Hepatitis virus induced autoimmunity

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Key words : autoantibodies, autoantigens, autoimmunity, cross-reaction, hepatitis B virus, homologous sequences, molecular mimicry.

We have recently demonstrated that anti-nuclear (ANA) and anti-smooth muscle (SMA) antibodies are part of the natural course of chronic hepatitis B virus (HBV) infection (1). Studying the presence and fluctuation of tissue autoantibodies in a cohort of HBV positive patients undergoing a three-arm interferon alpha (IFN-α) controlled trial (2), we noted that at least a third of the subjects in the control group, represented by patients with chronic HBV infection remaining untreated over the four-year period of the study, were seropositive for ANA and SMA. This seropositivity did not appear to be influenced by IFN-α treatment.

In order to investigate the mechanisms leading to autoantibody formation in chronic HBV infection and in particular the possible role of molecular mimicry, we have interrogated protein databases in search of similarities between HBV proteins and putative antigenic targets of ANA and SMA and found highly homologous sequences. Peptides encompassing these sequences were constructed and tested as targets of immune and cross-reactive autoimmune responses.

We scanned the PIR and SWISSPROT (GCG, Wisconsin, USA) protein databases using the motif search programme FINDPATTERNS to search for local sequence homology between hepatitis B virus, subtype *adr* and human nuclear and smooth muscle proteins (3,4). The complete sequences of the HBV proteins, pre-S, HBsAg, HB pre-C, HBcAg, HBX and HBV-DNA polymerase were serially divided into twelve amino acid segments, each overlapping the preceding segment by six amino acids. HBV-DNA polymerase (HBV-pol) was found to share 7-9 amino acid sequences with nuclear (MHCII transactivator, nuclear pore core protein, nuclear mitotic apparatus, polymyositis sclerosis antigen) and smooth muscle proteins (caldesmon, myosin). Twelve 20-mer amino acid peptides containing the relevant homologues between the HBV DNA polymerase and the antigenic targets of ANA and SMA and a 20-mer irrelevant control peptide were synthesised. Reactivity to the control and synthetic peptides was determined by an enzyme linked immunosorbent assay (ELISA). Competition ELISAs were performed using both peptides and native proteins as competitors in the liquid phase. Antibodies to native caldesmon and myosin were tested by immunoblot using the sera of HBV positive patients who had double reactivity to HBV-pol and corresponding self-homologues, myosin or caldesmon peptides.

A large series of children with chronic HBV infection (HBsAg positive (microparticle enzyme immunoassay (MEIA), Abbott, Illinois, USA)), of patients with other chronic liver diseases (CLD), extrahepatic autoimmune diseases and healthy controls were investigated. Comparison between categorical values was done using the $\chi 2$ test with Yates' correction, when necessary. A probability (P) value less than 0.05 was considered significant.

Double reactivity to HBV-pol peptides and corresponding self homologues was significantly more frequent in HBV positive patients than in those with other CLD, with extrahepatic autoimmune diseases and healthy controls ($P < 0.001$ for all). Double reactivity to myosin or caldesmon peptides and their HBV-pol homologues was associated with SMA positivity by immunofluorescence ($P < 0.05$ for both). HBV positive sera double reactive for myosin or caldesmon and their homologous HBV-pol peptides also reacted with the native proteins on immunoblot.

Antibody binding to individual HBV-pol peptides was inhibited 50-90% by pre-incubation with the HBV-pol peptide or self homologue. No inhibition was observed with the control peptide. Antibody binding to the HBV-pol peptides was also inhibited by 60-70% by preincubation with the native proteins (caldesmon and myosin) but not with the control protein.

As mentioned earlier, autoantibodies to smooth muscle and nuclear components are commonly detected in patients with chronic hepatitis B virus infection (1). These autoantibodies do not seem to be induced or influenced by IFN-α therapy, but appear to be a consequence of HBV infection. Reactivity to at least one of the HBV-pol peptides was observed in all of the patients with chronic HBV infection.

Antibody reactivity to all of the human nuclear and smooth muscle peptide homologues was remarkably restricted and strongly associated with chronic HBV infection, providing a clear indication that these an-

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tibodies are generated specifically as a consequence of HBV infection. Double reactivity to HBV-pol peptides and self-homologues was observed almost exclusively in patients with chronic HBV infection. Antibody recognition of HBV-pol/self peptide pairs was cross-reactive, as preincubation with self homologue inhibited antibody binding to its HBV-pol peptide, thus providing a mechanism for the simultaneous recognition of homologous viral/self peptide pairs. Significantly, antibody cross-reactivity between HBV-pol and the smooth muscle derived antigens, myosin and caldesmon was associated with SMA positivity in patients with chronic HBV infection, suggesting a central role for HBV-pol in the generation of SMA. This contention is further supported by two observations : firstly, the ability of the native proteins, myosin and caldesmon, to inhibit the antibody binding to the homologous HBV-pol peptide ; and secondly, the ability of the sera from HBV positive patients double reactive to HBV-pol peptide and corresponding self-homologues, myosin and caldesmon peptides to give particularly strong signals for native caldesmon and myosin on immunoblot. As far as antinuclear antibodies are concerned, double reactivity to one of the nuclear targets, nuclear mitotic apparatus, and corresponding HBV-pol peptide tended to be more common in HBV positive patients who are ANA positive.

Our finding of cross-reactive antibody responses between HBV-pol peptides and homologous regions of human nuclear and smooth muscle proteins suggests that these autoantibodies may have arisen as an inappropriate evolution of the anti-HBV-pol immune response, to include antigenically similar nuclear and smooth muscle proteins. Thus, "mimicry" between viral and "self" antigens may lead to failure of tolerance to self components resulting in autoimmunity (5).

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