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Arachnoid cell involvement in the mechanism of coagulation-initiated inflammation in the subarachnoid space after subarachnoid hemorrhage*

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Abstract: Objective: To assess if arachnoid cells have the capability to present antigen and activate T-lymphocytes after stimulation by bloody cerebrospinal fluid (CSF), and to illuminate the mechanism of coagulation-initiated inflammation in the subarachnoid space after subarachnoid hemorrhage (SAH). Methods: Arachnoid cells were cultured, characterized, and examined by immunofluorescence for the basal expression of human leukocyte antigen-DR (HLA-DR). Expression of HLA-DR, after co-culturing arachnoid cells in vitro with bloody CSF, was investigated by immunofluorescence and flow cytometry (FCM). The variation of arachnoid cells' ultrastructure was observed by transmission electron microscope (TEM). Arachnoid cells were co-cultured with peripheral blood mononuclear cells (PBMCs). The content of soluble interleukin-2 receptor (sIL-2r) in culture medium was detected by enzyme-linked immunosorbent assay (ELISA). Results: (1) Arachnoid cells were successfully cultured for many passages. The immunofluorescent staining was positive for HLA-DR in over 95% of the human arachnoid cells. The punctate HLA-DR was distributed in cytoplasm and not in the karyon. (2) After co-culturing arachnoid cells in vitro with bloody CSF, numerous particles with strong fluorescence appeared in the cytoplasm on Day 6. On Day 8, the quantity of particles and fluorescent intensity were maximal. FCM showed that the percentage of HLA-DR expressing cells was (2.5±0.4)% at the first 5 d, increasing to (60.8±3.6)% on Day 7. (3) After co-culturing arachnoid cells in vitro with bloody CSF, many lysosome and secondary lysosome particles were present in the cytoplasm. Hyperplasia of rough endoplasmic reticulum and enlarged cysts were observed, with numerous phagocytizing vesicles also observed at the edge of the arachnoid cells. (4) Arachnoid cells stimulated by bloody CSF were co-cultured in vitro with PBMCs. The content of sIL-2r in the culture medium, having been maintained at around 1.30 ng/ml during the first 3 d, had increased by Day 4. The content of sIL-2r peaked 7.53 ng/ml on Day 7 and then reduced gradually. Conclusions: (1) Basic HLA-DR expression is present in arachnoid cells. (2) After stimulation by bloody CSF, arachnoid cells have the potential to serve as antigen presenting cells (APCs) and the ability to activate T-lymphocytes, indicating that arachnoid cells are involved in the mechanism of coagulation-initiated inflammation in the subarachnoid space after SAH.

Key words: Arachnoid cells, Cell culture, Human leukocyte antigen-DR (HLA-DR), Soluble interleukin-2 receptor (sIL-2r)
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1 Introduction

Subarachnoid hemorrhage (SAH) is a frequent occurrence in cerebrovascular accidents, and cerebral vasospasm, especially delayed cerebral ischemia (DCI), is a serious complication after SAH (Kubota *et al*., 1993; Raymackers *et al*., 2000; Claassen *et al*., 2001; Gomis *et al*., 2005; Todo *et al*., 2008; Eddleman *et al*., 2009). The aetiology and pathophysiology of SAH-related vasospasm remain controversial (Chrapusta *et al*., 2000; Wada *et al*., 2002; Hendryk *et al*., 2004; Aydin *et al*., 2005; Wang

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et al., 2005; Nekludov *et al*., 2007). Some studies (Frank and Boyce, 1983; Akopov *et al*., 1992; Mathiesen *et al*., 1993; Massicotte and Del Bigio, 1999; Chrapusta *et al*., 2000; Ahn *et al*., 2002; Kim *et al*., 2002; Zubkov *et al*., 2002; Stein and Smith, 2004; Harrod *et al*., 2005) have shown that lymphocytes and macrophages are observed in the subarachnoid space during Days 2–5 after SAH. On Day 5, the quantities of T-lymphocytes and macrophages peak, and the proportion of CD4/CD8 is also increased. The activated mononuclear macrophages and lymphocytes secrete endothelin-1 and cytokines (e.g., interleukin-6 (IL-6), soluble interleukin-2 receptor (sIL-2r), tumor necrosis factor-α, adhesion molecules). Inflammation occurs in the subarachnoid space after SAH. The cerebral vasospasm after experimental SAH is prevented by lymphocyte-associated antigen-1 monoclonal antibody and humanized anti-CD11/CD18 monoclonal antibody despite the unaltered presence of hemoglobin (Frank, 1995; Ogihara *et al*., 2001; Clatterbuck *et al*., 2002; Pradilla *et al*., 2004). These data support the hypothesis that inflammation plays a role in cerebral vasospasm after SAH.

