

Alpha-fetoprotein is an autoantigen in hepatocellular carcinoma and juvenile Batten disease

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1. ABSTRACT

Failure of immune tolerance leads to production of autoantibodies to self-antigens. The repertoire of autoantibodies detected in cancer patients can indicate the presence of autoimmune disease. Alpha-fetoprotein (AFP) autoantibodies have been found in patients with hepatocellular carcinoma (HCC) and in juvenile Batten disease (BD), a neurodegenerative condition involving autoimmunity. Variant conformational forms of AFP together with exposed occult antigenic determinant sites on the AFP polypeptide resemble the features of a disordered protein which can impair central immune tolerance. These aberrant structural protein forms can lead to the persistence of autoantibody production by immune sensitized B-lymphocytes. Thus, it is not surprising that AFP, a self-antigen, can induce autoimmune responses in humans. Herein, we discuss the molecular and antigenic properties of AFP which make it a disordered protein, and its ability to induce autoantibody production to AFP cryptic epitopes in both HCC and BD patients. Such insights might aid in the future design of AFP-based vaccines and to discovery of novel pathogenic mechanisms of autoimmune

diseases which demonstrate the presence of denatured intermediate forms of AFP.

2. INTRODUCTION

Antigens are foreign substances targeted and bound either by antibodies in the circulation (humoral type) or by T-cell lymphocyte cell surface receptors (cell-mediated type) (1). In comparison, an autoantigen is a self antigen within a host organism that evokes an immune response (either humoral, cell-mediated, or both) against itself (2). Thus, an autoantigen, despite being an apparent internal “normal” component of the organism, could become a target of the humoral and/or cell-mediated immune response. In like fashion, an autoimmune disease (autoimmunity) is a disorder of an organism that has manifested an immune response against its own molecular components of cells and/or tissues (3).

3. AUTOANTIGENS AND AUTOIMMUNITY

The production of autoantibodies to self antigens is dependent on the failure of immune

tolerance (4-8). Autoantibodies to self antigens are found both in sera from patients with systemic autoimmune diseases and with cancer (7-8). The repertoire of autoantibodies detected in cancer patients to some extent encompasses that of patients with autoimmune diseases (7-8). Among these autoantibodies, AFP autoantibodies have been found in patients with hepatocellular carcinomas and juvenile Batten Disease (BD) a neurodegenerative condition in which autoimmunity is established as an involved component (9-10). Human autoimmune diseases comprise a large number of illnesses that include: Addison's disease, Hashimoto's thyroiditis, systemic lupus erythematosus, and many others. However, protein misfolding diseases are non-immune in origin (Table-1). In comparison, Tregs dysfunction has been observed in patients with paraneoplastic syndromes and/or with autoimmune diseases (11-13).

Antigens are molecular entities that are comprised of multiple subcomponents called antigenic determinants (ADs) sites or epitopes (14). An AD can constitute at least one or more specific part of an antigen that is recognized by the immune system as a foreign substance by antibodies, B-cells, and/or T-cell lymphocytes. Antibodies secreted by B-cell lymphocytes can bind to a specific epitope on the overall antigen, such as a protein. A "linear" antigenic epitope on a protein consists of a continuous amino acid (AA) sequence stretch on a polypeptide chain (15). Whereas, a "conformational" induced epitope consists of discontinuous segments of AA sequence stretches on a polypeptide chain. An antigenic determinant on a protein can interact with antigen-presenting cells bound to a Major Histocompatibility Complex (MHC); these can contain 8-11 AAs (MHC class I) or 13-17 AAs (MHC class II). A major antigenic site on a protein can consist of a molecular weight up to 10,000 Daltons each; thus, a full length protein of 60,000 Daltons, such as Albumin, would consist of a total of 6 major antigens (16).

4. DISORDERED SELF-PROTEINS AND AUTOIMMUNITY

Self-antigens' structural and immunological properties are driving forces for the selection of the autoantibody repertoire (17-18).

