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Relationship between protein C and antithrombin III deficiencies in sepsis without disseminated intravascular coagulation status

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Table 1 (abstract P39)

Portion of results displaying the systemic, lung, liver and coagulation responses of PI3Kγ WT, KO and KD mice to CLP-induced sepsis

	WT sham	KO sham	KD sham	WT CLP	KO CLP	KD CLP
Systemic response						
MIP-2 concentration (pg/ml in plasma x 10^4)	0 ± 0	0 ± 0	0 ± 0	13 ± 5**	2 ± 1*	2 ± 1*
IL-6 concentration (pg/ml in plasma x 10^3)	0 ± 0	0 ± 0	0 ± 0	18 ± 5*	19 ± 10*	22 ± 8*
Lung injury						
Pathology score	1.67 ± 0.42	1.8 ± 0.49	1.71 ± 0.42	4.17 ± 0.31*	2.00 ± 0.63	2.00 ± 0.45
Permeability (protein in BAL (μg/ml))	194 ± 7	199 ± 3	195 ± 8	234 ± 13*	191 ± 13	192 ± 10
Neutrophil infiltration (cell x 10^3/ml BAL)	0.3 ± 0.2	0.8 ± 0.4	0.2 ± 0.2	11.7 ± 5.9*	0.2 ± 0.1	0.8 ± 0.3
MIP-2 concentration (pg/ml in BAL x 10^2)	0 ± 0	0 ± 0	0 ± 0	21.6 ± 1.0*	1.1 ± 0.4	1.2 ± 0.5
IL-6 concentration (pg/ml in BAL x 10^2)	0 ± 0	0 ± 0	0 ± 0	14 ± 5*	1 ± 0	2 ± 0
Apoptosis (% apoptotic cells)	0.3 ± 0.1	1.2 ± 0.2*	1.0 ± 0.2*	7.7 ± 0.8**	1.3 ± 0.3*	1.2 ± 0.3*
Akt phosphorylation (fold increase over WT sham)	1.0 ± 0.1	0.7 ± 0.0*	0.7 ± 0.1*	1.4 ± 0.1**	0.8 ± 0.1*	0.8 ± 0.1*
Liver injury						
Pathology score	0.33 ± 0.21	0.20 ± 0.20	0.20 ± 0.20	2.83 ± 0.17**	1.20 ± 0.37*	1.83 ± 0.17*
Apoptosis (% apoptotic cells)	1.0 ± 0.2	4.6 ± 1.2*	4.3 ± 1.0*	23.9 ± 2.7**	5.6 ± 0.7*	4.9 ± 1.0*
Coagulation						
tPA (pg/ng protein)	404 ± 17	369 ± 53	423 ± 23	1 ± 1**	104 ± 36*	61 ± 31*
PAI-1 (pg/μg protein)	2.9 ± 0.2	2.7 ± 0.2	3.1 ± 0.4	84.8 ± 13.4**	50.5 ± 9.2*	52.0 ± 9.0*
Fibrinogen (ng/mg protein)	805 ± 35	807 ± 40	779 ± 33	1424 ± 89**	1043 ± 57*	1079 ± 58*

BAL, bronchoalveolar lavage; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor 1.

contribute to septic mortality. Phosphoinositide-3 kinase gamma (PI3Kγ) plays a dominant role in the inflammatory response; however, its role in the pathogenesis of sepsis, specifically SIRS, lung/liver inflammation and damage, apoptosis, coagulation and mortality remains unknown. We hypothesized that mice lacking PI3Kγ or possessing a kinase-dead enzyme will be protected against septic-induced injury.

Methods PI3Kγ wild-type (WT), knockout (KO) and kinase dead (KD) mice were randomized to cecal ligation and perforation (CLP)-induced sepsis or a sham laporatomy. After 18 hours, plasma and bronchoalveolar lavage and/or lung and liver tissue were collected. Plasma was assessed for inflammatory mediators and the lung/liver was analysed for pathology score, leukocyte infiltration, inflammatory mediators, edema, apoptosis, coagulation and downstream intracellular signalling of PI3Kγ. A separate cohort of WT and KO mice were used for evaluation of 7-day survival following CLP.

