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Progress report

Alpha-fetoprotein in primary liver cancer and other diseases

Alpha-fetoprotein (AFP) is an $alpha_1$ globulin normally present in high concentration in fetal serum but in only very small amounts thereafter. Although many of its physico-chemical properties have been determined[1], the role of AFP in ontogenesis is not yet known. In 1963 Abelev and his associates[2,3] noted the occurrence of a fetus-specific alpha globulin in the serum of adult mice with chemically induced hepatomas. When, shortly afterwards, Tatarinov[4,5] found AFP in the serum of patients with primary hepatocellular cancer, the exciting prospect appeared of an immunochemical marker which could be used in the diagnosis of this tumour. Its value in this regard has since been conclusively demonstrated and extensively documented in studies from many parts of the world[6-17]. However, relatively little is known about the dynamics of the protein during the course of the disease, and even less about the reason for the resumption of its synthesis by the tumour.

Early on it became apparent that AFP could also be demonstrated in the serum of some patients with malignant teratoblastomas of the testis and ovary[6,18], and later a minority of tumours of entodermal origin were found to share this property[8,19-29]. Moreover, with the introduction of more sensitive methods for its detection, raised levels of the globulin have also been shown in a variety of other liver diseases, so that its specificity for primary hepatocellular cancer is far from absolute.

AFP in Primary Hepatocellular Cancer

Using Ouchterlony double diffusion, AFP is demonstrable in the serum of between 28 and 87% of patients with primary hepatocellular cancer[6-17]. Its occurrence shows a striking geographical variation, the highest positivity rates being found in those areas where the tumour occurs most commonly, eg, 78% in South Africa[13], 72% in Senegal[8], 73% in Taiwan[17], and 87% in Indonesia[15], compared with 29% in Britain[11] and 28-50% in the United States[14,30], the latter being examples of countries in which primary hepatocellular cancer is rare. It is perhaps fortunate that the diagnostic usefulness of the test is least in those countries where primary hepatocellular cancer is rarely encountered.

The reason for the geographical variation in the proportion of AFP-positive primary hepatocellular cancer is not known. It may reflect differences in the aetiology of the tumour in endemic and non-endemic parts of the world, but may be explained, at least in part, by technical differences in carrying out the test, by the fact that AFP is more likely to be positive in younger and male patients with primary hepatocellular cancer[8,12,31-33], who predominate in those

areas where the condition is common, by lack of uniformity of diagnostic criteria, or by differing stages of presentation of the disease[34]. The different prevalences of the protein in primary hepatocellular cancer in Caucasians and Negroes in the United States of America[14,30] suggests that ethnic factors may also play a role in determining its occurrence. Experimental work has shown that the frequency with which AFP is found in animal hepatoma is probably not related to the chemical agent used to induce malignancy[1,35]. Persistence of the hepatitis-B antigen (HBAg) has recently been incriminated as a cause of primary hepatocellular cancer[36], and the possibility has been raised that the hepatitis virus may derepress the AFP genome as part of its oncogenic potential. The prevalence of the HBAg carrier state does vary in different parts of the world, but in three recent studies no correlation could be demonstrated between hepatitis-B antigenaemia and the presence of AFP in patients with primary hepatocellular cancer[17,37,38].

There is no obvious correlation between the serum AFP level in human primary hepatocellular cancer and any of the clinical or biochemical parameters of the disease, the size and stage of the tumour, or survival time after diagnosis[8,13,33]. Attempts to relate the degree of differentiation of the tumour to the presence of the globulin have produced conflicting results[6,8,11–13,17,39].