Long stretches or clusters of charged residues, and multivalent charges on autoantigens occur in several self-antigens targeted by humoral immune responses. According to the Carl and *et al* model, the structurally disordered proteins are poorly immunogenic because: (a) they might be hidden to the immune recognition due to their binding to other proteins; (b) their flexibility in conformational form might make it difficult for development of conformation-specific-antibodies; (c) their proteolytic instability might interfere with a strong binding to MHC II and thus hamper the induction of a T cell response amidst immune tolerance. Thus, autoreactive immune cells that bind with low affinity to disordered proteins and to their MHC-complexed peptides loaded on antigen presenting cells, respectively, are not removed during central tolerance development mechanisms and can remain as immunological constituents of the immune repertoire of healthy individuals (18).

In contrast to exposed epitopes, cryptic epitopes are concealed in molecular clefts or crevices of a tertiary-folded protein (19). Cryptic epitopes can be revealed by laboratory procedures, or in nature, by a denaturing and subsequent unfolding of the protein. Cryptic epitopes are capable of triggering inappropriate immune cell signaling events which can lead to inappropriate production of autoantibodies against self-antigens, most commonly proteins. Examples of human autoimmune diseases caused by antibodies against self-antigens include: 1) antibodies against 17-alpha-hydroxylase in Addison's disease; 2) antibodies directed against thyroglobulin in thyroiditis (Hashimoto's disease); 3) anti-wheat proteins (gliadin, gluten) in Celiac disease and many others (see list in Table-1, A, B) (14). In addition, there exists many non-immune associated unfolding, improper assembly, and misfolding of proteins in human diseases such as Cystic fibrosis, and Marfan's Disease (see list displayed in Table 1, C).

5. IS AFP A DISORDERED PROTEIN?

The human Alpha-fetoprotein is made by a single polypeptide chain with a molecular mass of 69 kD containing 3-5% carbohydrate content; it

Table 1. Selected human autoimmune diseases and their autoantigenic inducing substances and comparison with various nonimmune misfolded protein disorders

Autoimmune Disease	Autoantigenic Substance and/or Tissues/Cells
Celiac Disease	Tissue Transglutaminase, Gliadin, Gluten
Diabetes mellitus type-1	Pancreatic B-cell surface proteins
Sarcoidosis	Granulomatous tissues/cells
Eosinophilic granulomatosis (polyangiitis)	Small and medium-sized blood vessels; vasculitis, eosinophil influx
Hashimoto's Thyroiditis	Thyroglobulin protein
Grave's Disease	Toxic diffuse Goiter; thyroid stimulating immunoglobulin
Idiopathic thrombocytopenic purpura	Platelet cell surface proteins
Addison's Disease	17-alpha-hydroxylase enzyme
Systemic Lupus Erythematosus	Nuclear proteins; Ribonuclear proteins
Sjogren's syndrome	Salivary gland proteins
Rheumatoid arthritis	IgG-Fc Fragment
Ankylosing spondylitis	Joints of the spine (pelvis area, and shoulders)
Polymyositis	Skeletal muscle cells
Multiple Sclerosis	Myelin sheath coating on nerve cells
Primary sclerosis cholangitis	Cells of liver and gall bladder (scarring effect), bile ducts
Crohn's Disease	Inflammatory Bowel disease, Intestinal cells
Non-Immune Protein Misfolding Disorders/diseases (Misfolded Protein)¹	
Cystic Fibrosis (transmembrane conductance receptor TCR)	
Tay-Sachs disease (Hexosaminidase)	
Sickle cell disease (Hemoglobin)	
Marfan Syndrome (Fibrillin Protein)	
Alzheimer's Disease (Beta-Amyloid, tau)	
Lou Gehrig's Disease (superoxide-dismutase)	
Eye Cataracts (Crystallin Proteins)	
Protein-C deficiency (Protein-C)	
Von Willebrand Disease (Von Willebrand Factor)	
Creutzfeldt-Jacob Disease (Prion Proteins)	
Retinitis Pigmentosa (Rhodopsin Protein)	
Familial amyloidosis (transthyretin)	
Alpha-antitrypsin deficiency (alpha-antitrypsin)	
Familial hypercholesterolemia (LDL Receptor)	
Glanzmann's thrombasthenia (GPIIb Integrin)	
Scurvy disease (Collagen proteins)	
Note: Misfolded proteins do not induce an immune response, ¹ Misfolded protein is shown in parenthesis, LDL: low density lipoprotein	