The coagulation/inflammation interface participates in a variety of disease processes, and the interactions between coagulation and inflammation are complex. Inflammation cytokines are the major mediators involved in coagulation activation (Sanchez *et al*., 2004; Esmon, 2005; Muth *et al*., 2005; Recinos *et al*., 2006). In addition, the tissue factor-VIIa complex can induce pre-inflammatory effects on macrophage/monocyte, such as the expression of major histocompatibility complex (MHC) class II molecules (Motohashi *et al*., 1995b; Chrapusta *et al*., 1999). It is still unknown whether arachnoid cells are involved in the interactions between coagulation and inflammation in the subarachnoid space after SAH.

In the present study, arachnoid cells were co-cultured with bloody cerebrospinal fluid (CSF) and peripheral blood mononuclear cells (PBMCs) to simulate the microenvironment of the subarachnoid space after SAH. In addition to assessing if arachnoid cells have the capability to present antigen and activate T-lymphocytes after stimulation by CSF, it was the further aim of our study to preliminarily illuminate the mechanism of coagulation-initiated inflammation in the subarachnoid space after SAH.

2 Materials and methods

2.1 Culture and passage of human arachnoid cells

Under the operating microscope, we cut off the human arachnoid membranes which were transparent, colourless, and without adhesions. The membranes were cut into $\leq$1 mm^3 sections. These sections were plated in 25-cm^2 Falcon flasks with complete medium (Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% (v/v) fetal bovine serum, 100 U/ml penicillin, 100 U/ml streptomycin, and 0.1 mU/ml insulin). The flasks were maintained at 37 °C in a 95% humidity incubator with 5% CO_2 atmosphere. The medium was added into the flask 24 h later and changed every 3 to 4 d. Arachnoid cells were passaged on Days 20–24 from the bottom of the flask. After the medium was removed, the arachnoid cells were washed twice in Hanks' buffer and digested in 1:1 (v/v) dilution with 0.25% (w/v) trypsin and 0.02% (w/v) ethylenediamine tetra-acetic acid (EDTA) at room temperature for 8–10 min. The complete medium was added to stop the digestion, with an arachnoid cell suspension concentration of 4×10^5 cells/ml. The cell suspension was used for serial subcultivation. The arachnoid cells were passaged two to three times prior to testing.

2.2 Expression of cytokeratin 8&18 and HLA-DR in arachnoid cells by immunofluorescence staining assay

The passaged cells were fixed on coverslips in acetone-methanol (1:1, v/v) solution and then rinsed by 0.01 mol/L phosphate buffered saline (PBS). A total of 200 μl deactivated rabbit serum (1:20 (v/v) dilution) was used to block Fc receptor at 4 °C for 3 h. Arachnoid cells were stained for immunofluorescence. The primary antibody, monoclonal mouse anti-cytokeratin 8&18, was used at a 1:100 (v/v) dilution and the monoclonal mouse anti-human leukocyte antigen-DR (HLA-DR) was used at a 1:200 (v/v) dilution. The secondary antibody, fluorescein-conjugated goat anti-rabbit immunoglobulin G (IgG; Sigma, USA) was used at a 1:100 (v/v) dilution. The preparation was examined with fluorescence microscopy (Olympus BX50, Olympus Co., Tokyo, Japan) and photographed with Kodak film (Eastman Kodak Co., NY, USA).

2.3 Investigation of variation of HLA-DR expression of arachnoid cells co-cultured with bloody CSF by immunofluorescence and flow cytometry

Arachnoid cells were grown on coverslips in each well of the culture plate. We obtained the bloody CSF from patients 3 d after spontaneous SAH by lumbar puncture and maintained it at 4 °C. We diluted the bloody CSF by serum-free medium, keeping the number of red blood cells at 20000 cells/ml, and 0.3 ml of this CSF was added with each change of the medium. The cells of three independent wells were examined daily for 12 d.

Arachnoid cells were collected as scheduled. These cells were placed into cell suspension. Cell concentration was adjusted to 5×10^6–1×10^7 cells/ml. A total of 40 μl of cell suspension was placed into a centrifuge tube with 2 μg of monoclonal mouse anti-human HLA-DR. In addition, 2 μg of control murine antibody with the same isotype was added to control samples. A total of 50 μl deactivation cony serum 1:20 (v/v) dilution by Dulbecco's phosphate-buffered saline (DPBS; NaCl 80 g, KCl 2 g, Na_2HPO_4 11.5 g, KH_2PO_4 2 g, distilled water 1000 ml, 1:10 (v/v) dilution when used) was added to every sample. These samples were maintained at 4 °C for 30 min. After being washed, 50 μl 1:250 (v/v) diluted fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG was added and shaken, washed twice with 1 ml fixation fluid (DPBS 1000 ml, glucose 20 g, formaldehyde 10 ml, NaN_3 0.2 g, final concentration 0.02% (w/v)) before examination by flow cytometry (FCM; Elite ESP Type, Coulter Co., California, USA).

