SUPPRESSION OF THE IMMUNE RESPONSE BY α-FETOPROTEIN

II. The Effect of Mouse α-Fetoprotein on Mixed Lymphocyte Reactivity and Mitogen-Induced Lymphocyte Transformation*

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The synthesis of α-fetoprotein (AFP)[1] is associated with the processes of normal, restorative, and malignant growth as reflected by its synthesis by the fetal liver during embryogenesis, its expression during hepatic regeneration after various forms of injury (1–3), and its appearance in the sera of certain tumor-bearing hosts, most notably in primary hepatocellular carcinomas (4, 5) and malignant teratoblastomas (6). The use of more sensitive methods of detection have subsequently demonstrated abnormally high levels of AFP in several nonmalignant diseases (7–9) as well as malignant diseases (10–12). While much is now known about the pathological distribution of AFP and its potential diagnostic importance, the functional significance of AFP and the mechanisms involved in regulating its synthesis during ontogenesis and pathogenesis are not understood. In the preceding article (13), we have proposed that AFP has an immunoregulatory function based on our demonstration that this protein, either in the isolated form or as it occurs in amniotic fluid, exerts a noncytotoxic immunosuppressive effect on the primary and secondary antibody response. In the present study we extend this concept by demonstrating that AFP suppresses certain T-cell-dependent functions such as allogeneic and mitogen-induced lymphocyte transformation.

Materials and Methods

Animals. CBA/J and BALB/c mice, purchased from Jackson Laboratories, Bar Harbor, Maine, were used in all experiments. Pregnant HA/IRC mice, bred locally at the Mayo Clinic, were used as a source of amniotic fluid (MAF).

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[1] *Abbreviations used in this paper:* AFP, α-fetoprotein; Con A, concanavalin A; GVH, graft-vs.-host; HAA, hepatitis-associated antigen; IRA, immunoregulatory alpha globulin; LPS, lipopolysaccharide; MAF, mouse amniotic fluid; PHA, phytohemagglutinin; SI, stimulation index.

Antisera. Antiserum to MAF was prepared in rabbits by subcutaneous injection of MAF supplemented with Freund's complete adjuvant as previously described. The antiserum was rendered specific for AFP by absorption with lyophilized normal adult mouse serum.

Isolation of Mouse AFP, Albumin, and Transferrin. MAF was collected from HA/IRC mice in the late second or early third trimester of pregnancy as described elsewhere (13). Approximately 1 ml of fluid was collected form each pregnant mouse. AFP was isolated from pooled fluid by a two-step procedure of antibody-agarose affinity chromatography (14) followed by preparative polyacrylamide gel electrophoresis (15). MAF was passed over an affinity column consisting of the γ-globulin fraction of a rabbit antimouse serum coupled by cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) to selectively remove serum components. Pure AFP was obtained by applying the fall-through (nonretained protein) of the affinity column to a Shandon model SAE-2782 preparative disc electrophoresis apparatus (Shandon Scientific Co., London, England) as previously described (13). The criteria of purity was a single band on analytical disc gel electrophoresis (16, 17) and a single precipitin arc on gel diffusion and immunoelectrophoresis using antiwhole amniotic fluid. Mouse transferrin and albumin were isolated from pooled normal mouse serum by a two-step procedure of Geon-Pevikon block electrophoresis followed by sucrose density gradient isoelectric focusing (18).