of nearly 200 amino acids each (20). The triplicate domain structure of AFP is due to the intramolecular loops dictated by 32 disulfide bridges, resulting in a V- or U-shaped configuration (21, 22). Accordingly, AFP has been classified as a member of a three domain, cysteine-rich translated protein of the albuminoid gene family which currently consists of five members; albumin, vitamin D binding (Gc) protein, alpha-albumin, and the AFP-related gene (ARG) protein (23-25). Serum AFP binds and transports a variety of ligands such as bilirubin, fatty acids, retinoids, steroids, heavy metals, dyes, flavonoids, phytoestrogens, dioxin, and various drugs (26-27). Other ligands for AFP (rodent and human) are biological stains, L-tryptophan, warfarin, triazine dyes, phenylbutazone, streptomycin, phenytoin, anilinnaphthalene sulfate, heavy metals, low carbon chain alcohols, and polyunsaturated fatty acids (28). AFP has been demonstrated to induce immunosuppression in both B- and T-cells lectin stimulation, although AFP can also enhance immune cell activation under particular circumstances (29, 30). AFP has been reported to functionally impair dendritic cells thus leading to immune dysfunction and apoptosis of antigen processing cells (APCs) (30). In addition, molecular variants of AFP have been described. A cationic form of AFP has been demonstrated to complex with immunoglobulin M. Aberrant forms of AFP have been found in the reproductive and urinary tracts as well as in the sera of patients with breast cancer, reproductive organ and genital tract diseases (25-26, 31-33). Truncated forms of AFP have been detected in cell cultures comprising hepatomas, testicular embryonal carcinomas, and breast tumors (26, 31, 32). The denaturation of recombinant-derived AFP was found to be a reversible process independent of its starting source, fatty acid relationship, and glycosylated state (34-35). Accordingly, there are molecular variants of AFP that can be divided into multiple different classes: genetic variants, soluble-free or bound forms, pH Isoforms, carbohydrate heterogenic forms, enzymatic fragments, denatured intermediates. In accordance with the AFP structure and immunological properties, some forms of AFP appear to resemble the features of a disordered protein; thus, it is not surprising that AFP can induce immune response in humans.

6. SPONTANEOUS IMMUNE RESPONSE TO AFP IN HEPATOCELLULAR CANCER (HCC) PATIENTS

Autoantibodies and T cell immune responses to AFP have been reported to occur in patients with hepatocellular carcinoma or with liver diseases (36-53) (Table 2). The low immunogenicity of disordered self-antigens appears to be inconsistent with their capability to induce spontaneous immune response. However, intramolecular and intermolecular epitope spreading mechanisms may contribute to the development of immune responses targeting AFP (18, 54-56). In addition, the abnormal expression of AFP resulting from overexpression, conformational changes or post-translational modifications could have overwhelmed self-tolerance and induced autoreactive immune responses (57-59). The inflammatory status and the enhancement of lysosomal cathepsin B and L blood levels in liver diseases patients might have exposed hidden epitopes, thus inducing ex novo immune responses to conformationally modified forms of AFP (7-8). Indeed, to determine hepatocellular patients immunoglobulin reactivity with linear and/or conformational AFP epitopes, Bei *et al.* performed western blotting and immunoprecipitation analyses using denatured and nondenatured AFP (36). In order to determine whether the anti-denatured-AFP antibodies recognized protein or carbohydrate epitopes, AFP was deglycosylated with PNGase F to remove N-linked carbohydrate moieties. The authors analyzed patients with various liver disorders that demonstrated the breakdown of immune tolerance to AFP self-antigen. The analysis included 60 hepatocellular carcinoma patients; 15 liver cirrhotic patients; 15 chronic hepatitis patients; and 40 normal non-liver disease patients. Results indicated that anti-AFP antibodies were found in 14 of 60 (23%) hepatocellular carcinoma patients; 3 of 15 (20%) cirrhotic patients (CP); 1 of 15 (6.6%) chronic hepatitis patients (CHP); and 0 of 40 (0%) normal non-liver disease patients. The patient antibodies recognized cryptic epitopes in the denatured and deglycosylated forms of AFP. In patients with pathological conditions of liver disease and

