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Short communication

Asparaginase inhibits the lectin pathway of complement activation

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ABSTRACT

Oncological treatment has been associated with an increased risk of infection, most often related to therapy-induced pancytopenia. However, limited research has been conducted on the effect of oncological therapy on the complement system, being part of the non-cellular innate immune system. This became the rationale for an observational clinical study (C2012) in which we have investigated the prevalence of transient complement defects. Once we had observed such defects, a correlation of the complement defects to specific clinical parameters or to specific therapeutic regimens was investigated. A prominent defect observed in C2012 was the inhibition of the lectin pathway (LP) of complement activation during the treatment of acute lymphoblastic leukemia (ALL), which we could directly associate to the use of asparaginase (ASNase). *Ex-vivo* experiments confirmed a direct dose-dependent inhibitory effect of ASNase on the LP functionality.

1. Introduction

The introduction of ASNase has greatly improved the treatment outcome of ALL and has become an important component of ALL treatment regimens. ASNase rapidly depletes extracellular pools of asparagine by converting this non-essential amino acid into L-aspartic acid and ammonia. Depletion of asparagine in leukemic cells – which usually lack the enzyme asparagine synthase (Broome and Schwartz, 1967) – results in the inhibition of protein synthesis and nutritional deprivation and subsequent apoptotic cell death (Truelove et al., 2013). Although the efficacy of ASNase therapy has been proven in numerous clinical studies, severe side-effects have been observed including hepatic dysfunction, pancreatitis, hyperglycemia, and allergic reactions (Tong et al., 2014). The increased incidence of thrombosis with the use of ASNase has been attributed to reduced synthesis of pro- and anti-coagulation proteins by the liver resulting in an imbalance of these two processes (Payne and Vora, 2007; Bushman et al., 2000). The shared substrate specificity between complement components, especially MASPs, and some critical coagulation proteins (Factor Xa and Factor XIII) reinforces the overlap in infection and coagulation, as was suggested before (Krarup et al., 2007). Since most complement components are primarily produced by the liver a reduction in protein synthesis may have been responsible for the observed transient complement defects (Peng et al., 2008), although this has not been studied in more detail. Here we show that independent of the inhibition of protein synthesis, ASNase is able to directly interfere with functionality of the complement system, especially the LP, due to direct interaction with the effector-complexes.

2. Materials & methods

The initial study cohort C2012 comprised 48 pediatric oncology patients, independent of the underlying malignancy as described in detail elsewhere (Keizer et al., 2016). This longitudinal study investigated the functionality of the complement system before, during and after treatment with chemotherapy and/or subsequent admittance for (febrile) neutropenia by screen with the well-described commercially available Wielisa® Complement system Screen COMPL300 (Wieslab, Lund Sweden) (Palarasah et al., 2011). Using specific coated plates to activate the individual complement activation pathways the functionality is determined by reading out a terminal pathway complex. As part of the analysis of the C2012 study, the effect of ASNase on the complement system was further characterized in patients with ALL when paired samples were available (n = 4). The direct effects of the drug on complement LP and CP functionality was assessed and quantified following addition of ASNase to serum *ex-vivo*.

Specific assays to determine the functionality of the classical pathway (CP) (Wolbink et al., 1993) and LP (Keizer et al., 2014) were

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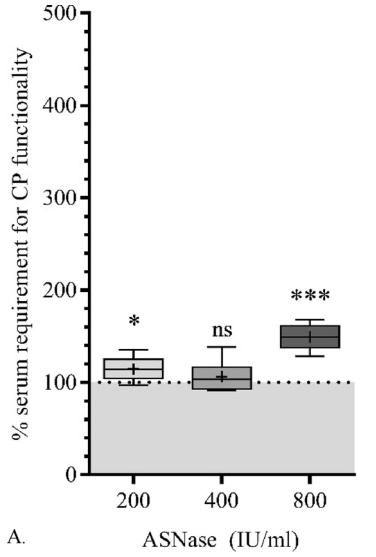
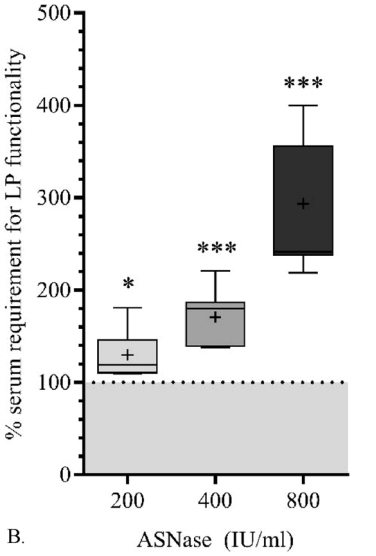


A.