Mixed Lymphocyte Reactions. One-way mixed lymphocyte reactions were performed by treating BALB/c spleen cells at a density of 100×10^6/5 ml with 25 μg mitomycin C/ml cell suspension as described by Peck and Bach (19). After an incubation of 30 min at 37°C the cells were washed twice in cold Hanks' balanced salt solution, incubated again for 10 min at 37°C, and washed before placing the mitomycin-treated cells in culture. Stimulator spleen cells (mitomycin-treated BALB/c) were suspended in RPMI 1640 medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with penicillin (50 U/ml), streptomycin (50 μg/ml), and L-glutamine (200 mM, 2 ml/100 ml of medium). Respondor spleen cells (CBA/J) were suspended in the same medium plus 10% fetal calf serum which had been heated at 56°C for 30 min. All tissue culture medium ingredients were obtained from GIBCO. Mixed lymphocyte cultures contained 2×10^6 respondor cells plus 5×10^6 stimulator cells in 0.2 ml of medium in flat-bottom Micro-Test tissue culture plates (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). Control cultures contained cells from either source alone. Three replicates of each cell type or combination were done. Preparations to be tested for immunosuppressive activity were added in 0.02 ml at the start of culture. Plates were incubated in a humidified 5% CO_2-95% air atmosphere for 72 h. 1 μCi of tritiated thymidine ([^{3}H]TdR; 2 Ci/mmol; New England Nuclear, Boston, Mass.) was added for the last 12 h of culture. The cells were harvested using a microculture harvesting device, placed in 2–3 ml of aquasol (New England Nuclear) and the trichloroacetic acid insoluble radioactivity measured in a liquid scintillation spectrometer. The arithmetic mean of the triplicate samples was determined and the results are expressed as counts per minute. The stimulation index (SI) is calculated by dividing the counts per minute in mixed cultures by the sum of counts per minute in cultures of each cell type alone.

Mitogen Reactivity. BALB/c or CBA/J spleen cells were cultured to assay for reactivity to the mitogens phytohemagglutinin (PHA), concanavalin A (Con A), and lipopolysaccharide (LPS) using a microculture system as previously described (20). 1.75×10^6 spleen cells were cultured in triplicate in 0.1 ml of RPMI 1640 supplemented as described above. 0.1 ml of purified PHA (Wellcome Reagents Limited, Beckenham, England) in concentrations of 4.5 and 9.0 μg/ml, 0.1 ml Con A (two times crystallized, Nutritional Biochemical Corp., Cleveland, Ohio) in concentrations of 4.5 and 9.0 μg/ml, and 0.1 ml LPS (LPS from *Escherichia coli* 0127, Difco Laboratories, Detroit, Mich.) in concentrations of 15.7, 31.25, and 62.5 μg/ml, or medium only was added. Preparations to be tested for immunosuppressive activity were added in 0.02 ml at the start of culture. The cell cultures were incubated in flat-bottom plates in a humidified atmosphere of 95% air and 5% CO_2 for 48 h. 1 μCi of [^{3}H]TdR was added for the last 12 h of culture and the cells were harvested and the amount of radioactivity incorporated into acid-precipitable material was determined as described above.

Results

Effect of Mouse Amniotic Fluid and Its Isolated Protein Component on Mitogen Reactivity. Initial experiments showed that MAF effectively sup-

pressed the DNA synthesis of normal BALB/c spleen cells cultured in the presence of various concentrations of PHA, Con A, and LPS. MAF did not suppress the amount of [^{3}H]TdR incorporation of cells cultured in medium alone which demonstrated an absence of cytotoxicity of MAF for the cultured lymphocytes. This is consistent with our previous study (13) in which MAF and AFP had no significant effect on the cell viability of cultured lymphocytes. As shown in Table I, MAF inhibited PHA stimulation by 85%, Con A stimulation by 70%, and LPS stimulation by 80%. Thus, MAF contains a factor(s) which is highly suppressive for the T-cell-dependent mitogenic effect of PHA and Con A on splenic lymphocytes. Since it is generally considered that LPS can be used as

TABLE I
*Suppression of Mitogen-Induced Lymphocyte Transformation by Mouse AFP**

Preparation	No. stimulant	[^{3}H]TdR incorporation		
		PHA	Con A	LPS
		mean cpm ± SEM		
Control (media)	829±50	37,789±285	40,735±1,407	29,481±950
MAF	950±78	6,432±1,115	12,126±750	600±95
AFP (200 μg/ml)‡	720±34	2,903±260	3,592±595	515±60
Albumin (200 μg/ml)	885±150	26,540±1,540	25,920±1,980	19,610±2,800
Transferrin (200 μg/ml)	1,225±260	38,553±4,100	53,366±5,670	28,034±1,125
Normal mouse serum (200 μg/ml)	790±65	38,149±2,410	47,104±1,550	23,682±955

* Spleen cells from adult CBA/J mice were cultured at 1.75×10^6 cells/0.2 ml in the presence of optimal mitogenic concentrations of PHA (4.5 μg/ml), Con A (4.5 μg/ml), or LPS (31.25 μg/ml). DNA synthesis was measured by the incorporation of [^{3}H]TdR and is expressed as the mean of triplicate cultures ± the standard error of the mean (SE).

