PREPARATION AND CHARACTERIZATION OF ANTIBODIES AGAINST HYDROXYL RADICAL MODIFIED THYMIDINE MONOPHOSPHATE

ABSTRACT

THESIS
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Systemic lupus erythematosus (SLE) is a prototype autoimmune disease characterised by the production of autoantibodies, in particular antibodies to native double-stranded DNA. These naturally occurring antibodies are heterogeneous, exhibiting wide antigen binding characteristics that includes a variety of nucleic acids and other biomolecules. While much has been learned in recent years of the fine specificity and cross reactivity of anti-DNA antibodies in human and murine lupus, neither their production and regulation nor the molecular genetics of their formation is known. Autoimmune phenomena manifested as antibodies to cellular components have also been described in cancer patients. These include anti-nuclear antibodies and antibodies to cytoplasmic antigens. Autoantibodies have been detected in patients with leukemias, malignant melanomas, lung, breast and hepatocellular carcinoma.

Reactive oxygen species are formed constantly in living organisms, as products of the normal metabolism, or as a result of different environmental influences. ROS have been implicated in a number of human degenerative diseases including SLE and cancer. Among the ROS, the 'OH is the most potent and can react with DNA, lipids and proteins. Of the five major DNA components, thymine and cytosine are most susceptible to 'OH damage, followed by adenine, guanine and deoxyribose moiety.

In the present thesis, thymidine monophosphate (TMP) was modified with hydroxyl radical, generated by H₂O₂ and UV light. UV absorption and difference spectra showed the characteristic loss in absorption pattern as a result of the damage caused by hydroxyl radical. Antibodies against TMP and ROS-TMP were induced in rabbits. Both the antigens were found to be non-immunogenic. TMP was conjugated to BSA as a carrier protein by
carbodiimide method to enhance its immunogenicity. TMP-BSA conjugate was characterized by UV spectra and PAGE. The results of PAGE reiterate the formation of conjugate. TMP-BSA conjugate was modified with hydroxyl radical. UV absorption and difference spectra showed hypochromicity in the modified conjugate. The formation of ROS-modified conjugate was analyzed by Sephadex G-100 column chromatography, densitometric scanning and agarose gel electrophoresis. The data conclusively demonstrates structural perturbations and formation of low molecular weight species.

Induced antibodies against TMP-BSA and ROS-TMP-BSA conjugates revealed their high immunogenicity. Antibodies thus formed were purified on Sepharose 4B column. Purified anti-TMP-BSA conjugate IgG showed 95% inhibition (at 10 μg/ml) with TMP-BSA conjugate. Almost similar inhibition of anti-ROS-TMP-BSA conjugate IgG was observed with ROS-TMP-BSA conjugate. The antigen binding characteristics of purified TMP-BSA conjugate IgG was investigated with various nucleic acid conformers. ROS-TMP-BSA conjugate showed a maximum inhibition of 42%. ROS-modified TMP, thymidine and thymine showed higher inhibitions of 69%, 56% and 82% as compared to their native forms. Similarly, ROS-adenine and ROS-guanine show higher inhibition of 57% and 61% than their native counterparts. Poly(dA-dT).poly(dA-dT) showed moderate inhibition while poly(I), ROS-poly(I) and ROS-poly(G) showed insignificant inhibition. Inhibition data showed crossreactivity of anti-ROS-TMP-BSA conjugate IgG with various conformers. The TMP-BSA conjugate gave a maximum inhibition of 89%. ROS-modified TMP, thymidine and thymine showed higher inhibitions (86%, 56% and 81%) than their unmodified counterparts (39%, 23% and 16%). Poly(dA-dU).poly(dA-dU) and poly(dI-dC).poly(dI-dC) showed inhibition of 35% each. ROS-guanine showed a
high inhibitory potential of 78% than native guanine (55%). The broad antigen binding characteristics of induced antibodies with a variety of polynucleotides might be due to recognition of the phosphodiester-backbone.

In the present study, anti-double stranded DNA autoantibodies from SLE patients were investigated for binding to native and modified conjugates. Results with purified SLE IgG showed appreciable binding with native DNA (inhibition varies from 32% to 55%). Results of direct binding ELISA show preferential binding of TMP-BSA conjugate as compared to ROS-modified conjugate. Competition ELISA of purified SLE IgG with TMP-BSA conjugate showed maximum inhibition ranging from 32% to 85% while ROS-modified form show inhibition from 10% to 52%. The data substantiated the results of direct binding ELISA.

The presence of autoantibodies in the sera of cancer patients were also studied. Direct binding ELISA of the serum samples from patients with breast, lung, liver and gall bladder cancer showed higher recognition of ROS-TMP-BSA conjugate as compared to its native form. Tonsil and oral cancer sera recognizes both native and ROS-modified conjugates. Out of the six breast cancer sera tested, two showed moderate inhibition with TMP-BSA conjugate while two gave similar binding with native and ROS-modified conjugates. Among seven serum samples from oral cancer three showed higher inhibition with TMP-BSA conjugate as compared to ROS-modified form. All three liver cancer sera had antibodies with a higher recognition to ROS-TMP-BSA conjugate as compared to its native form. Sera from lung and gall bladder cancer showed preferential recognition with ROS-TMP-BSA conjugate. Out of two sera from larynx one showed higher inhibition with TMP-BSA conjugate and the other with ROS-TMP-BSA conjugate. Purified cancer IgG was investigated for antibodies against
TMP-BSA and ROS-TMP-BSA conjugates. IgG isolated from breast cancer sera showed higher recognition of ROS-modified conjugate over that of native conjugate. One of them showed a maximum inhibition of 62% with ROS-TMP-BSA conjugate. Two IgG isolated each from liver and gall bladder cancer sera showed higher recognition with ROS-TMP-BSA conjugate. Out of the five IgGs isolated from oral cancer sera, four showed higher binding with TMP-BSA conjugate than towards ROS-modified conjugate.

In conclusion, the conjugation of TMP with BSA renders it immunogenic. The antibodies raised against TMP-BSA and ROS-TMP-BSA conjugates were found to be highly immunogenic and are precipitating in nature. The induced antibodies though highly specific for the immunogen, exhibited cross reactivity with various nucleic acid polymers, resembling the binding characteristics of SLE anti-DNA antibodies. TMP-BSA conjugate provides higher inhibitory potential to SLE anti-DNA autoantibodies which might serve as a diagnostic marker for SLE. IgG isolated from sera of breast, liver and gall bladder cancer recognize and bind to ROS-modified TMP-BSA conjugate.