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Short Report

Two-D Distance Distribution Analysis: An Application to HBcAg-Positive Hepatocytes and Its Relation to Septum Formation in Cirrhosis

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ENDOH, M., CHIBA, R. and TAKAHASHI, T. *Two-D Distance Distribution Analysis: An Application to HBcAg-Positive Hepatocytes and Its Relation to Septum Formation in Cirrhosis.* Tohoku J. Exp. Med.,1997, **182** (2), 181-184 —— The morphogenesis of cirrhotic septa in chronic hepatitis B was studied assuming that they arise at the sites of hepatocellular necrosis invoked by host immune reaction against HBcAg-expressing hepatocytes. Sections from three livers with chronic hepatitis B, all in cirrhotic stage, were immunostained with HBcAg and subjected to morphometry to analyze the distribution of HBcAg(+) hepatocytes and its relation with septa. HBcAg(+) cells were not distributed randomly over the nodules but forming focal areas. The septum formation along these foci was shown by 2-D distance distribution analysis, a technique we devised. Upon a sheet of color microphotograph of immunostained section, hundreds of test points each 400 μm apart were arranged by overlaying a tessellated grid. From each of the points hitting the nodules, the distance to the nearest nodulo-septal border D(min) was measured. Measurement was performed on a total of 2,585 test points. It was shown that the mean D(min) in the HBcAg(+) areas was significantly smaller than in HBcAg(−) areas. Thus, the cirrhotic septa are considered to arise at the places of HBcAg(+) foci, connecting them by postnecrotic collapsing. —— cirrhogenesis; HBcAg; chronic hepatitis B; morphometry; pathology

How can we explain the morphogenesis of cirrhosis from chronic hepatitis B ? We assume that interstitial septa arise at the sites of hepatocellular necrosis. With regard to the production of necrosis, the core antigen (HBcAg) has been incriminated as the target of host's immune response which is considered to destroy hepatocytes when the antigen is expressed over the membrane or in the cytoplasm (Chu and Liaw 1987). Thus, from a histopathological viewpoint, the way HBcAg is distributed in the tissue appears to define the pattern of necrosis and septum formation that follows. However, little attention has been paid to

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this aspect. We found that in cirrhosis from chronic hepatitis B, HBcAg(+) hepatocytes are not uniformly dispersed in the nodules but form clearly definable foci (Fig. 1). We also found that these foci are located in close association with septa and think that they are the very site where the septa develop and connect themselves into an interstitial belt. The association of HBcAg(+) foci with septa is shown applying the 2-D distance distribution analysis, a technique of morphometry we developed. This too, is to be explained.

Material and Methods

Material and specimen preparation

Used were liver specimens from three patients who had cirrhosis originating from chronic hepatitis B. In two, specimens were obtained at autopsy, and in the other, at surgery for hepatocellular carcinoma. The specimens were fixed in buffered formalin, embedded in paraffin, sectioned at 4 μm and immunostained for HBcAg according to avidin-biotin-complex method with an anti HBcAg antibody (Nichirei, Tokyo). All the livers proved to contain hepatocytes positive for HBcAg with cytoplasmic and/or membranous expression.

Two-D distance distribution analysis

Whether, in regenerative nodules, HBcAg(+) cells are located in the significant vicinity of septa, was examined applying a technique of morphometry. Several color microphotographs, each 24×20 cm, were prepared of each of the sections at $\times 25$. They were randomly overlaid with a tessellated grid as in Fig. 1, which was printed on a transparent plastic sheet. Here test points were defined by vertically crossing parallel lines with an interval of 1 cm, which corresponds to 400 μm in tissue section. When overlaid, some of the points come to hit the septa, while the other the nodules; of the latter, some hit HBcAg(+), and the other, HBcAg(−) areas. For each of the points hitting the nodules, the distance to the nearest nodulo-septal border D(min) was measured. The area contained 806 test points in Case 1, 1076 in Case 2 and 703 in Case 3. D(min)s from the points hitting HBcAg(+) and those from HBcAg(−) areas were separated and the distributions were compared. Mann-Whitney's test was applied to examine the significance of the difference.

Results

HBcAg positive cells found themselves in the nodules in essentially a focal fashion, with each of the foci variegated with hepatocytes showing cytoplasmic and/or membranous expression, those expressing the antigen only in the nuclei and those expressing none. Of the test points, those hitting an HBcAg(+) part occupied 26.8% (Table 1). This implies that the share of HBcAg(+) part is as much percent in terms of sectional area as well as volume of nodules. The distribution of D(min) is shown in Fig. 2 and Table 1. The mean D(min) in the

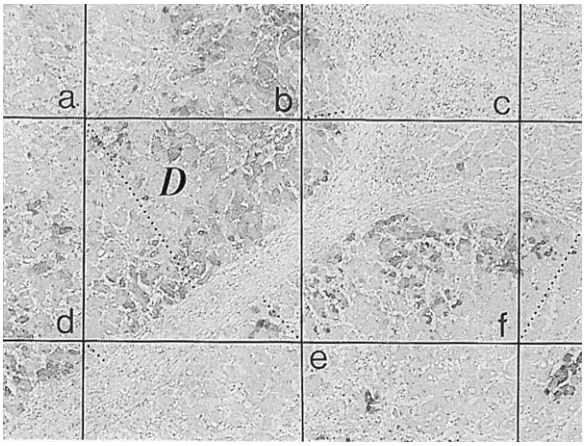

Fig. 1.