Results Systemically, KO and KD mice showed a reduction in five of 22 measured cytokines/chemokines (MIP1a, MIP2, RANTES, MCP1 and IL-10) compared with WT controls. In the lungs, KO and KD mice were significantly protected against septic damage, as observed by decreased pathology scores, edema/permeability, leukocyte infiltration, inflammation (all 22 measured mediators), apoptosis and Akt/mitogen-activated protein kinase activation, compared with WT lungs. Similarly, livers of CLP-exposed KO and KD mice had decreased pathology scores, leukocyte infiltration, apoptosis and coagulation derangements compared with WT controls. Furthermore, Kaplan–Meier analysis of 7-day survival following CLP showed KO mice had significantly reduced mortality compared with WT mice. See Table 1.

Conclusion The present study demonstrates that while PI3Kγ has a modest effect on SIRS during sepsis, its kinase activity is pivotal to the successive development of coagulation derangement and lung/liver inflammation and damage, probably through the modification of leukocyte recruitment and apoptosis. Furthermore, PI3Kγ is shown to effect CLP-septic-induced mortality, implying that it may be a possible therapeutic target in sepsis and multiple organ failure.

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Relationship between protein C and antithrombin III deficiencies in sepsis without disseminated intravascular coagulation status

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Background Recently, a reciprocal relationship has been known between anti-inflammation and anticoagulation responses to infection. In our previous studies, protein C (PC) deficiency and antithrombin III (AT III) deficiency have been shown in septic patients. Moreover, these AT III deficiencies in sepsis did not relate to their disseminated intravascular coagulation (DIC) status. We hypothesize that PC activity relates to AT III activity in septic patients without DIC status.

Materials Fifty ICU patients were included in this study and divided into three groups by primary diagnosis on admission; trauma patients, nonseptic patients, and septic patients. The patients who had already DIC on admission were excluded.

Methods Serum PC activity (%) (Diagnostica Stago®, Tokyo, Japan) and serum AT III activity (%) (Sysmex®, Kobe, Japan) were measured on admission. PC and AT III activities were compared between three groups. Values are expressed as the median. Data were analyzed by the Kruskal–Wallis test and the Mann–Whitney U test. Pearson's correlation coefficient was used for correlation. $P<0.05$ was considered statistically significant.

Results There were 23 trauma patients, 12 nonseptic patients and 15 septic patients. PC activity was significantly lower in septic patients than in trauma or in nonseptic patients (54.6 versus 85.6,

94.0% respectively, $P = 0.0006$). AT III activity was also lower in septic patients than in other groups (54.2 versus 94.4, 81.2% respectively, $P < 0.001$). There were correlations of PC activity with AT III activity in trauma patients ($r = 0.76$, $P < 0.0001$) and in non-septic patients ($r = 0.61$, $P = 0.048$). Especially, in septic patients, PC activity had significant correlation with AT III activity ($r = 0.91$, $P < 0.0001$).

Conclusion Both PC deficiency and AT III deficiency had already been shown in septic patients on admission to the ICU, but nevertheless no DIC status. The relationship between PC activity and AT III activity was found in all patients and there could be a definite correlation in septic patients.

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ELISpot analysis of lipopolysaccharide-stimulated leukocytes: human granulocytes selectively secrete IL-8, MIP-1β and TNFα

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Background Granulocytes or polymorphonuclear cells (PMN) represent the majority of leukocytes in peripheral blood. As terminally differentiated cells, they contain few ribosomes and assist innate immunity mainly through phagocytosis and degranulation. Whether or not they can release proinflammatory cytokines such as TNFα and IL-1β has been a controversial issue. To clarify the role of PMN in this aspect, lipopolysaccharide (LPS)-induced cytokine secretion from PMN was analyzed at the single-cell level with the ELISpot technique.