2.4 Investigation of variation of ultrastructure of arachnoid cells co-cultured with bloody CSF by transmission electron microscope

Arachnoid cells were co-cultured with bloody CSF under the previously mentioned concentration for 7 d. Cells of the control sample were cultured without bloody CSF. They were digested into monoplast suspension, washed by 0.15 mol/L PBS, centrifuged into cell cakes, fixed by 2.5% (w/v) glutaral phosphate buffer, washed by cold cane sugar buffer, fixed by osmic acid, dehydrated by acetone, and then dipped in araldite for rigidification. These samples were made into extra-thin sections for examination by transmission electron microscope (TEM; JEM-2000EX, Jed, Japan) and photos.

2.5 Variation of the sIL-2r content in medium after arachnoid cells were co-cultured with PBMCs and stimulated by bloody CSF

Abstraction, conservation, and culture of PBMCs: 20 ml venous blood was taken from the same patient when obtaining the arachnoid membrane. PBMCs were separated by Ficoll density gradient centrifugation. A concentration of 5×10^6 cells/tube within 90% (w/v) RPMI-1640/10% (v/v) dimethyl sulfoxide (DMSO) was maintained in liquid nitrogen. After revitalization, PBMCs were cultured in RPMI-1640 supplemented with 10% (v/v) fetal bovine serum for 48 h. Cell density was adjusted to 10^5 cells/ml.

Grouping: control group 1 (CG1), arachnoid cells were co-cultured with PBMCs; control group 2 (CG2), PBMCs were cultured alone; experimental group (EG), arachnoid cells stimulated by bloody CSF were co-cultured with PBMCs.

Cultivation of cells: arachnoid cells were cultured in a 96-well plate at a concentration of 10^2 cells/well. CG1: 100 μl PBMC suspension (about 1×10^5 cells/ml) was added into every well. EG: 0.3 ml bloody CSF with same concentration was added for stimulation for 3 d. This suspension was removed from the bloody CSF and co-cultured with PBMCs for 9 d. The supernatant of the culture was collected on Days 1–9 with the duplicates.

Human sIL-2r enzyme-linked immunosorbent assay (ELISA) kit (Antigenix, USA) and ELISA detector (Model 550, Bio-Rad Company, USA) were used in the examination of sIL-2r content of supernatant. Mean and standard deviation (SD) of every four optical density (OD) values at different times were calculated and compared with standard curve for sIL-2r content.

The statistical work-up was performed by using SPSS 11.0 computer programme to compare CG1 and CG2 with EG. The confidence interval and significance level were set at be 95% and $P<0.05$, respectively.

3 Results

3.1 Appearance of cultured arachnoid cells

The cells formed a monolayer (Fig. 1) and were polygonal in shape. The cells had larger nuclei and had one to two nucleoli each. Many granules existed

in the cytoplasm. The cells were compact and had good refraction. The appearance of the cells was some similar to epithelium. Cell proliferation increased during Days 8–15. After six to eight passages, cell growth declined.

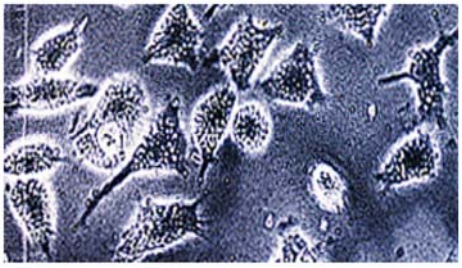

Fig. 1 Appearance of cultured arachnoid cells
The cells formed a monolayer and were polygonal in shape (×100)

3.2 Ultrastructure of cultured arachnoid cells

Keratin was uniformly distributed in the cytoplasm. A moderate quantity of rough endoplasmic reticula and chondriosomes were seen. Affluent Golgi bodies, ribosomes, and small quantities of cytolysis were also observed. Secretory granules existed in a few cells. The cystoskeleton was made up of intermediate filament and microfilament. The structure of cells was somewhat similar to epithelium.

3.3 Cytokeratin 8&18 and HLA-DR expression of cultured arachnoid cells

Cytokeratin 8&18 was expressed by the majority of cells (over 95%). They were silky and distributed in cytoplasm, but not in the karyons. HLA-DR was also basally expressed by the majority of cells (over 95%). They were punctiformly distributed in cytoplasm, but not in the karyons.

3.4 Immunofluorescence detection on variation of HLA-DR expression of cultured arachnoid cells after being stimulated by bloody CSF

From 1–120 h, the expression of HLA-DR had no obvious variation. By Day 6, many particles with strong fluorescence had begun to appear in the cytoplasm, mostly around the karyon. On Day 8, the quantity of the particles and their fluorescent intensity were at the vertex (Fig. 2). Thereafter, the fluorescent intensity became weak and fluorescent granules decreased.

