A151 APOPTOSIS AND THE SUBSEQUENT GENERATION OF AUTOANTIGENS AS INITIAL TRIGGERS IN THE PATHOGENESIS OF EXPERIMENTAL AUTOIMMUNE ARTHRITIS

S Herman, M Hoffmann, A Kny, J Smolen, G Steiner *Internal Medicine III, Department of Rheumatology, Medical University of Vienna, Austria*

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Background Rheumatoid arthritis (RA) has a definite autoimmune pathogenesis. Rising evidence suggests that deregulated and/or deficient clearance of apoptotic materials may lead to the release of potential autoantigens provoking a break in the self-tolerance mechanism. Major autoantigens in RA include immunoglobulin G, the target of rheumatoid factor (RF), citrullinated proteins and the heterogeneous nuclear ribonucleoprotein (hnRNP) A2. To gain more insight into the role of apoptotic processes in lymphoid tissues in the generation of arthritogenic autoantigens, we studied the autoimmune responses in the pristane-induced arthritis (PIA) model which shows some striking similarities with human RA, including symmetrical polyarthritis, massive bone erosion and the presence of RF and autoantibodies to hnRNP-A2.

Methods Pristane (2,6,10,14-tetramethylpentadecane) was injected intradermally at the base of the tail into DA.1F rats. Lymph nodes were analysed by immunohistochemistry and immunoblotting at various time points after pristane application. The fate of potential neoantigens, particularly hnRNP-A2, during apoptosis of lymphoid cells was studied in cultured cells by inducing apoptosis with agents such as etoposide or anti-Fas antibody, or by cultivating cells in the presence of pristane/cyclodextrine complexes. To investigate apoptotic cleavage of hnRNP-A2 in vitro, caspase digestion assays were performed.

Results Three days after pristane application the amount of apoptotic cells was strongly increased in draining inguinal lymph nodes of DA.1F rats compared with naïve animals. Immunoblotting analysis of inguinal lymph nodes revealed cleavage of hnRNP-A2. Furthermore, a cleaved product of hnRNP-A2 was also detected in cellular apoptosis assays performed with human peripheral blood cells and rat splenocytes. Cleavage was most pronounced after apoptosis induction via the Fas receptor. Interestingly, the cleaved product was preferentially detected in cell supernatants compared with cell lysates, whereas the intact protein was found in similar amounts in lysates and supernatants. Furthermore, both caspase 3 and caspase 6 were able to efficiently cleave recombinant hnRNP-A2 in vitro. The in vivo relevance of these findings was further strengthened by results obtained with pristane-cyclodextrine complexes which were able to induce apoptosis in both human and rat cells.

Conclusion Pristane induces apoptosis in draining lymph nodes leading to cleavage of hnRNP-A2 by proteolytic enzymes such as caspase 3 or caspase 6. This suggests a mechanism for the generation of neoepitopes which are subsequently targeted by arthritogenic T cells. These data further strengthen the view of hnRNP-A2 as a key player in the pathogenesis of PIA and possibly also human RA.