In striving both to decrease the number of 'false-negative' results, and to make earlier diagnosis of primary hepatocellular cancer possible, more sensitive methods for detecting the protein have been introduced, to the point where normal levels can now be detected. While the former objective has been realized there has inevitably been a lessening of the specificity of the test. Using radioimmunoassay, Ishii[40] has been able to increase the AFP-positivity rate in Chinese patients with primary hepatocellular cancer to 93% compared with 69% by the immunodiffusion method. With a somewhat less sensitive technique, McIntire *et al*[41] increased the number of positive results in Ugandan patients from 71 to 93%. Purves[42] has shown that the AFP concentration falls within the normal adult range in less than 1% of primary liver tumours in South African Negroes. However, in other series the use of the newer methods has not significantly improved the positivity rate[43]. Thus, even with the most sensitive techniques, it appears that in some patients primary hepatocellular cancer is not associated with AFP production. Synthesis of the globulin is therefore not essential for oncogenesis, or at least not for the maintenance of the neoplastic state.

Cumulative frequency distributions of AFP concentrations have been plotted in South African[44], West African[8], and Ugandan[41] Negroes with primary hepatocellular cancer. Quantitative values of the protein with sensitive techniques are spread over an extremely wide range (greater than six orders of magnitude), with the highest concentrations being about 7 mg/ml[8,33,41,44], the median figure 0·5-0·7 mg/ml[34,41,44] and the lowest levels only slightly above the normal adult concentrations of less than 30 ng/ml[34,45,46]. The diagnosis of primary hepatocellular cancer at low AFP concentrations is complicated by the fact that hepatitis and other hepatic diseases may cause slight or moderate elevations in the globulin level.

Several authorities in this field have recently stressed the need to go beyond simply reporting the serum AFP as being positive or negative, and to study the dynamics of the protein as a biochemical marker of disease activity in patients with primary hepatocellular cancer and germinal cell cancers of the

gonads. Unlike the situation in experimental animals, production of AFP by human primary hepatocellular cancer appears to be permanent[1]. In a particular patient the levels are usually remarkably stable, showing little fluctuation regardless of initial concentration[8,13]. With continued growth of the tumour and progression of the disease, the levels either remain the same or increase only slightly. This suggests that either the serum concentration is regulated in some way, or that the phase of rapidly increasing serum AFP levels occurs before the patient becomes symptomatic or the tumour clinically obvious. Occasionally, in a patient near death the serum concentration of the protein falls precipitously[8,44]. As might be expected, serum AFP levels fall rapidly after resection of the tumour, and with recurrence, the globulin reappears. Serial quantitation of AFP during this period has shown that the protein reaches the characteristic level for that particular tumour three to six months after first becoming detectable[41].

At present, it would seem reasonable to use the conventional, relatively insensitive methods for detecting AFP in the diagnosis of primary hepatocellular cancer, and the more sensitive techniques for following the course of the disease and assessing response to treatment.

The likely site of production of AFP in primary hepatocellular cancer is the tumour itself. The explanation for the resumption of synthesis by malignant hepatocytes is obscure. Two possible mechanisms have been considered. First, it has been suggested that with neoplastic transformation the hepatocytes dedifferentiate to a stage of development at which the AFP genome is not repressed, and production of the protein is resumed[47]. The second possibility is that the tumour arises from those cells which have the AFP genome in a non-repressed form[1,46]. With either view, the renewed production of the globulin reflects the presence of a population of hepatic parenchymal cells that is less mature than the hepatocytes which normally produce 'adult' proteins. Why some tumours synthesize and secrete AFP while others do not has not been established.

The introduction of highly sensitive methods for detecting and quantitating AFP has made meaningful epidemiological studies possible (although expensive and time consuming). Purves *et al*[46] have measured AFP concentrations in populations in areas which carry a high risk of primary hepatocellular cancer and have found raised levels in a higher proportion of apparently normal subjects than in other populations. Elevated levels diminished in individuals who left the high-risk areas, suggesting that they may reflect exposure to environmental carcinogens. There is some experimental support for this belief. By giving carcinogens, Kroes, Williams, and Weisburger[48] were able to induce AFP synthesis in rats before cancers were detectable histologically. With discontinuation of the chemical, the protein disappeared, possibly as a result of the immunological elimination of AFP-producing cells.

