CARFAX

Mediators of Inflammation, 7, 417–420 (1998)

Acidosis activates complement system *in vitro*

Michael Emeis,[1] Josef Sonntag,[1.CA] Carsten Willam,[2] Evelyn Strauss,[1] Matthias M. Walka and Michael Obladen[1]

Departments of [1]Neonatology and [2]Nephrology, Charité, Virchow-Hospital, Humboldt-University Berlin, 13353 Berlin, Germany

[CA]Corresponding Author
Tel: (+49) 30 45066463
Fax: (+49) 30 45066922

We investigated the *in vitro* effect of different forms of acidosis (pH 7.0) on the formation of anaphylatoxins C3a and C5a. Metabolic acidosis due to addition of hydrochloric acid (10 µmol/ml blood) or lactic acid (5.5 µmol/ml) to heparin blood (N=12) caused significant activation of C3a and C5a compared to control (both p=0.002). Respiratory acidosis activated C3a (p=0.007) and C5a (p=0.003) compared to normocapnic controls. Making blood samples with lactic acidosis hypocapnic resulted in a median pH of 7.37. In this respiratory compensated metabolic acidosis, C3a and C5a were not increased. These experiments show that acidosis itself and not lactate trigger for activation of complement components C3 and C5.

Key words: Acidosis, Alcalosis, Complement system, Complement activation, Anaphylatoxins

Introduction

Hypoxia and reperfusion cause complement activation in animal experiments and in clinical studies.[1–4] It could be shown, that after cytological damage, contact with cellular components such as mitochondria, or excess of hydroxyl radicals, was responsible for the activation of the complement system.[5,6] On the other hand, *in vitro* studies showed a complement activation by acidosis or hypoxia only.[7–9] In a previous study, we found complement activation induced by lactic acid.[10] The aim of the present *in vitro* study was to investigate: (1) whether lactate itself or metabolic acidosis is responsible for activation, and (2) whether respiratory acidosis is also able to activate the complement system.

Material and Methods

Blood samples (10 ml) from 12 healthy volunteers (6 male and 6 female, age: 28–40 years) were collected and 50 IE Heparin was added. Each sample was divided into eight portions and placed in polypropylen tubes.

To investigate the influence of respiratory as well as metabolic changes of pH on anaphylatoxin formation, the portions were equilibrated with different gas mixtures and supplemented with acids resulting in marked changes in pH, pCO_2 and base deficit (Table 1). Probes used as controls are No. 1–3. Four portions were equilibrated with different gas mixtures at 37°C to achieve normocapnia and normoxemia in control portions (No. 3; Table 1), respiratory acidosis (No 6; Table 1) or alcalosis (No. 7,8; Table 1). For this purpose we modified the method of Siriwardhana *et al.*[11] A 6 ml polypropylen tube (6 ml) was filled 1.5 to 2.0 ml blood and the end of a polypropylen tube with an inner diameter of 3 mm was placed into the blood a few millimetres above the bottom of the tube. Gas mixtures (O_2, N_2, CO_2) composed from medical gases by flowmeters were put through the tube into the blood sample. Gas flow was adjusted to one bubble per second for 20 min. Foam caused by bubbling was continuously removed by a suction catheter placed above the blood level. After equilibration, the blood samples were transferred into different tubes for further processing.

For metabolic acidosis 10 µmol hydrochloric acid (No. 4; Sigma-Aldrich, Deisenhofen, Germany) or 5.5 µmol lactate (No: 5; Sigma-Aldrich, Deisenhofen, Germany) were added per ml of blood. To achieve compensated metabolic acidosis, the sample was equilibrated with a hypocapnic gas mixture for 20 min and then 5.5 µmol lactate per ml of blood was added (No. 8).

