

5-5. Complement ©

Immunology. 1978 Mar;34(3):509-15.

Inhibition of the classical and alternative pathways by amino acids and their derivatives.

Takada Y, Arimoto Y, Mineda H, Takada A.

Effects of various aminoacids and their derivatives on the classical pathway and alternative pathway of the complement were studied. Leupeptin, acetyl-leucyl-leucyl-arginal, inhibited CH50 and Cl-esterase, but did not inhibit the alternative pathway. When aminoacids of carbon chains of the order of seven were used, arginine and lysine had stronger effects than trans-aminomethyl cyclohexane carboxylic acid (t-AMCHA), cis-aminomethyl cyclohexane carboxylic acid (cis-AMCHA) and epsilon aminocaproic acid (EACA). SH-compounds, cysteine, homocysteine and glutathione, had the strongest inhibitory effects among these aminoacids on both classical and alternative pathways. When effects on Cl esterase were compared, arginine, lysine, t-AMCHA, cis-AMCHA and EACA had weak inhibition while SH-compounds showed strong inhibition. Poly-L-lysine, which had extremely strong inhibition of CH50, had no inhibition of Cl esterase. The inhibitory effects of antifibrinolytic agents, EACA and t-AMCHA, were weak but when effects on early parts of the classical pathway, C(1,4,2)H50 were tested, some inhibitory activities were recognized. Thus inhibitory effects of these agents were due to their activities on the early parts of the classical pathway.

PMID: 305891 [PubMed - indexed for MEDLINE]

Mol Immunol. 2004 Jun;41(2-3):165-71.

Initiation of complement activation following oxidative stress. In vitro and in vivo observations.

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Ischemia and reperfusion of organs/tissues induce a state of inflammation that can lead to tissue injury. Focus on development of effective therapeutics based on sound pre-clinical work and the role of leukocytes in models of human disease has not lead to a successful clinical trial for anti-leukocyte technologies. For the past >30 years, it has been known that complement activation plays a role in the inflammation and tissue injury associated with ischemia/reperfusion (I/R) injury. In the last 10 years, several complement inhibitors have made their way from the bench to bedside. Will a complement inhibitor eventually be approved for clinical treatment of I/R type diseases? What pathway(s) are involved in I/R injury, and what role do they play? What specific complement components are needed for resolution of inflammation and what components need to be inhibited to decrease tissue injury? This short review will focus on the current state of the art knowledge about complement, complement pathways, complement components and several promising clinical biologics that inhibit complement activation. This review is not a complete review of complement in ischemia/reperfusion injury, but it raises important questions about the role of complement, its pathways and the current knowledge in the area of ischemia/reperfusion injury.

Publication Types:

- Review
- Review, Tutorial

PMID: 15159062 [PubMed - indexed for MEDLINE]

Eur J Cardiothorac Surg. 2003 Aug;24(2):260-9.

Biological effects of off-pump vs. on-pump coronary artery surgery: focus on inflammation, hemostasis and oxidative stress.

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Cardiopulmonary bypass (CPB) has been recognized as a cause of complex systemic inflammatory response, which significantly contributes to several adverse postoperative complications. In the last few years, off-pump coronary artery bypass grafting has gained widespread diffusion as an alternative technique to conventional on-pump coronary artery bypass grafting. Surgeons supporting off-pump surgery state that the avoidance of the CPB and myocardial ischemia-reperfusion significantly reduces the postoperative systemic inflammatory response and other biological derangements and, possibly, may improve the clinical outcomes. We review, here, the available evidence concerning possible differences between off-pump and on-pump procedures in terms of inflammation, hemostasis and oxidative stress. Consistent differences in the involvement of these systems are observed, but they are limited to the final steps of the surgical procedures and the early hours after. These findings suggest that the global surgical trauma may be as important, or even more, as the CPB in terms of systemic inflammatory and coagulation-fibrinolytic pathway activation. Further studies are needed in order to confirm this hypothesis.

Publication Types:

- Review

PMID: 12895618 [PubMed - indexed for MEDLINE]

Am J Pathol. 2001 Sep;159(3):1045-54.

Endothelial oxidative stress activates the lectin complement pathway: role of cytokeratin 1.