Alpha-fetoprotein has also been used as a screening test for early primary hepatocellular cancer in susceptible populations[34,42]. Unfortunately, these attempts have thus far proved unrewarding.

Other Liver Diseases

With the use of the more sensitive methods now available, raised levels of AFP have been detected in the serum of patients with various forms of liver

disease other than primary tumours. Liver cell damage and regeneration are common to all of these conditions. It is not yet known whether the AFP found in these conditions is identical with that present in foetal serum and patients with primary hepatocellular cancer. Although the exact source of the protein has not been determined, it is likely that synthesis takes place in hepatocytes.

The changes occurring in acute viral hepatitis have been most fully documented. Smith[49] reported that serum AFP levels increased only in patients with virus-B hepatitis, but other workers[50,51] have found the concentrations to be equally elevated in both forms of the disease. Maximum concentrations have usually been noted during the recovery phase of the illness[50,52], which is in keeping with the experimental observation that AFP is produced during the regeneration of hepatocytes[1]. Increased synthesis might then be due to retrodifferentiation of mature liver cells during regeneration to a stage at which AFP is again produced[1], or to proliferation or activation of those relatively undifferentiated hepatocytes that have the AFP genome in a non-repressed state[1,52]. In two recent studies[40,51] the AFP response has been related to the serum transaminase level, used as an index of hepatic damage. While in some patients the AFP concentration increased as the transaminase level returned to normal, suggesting synthesis during regeneration, in the majority of cases AFP and transaminase levels ran parallel courses, a pattern more in keeping with an acute-phase response to liver injury, or some change directly attributable to the presence of the virus. In most patients, mild or moderate elevations of the protein occur, but in some, particularly children, high serum concentrations are reached, and AFP can then be detected by conventional methods[51].

With the exception of Indian childhood cirrhosis, the changes in cirrhotic patients have been less marked. Using radioimmunoassay, Ruoslahti and Seppälä[52] have found moderately high levels of the protein in only two of 13 cirrhotic patients, and AFP has rarely, if ever, been detected by conventional methods in patients with cirrhosis or chronic active hepatitis. It is possible that with long-continued or recurrent regeneration of hepatocytes 'exhaustion' of AFP synthesis occurs. Alternatively, a minimal regeneration rate may be necessary for AFP to be 'induced'.

Using conventional techniques, Nayak and his associates[53] found AFP in the serum of 45% of patients with Indian childhood cirrhosis. They suggested that in infants who later develop this condition, the fetal hepatocyte fails to achieve the normal adult transformation, the defect being manifest in the continuing synthesis and secretion of the globulin. A similar explanation might account for the finding by radioimmunoassay of moderately raised levels of AFP in the serum of patients with ataxia telangiectasia[54]. Alpha-fetoprotein has also been demonstrated in the serum of patients with tyrosinosis[55]. The explanation is not known, but the presence of the protein is probably related in part to the severe liver damage which occurs in these children.

Alpha-fetoprotein has been found in the serum of infants with congenital biliary atresia and neonatal hepatitis[56,57,58]. The levels encountered in the latter condition are signficantly higher than in the former[58], and this may be of value in the differential diagnosis between the two diseases. Apart from the mechanisms already considered with respect to acute viral hepatitis, two possible causes for AFP synthesis in these conditions have been postulated.

One is that persistent deep jaundice may retard the differentiation of fetal and neonatal hepatocytes, leading to continued synthesis of the protein in large amounts. The other is that if viral infections are the cause of these diseases, the virus itself may be responsible for derepressing the AFP genome.

Tumours of Entodermal Origin

Alpha-fetoprotein has occasionally been demonstrated in the serum of patients with entodermal tumours[8,19–29]. With the use of the more sensitive methods, this association is encountered more frequently, eg, Abelev *et al*[59] found raised levels in 13% and Alpert[43] in 7% of such patients. The concentrations are, however, much lower than those seen in primary hepatocellular cancer. It was initially suggested that this phenomenon could be explained by increased synthesis of the protein in rapidly regenerating hepatic tissue adjacent to secondary deposits[20]. However, not all patients have had hepatic metastases. Moreover, tumour tissue of non-hepatic origin implanted into the liver of an experimental animal did not result in AFP synthesis[60].