Table 2. Evidence of immune responses to AFP in hepatocellular carcinoma (HCC) patients

Immune response	Target and stimulators	Positive Individuals ¹	Reference
Autoantibodies	Native AFP	14/60 HCC, 3/15 LC, 1/15 CH, 0/40 HD	(36)
Autoantibodies-IgM (IC) ²	Native AFP	30/50 HCC	(38)
Autoantibodies-IgM (IC)	Native AFP	48/74 HCC	(39)
Autoantibodies-IgM (IC)	Native AFP	77/118 HCC, 14/60 LC, 9/63 CH	(40)
Autoantibodies-IgM (IC)	Native AFP	28 HCC (Kazakh population) and 85 HCC (Han population)	(41)
T cells (CD8 ⁺)	AFP ₅₄₂₋₅₅₀ (immunodominant peptide)	HD, HLA-A2.1	(42)
T cells (CD8 ⁺)	AFP ₁₃₇₋₁₄₅ , AFP ₁₅₈₋₁₆₆ , AFP ₃₂₅₋₃₃₄ (immunodominant peptides)	HD, HLA-A*0201	(43)
T cells (CD8 ⁺)	AFP ₁₃₇₋₁₄₅ , AFP ₁₅₈₋₁₆₆ , AFP ₃₂₅₋₃₃₄ (immunodominant peptides) AFP ₁ , AFP ₄₉₂ , AFP ₅₄₇ , AFP ₅₅₅ (sub dominant peptides)	HD, HLA-A*0201 HCC, HLA-A*0201	(44)
T cells (CD8 ⁺)	AFP ₃₅₇ , AFP ₄₀₃ , AFP ₄₁₄ , AFP ₄₂₄ , AFP ₄₃₄	2-8/38 HCC, 0/11 HD, HLA-A*2402	(45)
T cells (CD8 ⁺)	AFP ₁₃₇ , AFP ₁₅₈ , AFP ₃₂₅ (immunodominant peptides)	1/18 HCC for AFP ₁₅₈ and AFP ₃₂₅ , 3/13 LC for AFP ₁₅₈	(46)
T cells (CD8 ⁺)	Overlapping peptides spanning the complete AFP sequence	19/40 HCC, 5/14 CH, 4/10 HD	(47)
T cells (CD4 ⁺)	AFP ₃₆₄₋₃₇₃	11/41 HCC	(48)
T cells (CD4 ⁺)	Panel of 60 AFP-derived peptides (10 pools of 6 peptides) and AFP ₃₆₄₋₃₇₃	15/20 HCC	(49)
T cells (CD4 ⁺)	AFP ₄₆₋₅₅	15/15 HCC, 10/10 HD (higher frequency in HCC than HD)	(50)
T cells (CD4 ⁺)	Soluble AFP Protein-fed DC Adenovirus AFP-engineered DC	0/6 HCC, 8/26 HD 3/6 HCC, 15/26 HD	(51)
T cells (CD4 ⁺) T cells (CD8 ⁺)	AFP derived-peptide pools	8/18 HCC, Child-Pugh A, 2 /13 HCC, Child-Pugh B or C, 3/18 HCC, Child-Pugh A, 6 /13 HCC, Child-Pugh B or C	(52)
T cells (CD8 ⁺)	AFP ₁₅₈₋₁₆₆	HD, HLA-A*0201	(53)

¹HCC = hepatocellular carcinoma, ²IC=immune complexes. LC = liver cirrhosis and CH = chronic hepatitis patients, HD = healthy individuals. *allelic group. When available, the number of positive subjects/total subjects is reported.

cancer, immune tolerance to AFP had been broken and/or circumvented. HCC patients were found to have higher AFP serum levels than CP or CHP (36). Thus, spontaneous immune responses to self-AFP antigens were significantly associated with hepatocellular carcinomas.