‡ Final concentration in the culture.

a functional marker of B-cell reactivity (21), there also appears to be suppressive activity in MAF for B cells although the specificity of LPS for B cells has recently been questioned (see Discussion).

To determine whether the immunosuppressive activity was associated with one of the three major protein components of MAF (albumin, transferrin, and AFP), these proteins were added to cultures individually in the isolated form. AFP was isolated from pooled MAF; however, insufficient amounts of albumin and transferrin for testing could be obtained from this source. For this reason, and also because the adult-type plasma proteins found in amniotic fluid are reported to be almost entirely of maternal origin (22), the albumin and transferrin used in this study were obtained from normal mouse serum. The results in Table I show that isolated AFP was highly suppressive for mitogen-stimulated cells. There was no significant effect of AFP on cultures to which just media or normal mouse serum were added. Equivalent amounts of albumin also suppressed but to a much smaller extent, whereas transferrin had a stimulatory effect that was particularly evident in the cells cultured in medium alone.

The results of dose-response studies with pure AFP, transferrin, and albumin on mitogen reactivity are listed in Table II. The suppressive activity of pure AFP diminished linearly over the 100-fold dilution range; however, suppression was still evident with a concentration of 1 μg/ml. In the experiment shown there was some depression of [^{3}H]TdR incorporation in media control cultures to which the higher concentrations of AFP were added. The reason for this is not clear since it was only noted when the background count in media control was relatively high and was not observed in other experiments where the background counts in the

TABLE II

*Dose-Response Effect of Mouse AFP, Albumin, and Transferrin on Mitogen-Induced Lymphocyte Transformation**

Preparation	No. stimulant	[^{3}H]TdR incorporation		
		PHA	Con A	LPS
		mean cpm ± SE		
Control (media)	2,948±327	40,582±5,625	45,020±7,223	28,385±1,860
AFP (100 μg/ml)‡	827±72	7,045±962	12,739±675	1,213±179
AFP (50 μg/ml)	1,432±137	8,817±3,305	13,331±4,677	2,138±154
AFP (10 μg/ml)	1,541±359	15,641±342	22,328±1,607	8,466±562
AFP (1 μg/ml)	2,383±130	26,658±2,239	33,432±1,335	17,878±875
Albumin (100 μg/ml)	4,923±853	40,106±4,527	43,588±261	29,393±2,979
Albumin (10 μg/ml)	5,648±163	44,453±2,619	49,526±5,035	35,915±3,818
Albumin (1 μg/ml)	4,820±369	49,179±2,387	53,029±14,000	31,412±3,747
Transferrin (100 μg/ml)	8,940±1,415	47,493±5,728	62,306±8,669	35,843±333
Transferrin (10 μg/ml)	6,574±327	48,351±2,092	61,638±1,811	46,238±1,146
Transferrin (1 μg/ml)	5,135±1,798	50,690±5,674	56,929±284	34,777±4,668

* Spleen cells from adult CBA/J mice were cultured at 1.75×10^6 cells/0.2 ml in the presence of optimal mitogenic concentrations of PHA (4.5 μg/ml), Con A (4.5 μg/ml), or LPS (31.25 μg/ml). DNA synthesis was measured by the incorporation of [^{3}H]TdR and is expressed as the mean of triplicate cultures ± the standard error of the mean (SE).

