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Short Communication Open Access

IdeS, a Bacterial IgG-cleaving Proteinase, as a Drug in Transplantation and Autoimmune Conditions

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Short Communication

For many years we have studied the significance of bacterial proteinases in relation to pathogenesis and virulence [1,2], and during the summer of 2001 we were performing a series of experiments where *Streptococcus pyogenes*, a major bacterial pathogen in the human population, was grown in the presence of 5% human blood plasma. When the growth medium containing plasma was analyzed by SDS-PAGE, we noticed a band of 31 kDa which was not present in the same medium containing plasma that had not been in contact with the bacteria. This band was identified as a fragment of the heavy chain of IgG, and the enzyme responsible for the cleavage was purified and named IdeS; Immunoglobulin G-degrading enzyme of *Streptococcus pyogenes* [3]. After the submission of this work, another group published a paper describing a protein, streptococcal protein Mac [4], identical to IdeS. The name Mac was based on a limited sequence homology to the α-subunit of the human β2-integrin Mac-1, but at that time our colleagues were not aware of the proteolytic activity of Mac. IdeS is therefore a more appropriate designation for this novel bacterial protease.

Analysis of the sequence of IdeS and the inhibition of its IgG-cleaving activity by cysteine proteinase inhibitors indicated that IdeS is a cysteine protease [3]. This was confirmed when the three-dimensional structure of the enzyme was determined by X-ray crystallography, showing a structure resembling the canonical papain fold [5]. The most striking and fascinating property of IdeS is the strict specificity for IgG (all four human subclasses are cleaved) which we noted in early experiments with human plasma proteins including the other classes of immunoglobulins; IdeS very rapidly and efficiently cleaved IgG in the lower hinge region but had no activity against IgM, IgA, IgD, IgE, or the additional proteins tested [3]. The extreme specificity makes IdeS a unique proteinase; apart from IgG no other substrate has been identified. In order to cleave IgG in the hinge region IdeS first has to bind to the Fc region, and the remarkable specificity for IgG is explained by the requirement for this initial protein-protein interaction [6]. The degradation of IgG occurs in two steps and the cleavage of the first heavy chain is much faster than the cleavage of the second chain [7-9]. This means that IdeS primarily generates single-cleaved IgG before the second Fc half is also removed and $F(ab')_2$ fragments are created. From a functional point of view it is important that the single-cleaved IgG and $F(ab')_2$ fragments both retain full antigen-binding activity, whereas already a single IgG cleavage by IdeS compromises the effector functions of IgG [8].

In many autoimmune conditions and in transplant rejection IgG antibodies play a pathogenic role. The efficient, and specific cleavage of IgG by IdeS, indicated that the enzyme could potentially be used to disarm pathogenic IgG antibodies *in vivo*. In a series of publications we could demonstrate that IdeS rapidly and effectively (one molecule of IdeS cleaves more than two thousand IgG molecules; Christian Kjellman and Lars Björck, unpublished data), cleaved the entire IgG pool in human blood (exemplified in Figure 1) and synovial fluid *in vitro* [7,10], completely but temporarily removed IgG from the circulation of rabbits [10] and cured mice with various IgG-driven autoimmune diseases; immune thrombocytopenia [10], rheumatoid arthritis induced by IgG antibodies against collagen type II [11], and experimental glomerulonephritis [12]. The effect of IdeS in all these *in vitro* and *in vivo* experiments was dramatic. Apart from human IgG, only rabbit IgG is completely cleaved by IdeS [3,10], and the entire intravascular pool of IgG was cleaved within six hours following a single dose of IdeS injected intravenously into rabbits [10]. This and the fact that IgG levels returned to normal levels after 10-14 days, and that the rabbits showed no adverse effects even after several IdeS injections [10], two of the animals were treated six times, were the first and clear indications that the bacterial enzyme could be used as a drug in humans.