The presence of AFP bound to IgM (AFP-IgM) as an immune complex (IC) has also been analyzed. In 1976, Norgaard-Petersen provided electrophoretic evidence that circulating human AFP was complexed to an immunoglobulin thought to be IgM; this was suggestive of an autoimmune

response to AFP (37). Subsequent reports in 2004 and thereafter confirmed that IgM complexed to circulating AFP was present in the sera of liver disease patients detected using ELISA methodology (38). In this latter study, the mean serum concentration of AFP-IgM IC was significantly higher in HCC patients than in cirrhotic patients and in patients with chronic hepatitis. HCC patients had AFP-IgM IC values above the cutoff in 60% of cases (30/50) (38). Jingting *et al.* found a significant difference between AFP-IgM serum levels of primary HCC patients and healthy subjects. They found that AFP-IgM was better than free AFP in the diagnosis of early primary HCC, although there was false positive reaction of AFP-IgM in patients suffering from cirrhosis and chronic hepatitis (39). Sheng *et al.* developed a novel time-resolved immunofluorometric assay. AFP-IgM and AFP were increased above the cutoffs in 65.25 and 45.76% of HCC, respectively (40). Wu *et al.* reported that serum AFP-IgM levels of hepatocellular carcinoma patients in the Han population and HCC patients in the Kazakh population were statistically higher than those of controls (41).

Numerous HLA I-restricted, CD8⁺ AFP epitopes have been identified and evaluated for their immunogenicity in HCC patients. Several AFP peptides were classified as “immunodominant” due to their robust binding to MHC I and *in vitro* high level IFN- γ production and cytotoxic T lymphocyte (CTL) induction from stimulated healthy individual T cells. It was reported that T-cell repertoire could recognize AFP in the context of HLA-A2.1. Dendritic cells (DCs) transduced with an adenovirus (AdV) producing AFP recognized the AFP_{542–550} (GVALQTMKQ) peptide (42). Three additional HLAA*0201-restricted immunodominant epitopes [i.e AFP_{137–145} (PLFQVPEPV), AFP_{158–166} (FMNKFYIEI) and AFP_{325–334} (GLSPNLNRFL)] and 10 hypothetically subdominant epitopes were then identified (43). It was reported that healthy individuals exhibited high-avidity T cell precursors with AFP₁ (MKWVESIFL), AFP₄₉₂ (PVNPGVGQC), AFP₅₄₇ (TMKQEFNLNL), AFP₅₅₅ (NLVKQKPQI) subdominant epitopes (44). Subdominant -specific

T cells recognized AFP-expressing tumor cells (44). Mizukoshi *et al.* reported that AFP₄₀₃ (KYIQESQAL), AFP₄₂₄ (EYYLQNAFL), AFP₄₃₄ (AYTKKAPQL), AFP₃₅₇ (EYSRRHPQL) and AFP₄₁₄ (RSCGLFQKL) peptides were able to stimulate CTLs to release IFN- γ and destroy AFP-producing hepatoma cells in culture (45).

A quantitative analysis was performed on the occurrence of T cells recognizing 3 MHC class I immunodominant AFP epitopes (i.e. AFP₁₃₇, AFP₁₅₈ and AFP₃₂₅) in the naive repertoire of peripheral blood mononuclear cells (PBMCs) from 37 cirrhotic and 54 HCC patients (46). The frequency of the naive T cells recognizing these peptides was minimal (46). Thimme R *et al.* identified 46 epitope regions targeted by AFP-specific CD8⁺ T cells in the background of diverse HLA types (47). Alisa *et al.* reported that the AFP_{364–373} (QLAVSVILRV) epitope was recognized by peptide specific CD4⁺ T cells from HCC patients in the context of a HLA-DR site (48). Specific CD4⁺ T cells to AFP_{137–145} (PLFQVPEPV) and AFP_{249–258} (KVNFTIEIQL) as well as to AFP_{364–373} epitope were detected in HCC patients as opposed to patients with chronic liver diseases or healthy donors (49). On the other hand a subset of CD4⁺ T cells recognizing the AFP_{46–55} epitope and releasing TGF- β with inhibitory effects on T cell proliferation *in vitro* were identified; their frequency was significantly higher in HCC patients than in healthy donors (50). It was then reported that the modality of antigen presentation had an important outcome on the detection of AFP T cell responses (51). Behboudi *et al.* detected an anti-AFP CD8⁺ T cell response in controls versus HCC patients, wherein an anti-AFP CD4⁺ T cell response was restricted only to HCC patients (52). Sun *et al.* reported efficient activation and expansion of AFP_{158–166}-specific CTLs by APCs (53). Accordingly, the human T cell repertoire is efficient for identifying AFP in the context of MHC Class-I immune responses, even in an environment of low to moderate circulating AFP levels, in both HCC and cirrhotic patients (60). Immune response to AFP occurring in HCC patients might induce biological and pathogenic effects that can modulate tumor growth and survival. Anti-AFP autoantibodies administered to cancer patients

could possibly interfere with the growth of AFP expressing tumor cells. Furthermore, Wang *et al.* reported that the enhanced proliferation of cancer cells by AFP was inhibited by the treatment with anti-AFP antibodies (61).