Blood gas analysis, potassium and lactate concentrations were measured by a Radiometer Copenhagen ABL 505 (Willich, Germany) using heparinised syringes after all samples were incubated at 37°C for 1 h. To stop complement activation after incubation, we added 1 mg EDTA dissolved in purified water to each sample. Plasma was separated by centrifugation at

Table 1. Sample characteristics with different forms of acidosis after gas equilibration, addition of acids and 1 h incubation time at 37°C (*N*=12). Values are given as medians with quartiles

No	Name	Treatment	Sample procedure	pH value	pCO_2 (mmHg)	Base excess (mEq/L)	Potassium (mmol/L)	C3a (μg/L)	C5a (μg/L)
1	baseline value (without incubation)	bubbling:	No	7.36	50	0.0	4.1	128	0.13
		additive:	No	7.35/7.36	46/51	0.0/0.0	4.0/4.3	89/144	0.10/0.42
2	incubation control	bubbling:	No	7.30	50	–3.0	3.5	1595	1.4
		additive:	No	7.27/7.33	44/53	–2.4/–4.4	3.4/3.7	972/2070	0.9/2.6
3	bubble control	bubbling:	78% N_2, 5% CO_2, 17% O_2, 20 min	7.30	51	–2.2	4.2	1025	1.4
		additive:	No	7.25/7.35	45/57	–0.8/–3.0	3.9/4.4	802/1257	0.8/2.8
4	HCl acidosis	bubbling:	No	7.01	78	–13.8	5.2	3360	21.8
		additive:	10 μmol HCl per 1 ml sample	7.00/7.05	71/85	–12.4/–14.9	4.5/5.7	2860/3850	17.8/35.2
5	lactic acidosis	bubbling:	No	6.98	57	–17.1	6.4	2390	4.4
		additive:	5.5 μmol lactate per 1 ml sample	6.90/7.00	50/76	–16.2/–19.6	6.4/6.8	1975/2670	3.2/6.6
6	respiratory acidosis	bubbling:	81% N_2, 19% CO_2, 20 min	7.01	137	–5.2	4.3	1625	3.3
		additive:	No	6.97/7.04	123/158	–3.9/–7.3	3.9/4.7	1080/1868	1.8/4.2
7	respiratory alcalosis	bubbling:	83% N_2, 17% O_2, 20 min	7.54	20	–3.5	4.4	917	1.4
		additive:	No	7.49/7.57	17/23	–2.4/–4.0	4.1/4.7	825/1425	0.8/2.8
8	respiratory alcalosis + lactate	bubbling:	83% N_2, 17% O_2, 20 min	7.37	28	–8.3	4.2	935	1.8
		additive:	5.5 μmol lactate per 1 ml sample	7.32/7.38	24/34	–7.3/–10.5	4.1/4.5	760/1150	1.3/5.4

4°C at 3000 g for 10 min and frozen immediately at –80°C.

Anaphylatoxins C3a and C5a were determined as previously described.[12] C3a enzyme immunoassay (EIA, Fa. Progen Biotechnik GmbH, Heidelberg, Germany) selectively detects C3a-desArg using monoclonal antibodies.[13] C5a was determined with a specific sandwich EIA (Fa. Behring, Marburg, Germany).[14]

Statistical analysis

As most of the data were not normally distributed, results were shown as medians with quartiles. Differences between controls and study samples were assessed using the Wilcoxon test. Statistical significance was assumed at $p<0.05$. All calculations and tests were performed with the SPSS-PC software (Chicago, Illinois, USA).

Results

Influence of incubation and equilibration procedures

The incubation (No. 2) and equilibration (No. 3) procedure led to a measurable complement activation compared to the baseline value (No. 1; Table 1). Therefore the results were compared to controls of the same handling method but without pH change.

Influence of metabolic and respiratory acidosis

Addition of hydrochloric as well as lactic acid caused a marked metabolic acidosis. Concentrations of C3a, C5a, and potassium were higher after incubation in acidic blood compared to incubation of control samples (No. 4 and 5 versus 2; Table 1). The potassium concentration was lower in hydrochloric acidosis and the anaphylatoxin concentrations were higher than

those from lactic acidosis (No. 4 versus 5). The anaphylatoxins were also higher in the hypercapnic samples compared to the normocapnic controls (No. 6 versus 3; Table 1).

