Neutrophil Elastase and Myeloid-Related Protein-8/-14 as Executors and Regulators of Tissue Damage in Autoimmune Bullous Diseases

DISSERTATION

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SUMMARY

The prevalence of chronic inflammatory diseases, especially autoimmune disorders (AID), has increased dramatically during the last decades in the world. Most diseases require a life-long non-curative treatment which is largely limited to the application of immunosuppressive drugs. The permanent and severe reduction in the quality of life of the patients as well as the enormous economical burden associated with these diseases emphasizes the high medical need for the development of novel therapeutic options. Since many years, the contribution of the different components of immune system to the pathogenesis of AID has been under intensive investigation. In the context of autoimmune bullous diseases (AIBDs), neutrophils have been identified as essential players in the effector phase of AIBDs. Neutrophil-derived mediators are involved in tissue damage and skin blister formation which is the hallmark of AIBDs including epidermolysis bullosa acquisita (EBA) and bullous pemphigoid (BP). However, although the principal participation of neutrophil proteases and ROS has clearly been shown in this course, the precise regulatory and executive mechanisms of neutrophils in the disease still remain unclear. In this study, it is hypothesized that neutrophils contribute to the effector phase by two ways, indirectly by the secretion of myeloid-related proteins (MRP)-8 and -14 leading to amplification and maintenance of the inflammatory loop as well as directly by the release of tissue-destructing elastase. Therefore, delineation of the mechanisms how neutrophils cause tissue damage will establish further information about the pathomechanisms of EBA and BP and provide a novel therapeutic avenue specifically targets the terminal phase of the disease.

MRP-8/-14 proteins belong to the group of alarmins or danger-associated molecular pattern (DAMPs) and their association with numerous human disorders, including acute and chronic inflammatory conditions and autoimmune diseases has been shown. In this study it is shown that elevated levels of MRP-8/-14 occur also in EBA and BP patients as well as in experimental disease. To clarify whether these functions are also relevant in the pathological process of the skin bullous diseases, Mrp-14-deficient mice, which are also functional deficient in MRP-8, were tested in antibody transfer models of EBA and BP. However, the knockout mice were not protected against disease development and their phenotype did not differ from that of the wild type controls. In contrast to the original hypothesis, these results indicate
that MRP-8/-14 proteins are not involved in the effector phase of EBA and BP. As a consequence, MRP-8/-14 do not appear as suitable therapeutic targets for the treatment of the effector phase of EBA or BP.

Neutrophil proteases such as elastase are essentially involved in many pathophysiological processes and uncontrolled proteolysis is a major reason for tissue damage and destruction in chronic inflammatory diseases like asthma, COPD, or AIDs. However, due to their low efficacy or severe side effects, antiproteinase therapies failed so far as treatment concept for these diseases. Therefore, the understanding of the mode of action of proteases under physiological conditions could help to develop new strategies to prevent the host from harmful attacks by these proteins. So far the role of these enzymes in disease has been shown only indirectly, either by appropriate knockout mice or inhibitor studies, determination of protease activity in cell extracts and supernatants, or by histological localization of the protein in the diseased tissue. For better understanding of the pathophysiological mechanism of neutrophil elastase in disease, a direct approach allowing their quantification and localization of the enzyme activity at its site of action in vitro and in vivo is required. Therefore, a method was established by using NE FRET reporter NEmo-2 to visualize NE activity on neutrophils in vitro as well as in experimental EBA in vivo. Using these novel tools, an interdependency between immune complex (IC)-induced neutrophil adhesion and elastase enzyme activity was discovered.

According to my results, exposure of neutrophils to immobilized IC induces their rapid activation in terms of cell adhesion, ROS production and the release of elastase. Surprisingly, although blocking of neutrophil adhesion with antibodies to CD18 did not affect the release of elastase or ROS, it has been shown in our group that IC-mediated tissue damage is significantly reduced under these conditions. Analysis of membrane-associated elastase activity in vitro by using the novel FRET-based protease sensor NEmo-2 revealed the presence of protease activity on adherent cells which is resistant to macromolecular protease inhibitors. Most likely, the escape of elastase from inhibitor access is enabled by the formation of a closed space between cell and surface. This fine cooperation among IC-induced neutrophil adhesion and elastase activity in tissue damage was further confirmed in a model of EBA in vivo, where elastase enzyme activity could be imaged on the surface of adherent neutrophils in presence of protease inhibitors.
Taken together, this study demonstrates that during the effector phase of experimental AIBD, MRP-8/-14 proteins do not contribute to these diseases and, therefore, may not represent suitable therapeutical targets in this stage. However, highly sensitive detection of neutrophil elastase protease activity reveals an essential dependency of protease activity in vitro and in vivo on IC-induced neutrophil adhesion. Therefore, prevention of adhesion process in neutrophils by breaking the protected space could facilitate the access of intrinsic as well as therapeutic inhibitors at the site of tissue damage. These results help to better understand the pathophysiological functions of neutrophils in the effector phase of the disease particularly the direct effects of NE on tissue degradation and enable us to open a previous unseen window for novel therapeutical approaches of AIBD and other neutrophil-mediated disorders.