However, the sensitization of T cells against AFP might induce hepatic cell damage. Geissler M *et al.*, after immunizing mice with DNA encoding mouse AFP, described a significant hepatocyte disruption in regenerating liver that correlated with the number of AFP-specific CD8(+) T cells, the activation of liver regeneration, and levels of AFP synthesis. Autoimmune liver damage was mediated by CD4(+) T cell-dependent CD8(+) cytotoxic T lymphocytes (62).

7. BATTEN DISEASE AND AFP AUTOANTIBODIES

Batten Disease (BD) is one of a cluster of names for a family of human diseases known as “neuronal ceroid lipofuscinoses” (NCL) (63). BD is a neurodegenerative disorder resulting from excessive accumulation and storage of lipopigments (lipofuscin) in body cells including nerve cells. The lipopigments are composed of fat and protein complexes. Ceroids themselves are wax-like golden yellow-brown fatty pigments abundantly found in fibrotic tissue such as liver cirrhosis. Such conditions lead to accumulations of lipopigments in cytoplasmic lysosomes, which in nervous tissue results in neuroinflammation, neurodegeneration, and subsequent nerve cell death (64).

The lipofuscin associated BD, first described in 1903, is a lethal disorder of the nervous system beginning largely in childhood, and occurring in 5 to 10 years old (child) patients (65). Its population incidence is 1 in 250,000 people. NCL is basically a lysosomal storage disease linked to a mutation in the human CLN3 gene on chromosome-16. The onset of BD, although most common in childhood, can occur at four life stages, namely; infantile, late infantile, juvenile, and more rarely, in adults.

The major cytoplasmic component of the

NCL disease is reported to affect the “C” subunit of the mitochondrial enzyme ATPase, present in both man and mice (66). Mutated genes in BD have further been localized in lysosomes of neurons at the junctional synapses of both cerebral and cerebellar cortex cells. Associated reports of altered arginine metabolism in BD have also been described in conjunction with the neuronal lysosome alterations (67). Many patients display defects in their GABAergic interneuron pathways. Finally, subtle changes in the urea cycle and the citrulline-nitric oxide cycle pathway often accompany the above cell biochemical alterations (68). The latter pathways are capable of influencing both mitochondrial oxidation and energy generation in various cells of BD patients.

The early signs of onset of BD in infancy involves largely vision problems, seizures, and personality changes (69). Such changes include slow learning, repetitive speech, clumsiness, stumbling, and slowed head-size growth. These characteristics are accompanied by poor blood circulation in the appendages, decreased body fat, and reduced muscle mass. BD progression can further induce curvature spine changes, holding one's breath, teeth grinding, hyperventilation, constipation, worsening seizures, and reduction in speech and mental skills (70).

Although the precise cause of BD onset is still unknown, mutation of the CLN3 gene is intimately involved (71, 72). The mutation causes production of two truncated variants of the CN3 protein. The normal, non-mutated protein contains a total of 438 amino acids, while the mutated truncated CLN3 protein displays 181 amino acids (73, 74). The non-mutated CLN3 gene encodes for a hydrophobic transmembrane protein normally present on the cytoplasmic lysosome membrane. The CLN3 protein has further been localized in membranes of the endoplasmic reticulum and the Golgi apparatus. While vision loss is the most common diagnostic sign, the exact function of the CLN3 protein has yet to be ascertained (75).

A null mutant mouse model of BD has been developed with the pathologic characteristics mimicking the human BD phenotype (76-77).