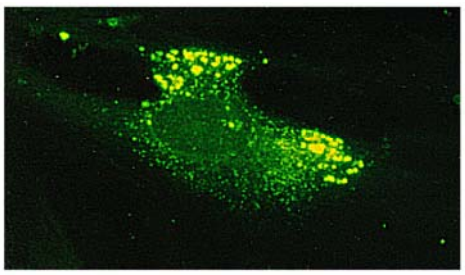

Fig. 2 Immunofluorescence expression of HLA-DR on Day 8
The quantity of the particles and their fluorescent intensity were at the vertex (×400)

3.5 FCM detection on variation of HLA-DR expression of cultured arachnoid cells after stimulation by bloody CSF

After stimulation by bloody CSF, the percentage of HLA-DR expressing cells remained at (2.5±0.4)% (n=3) for 1–120 h. On Day 6, the percentage increased to (9.2±1.2)%. On Day 7, the percentage increased to (60.8±3.6)% (Fig. 3), and then began to decrease. On Day 10, it was (12.5±2.5)%. The variation detected by FCM coincided in time with that of immunofluorescence.

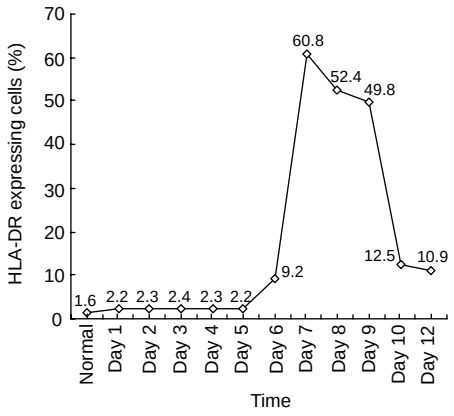

Fig. 3 FCM detection of HLA-DR expression of arachnoid cells after stimulation by bloody CSF

3.6 TEM detection on variation of arachnoid cells' ultrastructure after stimulation by bloody CSF

On Day 7, lysosomes and secondary lysosomes were greatly increased in number. Hyperplasia of rough endoplasmic reticulum and an enlarged cyst pool were observed. Many pinocytosis bubbles were observed at the edge of cells. Some bubbles were observed in the endocytic process (Fig. 4).

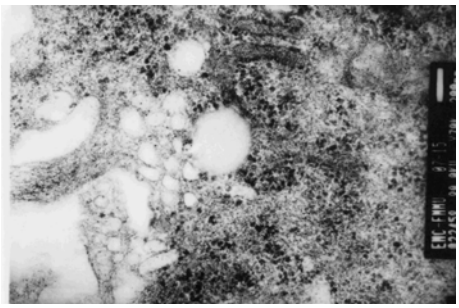

Fig. 4 Ultrastructure of arachnoid cells after stimulation by bloody CSF under TEM
Pinocytosis bubbles are observed at the edge of cells (×30000)

3.7 ELISA detection on variation of sIL-2r content in the culture supernatant

The sIL-2r contents in the culture supernatants of CG1 and CG2 were unchanged with time-lapse and were about 1.40 ng/ml, but it appeared different when arachnoid cells stimulated by bloody CSF were co-cultured with PBMCs. During Days 1–3, the sIL-2r content was around 1.30 ng/ml. On Day 4, the sIL-2r content increased to 2.57 ng/ml. On Day 6, the sIL-2r increased to 6.86 ng/ml. On Day 7, it peaked to 7.53 ng/ml. On Day 8, the content began to decrease. On Day 9, it was 5.30 ng/ml (Table 1, $P<0.01$, comparison of CG1 and CG2 with EG, respectively, from Day 4 to Day 9).

4 Discussion

The arachnoid membrane has been considered to be no more than a layer of connective tissue. Research shows, however, that arachnoid cells phagocytize foreign matter, express HLA-DR antigen, IgG receptor, and complement receotor, and have antigen presenting cell (APC)-like characteristics and activities (Frank, 1995; Bernstein *et al*., 1996; Prignano, 1998; McMenamin *et al*., 2003). Arachnoid cells can secrete many cytokines, e.g., fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β), and express glial fibrillary acidic proteins, the idio-proteins of gliocytes (Mathiesen and Lefvert, 1996). There are 18 kinds of CSF proteins secreted by cells of cultured leptomeninges (Raymackers *et al*., 2000). Thus, arachnoid cells may participate in antigen presentation in the subarachnoid space. SAH can induce proliferation of leptomeningeal cells (McMenamin, 1999).

Cytokeratin, a kind of keratin, is a molecular marker of epithelial differentiation. It is used to investigate the differentiation and function of epithelium and identify arachnoid cells (Murphy *et al*., 1991; Frank, 1995). In our research, arachnoid cells formed a monolayer and were polygonal in shape. The cells had large nuclei and many cytoplasmic granules. Approximately 95% of arachnoid cells expressed cytokeratin, in agreement with previous studies (Murphy *et al*., 1991; Frank, 1995).