‡ Final concentration in the culture.

media controls were low and higher concentrations of the protein were added (Table I). The AFP preparation did not affect cell viability as measured by the ability of cells to exclude trypan blue. Perhaps the effect is due to suppression by AFP of proliferation of lymphocytes by stimulatory factors present in the batches of fetal calf serum which give a high background count. The suppressive effect observed with 200 μg/ml of albumin (Table I) was lost when the preparation was diluted to 100 μg/ml and lower concentration appeared to be slightly stimulatory. Transferrin retained its augmenting effect at all concentrations tested. In experiments to be reported in more detail elsewhere[2] we have shown that the stimulatory effect of transferrin on mixed lymphocyte reactivity is optimal at a concentration of approximately 50 μg/ml and augmentation diminishes as the amount of transferrin is increased or decreased from this optimal level. These results demonstrated that AFP was highly suppressive, albumin was slightly

[2] Murgita, R. A., and T. B. Tomasi, Jr. The effect of normal serum proteins on the immune response. Manuscript in preparation.

suppressive at the highest concentration tested (200 μg/ml), and that transferrin had a stimulatory effect on B- and T-cell-dependent mitogen reactivity.

Suppression and Augmentation of the Mixed Lymphocyte Reaction by MAF and Its Isolated Protein Components. Shown in Table III are the results of a dose-response effect of MAF on CBA splenic lymphocyte proliferation in cultures stimulated with mitomycin-treated BALB/c spleen cells. DNA synthesis in unstimulated cultures of CBA respondor cells alone was increased in cultures containing all but the highest dilution of MAF. The augmenting effect diminished as the MAF was diluted and disappeared at a 1/110 dilution.

TABLE III
Dose-Response Effect of MAF on the Mixed Lymphocyte Reaction

CBA	BALB/c*	Preparation	Mean 72 h cpm ± SE	SI
2×10^6	0		845±122	
2×10^6	5×10^6	Media	4,754±568	4.8
0	5×10^6		147±35	
2×10^6	0		3,008±488	
2×10^6	5×10^6	MAF (1/12)‡	2,442±355	0.7
0	5×10^6		500±72	
2×10^6	0		1,500±196	
2×10^6	5×10^6	MAF (1/55)	2,696±210	1.6
0	5×10^6		185±47	
2×10^6	0		966±145	
2×10^6	5×10^6	MAF (1/110)	2,181±355	1.9
0	5×10^6		168±41	
2×10^6	0		454±187	
2×10^6	5×10^6	MAF (1/550)	2,547±342	4.3
0	5×10^6		133±38	

* Mitomycin C treated (stimulator cells).
‡ Final dilution of MAF in the culture.

However, the degree of suppression of the allogeneic mixture remained relatively constant over the 500-fold dilution range of MAF. The combination of the suppressing and augmenting effects of MAF resulted in an almost linear increase of the SI with decreasing amounts of MAF added to the cultures. Therefore, observations of the SI's alone indicated a suppressive effect of MAF on mixed lymphocyte reactivity which decreased proportionally to decreasing amounts of MAF in the culture. However, the individual counts per minute of the respondor cells and the allogeneic mixture more accurately reflected that MAF exerted both augmenting and suppressing effects on the cultures. Experiments comparing the effects of dialyzed vs. undialyzed MAF suggested that part of the augmenting activity of MAF was dialyzable.

The results shown in Table IV demonstrated that isolated AFP was highly

TABLE IV
Suppression of the Mixed Lymphocyte Reaction by Purified Mouse AFP

CBA	BALB/c*	Preparation	Mean 72 h cpm ± SE	SI
2×10^6	0		780±98	
2×10^6	5×10^6	Media	3,846±121	4.31
0	5×10^6		111±25	
2×10^6	0		550±110	
2×10^6	5×10^6	AFP (200 μg/ml)	301±86	0.46
0	5×10^6		95±21	
2×10^6	0		355±75	
2×10^6	5×10^6	Albumin (200 μg/ml)	1,351±112	2.10
0	5×10^6		287±56	
2×10^6	0		227±18	
2×10^6	5×10^6	Albumin (100 μg/ml)	3,074±234	7.51
0	5×10^6		182±45	
2×10^6	0		514±45	
2×10^6	5×10^6	Transferrin (200 μg/ml)	6,965±327	10.29
0	5×10^6		163±10	
2×10^6	0		717±156	
2×10^6	5×10^6	Normal mouse serum (200 μg/ml)‡	3,239±787	3.63
0	5×10^6		175±37	

