

REVIEW ARTICLE

Alpha-Fetoprotein: A Review

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Alpha-fetoprotein (AFP) is an oncofetal protein found in high concentrations in fetal and maternal blood and in patients with certain neoplastic and non-neoplastic disorders [1]. AFP was first identified in 1956, in 2 separate laboratories during electrophoretic experiments on plasma proteins of neonates, as a protein which migrated between albumin and α 1-globulin [2]. Clinical interest in AFP developed when it was discovered that transplantable hepatocellular carcinoma of the mouse synthesized and secreted AFP into the blood [3]. High serum levels of AFP were subsequently detected in patients with hepatoma, germ cell tumors and certain other neoplastic and non-neoplastic disorders [1,4,5]. In addition to mammals, birds and even several species of sharks have been found to synthesize a fetal-specific plasma alpha-globulin analogous to mammalian AFP [5]. The main site of AFP synthesis has shifted during evolution from fetal stomach in sharks to yolk sac in birds to liver in mammals, all derived from the same endodermal origin [5]. These ancient evolutionary ancestors of human AFP suggest a necessary function of AFP in developmental biology that still remains poorly defined.

Interest in AFP currently exists in areas such as developmental biology, immunoregulation, oncology and prenatal diagnosis of certain congenital anomalies. This brief review covers some of the current knowledge about AFP and is intended to kindle interest in the subject among dermatologists and other investigators in the field of cutaneous biology.

STRUCTURAL AND BIOCHEMICAL PROPERTIES OF AFP

AFP is a single-chain sialylated glycoprotein composed of approximately 580 amino acid residues and 3-4% carbohydrate [1]. The molecular weight of AFP has been estimated to be about 67,000 Daltons by SDS gel electrophoresis. AFP is a negatively charged protein with an isoelectric point of pH 4.57 to 5.08. These variations in charge, due only in part to sialic acid content, do not affect the antigenic properties of this protein. Several (at least 3) species of AFP have been isolated by extended agarose gel electrophoresis and crossed immunoelectrophoresis. There is a correlation between the relative amounts of the most negatively charged species and the inhibitory effect of AFP on *in vitro* transformation of lymphocytes [1]. Treatment with neuraminidase to remove completely the sialic acid residues does not alter the biologic potency. Thus differences in sialic acid content are only partly responsible for the microheterogeneity of AFP and variability of other charged regions is also present [6]. In addition to the monomeric forms, polymers including dimeric and trimeric forms have been identified

which dissociate into the monomer upon exposure to disulfide reducing agents, implying that their formation is dependent upon intermolecular sulfide bonds.

The chemical and antigenic structures of AFP's isolated from various mammals are closely related. In addition, no antigenic differences have been identified between AFP's isolated from tumor bearing adults and from fetal sources [1,4]. Immunization of an animal with its own AFP does not lead to antibody formation because of tolerance. Tolerance can be broken by altering AFP or by immunization with a cross-reacting AFP from another species. These antibodies can then eliminate the animal's normal serum AFP [4].

Another interesting and not yet fully explored property of AFP is the high affinity of murine AFP for certain estrogens by means of which AFP could influence cell growth. Human AFP has a much lower estrogen binding ability [7].

BIOSYNTHESIS AND SERUM LEVELS OF AFP

Synthesis of AFP by the human embryo can be detected as early as 29 days after conception [1]. As the yolk sac becomes atretic by 11 to 12 weeks of gestation, AFP synthesis occurs predominantly in fetal hepatocytes although a small amount may be produced by the fetal gut. AFP levels in fetal serum reach a maximum of 3,000 μ g per ml at about 14th week of gestation, exceeding in its concentration all other fetal plasma proteins including albumin. Serum concentrations decrease thereafter to 200 to 300 μ g per ml at age 32 weeks of gestation, to 20 to 120 μ g per ml at term and then drop sharply after birth. During the first 2 mo of life serum levels are about 400 ng per ml, fall to 30 ng per ml by 6 mo of age and to less than 15 ng per ml by 1 to 2 yr. Thereafter, serum AFP levels are maintained between 3 and 15 ng per ml due to synthesis of AFP which has a biological half life of about 6 days. During adult life serum levels can rise again in the event of occurrence of regenerative or neoplastic proliferation of hepatocytes and of a number of extrahepatic tumors [1].