Similar to man, the null CLN3 mice have alterations in their GABAergic interneuron cell pathways and display circulating autoantibodies against AFP and glutamic acid decarboxylate (GAD65) among other proteins (78). The injection of serum from BD patients into the mouse models is capable of inhibiting the catalytic activity of GAD65 that converts glutamic acid to the neurotransmitter GABA (78, 79). However, autoantibody interference to nerve degeneration and cell death has yet to be explained as is the precise function of the CLN3 transmembrane protein in both man and rodents (80).

The most common onset of BD occurs shortly after birth in infants (81). Unfortunately, no therapeutic strategy intervention procedures are yet available (63). As mentioned above, visual failures accompanied by seizures occur first; followed by progressive declines in cognitive motor skills and performances, eventually leading to premature death of the child (69). The cause of death is largely reported as deficiencies in soluble lysosomal enzymes. However, behind the scenes, multiple mutation genes and their resultant foreign proteins are generated which can induce autoantibodies against several brain protein antigens (see below) (82). Such autoantibodies are deposited in the cerebral spinal fluids of the central nervous system, causing inflammation leading to a breach in the blood brain barrier. This allows a vast insurgence of normally-restricted compounds into the brain (83).

Using 2-dimensional gel electrophoresis, immuno-blotting, and mass spectroscopy, investigators have detected multiple brain occult antigens using BD patient serum-containing autoantibodies. These investigators have detected autoantibodies that recognized denatured epitopes of AFP (10). Of the 13 male Batten disease patients analyzed, 12 (92%) tested positive for the presence of anti-AFP antibodies, whereas 8 out of 18 (44%) of females were positive for the presence of anti-AFP antibodies. Overall, of 31 Batten disease patients tested, 19 (61%) were positive for the presence of autoantibodies against AFP (10). During human fetal development, AFP has been reported to localize in the cytoplasm of

neurons; however, it has been shown that AFP is not synthesized and/or produced in nerve and glial-associated brain cells (84, 85). The data from BD patients demonstrated that immune tolerance to human AFP had been circumvented in the neuronal degenerative disorder in both human BD and its mouse models. Following mass spectroscopy analysis in samples of serum from BD patients, 61% of patient further involved at least 4 additional human non-AFP proteins including; 1) dynactin-2; 2) Hsp 70 (heat shock protein-70); 3) GRP58; and 4) peptidase-D (see Table-2). Furthermore, the serum reactivity for each AFP-antibody positive patient was completely inhibited by inclusion of denatured AFP in the mixed test solution. Thus, adding purified denatured AFP to the reactant samples was effective in neutralizing the serum reaction mixture against human AFP (10). In the mutant mouse model, total brain and liver homogenates (extracts) displayed higher levels of circulating serum AFP in age-matched mutant mice compared to wild-type mice suggestive of increased AFP presence (86).

8. CONCLUSION

AFP has been reported to be a significant contributor to normal human brain development (87, 88). This oncofetal protein has been detected in the cytoplasm of developing brain neurons being abundant in nerve cells specific to both symmetrical halves of the brain. However, AFP was not found in limbic, preoptic, and hypothalamic brain regions; in addition, no coding of mRNA was present, and no syntheses of AFP occurred in the brain cells (89, 90). The fetal protein detected in brain cells was engulfed and internalized into the cytoplasm by non-energy procedures of macropinocytotic and endocytotic pathways which transport and deliver essential nutrients (lipids, estrogens) to the developing brain. Moreover, AFP has been found crucial for normal feminization of the female brain and defeminization of the male brain in rodents. In previous reports, it has been confirmed that AFP plays a significant role being involved in regulating the hypothalamic-pituitary reproductive axis (89). In studies of knock-out AFP models in mice, AFP has been demonstrated to not be required for full-term pregnancies; however,

