blood-CSF-barrier
- Production of CSF
- Transport of vitamins
- Secretion of growth Factors and hormones

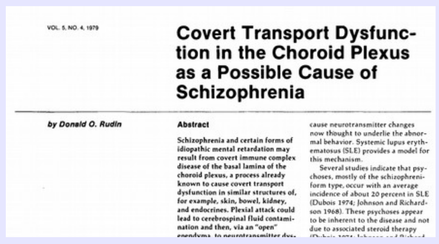
Figure 01

Choroid plexus morphology and functions are changed in schizophrenic patients



„The three cases of *dementia proecox* examined did not show such marked changes as those met with in general paralysis and other cases. The epithelium was well preserved. The cells were rather smaller and longer than normal and showed a diminution of basophil chromatin matter with a correspondingly increased acidophil reaction. The epithelial cells showed more granular chromatin substance, especially of cholesterol character, and fat droplets. The connective tissue stroma and endothelium show a moderate proliferative fibrous degeneration around the large vessels and villi of the choroid plexus. They show also hyaline and concentric amyloid bodies. Even the surfaces of large cystic formations met with in these cases are usually covered with flourishing epithelial cells, while the stroma shows an embryonic mucoid metamorphosis of the connective tissue cells and fibres accompanied with forms of fat degeneration. The cells of the stroma are especially abundant in lipoid of cholesterol nature and often neutral fat."

Morowoka T. 1921. The Microscopical Examination of the Choroid Plexus in General Paralysis of the Insane, and other forms of Mental Disease. Proc R Soc Med. 14:23-33.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Covert Transport Dysfunction in the Choroid Plexus as a Possible Cause of Schizophrenia

by Donald O. Rudin

Abstract

Schizophrenia and certain forms of idiopathic mental retardation may result from covert immune complex disease of the basal lamina of the choroid plexus, a process already known to cause covert transport dysfunction in similar structures of, for example, skin, bowel, kidney, and endocrines. Plexial attack could lead to cerebrospinal fluid contamination and then, via an "open" ependyma, to neurotransmitter dys- cause neurotransmitter changes now thought to underlie the abnormal behavior. Systemic lupus erythematosus (SLE) provides a model for this mechanism.

Several studies indicate that psychoses, mostly of the schizophrenia form type, occur with an average incidence of about 20 percent in SLE (Dubois 1974; Johnson and Richardson 1968). These psychoses appear to be inherent to the disease and not due to associated steroid therapy

Rudin DO. 1979. Covert transport dysfunction in the choroid plexus as a possible cause of schizophrenia. Schizophr Bull 5:623-626.

