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The Role of Macrophages in Regulating Inflammation by Oxidative Burst

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i hörsal Farmakologi, Solna

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ABSTRACT

The production of reactive oxygen species (ROS) through a process called oxidative burst is an essential defence mechanism against pathogens. In phagocytes, such as neutrophils and macrophages, the NADPH oxidase 2 (NOX2) complex is the main source of ROS. Genetic alterations in any of the components of the NOX2 complex that impair the ROS production are at the origin of a condition called chronic granulomatous disease (CGD), characterized by recurrent life threatening bacterial and fungal infections. Recently, natural occurring mutations in *Ncf1*, a regulatory component of the NOX2 complex, were described to compromise the protein function and to increase arthritis severity in rats and mice. Macrophages are phagocytes that express NCF1 and are able to kill pathogens. At the same time, they are antigen-presenting cells known to play an important role in arthritis. We therefore hypothesized that expression of NCF1 in macrophages would have an impact on the immune response during arthritis and bacterial infections. The aim of the studies presented in this thesis is to evaluate the influence of NCF1 expressed by macrophages on development of arthritis and resolution of bacterial infections.

Using transgenic mouse models we could describe a role for macrophages in both priming and activation of arthritogenic T cells. In a first transgenic mouse, expression of functional NCF1 restricted to macrophages reduced arthritis severity, priming of Th1 T cells and T cell proliferation, therefore limiting the T cell-dependent autoimmune outbreak. In a second transgenic mouse strain, where macrophages were the only cells expressing the arthritis-prone MHC class II A^g molecule, macrophages could prime arthritogenic T cells and mediate arthritis development, but only in NCF1 deficient setting. We could conclude that ROS production by macrophages is important in determining the activation state of T cells and in regulating the severity of arthritis

As in the human CGD situation, mice carrying the *Ncf1* mutation were more susceptible to spontaneous and induced bacterial infections. Using the transgenic mouse where macrophages expressed the functional NCF1, we observed that macrophage-derived ROS effectively protected mice from bacterial infections, a function believed to be executed mainly by neutrophils.

Finally, we tested a new model of arthritis where the disease was induced with a peptide of a glycolytic enzyme. We found that the symptoms and pathogenesis of the disease resembled the one of the most common arthritis model, collagen-induced arthritis, which is induced with the full collagen protein. Both diseases are dependent on an intact adaptive immune system and their severity is influenced by *Ncf1*. We were also able to identify one of the important residues causing the peptide's arthritogenicity.

In summary, our data highlight the crucial role of NCF1, and consequently of NOX2 complex, in regulating both innate and adaptive immune responses. NCF1-dependent ROS in macrophages was important during both phagocytosis and antigen presentation, resulting in clearance of bacterial infection and suppression of chronic inflammation. These findings will facilitate further investigations of the molecular pathways through which ROS influence arthritis pathogenesis and hopefully lead to identification of new therapeutic targets.