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SHORT REPORT

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Studies on Tissue Expression of HCV Proteins (NS3 and C) in Chronic Hepatitis C Using the ImmunoMax Technique

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Apart from serological tests for anti-HCV antibody detection, the complete diagnosis of hepatitis C virus (HCV) infection requires application of techniques to detect HCV genetic material (1, 2). Elaboration of techniques which would allow detection of the viral proteins in paraffin-embedded liver biopsies continues to be a diagnostic and cognitive challenge (3).

The aims of the studies reported on here were to compare detection of two different HCV proteins (NS3 and C protein) in liver biopsies from 9 children and 12 adults with chronic hepatitis C and 6 control liver biopsies from HCV-negative patients using the ImmunoMax technique and to compare the protein expression with grading and staging in the respective groups of patients. Histological features of all the liver specimens were examined using a numerical scoring system (4). For immunocytochemistry according to the classical ABC (avidin-biotin-peroxidase complex) technique alone or in association with the ImmunoMax technique (5), mouse MAbs against the NS3 protein (NOVO CASTRA Labs.) and against the capsid C protein (CHEMICON International, Inc.) were used. The results obtained using the ImmunoMax technique were calculated with the semiquantitative technique relating the score of 0 to 4 points to the fraction of stained cells, evaluated separately in the cytoplasm and in cell nucleus under a light microscope (five fields, at $\times 400$ magnification). To determine the statistical significance of variations in the number of immunopositive cells studied, we first calculated the mean values of staining scores for children and adult liver biopsies. These values were compared using the Mann-Whitney U test for non-parametric data. Correlation analysis between expression of detected proteins and expression of their respective grading and staging was done using the Spearman rank correlation index.

The ABC technique alone allowed the detection of NS3 protein only in hepatocytes of all biopsies of HCV-infected patients. The ImmunoMax technique also allowed the detection of C protein. All control biopsies were negative for both of the HCV proteins. In both groups of HCV-infected patients, cytoplasmic localization of NS3 protein dominated over nuclear localization ($P < 0.001$). Cytoplasmic localization of protein C prevailed over its nuclear localization in adults only ($P < 0.05$). Semiquantitative analysis of the two proteins in liver did not show significant differences between children and adults. The amount of the two proteins did not correlate with grading or with staging in either group of patients, although adults manifested significantly higher grading and staging (Table I).

Application of the ImmunoMax technique in detection of HCV proteins significantly augmented the potential for diagnosis of chronic type C hepatitis in paraffin-embedded liver biopsies. We observed more frequent cytoplasmic than nuclear localization of the two HCV proteins and the average

Table I. Histological score, localization and semiquantitative appraisal of NS3 and C proteins in liver biopsies of children (1–9) and adults (10–21) with chronic hepatitis C

	Histological score[1]			NS3 protein[2]		C protein[2]	
Patient	G1	G2	S	N	C	N	C
1.	1	0	0	0	1	0	0
2.	1	2	3	0	4	1	2
3.	1	0	2	0	2	2	0
4.	1	0	0	0	1	3	2
5.	1	2	1	0	2	1	1
6.	1	0	1	0	4	1	0
7.	1	0	1	0	4	2	2
8.	2	1	2	0	1	0	1
9.	1	2	0	0	1	1	1
10.	2	2	2	0	2	0	0
11.	1	2	2	0	3	0	0
12.	1	2	2	0	2	1	1
13.	3	2	4	1	4	2	3
14.	2	3	4	0	4	1	3
15.	2	1	3	0	1	1	1
16.	1	2	3	0	2	0	2
17.	1	1	1	nt	nt	1	1
18.	2	2	2	0	3	1	3
19.	3	3	4	0	3	0	3
20.	2	2	2	0	3	0	2
21.	1	1	1	0	3	2	2

[1]Scoring system according to (4): G1 = portal/periportal activity; G2 = lobular activity; S = staging; N = nuclear localization; C = cytoplasmic localization.

[2]Score: 0 = negative signal; 1 = less than 5% positive cells; 2 = 5%–20% positive cells; 3 = 20%–40% positive cells; 4 = more than 40% positive cells; nt = not tested.

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proportions of immunopositive hepatocytes (from 5% to over 20%) are consistent with the data found elsewhere (2, 3). Higher grading and staging levels in adults were not reflected in the total number of cells with detectable HCV proteins in the liver biopsies, which was similar in the two groups of patients.

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