Figure 02

Crossreactivity of α-NG and α-NM with human choroid plexus epithelium

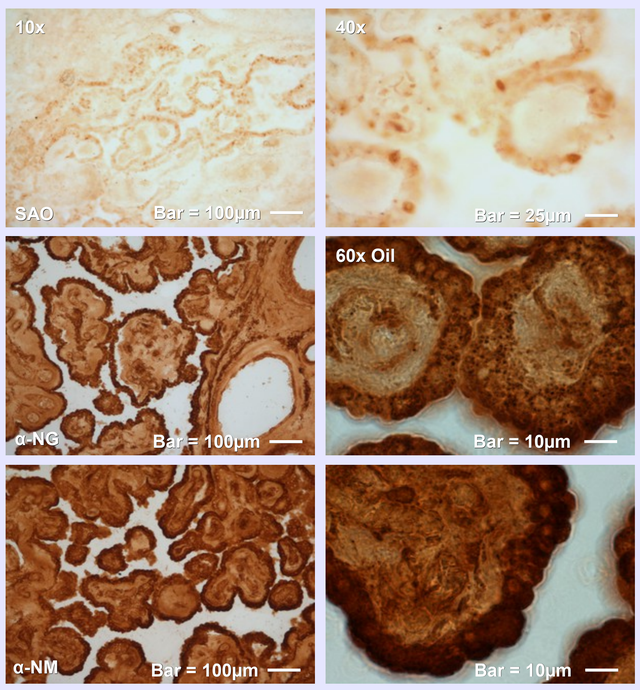


Figure 03

Crossreactivity of α-NG and α-NM in HIBCPP-cells: IHC and WB

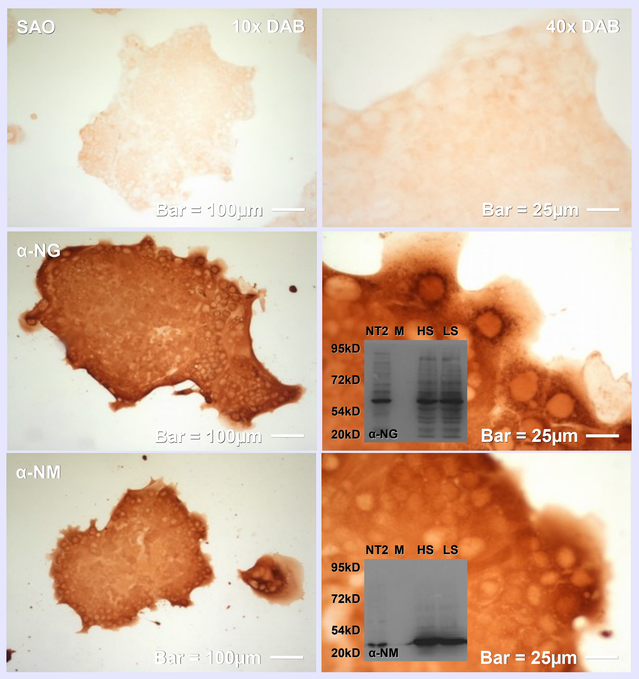
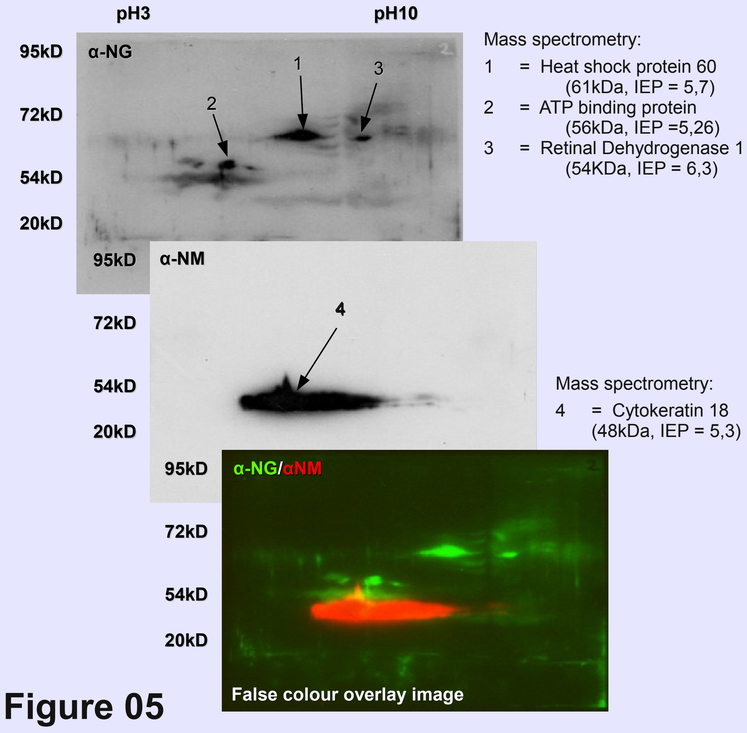


Figure 04

Crossreactivity of α-NG and α-NM in HIBCPP-cells: 2D-WB



Mass spectrometry:
1 = Heat shock protein 60 (61kDa, IEP = 5,7)
2 = ATP binding protein (56kDa, IEP =5,26)
3 = Retinal Dehydrogenase 1 (54KDa, IEP = 6,3)

Mass spectrometry:
4 = Cytokeratin 18 (48kDa, IEP = 5,3)

Figure 05

Specific crossreactivity of α-NG and α-NM with HIBCPP, ADH1, ADH2, and Hsp60: WB

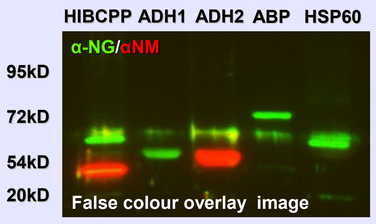


Figure 06

Results

Immunocytochemistry revealed α-NG to label both in human choroid plexus (Fig. 03) and HIBCPP cells (Fig. 04), antigens located in an intracellular organelle, which by Western blot revealed a prominent band of around 60kDa, as several weaker bands of higher and lower molecular weights (Fig. 04). In contrast, an antiserum directed to Neisseria meningitidis (α-NM) reacted with a cytoplasmic antigen with a molecular weight of around 40kDa (Fig. 03 and 04). By 2D-immunoblot (Fig. 05), several α-NG-immunoreactive protein spots could be localized, and identified by LC-Q-TOF MS/MS analysis as human mitochondrial heat-shock protein Hsp60, ATP binding protein ABP, and Aldehyde Dehydrogenase ADH1. The protein interacting with α-NM was identified by mass spectrometry as Cytokeratin 18. For ADH1, ABP, and Hsp60, this interaction could be confirmed by Western blot analysis (Fig. 06) demonstrating crossreactivity of α-NG with commercially available recombinant proteins. Surprisingly also an interaction of α-NM with ADH2 could be detected, which otherwise failed to bind α-NG.

Conclusions

These results demonstrate that in human choroid plexus epithelium, antibodies directed to Neisseria gonorrhoeae interact with cellular proteins such as Hsp60, ABP and ADH1. Known functions of these proteins suggest impaired production of energy rich substrates such as ATP, as well as of retinoic acid to be a consequence. Whereas impaired mitchondrial functioning could directly affect choroid plexus transport functions, diminished retinoic acid production could indirectly affect neuronal differentiation during brain development, functional consequences of, i.e. with regard to schizophrenia pathogenesis will have to be studied at more detail in the future.

Acknowledgements

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