It is known that T cells cannot discriminate the determinant groups of native protein antigen and need the presentation of APCs. MHC-I and MHC-II are considered to be the carriers of antigen polypeptides. HLA-DR is one kind of MHC-II and is primarily expressed on APCs (e.g., macrophages, B lymphocytes, dendritic cells, monocytes, activated T cells, Langhans' cells, and spermatozoons). Cells which can express HLA-DR are considered to be functional APCs. The level of expression is related to activation stimulation by antigen.

Our research shows that, after being stimulated by bloody CSF, the HLA-DR expression of arachnoid cells increased, and the results of immunofluorescence and FCM were temporally consistent. On Day 6, the expression of HLA-DR began to increase. On Day 7, the expression significantly increased. The hemolyses of red blood cells (RBCs) and other cells are

Table 1 Variation of sIL-2r content in medium by ELISA

Group	sIL-2r content (ng/ml)								
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
CG1	1.34±0.010	1.33±0.013	1.31±0.006	1.35±0.009	1.30±0.007	1.39±0.006	1.37±0.004	1.40±0.013	1.40±0.010
CG2	1.30±0.013	1.33±0.015	1.31±0.014	1.38±0.006	1.36±0.006	1.40±0.011	1.41±0.005	1.42±0.004	1.48±0.012
EG	1.28±0.009	1.31±0.006	1.45±0.007	2.57±0.010	3.61±0.017	6.86±0.009	7.53±0.017	6.23±0.010	5.30±0.021

$P<0.01$, comparison of the control group 1 (CG1) and the control group 2 (CG2) with the experimental group (EG), respectively, from Day 4 to Day 9. $n=3$

endogenous antigens which may be presented to arachnoid cells. Arachnoid cells phagocytize and elaborate these antigens and combine the antigen determinants with HLA-DR. Time is needed to express the combination of antigen-polypeptide-HLA-DR on the surface of arachnoid cells. In the first 5 d, the expression is maintained at basal level. On Day 6, the combination is expressed on the cell surface and can be detected by FCM.

The morphologic appearance is that of activation. The increase of lysosomes, endoplasmic reticulum, and enlargement of the cyst-pool are evidence indicating that cells phagocytize and elaborate antigens and synthetize proteins (e.g., the lysosomal hydratase and HLA-DR molecule). Increased pinocytosis bubbles were also observed in the cells. These morphologic variations indicate that arachnoid cells have macrophage-like characteristics and activation capabilities.

IL-2r exists in two forms: membrane IL-2r (mIL-2r) and sIL-2r. After synthesis, sIL-2r falls from the cellular membrane into blood or culture supernatant. In this study the content of sIL-2r was measured to reflect the activation degree of T-lymphocytes (Jackowski *et al*., 1990). After being stimulated by bloody CSF, arachnoid cells were co-cultured with PBMCs. The content of sIL-2r in the supernatant began to rise from Day 4, indicating that the MHC-antigen combination provides activation signals to T cells acting with T-lymphocyte receptor (TCR). Activated T lymphocytes express IL-2r, which can then be detected. On Day 7, sIL-2r content arrived at the vertex, indicating T cell activation.

CG1 and CG2 showed cultured PBMCs alone or with arachnoid cells, and the content of sIL-2r in supernatant was maintained at a basal level because MHC-antigen combination was not presented on unstimulated arachnoid cells. These results indicate that not only the origin of bloody CSF but also the white cells in the bloody CSF confound the issue. Arachnoid cells and PBMCs in our research were obtained from single patient, thus avoiding lymphocyte antigen activation.

The mechanism of coagulation-initiated inflammation has not been fully illuminated. Thrombin is a multifunctional enzyme which generates procoagulant, anticoagulant, inflammatory, and mitogenic responses (Sanchez *et al*., 2004; Esmon, 2005; Muth *et al*., 2005; Recinos *et al*., 2006). Platelet activation also appears to contribute to the inflammatory response (Kwon and Jeon, 2001; André *et al*., 2002; Esmon, 2005). The tissue factor-VIIa complex can induce preinflammatory effects on the macrophage/monocyte, such as the expression of MHC-II molecules (Motohashi *et al*., 1995b; Chrapusta *et al*., 1999). Thrombin, TGF-β, and granulocyte-macrophage colony-stimulating factor can promote leptomeninggeal cell proliferation (Motohashi *et al*., 1995a; Takizawa *et al*., 2001; Jin *et al*., 2006). Additionally, clinical DCI usually happens on Day 3, peaks 1–2 weeks after SAH, and then subsides gradually (Claassen *et al*., 2001; Todo *et al*., 2008; Eddleman *et al*., 2009). The results of the present study are consistent with the time of DCI. Early on Day 2 post-SAH, a dramatic increase in the number of subarachnoid macrophages arose from a transformation of cells of the pia-arachnoid. This period was characterized by intense phagocytic activity, erythrocytes, fibrin, and other debris being largely cleared over the next 24 h. At 5 d post-SAH, the subarachnoid macrophage population declined, cells losing their mobile active features to assume a more typical pia-arachnoid cell appearance once more (Iuliano *et al*., 2004; Lan *et al*., 2005).