Influence of respiratory alkalosis

Respiratory alkalosis had no influence on anaphylatoxin concentration (No. 7 versus 3; Table 1).

Influence of respiratory compensated metabolic acidosis

Although measured median lactate (13.1 versus 11.6 mmol/l) was not different in the lactic samples (No. 5 versus 8; $p=0.75$), anaphylatoxins were higher in acidic samples compared to respiratory compensated normacidic samples (No. 5 versus 8; Table 1).

Discussion

The present *in vitro* study confirms the results of our previous study that acidosis activates complement systems in heparin blood. The study shows that the acidosis itself is the trigger for activation, because all three forms of acidosis (hydrochloric or lactic acid or carbon dioxide) lead to a significant increase of anaphylatoxin concentrations. Lactate did not lead to an activation, when acidosis was prevented by previous respiratory alcalosis (No. 8; Table 1).

Heparin was chosen as anticoagulant, because the complement may be markedly spontaneously activated in serum and both EDTA and sodium citrate possibly influence pH values and impair complement activation by calcium binding. Heparin however did not completely prevent spontaneous complement activation even after incubation for 1 h at 37°C and using a bubble oxygenator for gas equilibration. Therefore we could only compare the extent of complement activation by acidosis to the spontaneous activation that occurred during the sample preparation procedure. As pH levels were similar in lactic and hydrochloric acidosis, the activation of the complement system was stronger for hydrochloric acid. The effects of the two acids on the blood gas analysis were different. Whereas lactate led to a larger increase in base deficit, hydrochloric acid caused a stronger release of carbon dioxide. This difference may explain the stronger complement activation by hydrochloric acid. Respiratory acidosis following equilibration of blood samples with elevated carbon dioxide concentrations also led to an activation of the complement system compared to equilibration control. In contrast to acidosis, respiratory alcalosis did not have an influence on the complement system.

With the used *in vitro* setting, we could not clarify the mechanism of anaphylatoxin formation by acidosis. Complement activation in acidosis mediated by membrane fragments of damaged erythrocytes[6] is improbable, as we could show that activation also occurs in plasma without cell components.[10] There are other arguments against membrane fragment-induced complement activation in acidosis, increased potassium values and a slight visible hemolysis occured only for metabolic but not for respiratory acidosis, but both forms of acidosis activate the complement system. A probable mechanism is that acidification of plasma inactivates complement protease inhibitors, this inhibition then results in complement activation in the absence or decreased presence of functional inhibitors.

The activation of the complement system by acidosis may explain the elevation of anaphylatoxins in different diseases, such as perinatal asphyxia, myocardial infarction and shock.[3,4,15–17] These processes probably participate in the pathogenesis of reperfusion injury.[4,17–19]

Conclusion

We have shown evidence that acidosis activates complement factors C3 and C5. This is independent from the type of acidosis which occurs.