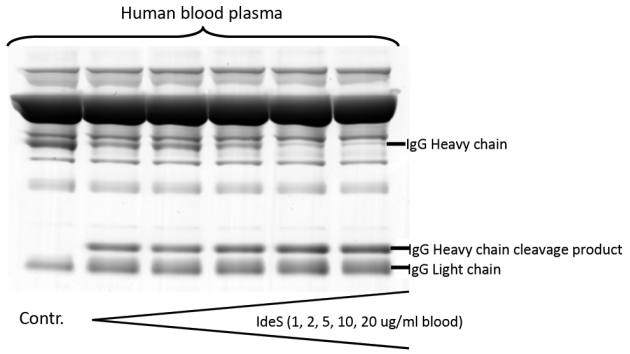

Figure 1: One ml samples of human blood were incubated with increasing amounts of IdeS for three hours at 37°C. Blood cells were spun down and the resulting supernatants were subjected to SDS-PAGE under reducing conditions. At 10 µg IdeS/ml blood (lane 5 from the left) the heavy chain of IgG is completely degraded. The 31 kDa fragment of the heavy chain (IgG Heavy chain cleavage product) is clearly visible already at 1 µg IdeS.

The studies described above and additional data generated by Hansa Medical, a clinical stage drug development company in Lund, Sweden (for more information about Hansa Medical and IdeS, see hansamedical.com), made it possible to take the decisive step to test IdeS in humans. Following approval from the Swedish Medical

Products Agency, a clinical Phase 1 study on IdeS was performed. The study, including 29 healthy Swedish volunteers, showed that a single dose of IdeS injected intravenously at 0.24 mg/kg bodyweight, within minutes cleaved the entire pool of extracellular IgG. After 3-4 days intact IgG antibodies started to reappear in the circulation and the levels were back to normal 2-3 weeks later. A short and transient removal of extracellular IgG should have little impact on immunity in general, and no significant adverse effects were recorded in any of the twenty individuals injected with IdeS, taken together, the results of the study [13] emphasized the therapeutic potential of IdeS. As mentioned, there are many clinical conditions where IgG autoantibodies contribute to the pathology. One possible indication for IdeS that was discussed already in 2007 after the successful *in vivo* experiments in rabbits [10] was to use the enzyme as a drug against allograft rejection, especially in sensitized dialysis patients. Due to previous transplantations, blood transfusions or pregnancies, approximately 30% of patients on the waiting list for kidney transplantation have IgG antibodies against donor MHC antigens. About half of these patients are classified as highly sensitized, which is a contraindication to transplantation due to the risk of IgG-mediated acute rejection. A phase II study in Sweden to remove donor specific IgG antibodies in dialysis patients awaiting kidney transplantation was recently completed, and the results showed that IdeS given prior to transplantation has the potential to enable transplantation of thousands of patients with donor specific IgG antibodies. Two additional phase II studies with IdeS are ongoing, one in Sweden and one in the USA, to investigate whether IdeS can replace plasmapheresis in the desensitization protocols that are currently used.

Apart from organ transplantation, other indications for IdeS therapy are also under consideration; haemophilia patients with IgG antibodies against coagulation factors VIII or IX resulting in intractable bleedings, patients with Guillain-Barrés syndrome, Goodpastures syndrome, anti-NMDA receptor encephalitis, neuromyelitis optica, systemic lupus erythematosus with antiphospholipid syndrome, acute and severe onset or relapse of multiple sclerosis, and various other IgG-driven autoimmune conditions and episodes. Compared to plasmapheresis the very rapid and complete cleavage of IgG by IdeS offers an attractive therapeutic alternative. Another advantage of IdeS treatment is that the enzyme due to its size (35 kDa) will leave the circulation and cleave IgG also in the extravascular space [11-13]. In addition, single-cleaved IgG and $F(ab')_2$ fragments of IgG, generated by IdeS cleavage of IgG in solution or when the antibodies are already bound to a cell surface antigen, will still bind their antigen with retained affinity. As long as the antibody fragments are bound, the antigens will be masked for new intact IgG produced against these antigens. Finally, it was recently reported that IdeS cleaves IgG also when IgG is part of the B cell receptor complex with CD79a/b. As a consequence, memory B cells do not respond to antigenic stimulation leading to their transition into antibody-producing cells [14]. The temporarily silencing of memory B cells by IdeS is also a potentially important and favorable property of IdeS in transplantation and autoimmune conditions.

S. pyogenes is one of the most significant bacterial pathogens in the human population (the bacterium has gained notoriety in the public as the "flesh-eating killer bug") responsible for more than 0.5 million deaths and causing at least 700 million cases of pharyngitis and skin infections annually [15]. The demonstration that a proteolytic enzyme which has evolved to protect the pathogen against the human immune system [3,16], now shows promise as a drug against transplant rejection and serious autoimmune conditions, represents an unusual scientific story.

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