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Oxidative stress increases endothelial mannose-binding lectin (MBL) binding and activates the lectin complement pathway (LCP). However, the molecular mechanism of MBL binding to the endothelium after oxidative stress is unknown. Intermediate filaments have been previously reported to activate the classical complement pathway in an antibody-independent manner. We investigated whether oxidative stress increases human umbilical vein endothelial cell (HUVEC) cytokeratin 1 (CK1) expression and activates the LCP via MBL binding to CK1. Reoxygenation (3 hours, 21% O₂) of hypoxic HUVECs (24 hours, 1% O₂) significantly increased CK1 mRNA (in situ hybridization) and membrane protein expression [enzyme-linked immunosorbent assay (ELISA)/confocal microscopy]. Incubating human serum (HS) with N-acetyl-D-glucosamine or anti-human MBL monoclonal antibody attenuated MBL and C3 deposition on purified CK1 (ELISA). CK1 and MBL were co-immunoprecipitated from hypoxic HUVECs reoxygenated in HS. Treatment with anti-human cytokeratin Fab fragments attenuated endothelial MBL and C3 deposition after oxidative stress (ELISA/confocal microscopy). We conclude that: 1) endothelial oxidative stress increases CK1 expression, MBL binding, and C3 deposition; 2) inhibition of MBL attenuates purified CK1-induced complement activation; and 3) anti-human cytokeratin Fab fragments attenuate endothelial MBL and C3 deposition after oxidative stress. These results suggest that MBL binding to endothelial cytokeratins may mediate LCP activation after oxidative stress.

PMID: 11549596 [PubMed - indexed for MEDLINE]

Science. 2005 Apr 15;308(5720):385-9. Epub 2005 Mar 10.

Complement factor H polymorphism in age-related macular degeneration.

Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, Sangiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J.

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Age-related macular degeneration (AMD) is a major cause of blindness in the elderly. We report a genome-wide screen of 96 cases and 50 controls for polymorphisms associated with AMD. Among 116,204 single-nucleotide polymorphisms genotyped, an intronic and common variant in the complement factor H gene (CFH) is strongly associated with AMD (nominal P value $<10^{-7}$). In individuals homozygous for the risk allele, the likelihood of AMD is increased by a factor of 7.4 (95% confidence interval 2.9 to 19). Resequencing revealed a polymorphism in linkage disequilibrium with the risk allele representing a tyrosine-histidine change at amino acid 402. This polymorphism is in a region of CFH that binds heparin and C-reactive protein. The CFH gene is located on chromosome 1 in a region repeatedly linked to AMD in family-based studies.

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Science. 2005 Apr 15;308(5720):421-4. Epub 2005 Mar 10.

Complement factor H polymorphism and age-related macular degeneration.

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Age-related macular degeneration (AMD) is a common, late-onset, and complex trait with multiple risk factors. Concentrating on a region harboring a locus for AMD on 1q25-31, the ARMD1 locus, we tested single-nucleotide polymorphisms for association with AMD in two independent case-control populations. Significant association ($P = 4.95 \times 10^{-10}$) was identified within the regulation of complement activation locus and was centered over a tyrosine-402 --> histidine-402 protein polymorphism in the gene encoding complement factor H. Possession of at least one histidine at amino acid position 402 increased the risk of AMD 2.7-fold and may account for 50% of the attributable risk of AMD.

PMID: 15761121 [PubMed - in process]

Science. 2005 Apr 15;308(5720):419-21. Epub 2005 Mar 10.

Complement factor H variant increases the risk of age-related macular degeneration.

Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Nouredine M, Gilbert JR, Schnetz-Boutaud N, Agarwal A, Postel EA, Pericak-Vance MA.

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Age-related macular degeneration (AMD) is a leading cause of visual impairment and blindness in the elderly whose etiology remains largely unknown. Previous studies identified chromosome 1q32 as harboring a susceptibility locus for AMD. We used single-nucleotide polymorphisms to interrogate this region and identified a strongly associated haplotype in two independent data sets. DNA resequencing of the complement factor H gene within this haplotype revealed a common coding variant, Y402H, that significantly increases the risk for AMD with odds ratios between 2.45 and 5.57. This common variant likely explains approximately 43% of AMD in older adults.

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JAMA. 2004 Feb 11;291(6):704-10.

Association between C-reactive protein and age-related macular degeneration.