5 Conclusions

After stimulation by bloody CSF, arachnoid cells have APC-like functions and mediate the immune reactions, this research indicating for the first time that arachnoid cells are probably involved in the interactions between coagulation and inflammation in subarachnoid space after SAH.

References

Ahn, Y.M., Gajdusek, C., London, S., Moon, C.T., Oh, C.W., Mayberg, M.R., 2002. Sustained arterial narrowing after prolonged exposure to perivascular endothelin. *Neurosurgery*, **50**(4):843-849. [doi:10.1097/00006123-200204000-00029]

Akopov, S.E., Grigorian, M.R., Gabrielian, E.S., 1992. The endothelium-dependent relaxation of human middle cerebral arteries: effects of activated neutrophils. *Cellular and Molecular Life Sciences*, **48**(1):34-36. [doi:10.1007/BF01923601]

André, P., Prasad, K.S., Denis, C.V., He, M., Papalia, J.M., Hynes, R.O., Phillips, D.R., Wagner, D.D., 2002. CD40L

stabilizes arterial thrombi by a β3 integrin-dependent mechanism. *Nature Medicine*, **8**(3):247-252. [doi:10.1038/nm0302-247]

Aydin, M.D., Akyol-Salman, I., Sahin, O., 2005. Histopathological changes in ciliary ganglion of rabbits with subarachnoid hemorrhage. *International Journal of Neuroscience*, **115**(11):1595-1602. [doi:10.1080/00207450590958079]

Bernstein, J.J., Karp, S.M., Goldberg, W.J., DeGiorgio, L.A., Blass, J.P., 1996. Human leptomeningeal-derived cells express GFAP and HLA-DR when grafted into rat spinal cord. *International Journal of Developmental Neuroscience*, **14**(5):681-687. [doi:10.1016/0736-5748(95)00096-8]

Chrapusta, S.J., Gorski, A., Mrowiec, T., Grieb, P., Andrychowski, J., Ryba, M.S., 1999. Tissue factor and factor VIIa receptor/ligand interactions induce proinflammatory effects in macrophages. *Blood*, **94**(10):3413-3420.

Chrapusta, S.J., Gorski, A., Mrowiec, T., Grieb, P., Andrychowski, J., Ryba, M.S., 2000. Immune abnormalities in aneurysmal subarachnoid haemorrhage patients: relation to delayed cerebral vasospasm. *Scandinavian Journal of Immunology*, **51**(4):400-407. [doi:10.1046/j.1365-3083.2000.00694.x]

Claassen, J., Bernardini, G.L., Kreiter, K., Bates, J., Du, Y.E., Copeland, D., Sander Connolly, E., Mayer, S.A., 2001. Effect of cisternal and ventricular blood on risk delayed cerebral ischemia after subarachnoid hemorrhage: the fisher scale revisited. *Stroke*, **32**(9):2012-2016. [doi:10.1161/hs0901.095677]

Clatterbuck, R.E., Oshiro, E.M., Hoffman, P.A., Dietsch, G.N., Pardoll, D.M., Tamargo, R.J., 2002. Inhibition of vasospasm with lymphocyte function-associated antigen-1 monoclonal antibody in a femoral artery model in rats. *Journal of Neurosurgery*, **97**(3):676-682. [doi:10.3171/jns.2002.97.3.0676]

Eddleman, C.S., Hurley, M.C., Naidech, A.M., 2009. Endovascular options in the treatment of delayed ischemic neurological deficits due to cerebral vasospasm. *Neurosurgical Focus*, **26**(3):6-8. [doi:10.3171/2008.11.FOCUS08278]

Esmon, C.T., 2005. The interactions between inflammation and coagulation. *British Journal of Haematology*, **131**(4):417-430. [doi:10.1111/j.1365-2141.2005.05753.x]

Frank, E., 1995. HLA-DR expression on arachnoid cells. A role in the fibrotic inflammation surrounding nerve roots in spondylotic cervical myelopathy. *Spine*, **20**(19):2093-2096. [doi:10.1097/00007632-199510000-00004]

Frank, H., Boyce, W., 1983. Cytokeratin provides a specific marker for human arachnoid cells grown in vitro. *Experimental Cell Research*, **146**(2):371-376. [doi:10.1016/0014-4827(83)90138-6]

Gomis, P., Tran-Dinh, Y.R., Sercombe, C., Sercombe, R., 2005. Dexamethasone preventing contractile and cytoskeletal protein changes in the rabbit basilar artery after subarachnoid hemorrhage. *Journal of Neurosurgery*, **102**(4):715-720. [doi:10.3171/jns.2005.102.4.0715]

Harrod, C.G., Bendok, B.R., Batjer, H.H., 2005. Prediction of cerebral vasospasm in patients presenting with aneurysmal subarachnoid hemorrhage: a review. *Neurosurgery*, **56**(4):633-654. [doi:10.1227/01.NEU.0000156644.45384.92]

Hendryk, S., Jarzab, B., Josko, J., 2004. Increase of the IL-1 beta and IL-6 levels in CSF in patients with vasospasm following aneurysmal SAH. *Neuroendocrinology Letters*, **25**(1/2):141-147.