* Mitomycin C treated (stimulator cells).
‡ Final concentration in the culture.

suppressive, albumin inhibited at 200 μg/ml but not at 100 μg/ml, and that transferrin exerted an augmenting effect on the mixed lymphocyte reaction. As demonstrated in Table V, AFP strongly inhibited the mixed lymphocyte reaction at 100 and 10 μg/ml and was still slightly suppressive at a concentration of 1 μg/ml. In general, therefore, the effects of AFP and the other constituents of MAF on mixed lymphocyte reactivity are similar to the effects of these same proteins on mitogen-induced lymphocyte transformation.

Discussion

AFP, in concentrations comparable to those we have shown to suppress antibody synthesis (13), effectively inhibits normal splenic lymphocyte stimulation by T-cell mitogens (PHA and Con A), a presumed B-cell mitogen (LPS), and mitomycin-treated allogeneic spleen cells in a one-way mixed lymphocyte reaction. Dose-response studies demonstrated that AFP was still suppressive at 1 μg/ml. The addition of MAF, shown previously to contain approximately 36% albumin, 50% AFP, and 14% transferrin (13), also inhibited allogeneic and mitogen-induced lymphocyte transformation. However, a simple quantitative

TABLE V
Dose-Response Effect of Mouse AFP on the Mixed Lymphocyte Reaction

CBA	BALB/c*	Preparation	Mean 72 h cpm ± SE	SI
2×10^6	0		505 ± 64	
2×10^6	5×10^6	Media	3,027 + 152	5.07
0	5×10^6		92 ± 14	
2×10^6	0		609 ± 192	
2×10^6	5×10^6	AFP (100 μg/ml)‡	1,136 ± 270	1.59
0	5×10^6		103 ± 13	
2×10^6	0		372 ± 63	
2×10^6	5×10^6	AFP (10 μg/ml)	1,972 ± 129	3.73
0	5×10^6		156 ± 73	
2×10^6	0		559 ± 88	
2×10^6	5×10^6	AFP (1 μg/ml)	2,313 ± 225	3.29
0	5×10^6		144 ± 10	
2×10^6	0		662 ± 97	
2×10^6	5×10^6	AFP (0.1 μg/ml)	2,847 ± 163	3.77
0	5×10^6		93 ± 7	

* Mitomycin C treated (stimulator cells).
‡ Final concentration in the culture.

comparison between the suppressive effects of isolated AFP with an equivalent amount of MAF was not possible because of the presence of stimulatory factor(s) also present in MAF (Table III). The basis of the augmenting effect of MAF is not known; however, it was only partly removed by exhaustive dialysis of MAF. The nondialyzable augmenting factor may be transferrin since this protein, when isolated, had a significant stimulatory effect on DNA synthesis in control as well as mitogen and allogeneically stimulated cultures. Albumin, at a concentration of 200 μg/ml, partially inhibited mitogen stimulation (Table I) and the mixed lymphocyte reaction (Table IV); however, this effect was no longer evident at 100 μg/ml, in fact, lower concentrations appeared to be slightly stimulatory. It is not entirely clear whether the effect of LPS on mouse lymphocytes is exclusively on B cells as originally reported (23, 24) or if T cells may also be involved. Recent data (25) suggest that the mitogenic effect of LPS on B cells may require the participation of T cells. Therefore, it is difficult to be certain at this time if the suppressive effect of AFP on LPS stimulation is due to a direct effect of AFP on B cells or whether it is acting via a T cell.

An α-globulin fraction isolated from normal plasma has been shown to suppress several T-cell-mediated immune reactions, including graft rejection (26), the elaboration of macrophage immobilization factor (27), lymphocyte transformation induced by PHA and allogeneic stimulation (28), and sheep erythrocyte rosette formation with human lymphocytes (29). It is suggested that the immunosuppressive α-globulin (termed immunoregulatory α-globulin or IRA) represents a naturally occurring feed-back control mechanism which exists

to regulate T-cell-dependent immune reactions (29). Since the active fractions of IRA contain primarily α_1- and α_2-globulins (28, 29) and since AFP is present in normal adult sera (3) it has not been excluded that the suppressive effects observed with IRA preparations are partly due to low levels of AFP in these fractions. However, recent studies (30) suggest that the active moiety of IRA is a peptide which is noncovalently bound to proteins with an electrophoretic mobility in the α-region. Although our findings do not exclude the possibility of a suppressive peptide tightly bound to AFP, for reasons previously discussed (13) we believe that AFP is not serving as a carrier and is probably not related to IRA.