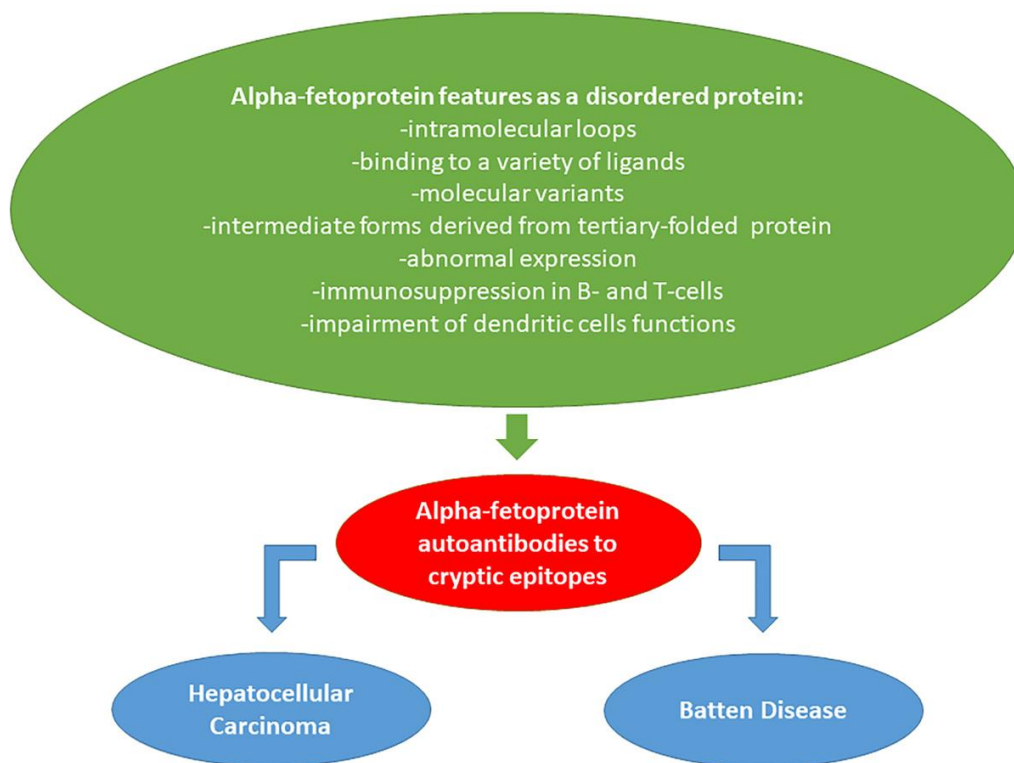


Figure 1. AFP intermediate forms and immune response. Given that denatured intermediate forms derived from tertiary-folded AFP resemble those of a disordered protein, AFP might induce immune responses in various human diseases including hepatocellular carcinoma and Batten disease.

second generation female mice were found to be infertile. It is germane to this discussion that hyperandrogenization has also been reported in human female BD patients (10).

In summation, elevated AFP levels are known to be associated with prenatal nervous system pathologies involving birth defects such as neural tube defects and anencephaly. On the other hand, AFP represents one of the most useful markers for hepatocarcinomas and teratocarcinomas and for the monitoring of the patient response to therapy (27). In addition, postnatal and juvenile neurodegenerative disorders such as ataxia telangiectasia and autosomal recessive ataxia-ocular apraxia (brain/eye) disorders have been reported (91). Serum from patients with autoimmune disorders in pregnancy are known to display high circulating levels of both fetal and maternal serum (MS) AFP levels (92). In fact, during pregnancy, MS-AFP

screening programs are in routine clinical use for the above mentioned fetal disorders. In keeping with such screening, abnormal serum AFP levels obtained from newborns, infantile, and juvenile patients with BD have been reported: these observation were also observed in BD mouse models (10). It is now apparent that AFP among other proteins, can serve as targets of autoimmune responses to exposed, hidden epitopes exposed on unfolded proteins in the brain of BD patients. Similarly, autoantibodies to AFP-hidden epitopes were detected in hepatocarcinoma patients. Cryptic epitopes may become exposed to the immune system secondary to events, such as trauma and necrosis, which may act by causing conformational change modifications of the protein and/or in the processing machinery (19). Human and rodent AFP are immunogenic in xenogenic animals, while they do not induce immune responses in the species of origin (93, 94). In summary, the unveiling of hidden epitope(s) on

AFP may result in an induction of autoantibodies, indicating the activation of B and T lymphocyte clones which were not eliminated during the immune tolerance development (95). Since denatured intermediate forms derived from tertiary-folded AFP resemble those of a disordered protein (Figure 1), it is logical to predict that AFP could indeed induce immune responses in various human diseases.

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