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CONTEXT: C-reactive protein (CRP) is a systemic inflammatory marker associated with risk for cardiovascular disease (CVD). Some risk factors for CVD are associated with age-related macular degeneration (AMD), but the association between CRP and AMD is unknown. **OBJECTIVE:** To test the hypothesis that elevated CRP levels are associated with an increased risk for AMD. **DESIGN, SETTING, AND PARTICIPANTS:** A total of 930 (91%) of 1026 participants at 2 centers in the Age-Related Eye Disease Study (AREDS), a multicenter randomized trial of antioxidant vitamins and minerals, were enrolled in this case-control study. There were 183 individuals without any maculopathy, 200 with mild maculopathy, 325 with intermediate disease, and 222 with advanced AMD (geographic atrophy or neovascular AMD). The AMD status was assessed by standardized grading of fundus photographs, and stored fasting blood specimens drawn between January 1996 and April 1997 were analyzed for high-sensitivity CRP levels. **MAIN OUTCOME MEASURE:** Association between CRP and AMD. **RESULTS:** The CRP levels were significantly higher among participants with advanced AMD (case patients) than among those with no AMD (controls; median values, 3.4 vs 2.7 mg/L; $P = .02$). After adjustment for age, sex, and other variables, including smoking and body mass index, CRP levels were significantly associated with the presence of intermediate and advanced stages of AMD. The odds ratio (OR) for the highest vs the lowest quartile of CRP was 1.65 (95% confidence interval [CI], 1.07-2.55; P for trend = .02). The OR for CRP values at or above the 90th percentile (10.6 mg/L) was 1.92 (95% CI, 1.20-3.06), and the OR for CRP values at or above the mean plus 2 SDs (16.8 mg/L) was 2.03 (95% CI, 1.03-4.00). A trend for an increased risk for intermediate and advanced AMD with higher levels of CRP was seen for smokers (OR, 2.16; 95% CI, 1.33-3.49) and those who never smoked (OR, 2.03; 95% CI, 1.19-3.46) with the highest level of CRP. **CONCLUSION:** Our results suggest that elevated CRP level is an independent risk factor for AMD and may implicate the role of inflammation in the pathogenesis of AMD.

Publication Types:

- Multicenter Study

PMID: 14871913 [PubMed - indexed for MEDLINE]

Arterioscler Thromb Vasc Biol. 1999 Nov;19(11):2623-9.

Endothelial nuclear factor-kappaB translocation and vascular cell adhesion molecule-1 induction by complement: inhibition with anti-human C5 therapy or cGMP analogues.

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We have previously shown that reoxygenation of hypoxic human umbilical vein endothelial cells (HUVECs) leads to the activation and deposition of complement. In the present study, we investigated whether the terminal complement complex (C5b-9) influences HUVEC nuclear factor-kappaB (NF-kappaB) translocation and vascular cell adhesion molecule-1 (VCAM-1) protein expression after hypoxia/reoxygenation by decreasing endothelial cGMP. Additionally, we investigated the action of anti-human C5 therapy on endothelial cGMP, NF-kappaB translocation, and VCAM-1 protein expression. Reoxygenation (0.5 to 3 hours, 21% O₂) of hypoxic (12 hours, 1% O₂) HUVECs in human serum (HS) significantly increased C5b-9 deposition, VCAM-1 expression, and NF-kappaB translocation compared with hypoxic/reoxygenated HUVECs treated with the recombinant human C5 inhibitor h5G1.1-scFv. Acetylcholine (ACh)-induced cGMP synthesis was significantly higher in normoxic HUVECs compared with hypoxic HUVECs reoxygenated in HS but did not differ from hypoxic HUVECs reoxygenated in buffer or HS treated with h5G1.1-scFv. Treatment of hypoxic/reoxygenated HUVECs with h5G1.1-scFv or cGMP analogues significantly attenuated NF-kappaB translocation and VCAM-1 protein expression. Treatment with NO analogues, but not a cAMP analogue, cGMP antagonists, or an NO antagonist, also significantly attenuated VCAM-1 expression. We conclude that (1) C5b-9 deposition, NF-kappaB translocation, and VCAM-1 protein expression are increased in hypoxic HUVECs reoxygenated in HS; (2) reoxygenation of hypoxic HUVECs in HS, but not buffer alone, attenuates ACh-induced cGMP synthesis; and (3) treatment of hypoxic/reoxygenated HUVECs with h5G1.1-scFv attenuates C5b-9 deposition, NF-kappaB translocation, and VCAM-1 expression while preserving ACh-induced cGMP synthesis. C5b-9-induced VCAM-1 expression may thus involve an NO/cGMP-regulated NF-kappaB translocation mechanism.

PMID: 10559004 [PubMed - indexed for MEDLINE]

Immunopharmacology. 1998 Mar;39(1):39-50.

Complement activation following reoxygenation of hypoxic human endothelial cells: role of intracellular reactive oxygen species, NF-kappaB and new protein synthesis.