The dynamics of AFP and albumin synthesis have been studied by a number of investigators [8,9]. The serum levels seem to correlate with the number of hepatocytes which produce this protein. In experiments with rat hepatoma lines, it has been shown that some clones produce high levels of AFP and low levels of albumin and others the reverse. Moreover albumin and AFP are produced during different phases of cell cycle. AFP is synthesized during G1 and S phase while albumin is produced from mid-S through G2 [4,10]. Immunofluorescence studies have shown that AFP and albumin are probably synthesized in different hepatic cells. The synthesis of AFP in the developing liver is regulated at the level of m-RNA. The level of AFP mRNA in adult liver is greatly reduced [11]. The synthesis of serum AFP was studied in 16 human embryos and fetuses between 4.2 and 18 weeks of gestation by incubation of selected tissues with ¹⁴C-labeled amino acids followed by im-

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Abbreviations:

AFP: alpha-fetoprotein

munoelectrophoresis of the culture fluids and radioautography. Relatively large amounts of radioactive AFP were found in each of the liver cultures and in cultures of the developing yolk sac. Smaller amounts of labeled AFP were observed in almost all of the gastrointestinal tract cultures. Labeled AFP was formed in the kidney cultures from 1 of 9 conceptuses and in only 1 of 14 placentas cultured. None of the cultures of lung, thymus, pancreas, skeletal muscle, amnion or chorion produced detectable amounts of AFP.

The immunological techniques for the quantitation of AFP vary greatly in their sensitivity. High levels of AFP can be measured by single radial immunodiffusion or rocket immunoelectrophoresis. Smaller amounts (5 to 500 ng per ml) are commonly quantitated by radioimmunoassay [1].

PHYSIOLOGICAL PROPERTIES OF AFP

The exact physiological function of AFP remains unknown [1,4]. Some consider it to be a form of fetal albumin. Speculation exists that AFP may be involved in immunoregulation during pregnancy. The supportive evidence is mostly based on *in vitro* experiments. Laboratory studies have demonstrated that murine AFP exerts an immunosuppressive effect on antibody synthesis [12]. AFP has been shown to induce the formation of highly efficient suppressor T cells with capacity to inhibit helper T cells, but with no effect on B cells responding to thymus independent antigens [1,4]. AFP has been shown to suppress the mitogenic response of human lymphocytes to phytomitogens, antihuman thymocyte antiserum and mixed lymphocyte culture. In order to achieve maximum inhibition AFP must be present at the time of mitogen addition [13]. Pre-exposure of lymphocytes to AFP followed by washing does not result in lymphocyte suppression. Increased concentrations of mitogen cannot overcome the inhibitory effects of AFP. This implies that AFP does not act by simple competition with the lymphocyte membrane for the mitogen combining site. Also the lymphocyte response to phytomitogens or anti-human thymocyte antiserum cannot be totally suppressed by increasing doses of AFP, suggesting the presence of a subpopulation of T lymphocytes [13]. Murine AFP has been reported to bind to a subpopulation of T lymphocytes [14]. We have not been able to detect similar high binding affinity of human AFP to human lymphocytes.

On the basis of these findings it has been suggested that AFP helps maintain the fetus as an allograft in a genetically incompatible environment. Administration of anti-AFP antisera to pregnant mice and rabbits has been abortogenic [15]. Although the mechanism of the induced abortion is unknown it is conceivable that neutralization of AFP at the maternal/fetal interface might initiate rejection of the fetus through a cell-mediated immune reaction [15].

CLINICAL SIGNIFICANCE OF AFP

Liver Disorders

Approximately 10 to 20% of patients with nonmalignant liver diseases including infectious hepatitis and cirrhosis have elevated serum AFP levels ranging from 25 to 500 ng per ml. In serial determinations these levels tend to be fluctuant and transient. On the other hand steady or rising serum AFP levels particularly exceeding 500 ng per ml are indicative of primary liver cell cancer. In screening a group of cirrhotics, who have a higher than normal risk for developing hepatoma, 22% had elevated serum AFP levels. Of these, about 10% were ultimately diagnosed as having hepatoma [1,3-5].