References

1. Hill J, Lindsay TF, Ortiz F, Yeh CG, Hechtman HB, Moore FD. Soluble complement receptor type 1 ameliorates the local and remote organ injury after intestinal ischemia reperfusion in the rat. *J Immunol* 1992; **149**: 1723–1728.
2. Brus F, van Oeveren W, Okken A, Oetomo SB. Activation of circulating polymorphonuclear leukocytes in preterm infants with severe idiopathic respiratory distress syndrome. *Pediatr Res* 1996; **39**: 456–463.
3. Sonntag J, Wagner MH, Strauss E, Obladen M. Complement and contact activation in term newborns after fetal acidosis. *Arch Dis Child Fetal Neonatal Ed* 1998; **78**: F125–F128.
4. Horstick G, Heimann A, Götze O, Hofner G, Berg O, Boehmer P, *et al.* Intracoronary application of C 1 esterase inhibitor improves cardiac function and reduces myocardial necrosis in an experimental model of ischemia and reperfusion. *Circulation* 1997; **95**: 701–708.
5. Turnage RH, Magee JC, Guice KS, Myers SI, Oldham KT. Complement activation by hydroxyl radicals during intestinal reperfusion. *Shock* 1994; **2**: 445–450.
6. Rossen RD, Michael LH, Kagiyima A, Savage HE, Hanson G, Reisberg MA, *et al.* Mechanism of complement activation after coronary artery occlusion: Evidence that myocardial ischemia in dogs causes release of constituents of myocardial subcellular origin that complex with human C1q *in vivo*. *Circulation Research* 1988; **62**: 72–584.
7. Fishelson Z, Horstman RD, Müller-Eberhard HJ. Regulation of the altemative pathway of complement by pH. *J Immunol* 1987; **138**: 3392–3395.
8. Hammer CH, Hansch G, Gresham HD, Shin ML. Activation of the fifth and sixth components of the human complement system: C6-dependent cleavage of C5 in acid and the formation of a bimolecular lytic complex. *J Immunol* 1983; **131**: 892–898.
9. Collard CD, Vakeva A, Bukusoglu C, Zund G, Sperati CJ, Colgan SP, *et al.* Reoxygenation of hypoxic human umbilical vein endothelial cells activates the classic complement pathway. *Circulation* 1997; 96: 226.
10. Sonntag J, Emeis M, Strauss E, Obladen M. *In vitro* activation of complement and contact system by lactic acidosis. *Mediat Inflamm* 1998; 7: 49–51.
11. Siriwardhana SA, Kawas A, Yates S, Gulden H, Lipton JM, Giesecke AH. Oxygen mediated complement activation: an in-vitro study. *Can J Anaesth* 1990; **37**: 116.
12. Sonntag J, Stiller B, Walka MM, Maier R. Anaphylatoxins in fresh frozen plasma. *Transfusion* 1997; **37**: 798–803.
13. Burger R, Zilow G, Bader A, Friedlein A, Naser W. The C terminus of the anaphylatoxin C3a generated upon complement activation represents a neoantigenic determinant with diagnostic potential. *J Immunol* 1988; **14**: 553–558.

14. Klos A, Ihrig V, Messmer M, Grabbe J, Bitter-Suermann D. Detection of native human complement components C3 and C5 and their primary activation peptides C3a and C5a (anaphylatoxic peptides) by ELISAs with monoclonal antibodies. *J Immunol Methods* 1988; **111**: 241–252.
15. Heideman M, Hugli TE. Anaphylatoxin generation in multisystem organ failure. *J Trauma* 1984; **24**: 1038–1043.
16. Weiser MR, Williams JP, Moore FD, Kobzik L, Ma M, Hechtman HB, *et al.* Reperfusion injury of ischemic skeletal muscle is mediated by natural antibody and complement. *J Exp Med* 1996; **183**: 2343–2348.
17. Buerke M, Murohora T, Lefer AM. Cardioprotective effects of a C1 esterase inhibitor in myocardial ischemia and reperfusion. *Circulation* 1995; **91**: 393–402.
18. Ikai M, Itoh M, Joh T, Yokoyama Y, Okada N, Okada H. Complement plays an essential role in shock following intestinal ischemia in rats. *Clin Exp Immunol* 1996; **106**: 156–159.
19. Crawford MH, Grover ML, Kolb WP, McMahan A, O'Rourke RA, McManus LM, *et al.* Complement and neutrophil activation of the pathogenesis of ischemic myocardial injury. *Circulation* 1988; **78**: 144–148.

ACKNOWLEDGEMENTS. This work was supported by the German Research Society (DFG-0b 43/6–2).

Received 10 September 1998; accepted 5 October 1998