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Complement plays an important role in ischemia-reperfusion injury. We recently demonstrated that reoxygenation of hypoxic human umbilical vein endothelial cells (HUVECs) activated the classical complement pathway and augmented iC3b deposition. In the present study, we investigated the potential role of oxygen-derived free radicals, NF-kappaB and new protein synthesis in this model. HUVECs subjected to 12 or 24 h hypoxic stress (1% O₂) and then reoxygenated (0.5, 1, 2 or 3 h; 21% O₂) in 30% human serum activated complement and deposited iC3b. Addition of hydrogen peroxide (H₂O₂; 1-100 micromol/l) to normoxic HUVECs increased iC3b deposition in a concentration-dependent manner. H₂O₂ (10 micromol/l), a concentration that did not significantly increase iC3b deposition on normoxic HUVECs, augmented iC3b deposition on hypoxic/reoxygenated HUVECs. We observed a significant increase in intracellular H₂O₂ and hydroxyl radical (OH \cdot) production in hypoxic/reoxygenated HUVECs using dihydrorhodamine 123. Further, treatment of HUVECs with dimethylthiourea (DMTU, 1-100 micromol/l), deferoxamine (DEF, 1-100 micromol/l), or oxypurinol (10 micromol/l), but not superoxide dismutase (SOD, 500 U/ml), catalase (300 U/ml) or iron-loaded DEF, attenuated iC3b deposition following hypoxia/reoxygenation in a concentration-dependent manner. Western analysis demonstrated hypoxia-induced nuclear NF-kappaB translocation that increased with reoxygenation. Inhibition of new protein synthesis (i.e. cycloheximide) or inhibition of NF-kappaB (ALLN or SN-50) also significantly decreased iC3b deposition on hypoxic/reoxygenated HUVECs. We conclude that (1) hypoxic/reoxygenated HUVECs generate H₂O₂ and OH \cdot ; (2) treatment of HUVECs with cell permeable reactive oxygen species inhibitors/scavengers (i.e. DEF, DMTU, oxypurinol) but not large molecular weight inhibitors (i.e. catalase or SOD) significantly reduces iC3b deposition and (3) inhibition of new protein synthesis or NF-kappaB activation attenuates iC3b deposition. These data suggest that iC3b deposition on the vascular endothelium may be regulated by intracellular oxygen-derived free radical-induced activation of NF-kappaB, new protein synthesis and activation of the classical complement pathway during ischemia/reperfusion.

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J Thorac Cardiovasc Surg. 2003 Jan;125(1):165-71.

Nuclear factor kappaB mediates a procoagulant response in monocytes during extracorporeal circulation.

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OBJECTIVE: The objective of this study was to examine the mechanism of procoagulant activity and inhibition in whole blood during extracorporeal circulation. **METHODS:** In this study we examine the development of procoagulant activity and monocyte activation in heparinized whole blood passing through a closed circuit consisting of a pump and silicone envelope membrane oxygenator for 6 hours. **RESULTS:** Anaphylatoxins, C3a and C5a, determined by means of enzyme-linked immunosorbant assay, appeared in the blood within 30 minutes of circulation. Circulated blood developed a marked potential for coagulation demonstrated in a 1-step clotting assay that reached maximal activity by 4 hours of circulation. This procoagulant activity was neutralized by anti-tissue factor antibody, suggesting a prominent role for the extrinsic pathway in pump-induced intravascular coagulation. Isolation of monocytes from circulated blood revealed that tissue factor expression is upregulated on the cell surface. Furthermore, we observed nuclear factor kappaB nuclear translocation in monocytes from blood passing through the circuit, suggesting that tissue factor expression was due to monocyte stimulation and transcriptional activation of the tissue factor gene. Tissue factor expression resulted in an approximately 30-fold increase in thrombin generation. Monocyte nuclear factor kappaB activation, monocyte tissue factor expression, thrombin generation, and the procoagulant activity of blood in extracorporeal circulation were all blocked by the proteasome inhibitor MG132. **CONCLUSIONS:** We conclude that intravascular tissue factor expression during extracorporeal circulation of blood is due to nuclear factor kappaB-mediated activation of monocytes (possibly by complement), which can be controlled pharmacologically.

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J Immunol. 2005 Jan 1;174(1):491-7.

Role of complement and complement membrane attack complex in laser-induced choroidal neovascularization.

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Choroidal neovascularization (CNV), or choroidal angiogenesis, is the hallmark of age-related macular degeneration and a leading cause of visual loss after age 55. The pathogenesis of new choroidal vessel formation is poorly understood. Although inflammation has been implicated in the development of CNV, the role of complement in CNV has not been explored experimentally. A reliable way to produce CNV in animals is to rupture Bruch's membrane with laser photocoagulation. A murine model of laser-induced CNV in C57BL/6 mice revealed the deposition of C3 and membrane attack complex (MAC) in the neovascular complex. CNV was inhibited by complement depletion using cobra venom factor and did not develop in C3(-/-) mice. Anti-murine C6 Abs in C57BL/6 mice inhibited MAC formation and also resulted in the inhibition of CNV. Vascular endothelial growth factor, TGF-beta2, and beta-fibroblast growth factor were elevated in C57BL/6 mice after laser-induced CNV; complement depletion resulted in a marked reduction in the level of these angiogenic factors. Thus, activation of complement, specifically the formation of MAC, is essential for the development of laser-induced choroidal angiogenesis in mice. It is possible that a similar mechanism may be involved in the pathophysiology of other angiogenesis essential diseases.

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