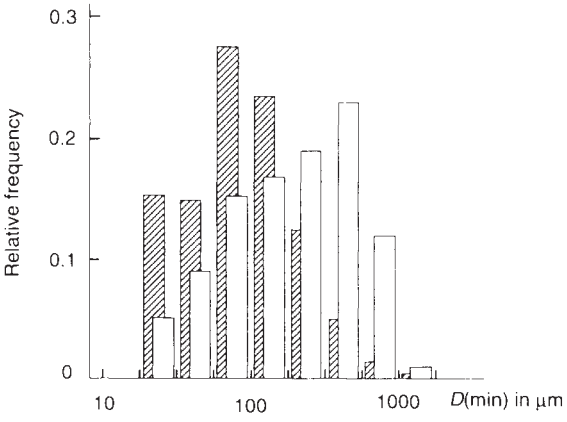

Fig. 2.

Fig. 1. An HBcAg-positive focus overriding a cirrhotic septum, shown to visualize the principle of 2-D distance distribution analysis. Of the six crossing points, *c* hit the septum and was excluded. The shortest distance $D(\min)$ to the septum was measured for the other five points, of which *a* and *b* were in the inside, *d*, *e* and f in the outside, of the antigen positive foci. Immunostain for HBcAg.

Fig. 2. $D(\min)$, the distance from the intranodular test points to the nearest septa, was shown on a log scale. Note that $D(\min)$ from the test points hitting the HBcAg(+) areas (▨) is smaller than from HBcAg(−) areas (□).

HBcAg(+) areas is apparently smaller than in HBcAg(−) areas, a result confirmed not only in each of the three cases but also when all the data were pooled. By Mann-Whitney's test, the differences were confirmed to be significant at $p<0.001$.

Discussion

In the above, we introduced a technique of morphometry which we call 2-D distance distribution analysis. This allows us to analyze whether in the tissue, lesions of interest are associated with a structural component, and to what degree. This time, it was applied to the relation of HBcAg(+) areas to the septa. When a test point came to hit an HBcAg(+) area, $D(\min)$, the shortest distance from it to the nodulo-septal border, proved to be smaller than when it hit a negative area. Obviously, this implies that the HBcAg(+) areas are located more closely to the septa than the HBcAg(−) areas. This conclusion is valid statistically because we

Table 1. *Mean $D(min)$[a] in μm, the distance to the nearest septum*

Case	from HBcAg(−) areas	from HBcAg(+) areas	
1	625.4 ($n=636$)	325.3 ($n=170$)	($p<0.001$)
2	356.4 ($n=822$)	172.4 ($n=254$)	($p<0.001$)
3	461.0 ($n=435$)	217.7 ($n=268$)	($p<0.001$)
Total	470.8 ($n=1893$)	225.3 ($n=692$)	($p<0.001$)

[a]Caluculated upon logarithmic scale. n, the number of hit points.

used a system of test points uniformly arranged and completely randomized with regard to the liver structure.

So far, little attention has been paid to the way hepatitis B virus-related antigens are distributed in the liver with chronic hepatitis. Some authors deny the possibility that HBcAg(+) cells take a characteristic pattern in their location (Gudat et al. 1975; Hsu et al. 1987). Some have gone even so far as to say that the distribution of HBV antigens does not correlate with hepatocellular necrosis (Peters et al. 1991). Today, however, there seems to be no doubt that HBcAg, when expressed in a cytoplasmic and/or membranous fashion, is responsible for inducing host's immune response against the antigen-bearing hepatocytes (Chu and Liaw 1987; Naoumov and Eddeleston 1994). Hepatocellular necrosis, if it involves a parenchymal area larger than a certain extent, leaves an interstitial septum. Accordingly, the pattern of distribution in the liver of HBcAg(+) hepatocytes has to be regarded as being essential in deciding the distribution of necrosis and, hence, that of septum formation.

The present analysis has disclosed an aspect of cirrhogenesis. HBcAg(+) hepatocytes are by no means randomly dispersed in the nodules, but aggregated so as to form focal areas. Such foci are located in the vicinity of septa, as demonstrated. This, in other words, appears to be showing that the foci are the site of septum formation. It may arise when neighboring foci of necrosis, collapsed by enlarging regenerating nodules, come to link one to another. Why HBcAg(+) areas remain even in cirrhosis where fibrous septa have already been precipitated may be understandable from the smoldering character of host's reaction in chronic hepatitis. HBcAg(+) areas, only partly destroyed at the bout of necrosis, are likely to remain as antigen-positive foci along the septa. However, HBcAg(+) areas, necroses and septa are all lesions distributed in the 3-D space. Accordingly, some 3-D structural analysis is necessary to examine whether the above model can really work.

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