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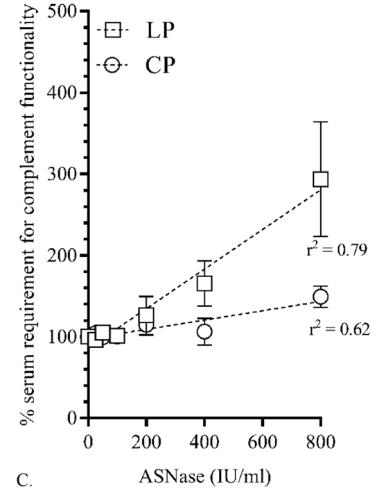

C.

Fig. 1. Asparaginase is able to inhibit the classical and lectin pathway of complement. (A) C4-deposition on AHG-coated plates for CP functionality or (B) mannan-coated plates for LP functionality of pooled serum from healthy donors (NHS) incubated with different amounts of ASNase. Data are normalized to NHS incubated at 37 °C (shown as a dotted line at 100%). Data are shown as mean ± SD (n = 9). *p < 0.05; ***p < 0.001; ****p < 0.0001 one-way ANOVA on paired samples with post-hoc Holm-Sidak multi-comparison test. (C) Correlation between the increase in serum requirement as determined byan increase of serum concentrationof NHS to ASNase (n = 7 concentrations, with four independent replicates). The correlation for the LP functionality (r^2 = 0.79) is stronger than for CP functionality (r^2 = 0.62). Data are shown as mean ± SD.

used. Briefly, pre-coated plates for the activation of the individual complement activation pathways were incubated with a titration of patient serum and compared to a serum pool from healthy donors (normal healthy serum (NHS)). In addition, NHS was incubated with increasing concentrations of ASNase (Paronal®) to show the specific inhibitory effect on the LP and CP. All samples were incubated with ASNase at 37 °C to mimic the *in vivo* situation, all other steps were performed at room temperature. Incubation with a pegylated form of ASNase (Oncaspar®) gave similar inhibition (data not shown). The functionality of the CP was further investigated by detecting C4 deposition on plates coated with aggregated human immunoglobulines (AHG), whereas the functionality of the LP was determined using mannan-coated plates hereby circumventing possible effects of ASNase on the terminal pathway.

To quantify an effect of ASNase on the binding of MBL, or an effect on the MBL/MASP-complexes, specific monoclonal antibodies (mAbs) were used. MBL-binding to mannan-coated plates was measured as described previously by Brouwer et al. (2009). Briefly, mannan-coated plates were incubated for 60 min with NHS, containing increasing concentrations of ASNase. After washing, plates were developed with specific monoclonal antibodies. MBL-binding was detected using biotinlylated mAb αMBL-1. MBL/MASP-1 and MBL/MASP-2 complexes were detected using biotinylated αMASP-1 4H2A9 (Hycult Biotech, Uden, the Netherlands) and αMASP-2 12D12 (in house mAb), respectively. To quantify the effect on the functionality, samples were compared to NHS, which was corrected for incubation time and temperature and normalized to the control conditions (NHS, 37 °C).

ASNase activity levels in the patient sera were measured as described earlier (Kloos et al., 2016). Briefly, patient samples were incubated with an excess amount of L-aspartic acid ß-hydromate (AHA) at 37 °C. Hydrolyzation of AHA to L-aspartic acid and hydroxylamine by ASNase can be detected at 690 nm after condensation with 8-hydroxyqinoline and oxidation to indo-oxine.

Inhibition of functionality is shown as an increase in serum requirement to obtain similar complement activation as the control situation (Keizer et al., 2014). The half maximum activation (EC50) of CP in NHS was 0.056% ± 0.023 (mean ± SD; n = 9) and 0.31% for LP activation (data not shown). Pre-incubation of serum at 37 °C for 1 h did not change the EC50 (data not shown).

3. Results & discussion

We observed a reduction of the C4-converting capacity of NHS in both the CP and, more strongly, the LP after incubation of NHS with increasing ASNase concentrations, as indicated by a significant, dose-dependent increase in serum concentration to obtain a similar functionality to the control (Fig. 1A, B). This effect was much more pronounced for the LP, which showed a five-fold increase in serum required to reach the same response, than for the CP, which only required twice the normal serum amount. This was also underlined by a stronger correlation between the ASNase concentration and the amount of serum required for the LP (r^2 = 0.79) compared to the CP (r^2 = 0.62) (Fig. 1C).

Because the effect of ASNase was most pronounced on mannan-binding lectin (MBL)-dependent complement activation, we studied the