The incidence of elevated serum AFP levels in hepatoma ranges from 50 to 90% depending on the geographical location. Certain pathologic features are correlated with the appearance of AFP in liver cancer. Size of the tumor is related to the number of samples positive for AFP but not to the level of AFP. AFP is synthesized more frequently and in higher amounts by faster growing tumors and more undifferentiated

cancer cell lines. Also younger patients with hepatoma are more frequently AFP-positive than elderly ones. Only a small proportion of tumor cells make AFP. Less than 5 to 20% of cells have been found by immunofluorescence to be positive for AFP. Exposure to even small amounts of hepatocarcinogenic substances such as 3'-methyl-4-dimethylaminoazobenzene and N-2-fluorenylacetylacetamide can cause a rapid and significant elevation of serum AFP levels in rats [16]. In patients with hepatoma, if there is no therapeutic intervention, the serum AFP usually increases gradually as the tumor progresses. Complete surgical resection of the tumor produces an immediate exponential fall that parallels the catabolic decay rate for AFP. Recurrence of elevated AFP levels will almost certainly mean tumor recurrence [1].

Other Tumors and Diseases

Elevated serum AFP levels have been associated with germ cell tumors containing elements of yolk sac or endodermal sinus components [1]. In mixed germ cell tumors and in pure tumors of extraembryonic origin derived from yolk sac AFP elevations are present. Pure seminomas of the testis or dysgerminomas of the ovary are not associated with serum AFP elevations. Elevated serum AFP levels have been reported in association with various carcinomas of the gastrointestinal tract particularly gastric and pancreatic cancers, in retinoblastoma and in some nonmalignant conditions such as cystic fibrosis, Turner's syndrome, hereditary tyrosinemia and ataxia telangiectasia [1,4,17].

Skin Tumors

Elevated serum AFP levels have been reported in a few cases of malignant melanoma and in a patient with basal cell carcinoma [17,18]. AFP was also detected in tissue of a case of Bowen's disease by immunofluorescence techniques [19]. A systematic study of serum and tissue samples in large numbers of patients with various cutaneous disorders would be needed to assess the significance of AFP in dermatology.

AFP in Pregnancy

Elevation of AFP levels in maternal blood and in amniotic fluid has been found helpful in the detection of multiple pregnancy, congenital nephrosis, intrauterine fetal death, hydrocephalus, hemolytic disease secondary to Rh isoimmunization, omphalocele, duodenal or esophageal atresia, threatened or missed abortion, placental separation and open neural tube defects such as anencephaly, meningocele and myelocele [1,4,5].

SUMMARY

Detection and measurement of serum AFP levels have been found useful in the diagnosis, prognosis and follow up of patients with hepatoma and germ cell tumors [20]. Monitoring maternal and amniotic fluid AFP concentration helps detect a variety of fetal disorders. The *in vitro* evidence for immunosuppressive effects of AFP suggests a possible role of AFP in immunoregulation.

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Announcement

Pacific Dermatologic Association Essay Contest

The 31st Annual Meeting of the Pacific Dermatologic Association will be held in San Francisco, California, September 16-20, 1979. Each year the Pacific Dermatologic Association encourages young dermatologists to submit essays on original work. The Nelson Paul Anderson Memorial Essay Contest is open to all physicians in graduate dermatologic training or those who are not more than 5 years out of residency training. Essays may be submitted by residents of the geographical area of the Pacific Dermatologic Association: The Western United States (California, Oregon, Alaska, Nevada, Washington, Idaho, Utah, Arizona, Hawaii, Montana, Wyoming, Colorado, New Mexico), British Columbia and Alberta, Canada, Mexico, Australia, New Zealand, Japan, and the Philippines. The winning essayist will receive a cash prize of \$500 plus expenses to the next Annual Meeting. His/her sponsoring department will receive \$250 for educational materials.

Essays will be judged on the following considerations: A. Originality; B. Potential importance of work; C. Evaluation of results; D. Experimental methods and use of control; and E. Clarity of presentation. Six copies of the essay should be submitted under a non-de-plume, with no information in the paper which will lead to recognition by the judges of the institution or clinic where the work was done. The essay with non-de-plume should be accompanied by a plain sealed envelope enclosing the name and address and non-de-plume of the author. Entries must be received by the Secretary-Treasurer, Gerald A. Gellin, M.D., 3838 California St., San Francisco, California 94118, no later than July 15, 1979.