Methods PMN and peripheral blood mononuclear cells (PBMC) from healthy human donors were prepared by gradient-based centrifugation to a purity >98%. ELISpot assays were used for detection of a large panel of inflammatory mediators. Cells were stimulated with endotoxin (LPS, 100 ng/ml) for 20 hours and the numbers of secreting cells were quantified with an ELISpot reader. For comparison, cytokine production was also analyzed by ELISA. In some experiments, PBMC were depleted of monocytes using anti-CD14 magnetic beads.

Results Purified PMN secreted IL-8 and MIP-1β and a subpopulation also released TNFα after LPS stimulation. In contrast and different from some earlier reports, we were unable to detect secretion of IL-1β, IL-12, granulocyte–macrophage colony-stimulating factor, IL-6 or IFNγ. Furthermore, granulocytes did not secrete the cytotoxic molecules perforin or granzyme B in response to LPS. Compared with the limited cytokine production by PMN, PBMC secreted significant amounts of all substances investigated and were found to require a 100x lower concentration of LPS than granulocytes to obtain the maximum number of responding cells. In addition, $CD14^+$ monocytes were found to be the primary source of production.

Discussion By use of the ELISpot method we could establish the cytokine profiles for both PBMC and PMN based on the frequency and pattern of cytokine secreting cells, rather than the amount of produced cytokine as by ELISA. This way, low levels of contaminating monocytes present in our PMN preparations could be discriminated from the granulocytes. Additionally, we could demonstrate that ELISpot, compared with ELISA, not only provides a more sensitive means of detection but potentially gives biologically more relevant information.

Conclusion LPS-stimulated PMN were shown to secrete IL-8, MIP-1β and TNFα but not IL-1β, IL-6, IL-10, IL-12, granulocyte–macrophage colony-stimulating factor, IFNγ, perforin or granzyme B. Our findings suggest that the ELISpot assay may be a suitable tool in further studies of cellular signaling.

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Prognostic factors of severe sepsis: a result of Korean sepsis registry system

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Background Severe sepsis is a highly fatal condition, but the prognostic factors of severe sepsis are not yet fully understood.

Materials One thousand and twenty-six severe sepsis patients (community-acquired infection only) were registered in the Korean Sepsis Registry System from May 2005 to November 2007. The Korean Sepsis Registry System is a web-based ongoing prospective data collection system from 12 tertiary referral hospitals in Korea.

Methods The Acute Physiology and Chronic Health Evaluation II score, Serial Organ Failure Assessment (SOFA) at admission and serial 1 to 4 days after admission, demographic characteristics, comorbidity conditions with the Charlson score, Glasgow coma scale, organ dysfunction index, infection site, organism, and laboratory data at admission of 1,026 severe sepsis patients were analysed and evaluated to determine the association with 7-day mortality respectively. To develop a prognostic model, decision tree analysis was carried out with SAS 9.1.

Results The 7-day mortality rate was 13.6/100 patients. Age was an independent risk factor, but the highest mortality (25.3%) was seen in the 60 to 69 years age group. The greater the number of organ dysfunctions, the higher the mortality. The underlying conditions were not statistically significant as a risk factor of 7-day mortality except liver diseases ($P = 0.0015$). The blood pressure, Charlson score, Acute Physiology and Chronic Health Evaluation II score and SOFA score at admission were all significantly associated with mortality. The initial laboratory values of hemoglobin, white blood cells, platelets, fibrinogen, prothrombin time, partial prothrombin time, arterial pH, potassium and albumin at admission were also statistically significant in bivariate analysis. Systemic infection and central nervous system infection showed 26.7% and 25.0% 7-day mortality. In a prognostic model by decision tree analysis, the blood coagulation factors (prothrombin time, platelet) and SOFA at 5 days after admission were the most significant prognostic factors of 7-day mortality. The sensitivity and specificity of this model were 67.5% and 96.8%, respectively.

Conclusion The blood coagulation factors and SOFA were the most significant prognostic factors of 7-day mortality.