The synthesis of AFP is a normal physiological event during embryonic development, and if this glycoprotein has immunoregulatory activity as we postulate, it may serve as a natural mechanism to protect the fetus from the potentially harmful effects of the maternal immune mechanism. There appears to be no intrinsic defect in the maternal cellular immune mechanism in most reports (31, 32). Moreover, there is some evidence (33) for an extrinsic factor present in maternal serum which has an inhibitory effect on the response of normal adult lymphocytes to nonspecific mitogen stimulation and the authors noted a positive correlation between the level of AFP and the inhibitory activity in the fetal plasma. Therefore, further studies would be in order to determine whether the lowered maternal cell-mediated immunity during pregnancy manifested by impaired lymphocyte responses to nonspecific mitogen (34, 35), infectious agents (36), and homografts (37) may result, at least in part, from immunosuppression levels of AFP.

Although it is known that the mammalian fetus is capable of expressing an active immunological response to certain antigenic stimuli in utero (38), numerous studies have demonstrated a reduced capacity of the fetus and newborn to respond immunologically (reviewed in 39). The gradual acquisition of full immunological responsiveness by the fetus certainly depends on quantitative changes in the lymphoid cell populations required for the immune response. However, in addition, there is evidence which suggests that the cellular constituents necessary to initiate an immune response are present in the developing young before active immune reactivity normally occurs. In mice, B cells, T cells, and antigen-binding cells all appeared in the fetal spleen at about 15–16 days of a 19-day gestation period and their numbers increased rapidly reaching a plateau by 1 wk after birth (40). However, the capacity to response to antigen did not arise until about 2 wk after birth despite the fact that there were no quantitative changes in the total numbers of T, B, and antigen-binding cells between 1 and 2 wk of age. Other studies have shown that while neonatal thymus cells are poorly responsive to Con A stimulation, adult thymocytes are markedly stimulated by this mitogen, despite the fact that both neonatal and adult mice possess similar numbers and frequency of θ-bearing cells (41). Full reactivity to Con A is only achieved after the first 3 wk of life. Although these results can be interpreted as indicating the necessity for subtle pre- and postnatal maturational events necessary for the full development of immune capacity, they are also consistent with the presence of a fetal and neonatal circulating suppressive factor(s). Appropriate to this point is an earlier report by Dixon and Weigle (42) demonstrating the relatively poor immune response of sensitized adult lymphoid

cells when transferred to neonatal recipients whereas such cells were capable of strong antibody responses when transferred to X-irradiated adult recipients. The authors suggested that the neonatal rabbit did not offer the transferred immunocompetent adult cells an "environment" conducive to antibody formation. Conversely, they showed that while immune cells from neonatal rabbits did not function optimally in antibody formation in their natural environment, they did when transferred to X-irradiated adult recipients (43).

Fetal cells have been reported (44) to undergo cellular reactions and this has been cited as important in preventing graft-vs.-host (GVH) reactions from transmitted maternal cells. Although this may be valid, an alternate explanation is that the fetal cells have been removed from the suppressive effects of AFP during isolation and washing. Moreover, it is conceivable that both fetal and transmitted maternal cells are simultaneously suppressed in vivo, thus preventing the development of a GVH reaction. According to this hypothesis, after birth as the AFP concentration decreases and immune reactivity appears, transmitted maternal cells would then be rejected. This thesis suggests several experimental approaches such as studies of the time of rejection of transmitted maternal cells and the relative sensitivity of fetal vs. maternal lymphocytes to suppression by AFP. For example, if fetal lymphocytes escape suppression at higher concentrations of AFP than maternal cells this would lead to the rejection of maternal cells at the point in development (either before or after birth) when the AFP levels dropped to a critical level. Other survival advantages to the fetus and young neonates of immune suppression during ontogenetic development have already been discussed (13).