With two exceptions, one with malignant lymphogranulomatosis[32] and the other with a Wilm's tumour[61], the primary tumours in all the patients reported have been either in the gastrointestinal tract or in organs derived from the primitive foregut. In the former group, the primary tumour has, with rare exceptions[25,26], been in the stomach. Of the foregut derivatives, pancreas[24,25], gallbladder[40], and lungs[29] have been reported as sites of primary lesions. The fetal gastrointestinal tract is known to synthesize AFP[62], which suggests that the globulin in these patients is secreted by the tumour itself, in much the same way as occurs in primary hepatocellular cancer. This belief is strengthened by a recent case in which AFP synthesis was demonstrated in both the primary gastric tumour and in the hepatic metastasis[22].

Germinal Cell Cancers of the Testis and Ovary

The presence of AFP in the serum of patients with undifferentiated teratocarcinomas of gonadal origin was first reported by Abelev and his associates[6] and Masopust *et al*[18] in the late 1960s. Subsequent studies using double diffusion have shown a positive correlation in up to 50% of patients[14,63–66], and a greater number of positive cases can be expected when more sensitive methods for detecting the protein are used[65,67]. As is the case in both primary hepatocellular cancer and acute hepatitis, AFP is more frequently encountered in children with these tumours than in adults[55]. Neither well differentiated embryonal cancers nor other types of testicular or ovarian tumours appear to share this property. Alpha-fetoprotein has occasionally been found in the serum of patients with primary mediastinal, retroperitoneal, or presacral teratocarcinomas[43,68,69].

Some correlation appears to exist between the presence of AFP in patients with these cancers and the degree of malignancy of the tumour, those synthesizing the globulin being more malignant and less likely to respond to treatment[63]. There seems also to be a relationship with the size of the tumour, and in some patients AFP could be demonstrated in the serum only after extensive metastases had occurred[65]. Serial quantitation of the protein level may be useful in following the course of the disease[70], as a

test of completeness of resection[63], and in assessing response to therapy[63,71], far more so than has been possible with primary hepatocellular cancer.

The likely site of AFP synthesis in these patients is the tumour itself. Teratocarcinomas are capable of forming dedifferentiated tissues including yolk sac and, less frequently, hepatocytes, and both have the potential to produce AFP[62]. Undifferentiated teratoma cells or yolk sac tissue seems the more probable origin[71,72] in view of the tissue culture experiments of Kahan and Levine[73], as well as the recent reports of AFP production by yolk sac carcinomas of the ovary[69,71,74].

AFP in Antenatal Diagnosis

With the introduction of more sensitive techniques for measuring AFP levels, a further diagnostic use for this protein has become apparent. The normal values of AFP in amniotic fluid[75,76] and in maternal serum[75,77] have been established, and it has been realized that increased levels may be associated with foetal distress, intrauterine death, and severe congenital anomalies of the neural tube[75,76,78,79]. In the latter instances, AFP presumably gains access to the amnion through cerebrospinal fluid leakage[76], while in the other circumstances, it has been suggested that the globulin is secreted into the amnion in fetal urine[75,80]. High maternal serum concentrations probably originate from the fetus. They may result from increased synthesis by the liver of the distressed fetus, placental leakage of fetal elements, or by way of the amniotic fluid[78].

The finding of abnormally high amniotic fluid AFP levels has made early diagnosis of serious neural tube defects and termination of pregnancy possible[81–83]. Examination of maternal serum levels, which has the advantage of ease and safety, has been less useful, because a clear separation between normal values and those found with an abnormal or distressed fetus have not always been found[84,85].

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