Iuliano, B.A., Pluta, R.M., Jung, C., 2004. Endothelial dysfunction in a primate model of cerebral vasospasm. *Journal of Neurosurgery*, **100**(2):287-295. [doi:10.3171/jns.2004.100.2.0287]

Jackowski, A., Crockard, A., Burnstock, G., Russell, R.R., Kristek, F., 1990. The time course of intracranial pathophysiological changes following experimental subarachnoid haemorrhage in the rat. *Journal of Cerebral Blood Flow & Metabolism*, **10**(6):835-849.

Jin, H., Gong, D., Adeegbe, D., Bayer, A.L., Rolle, C., Yu, A., Malek, T.R., 2006. Quantitative assessment concerning the contribution of IL-2Rβ for superantigen-mediated T cell responses in vivo. *International Immunology*, **18**(4):565-572. [doi:10.1093/intimm/dxh398]

Kim, K.Y., Kim, M.Y., Choi, H.S., 2002. Thrombin induces IL-10 production in microglia as a negative feedback regulator of TNF-α release. *NeuroReport*, **13**(6):849-852. [doi:10.1097/00001756-200205070-00022]

Kubota, T., Handa, Y., Tsuchida, A., Kaneko, M., Kobayashi, H., Kubota, T., 1993. The kinetics of lymphocyte subsets and macrophages in subarachnoid space after subarachnoid hemorrhage in rats. *Stroke*, **24**(12):1993-2000.

Kwon, K.Y., Jeon, B.C., 2001. Cytokine levels in cerebrospinal fluid and delayed ischemic deficits (DCI) in patients with aneurysmal subarachnoid hemorrhage. *Journal of Korean Medical Science*, **16**(4):774-780 (in Korean).

Lan, W., Harmon, D.C., Wang, J.H., 2005. Activated endothelial interleukin-1, -6, and -8 concentrations and intercellular adhesion molecule-1 expression are attenuated by lidocaine. *Anesthesia & Analgesia*, **100**(2):409-412. [doi:10.1213/01.ANE.0000142113.39092.87]

Massicotte, E.M., Del Bigio, M.R., 1999. Human arachnoid villi response to subarachnoid hemorrhage: possible relationship to chronic hydrocephalus. *Journal of Neurosurgery*, **91**(1):80-84. [doi:10.3171/jns.1999.91.1.0080]

Mathiesen, T., Lefvert, A.K., 1996. Cerebrospinal fluid and blood lymphocyte subpopulations following subarachnoid haemorrhage. *British Journal of Neurosurgery*, **10**(1):89-92. [doi:10.1080/02688699650040584]

Mathiesen, T., Andersson, B., Loftenius, A., von Holst, H., 1993. Increased interleukin-6 levels in cerebrospinal fluid following subarachnoid hemorrhage. *Journal of Neurosurgery*, **78**(4):562-567. [doi:10.3171/jns.1993.78.4.0562]

McMenamin, P.G., 1999. Distribution and phenotype of dendritic cells and resident tissue macrophages in the dura mater, leptomeninges, and choroid plexus of the rat brain as demonstrated in wholemount preparations. *The Journal of Comparative Neurology*, **405**(4):553-562. [doi:10.1002/(SICI)1096-9861(19990322)405:4<553::AID-CNE8>

3.3.CO;2-Y]

McMenamin, P.G., Wealthall, R.J., Deverall, M., Cooper, S.J., Griffin, B., 2003. Macrophages and dendritic cells in the rat meninges and choroid plexus: three-dimensional localisation by environmental scanning electron microscopy and confocal microscopy. *Cell and Tissue Research*, **313**(3):259-269. [doi:10.1007/s00441-003-0779-0]

Motohashi, O., Suzuki, M., Shida, N., Umezawa, K., Ohtoh, T., Sakurai, Y., Yoshimoto, T., 1995a. Subarachnoid haemorrhage induced proliferation of leptomeningeal cells and deposition of extracellular matrices in the arachnoid granulations and subarachnoid space. *Acta Neurochirurgica*, **136**(1/2):88-91. [doi:10.1007/BF01411441]