Serum factors which inhibit lymphocyte transformation in vitro in response to nonspecific mitogens and/or specific antigens have been described in a number of pathological conditions (45) which include ataxia telangiectasis (46), hepatitis (47–49), alcoholic and primary biliary cirrhosis (50, 51), and gastrointestinal cancer (52). This group of pathological conditions is of particular interest here since abnormally high AFP levels have been reported in most of these diseases (7–11). Based on our demonstration of the immunosuppressive activity of AFP, we postulate that AFP may be one, and possibly an important factor causing the nonspecific immune suppression reported in many of the diseases mentioned above. Furthermore, as we have stated before (13) and stress here, it is possible that local immune suppression in the absence of elevated serum levels of AFP could be caused by the production of suppressive concentrations of AFP within and around the microenvironment of the disease process (i.e., proliferating tumor cells). The presence of extremely low (nanogram) levels of AFP in normal adult sera (53) together with our demonstration that AFP is immunosuppressive in vitro at concentrations of the order of 1 μg/ml makes the suggestion tenable that immune suppression accompanying certain diseases may be attributable to functionally significant levels of AFP at the local level.

The fact that AFP reappears in postnatal life with regularity in association with certain malignant diseases (4–11) has led to its classification as a tumor-associated embryonic antigen (54). At present at least three situations are known to exist showing a relationship between the occurrence of unusually high AFP levels with subsequent development of malignancy. Firstly, in different

strains of mice an association between the level of AFP in adult serum and susceptibility to spontaneous and carbon tetrachloride-induced hepatomas has been noted (3). Secondly, in humans an association has been reported between an abnormal thymus, immune deficiency, and malignancy in patients with ataxia telangiectasia (55), and in one report (9) all patients had raised serum AFP concentrations. Thirdly, it has been suggested (56) that cell-mediated immunity is an important factor in determining the clinical course of the liver disease associated with the presence of Australian or hepatitis-associated antigen (HAA). It has been postulated that viral hepatitis associated with HAA can progress through postnectoric cirrhosis to the development of hepatomas and there is a tendency for HAA-positive individuals with hepatocellular carcinoma to be AFP positive and to have underlying cirrhosis (57). These circumstances are consistent with a relationship between AFP, the persistence of HAA, depressed cellular immunity, and its progression to cirrhosis and/or malignant disease. We recognize fully that the intriguing suggestions raised above concerning the role of AFP in normal development and in certain disease states are at present speculative but hope that they will stimulate further investigation of these potentially important interrelationships.

Summary

α-fetoprotein (AFP), both in the isolated form and as it occurs in amniotic fluid, was shown to suppress the mitogenic effects of phytohemmaglutinin, concanavalin A, and lipopolysaccharide on mouse spleen cells and to inhibit allogeneic lymphocyte stimulation in the one-way mixed lymphocyte reaction. Dose-response experiments demonstrated that the suppression effect of AFP on the response to mitogens and to allogeneic cells diminished linearly with inhibitory activity still evident at 1 μg/ml. There was no significant effect of AFP on control cultures to which media alone was added and the cell viabilities of cultures containing mouse amniotic fluid (MAF) or AFP did not differ from control cultures. Unfractionated MAF had in addition to suppressive activity, a stimulatory effect on lymphocyte reactions in vitro which was particularly evident in the mixed lymphocyte reaction. The augmenting activity in MAF was partly removed by dialysis. The addition of mouse transferrin to the cultures had a stimulatory effect whereas albumin was slightly suppressive at 200 μg/ml but not at lower ($\leq$100 μg/ml) concentrations. Since, in addition to AFP, albumin and transferrin are the two major protein components of MAF it is concluded that the suppressive activity of MAF is attributable to AFP while the stimulatory activity is due in part to low molecular weight (dialyzable) factors and in part to transferrin. The results of this investigation are discussed in terms of the concept that AFP has an immunoregulatory function in vivo.

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