Motohashi, O., Suzuki, M., Yanai, N., Umezawa, K., Shida, N., Yoshimoto, T., 1995b. Thrombin and TGF-beta promote human leptomeningeal cell proliferation in vitro. *Neuroscience Letters*, **190**(2):105-108. [doi:10.1016/0304-3940(95)11513-V]

Murphy, M., Chen, J.N., George, D.L., 1991. Establishment and characterization of a human leptomeningeal cell line. *Journal of Neuroscience Research*, **30**(3):475-483. [doi:10.1002/jnr.490300304]

Muth, H., Kreis, I., Zimmermann, R., Tillmanns, H., Hölschermann, H., 2005. Differential gene expression in activated monocyte-derived macrophages following binding of factor VIIa to tissue factor. *Thrombosis and Haemostasis*, **94**(5):1028-1034.

Nekludov, M., Antovic, J., Bredbacka, S., Blombäck, M., 2007. Coagulation abnormalities associated with severe isolated traumatic brain injury: cerebral arterio-venous differences in coagulation and inflammatory markers. *Journal of Neurotrauma*, **24**(1):174-180. [doi:10.1089/neu.2006.0173]

Ogihara, K., Aoki, K., Zubkov, A.Y., Zhang, J.H., 2001. Oxyhemoglobin produces apoptosis and necrosis in cultured smooth muscle cells. *Brain Research*, **889**(1-2):89-97. [doi:10.1016/S0006-8993(00)03120-6]

Pradilla, G., Wang, P.P., Legnani, F.G., 2004. Prevention of vasospasm by anti-CD11/CD18 monoclonal antibody therapy following subarachnoid hemorrhage in rabbits. *Journal of Neurosurgery*, **101**(1):88-92. [doi:10.3171/jns.2004.101.1.0088]

Prignano, F., 1998. Immunophenotype analysis of normal human dendritic cells isolated from epidermis and dermis. *International Journal of Dermatology*, **37**(2):116-119. [doi:10.1046/j.1365-4362.1998.00116.x]

Raymackers, J., Daniels, A., de Brabandere, V., Missiaen, C., Dauwe, M., Verhaert, P., Vanmechelen, E., 2000. Identification of two-dimensionally separated human cerebrospinal fluid proteins by *N*-terminal sequencing, matrix-assisted laser desorption/ionization-mass spectrometry, nanoliquid chromatography-electrospray ionization-time of flight-mass spectrometry, and tandem mass spectrometry. *Electrophoresis*, **21**(11):2266-2283. [doi:10.1002/1522-2683(20000601)21:11<2266::AID-ELPS2266>3.0.CO;2-Z]

Recinos, P.F., Pradilla, G., Thai, Q.A., 2006. Controlled release of lipopolysaccharide in the subarachnoid space of rabbits induces chronic vasospasm in the absence of blood. *Surgical Neurology*, **66**(5):463-469. [doi:10.1016/j.surneu.2006.04.010]

Sanchez, C.M., Suarez, M.A., Nebra, A., 2004. Early activation of coagulation and fibrinolysis in traumatic brain injury and spontaneous intracerebral hemorrhage: a comparative study. *Neurologia*, **19**(2):44-52.

Stein, S.C., Smith, D.H., 2004. Coagulapathy in traumatic brain injury. *Neurocritical Care*, **1**(4):479-488. [doi:10.1385/NCC:1:4:479]

Takizawa, T., Tada, T., Kitazawa, K., 2001. Inflammatory cytokine cascade released by leukocytes in cerebrospinal fluid after subarachnoid hemorrhage. *Neurological Research*, **23**(7):724-730. [doi:10.1179/016164101101199243]

Todo, K., Kitagawa, K., Sasaki, T., Omura-Matsuoka, E., Terasaki, Y., Oyama, N., Yagita, Y., Hori, M., 2008. Granulocyte-macrophage colony-stimulating factor enhances leptomeningeal collateral growth induced by common carotid artery occlusion. *Stroke*, **39**(6):1875-1882. [doi:10.1161/STROKEAHA.107.503433]

Wada, H., Hashimoto, K., Wada, Y., Kobayashi, M., Izumi, A., Sugiyama, A., Kohro, T., Hamakubo, T., Kodama, T., 2002. Extensive oligonucleotide microarray transcriptome analysis of the rat cerebral artery and arachnoid tissue. *Journal of Atherosclerosis and Thrombosis*, **9**(5):224-232.

Wang, L., Shi, J.X., Yin, H.X., 2005. The influence of subarachnoid hemorrhage on neurons: an animal model. *Annals of Clinical and Laboratory Science*, **35**(1):79-85.

Zubkov, A.Y., Tibbs, R.E., Clower, B., 2002. Morphological changes of cerebral arteries in a canine double hemorrhage model. *Neuroscience Letters*, **326**(2):137-143. [doi:10.1016/S0304-3940(02)00188-X]